SYSTEMATICS

Incipient Speciation in