

Evidence of Cryptic Species Within the Pest *Copitarsia decolora* (Guenée) (Lepidoptera: Noctuidae)

REBECCA B. SIMMONS¹ AND SONJA J. SCHEFFER²

Ann. Entomol. Soc. Am. 97(4): 675–680 (2004)

ABSTRACT *Copitarsia decolora* (Guenée) is a widely distributed, highly polyphagous pest found throughout Central and South America. We examined multiple populations of *C. decolora* with the mitochondrial gene cytochrome oxidase I (COI). We also investigated morphological variation in adult males sampled from these populations. COI evidence suggests that *C. decolora* is comprised of two cryptic lineages and is paraphyletic with respect to the Chilean *C. naenoides* (Butler). These two lineages, which are not completely geographically isolated, display high divergence in COI sequences compared with other members of *Copitarsia* and other insect pest species in general. Furthermore, these lineages also possess slight morphological differences in male genitalia; however, supporting information is necessary before the taxonomy of this species is revised.

KEY WORDS cryptic species, genetic variation, morphology, phylogeny, paraphyly

MITOCHONDRIAL DNA HAS BECOME a common tool for examining species limits, intraspecific relationships of populations, and haplotype distribution (Sperling et al. 1999, Scheffer 2000, Scheffer and Lewis 2001). Such data are also capable of revealing cryptic lineages representing distinct species within geographically widespread and apparently morphologically homogeneous organisms (Scheffer 2000, Weller et al. 2004). In some cases, mitochondrial evidence of morphologically cryptic species is later corroborated with other data sources (Scheffer and Lewis 2001). Information about cryptic species is important when determining control strategies for insect pests; cryptic species may differ from known species in many attributes, such as host preferences, insecticide resistance, or responses to varying environmental conditions (Miller and Rossman 1995).

Members of the moth genus *Copitarsia* (Noctuidae) are polyphagous pests on at least 39 crop plants from 19 plant families in Mexico, Central America, and South America. The most economically important member of *Copitarsia* is *Copitarsia decolora* (Guenée) (Fig. 1), which feeds on a variety of crops, including cut flowers, lettuce, peas, beets, carrots, beans, and potatoes (Castillo and Angulo 1991, Arce de Hamity and Neder de Roman 1992). *C. decolora* seems to be widely distributed in Central America and South America and has been reported from Mexico to Chile and east to Argentina. *C. decolora* is routinely intercepted on produce at U.S. ports of entry. Because of its polyphagy and wide geographic distribution, *C. decolora* is considered to represent a threat to U.S. agriculture (Venette and Gould 2003). This species

historically has been misidentified as *C. incommoda* (Walker) in both the agricultural and taxonomic literature (Castillo and Angulo 1991, Arce de Hamity and Neder de Roman 1992, Angulo and Olivares 2003); this issue will be addressed elsewhere.

Another pest species within *Copitarsia*, *C. naenoides* (Butler), is more restricted both geographically and in host plant preference. Known as the brown flax armyworm, *C. naenoides* feeds on a variety of commodities: radish, asparagus, beetroot, potato, sugar beet, tobacco, and linen (Klein Koch and Waterhouse 2000, Angulo and Olivares 2003). *C. naenoides* is found in the central and southern regions of Chile and in northern Argentina (Klein Koch and Waterhouse 2000, Angulo and Olivares 2003). *C. naenoides* can be distinguished from *C. decolora* by its smaller overall size and its overall reddish-brown wing coloration (personal observations). We included *C. naenoides* to examine intraspecific relationships within *C. decolora* and to compare levels of genetic divergence.

Copitarsia decolora may consist of a cluster of unrecognized cryptic species that may or may not be morphologically distinct. With the mitochondrial gene cytochrome oxidase I (COI), we examine mitochondrial variation within *C. decolora* to evaluate whether distinct host races or cryptic lineages are present. From this information, we found that *C. decolora* is comprised of two distinct genetic lineages that are morphologically similar. We make no taxonomic changes at this time; further morphological sampling is needed to corroborate these results and to better understand the geographic range of each lineage.

Materials and Methods

Gene Region and Analysis. Individuals were collected in the field by R. B. S. or as border interceptions

¹ USDA/ARS/PSI/SEL, Smithsonian Institution, PO Box 37012, Natural History Museum, MRC-0168, Washington, DC 20013-7012 (e-mail: rsimmons@sel.barc.usda.gov).

² USDA/ARS/PSI/SEL, 10300 Baltimore Ave., Bldg. 005, Beltsville, MD 20705.



Fig. 1. *Copitarsia decolora* (Guenée) from Mexico, adult habitus.

by USDA/APHIS personnel (Table 1). Larval abdominal segments or one to two adult legs were used to extract DNA from fresh material. Most of the representatives sequenced in this study were larvae. Adult museum specimens were used to obtain further DNA

samples from Chile and Peru (12 individuals). DNA was extracted from two to three legs per museum specimen, which were <32 yr old. A total of 75 individuals of *C. decolora* were sampled from four countries: Colombia, Ecuador, Peru, and Mexico. Repre-

Table 1. List of specimens used in mtDNA study

Genus species	Stage	Country/year	Host plant (family)	N	Haplotype no.	GenBank no.	
<i>Agrotis</i> sp.	A	Chile/2001	—	1	—	AY375158	
	L	Peru/2001	—	2	—	AY375157, AY375159	
<i>C. decolora</i>	L	Colombia/2001-2	<i>Alstroemeria</i> (Liliaceae)	13	1, 2, 7	AY203877, AY203882, AY203875, AY203873, AY203871, AY203868, AY203866, AY203850, AY203847, AY203894, AY203893, AY203887, AY203884	
	L	Colombia/2002	<i>Limonium</i> (Plumbaginaceae)	2	1	AY203883, AY203892	
	L	Colombia/2002	<i>Molucella</i> (Lamiaceae)	1	1	AY203876	
	L	Colombia/2002	<i>Callostephus</i> (Asteraceae)	2	7	AY203867, AY203888	
	L	Colombia/2002	<i>Aster</i> (Asteraceae)	3	7	AY203865, AY203846, AY203885	
	L	Colombia/2002	Mixed flowers	2	1	AY203864, AY203890	
	L	Colombia/2002	<i>Lysimachia</i> (Primulaceae)	1	7	AY203890	
	L	Ecuador/2002	Mixed flowers	1	7	AY203879	
	L	Ecuador/2002	<i>Limonium</i> (Plumbaginaceae)	3	1, 6	AY203880, AY203891, AY203874	
	L	Ecuador/2001-2	<i>Hypericum</i> (Clusiaceae)	3	3, 4	AY203881, AY203849, AY203848	
	L	Ecuador/2002	<i>Alstroemeria</i> (Liliaceae)	2	1, 7	AY203878, AY203870	
	L	Ecuador/2002	<i>Campanula</i> (Campanulaceae)	1	7	AY203886	
	L	Ecuador/2002	<i>Ammi</i> (Apiaceae)	1	6	AY203872	
	L	Ecuador/2002	<i>Amaranthus</i> (Amaranthaceae)	1	6	AY203869	
	L	Mexico/2001	Husk tomato (Solanaceae)	6	1, 3, 5	AY203835, AY203836, AY203830, AY203824, AY203821, AY203819	
	A	Mexico/1999	<i>Argemone</i> (Papaveraceae)	2	1, 3	AY203853, AY203852	
	L	Mexico/2001	<i>Apium</i> (Apiaceae)	1	3	AY203837	
	L	Mexico/2001	<i>Brassica</i> (Brassicaceae)	6	1, 3, 5	AY203834, AY203832, AY203831, AY203828, AY203826, AY203816	
	L	Mexico/2001	<i>Coriandrum</i> (Apiaceae)	6	3, 5	AY203833, AY203829, AY203822, AY203820, AY203817, AY203813	
	L	Mexico/2001	<i>Chenopodium</i> (Chenopodiaceae)	2	1, 3	AY203830, AY203827	
	L	Mexico/2001	Chamomile (Asteraceae)	1	3	AY203825	
	L	Mexico/2001	<i>Suaeda</i> (Lamiaceae)	3	3	AY203823, AY203818, AY203814	
	L	Peru/2001	Asparagus (Liliaceae)	6	6, 7, 8	AY203896, AY203838, AY203842, AY203841, AY203840, AY203839	
	A	Peru/2001	Asparagus (Liliaceae)	6	6, 7, 8	AY203863, AY203862, AY203860, AY203861, AY203859, AY203858	
	<i>C. humilis</i>	A	Chile/2002	?	1	—	AY203812
	<i>C. naenoides</i>	A	Chile/1981	?	3	—	AY203855, AY203856, AY203857
	<i>Peridroma saucia</i>	L	Peru/2001	Artichoke (Asteraceae)	1	—	AY203844
L		Peru/2001	Potato (Solanaceae)	1	—	AY203843	
L		Ecuador/2002	<i>Dianthus</i> (Caryophyllaceae)	1	—	AY203811	
A		Peru/?	?	1	—	AY203845	

N, sample size; —, not applicable; L, larva; A, adult.

Table 2. Geographic distribution and host plant preference of *C. decolora* haplotypes

Haplotype	<i>n</i>	Country	Host plant	
1	1	Colombia	Aster	
	11	Colombia	Alstroemeria	
	2	Colombia	Limonium	
	1	Colombia	Mixed flowers	
	1	Colombia	Mollucella	
	1	Ecuador	Alstroemeria	
	2	Ecuador	Limonium	
	1	Mexico	Argemone	
	2	Mexico	Brassica	
	1	Mexico	Chenopodium	
	1	Mexico	Husk tomato	
	2	1	Colombia	Alstroemeria
		3	Mexico	Apium
	3	1	Mexico	Argemone
		3	Mexico	Brassica
1		Mexico	Chamomile	
1		Mexico	Chenopodium	
5		Mexico	Coriander	
4		Mexico	Husk tomato	
3		Mexico	Suaeda	
4		2	Ecuador	Hypericum
		1	Mexico	Brassica
5		1	Mexico	Coriander
	1	Mexico	Husk tomato	
	1	Ecuador	Amaranthus	
6	1	Ecuador	<i>Ammi majus</i>	
	1	Ecuador	Limonium	
	2	Peru	Asparagus	
	1	Colombia	Alstroemeria	
7	2	Colombia	Aster	
	2	Colombia	Callostephus	
	1	Colombia	Lysimachia	
	1	Ecuador	Alstroemeria	
	1	Ecuador	Campanula	
8	1	Ecuador	Mixed flowers	
	3	Peru	Asparagus	
	6	Peru	Asparagus	

n, number of individuals.

cies). To assess the confidence of the resulting trees, we resampled both data sets using 500 pseudoreplicates of bootstrapping (Felsenstein 1985). We then used maximum likelihood (ML) to investigate relationships among haplotypes. Tree searches were performed heuristically with TBR branch swapping in a random stepwise addition of taxa repeated 100 times. Nucleotide frequencies used were found empirically with the HKY85 model to address unequal base frequencies. Branch support was assessed with 100 replicates of nonparametric bootstrapping.

Morphology. Standard genitalia dissections were performed for the 12 adult specimens (Table 1) that were amplified and sequenced in this study to verify identifications (Winter 2000). Abdomens were softened in warm 10% KOH for 5–15 min and cleaned (scales and viscera removed) in several rinses of 40% ethanol. Abdominal sclerites and genitalia were stained with chlorazol black E (Sigma, St. Louis, MO) dissolved in deionized distilled water (saturated). Specimens were viewed in 40% ethanol. Permanent slide mounts (Euparal, Bioquip Gardenia, CA) were made of abdominal pelts and genitalia (Winter 2000). Terminology for abdominal and genital morphology follows Klots (1970) and Forbes (1939).

Collections consulted include The Natural History Museum, London (BMNH); Muséum National d'Histoire Naturelle, Laboratoire d'Entomologie, Paris (MNHP); Museo Zoología de Universidad de Concepción, Chile (MZUC); and the U.S. National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM). The type specimen of *C. decolora* and those of recognized synonyms were examined to verify identifications of adult specimens (Poole 1989) including the following: *Agrotis hostilis* Walker (BMNH), *Graphiphora sobria* Walker (BMNH), *Mamestra decolora* Guenée (MNHP), *Mamestra inducta* Walker (BMNH), and *Spaelotis subsignata* Walker (BMNH). Digital photographs were made of selected specimens.

Results

COI in *Copitarsia*. The obtained 453-bp fragment confirmed COI sequence for a majority of the individuals listed in Table 1. In *Copitarsia*, COI has an A/T bias, typical of insects (A: 31%, T: 39%); this bias is especially pronounced in third codon positions (A: 44%, T: 47%). Of the 453 characters in the final dataset, 376 are constant, 29 are noninformative, and

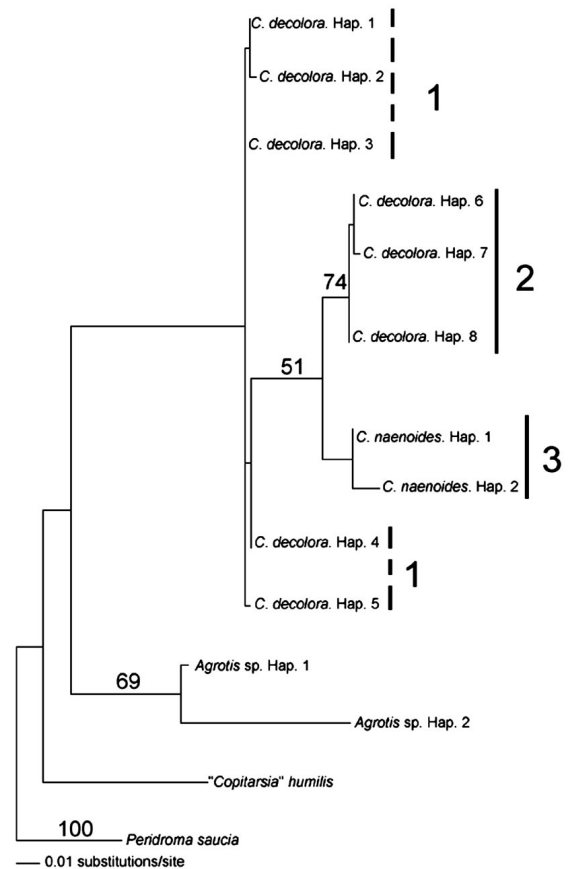


Fig. 3. Maximum likelihood topology of unique haplotypes (-Ln likelihood = 1186.93; bootstrap values >50% are given above supported branches; numbers 1, 2, and 3 refer to clades discussed in text).

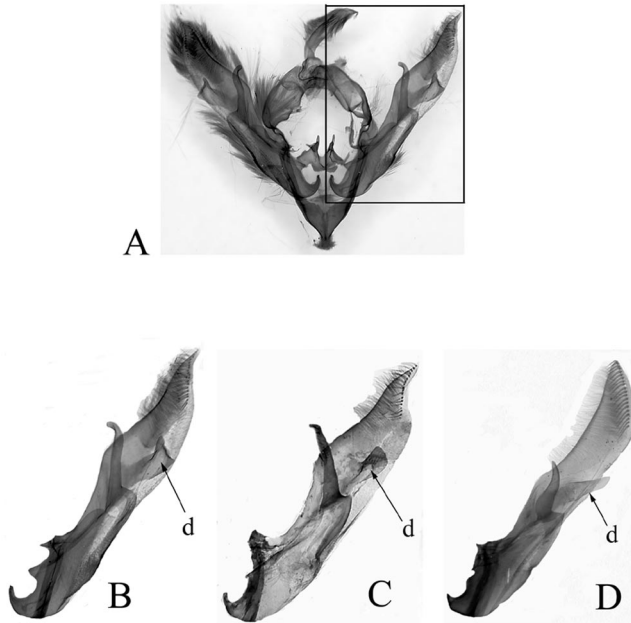


Fig. 4. Male genitalia. (A) *C. decolora* (clade 1; Mexico), entire capsule. (B) *C. decolora* (clade 1; Mexico), valve. (C) *C. decolora* (clade 2; Peru), valve. (D) *C. naenoides* Butler (clade 3; Chile), valve (box = magnified area of valve, d = digitus).

48 are phylogenetically informative. Again, the majority of the informative characters are found in third positions (46 versus 2 and 0 in the first and second positions, respectively). Within *C. decolora* there is a maximum of 4.2% divergence in COI sequences (AY203881 versus AY203880). In contrast, there is 0.7% divergence in the sampled *C. naenoides*; sequences were identical for *P. saucia*.

Eight haplotypes were recovered from *C. decolora* (Table 1; Fig. 2). The most common haplotype (1) was found in 25 samples from Colombia, Ecuador, and Mexico (Table 1). No clear host plant partitions are displayed by haplotype distribution (Table 2).

Phylogenetic Trees Based on COI. Analysis of these data by parsimony resulted in six trees ($L = 114$ steps, $CI = 0.86$, $RI = 0.95$, phylogram shown in Fig. 2). *Copitarsia humilis* is not found within the ingroup, indicating that it is not congeneric with other species of *Copitarsia*. This observation is supported by both morphological data and evidence from the nuclear gene elongation factor-1 α (EF-1 α) (unpublished data). The monophyly and species composition of *Copitarsia* will be examined in future studies.

Two lineages within *C. decolora* are present (clades 1 and 2; Fig. 2). Clade 1 contains haplotypes 1–5 and has a mixed distribution within Colombia, Ecuador, and Mexico. This clade has strong bootstrap support (93%). Clade 2, containing haplotypes 6–8, is exclusively South American and has high bootstrap support (80%; Fig. 1). Clade 2 is a sister to *C. naenoides* (clade 3), rendering *C. decolora* paraphyletic. This relationship has moderate bootstrap support (60%; Fig. 2).

ML analyses support the parsimony results (Fig. 3). Although clade 1 has high bootstrap support in the

parsimony trees, it is not supported by likelihood methods (Fig. 3). This result is likely because of the bias of transitions relative to transversions, particularly in the third codon position. This bias results in downweighting of these characters during the ML analysis, which in turn downweights characters supporting clade 1. Clades 2 (*C. decolora* in part) and 3 (*C. naenoides*) are again found to be sisters, although this relationship has low bootstrap support (Fig. 3).

Discussion

Uncorrected pairwise divergences within *C. decolora* suggest the presence of two distinct lineages. Within *C. decolora*, variation in COI is higher (4.2%) than that previously reported for many other pest species (e.g., Andreev et al. 1998, Sperling et al. 1999, Evans et al. 2000, Scheffer and Wiegmann 2000, Scheffer and Lewis 2001). Considerably less mitochondrial variation was found within the species *C. naenoides* (0.7%) and *P. saucia* (0%), although these species were not as extensively sampled as *C. decolora*. High levels of divergence within *C. decolora* and its paraphyly with respect to *C. naenoides* suggest that cryptic species may be present.

The geographic overlap between the cryptic lineages in *C. decolora* raises the issue of identifying sympatric, cryptic species. Representatives of clades 1 and 2 not only overlap geographically (Colombia and Ecuador) but also use the same hosts within these areas (*Limonium*, *Alstroemeria*, *Aster*; Table 2). Presumably, these representatives could be present in the same community. Unfortunately, specific locality information is not available for most of these specimens, because they were collected at U.S. ports of entry

by APHIS personnel. Further morphological studies will examine the possibility of sympatry of clades 1 and 2.

Despite the strong mitochondrial pattern of divergence, a single gene tree is not indisputable evidence for the presence of cryptic species (Sperling et al. 1999, Scheffer 2000). It is possible that the mitochondrial divergence instead reflects intraspecific polymorphism (Sperling et al. 1999). Preliminary morphological evidence from male genitalia, however, seems to support the presence of cryptic lineages (Fig. 4). Males from Mexico (clade 1) have a blunt, square-shaped digitus on the male valve (Fig. 4, A and B) that match the genitalia of the type specimen for *C. decolora* (R. B. S., personal observation), whereas males from Peru (clade 2) possess a digitus that is more rounded terminally (Fig. 4C). Male *C. naenoides* (Clade 3) have a digitus that is more rounded apically (Fig. 4D); members of clade 2 seem to be morphologically intermediates between clades 1 and 3 for this character. Members of clade 2 are distinct from *C. naenoides* (clade 3) both in external appearance and in female genitalia (data not shown). *C. naenoides* tend to be reddish brown in color, and females have a pleated antevagellar plate; members of clade 2 are gray brown in color, and females have a spinose antevagellar plate. While these slight differences may be further evidence of an undescribed species within *C. decolora*, a larger geographic sample is necessary to confirm the consistency of these morphological differences. Hence, any revision to the current taxonomy would be premature because our observations are based on <10 adult specimens.

This study represents a starting point for investigating cryptic lineages in this widely distributed and polyphagous noctuid. Subsequent analysis using nuclear markers and greater morphological sampling will be necessary to fully determine whether the clades uncovered by this study reflect intraspecific polymorphism or interspecific divergence.

Acknowledgments

M. L. Lewis (USDA/ARS/SEL) provided valuable technical advice and assistance for the molecular portion of this study. We also thank D. Carter (BMNH), D. Goodger (BMNH), J. Minet (MNHP), A. Angulo (MZUC), and T. Olivares (MZUC) for the loan of specimens and hospitality while visiting collections. J. Gould, T. Scarlinsky, T. Dobbs, C. Bodell, and many other USDA/APHIS personnel provided fresh specimens of *Copitarsia*. L. Castlebury, T. Henry, and two anonymous reviewers provided thoughtful insights that clarified and improved the manuscript. This work was supported by USDA/ARS/PSI/SEL and USDA/APHIS/PPQ.

References Cited

- Andreev, D., H. Breilid, L. Kirkendall, L. O. Brun, and R. H. French-Constant. 1998. Lack of nucleotide variability in a beetle pest with extreme inbreeding. *Insect Mol. Biol.* 7: 197–200.
- Angulo, A. O., and T. S. Olivares. 2003. Taxonomic update of the species of *Copitarsia* Hampson (Lepidoptera: Noctuidae: Cucullinae). *Guyana*. 67: 33–38.
- Arcé de Hamity, M. G., and L. E. Néder de Roman. 1992. Aspectos bioecológicos de *Copitarsia turbata* (Herrich-Schäffer) (Lepidoptera: Noctuidae) importantes en la determinación del daño económico en cultivos de *Latuca sativa* L. de la quebrada de Humahuaca, Jujuy, Argentina. *Revta. Soc. Entomol. Argent.* 50: 73–87.
- Castillo, E. E., and A. O. Angulo. 1991. Contribution to the knowledge of the genus *Copitarsia* Hampson, 1906 (Lepidoptera: Glossata: Cucullinae). *Guyana Zool.* 55: 227–246.
- Clary, D. O., and D. R. Wolstenholme. 1985. The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetics. *J. Molec. Evol.* 22: 252–271.
- Evans, J. D., J. S. Pettis, and H. Shimanuki. 2000. Mitochondrial DNA relationships in an emergent pest of honey bees: *Aethina tumida* (Coleoptera: Nitidulidae) from the United States and Africa. *Ann. Entomol. Soc. Am.* 93: 415–420.
- Felsenstein, J. F. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*. 39: 783–791.
- Forbes, W. T. M. 1939. The muscles of the lepidopterous male genitalia. *Ann. Entomol. Soc. Am.* 32: 1–10.
- Hampson, G. F. 1906. Catalogue of the Lepidoptera Phalaenae in the British Museum, vol. 6. Taylor and Francis, London, United Kingdom.
- Klots, A. B. 1970. Lepidoptera, pp. 115–130. In S. L. Tuxen (ed.), *Taxonomist's glossary of genitalia in insects*, 2nd ed. Munksgaard, Copenhagen, Denmark.
- Klein Koch, C., and D. F. Waterhouse. 2000. Distribución e importancia de los artrópodos asociados a la agricultura y silvicultura en Chile. ACIAR Monograph 68. Australian Government, Canberra, Australia.
- Miller, D. R., and A. Y. Rossman. 1995. Systematics, biodiversity, and agriculture. *Bioscience*. 45: 680–686.
- Poole, R. W. 1989. Lepidopteran catalogus, fascicle 118: Noctuidae, part 1. E. J. Brill, Leiden, Netherlands.
- Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*. 239: 487–491.
- Scheffer, S. J. 2000. Molecular evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *J. Econ. Entomol.* 93: 1146–1151.
- Scheffer, S. J., and B. M. Wiegmann. 2000. Molecular phylogenetics of the holly leafminers (Diptera: Agromyzidae: *Phytomyza*): species limits, speciation, and dietary specialization. *Mol. Phylo. Evol.* 17: 244–255.
- Scheffer, S. J., and M. L. Lewis. 2001. Two nuclear genes confirm mitochondrial evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Ann. Entomol. Soc. Am.* 94: 648–653.
- Sperling, F. A. H., A. G. Raske, and I. S. Otvos. 1999. Mitochondrial DNA sequence variation among populations and host races of *Lambdina fiscellaria* (Gn.) (Lepidoptera: Geometridae). *Insect Mol. Biol.* 8: 97–106.
- Swofford, D. L. 2000. PAUP*: phylogenetic analysis using parsimony (version 4.0). Sinauer, Sunderland, MA.
- Venette, R. C., and J. R. Gould. 2004. A pest risk assessment for *Copitarsia* spp., cutworms of economic importance south of the U.S. border. *Euphytica*. (in press).
- Weller, S. J., R. B. Simmons, and A. L. Carlson. 2004. *Emphyreuma* species and species limits: evidence from morphology and molecules (Arctiidae: Arctiinae: Ctenuchini). *J. Lep. Soc.* 58: 21–32.
- Winter, W. D. 2000. Basic techniques for observing and studying moths and butterflies. Lepidopterists' Society, New Haven, CT.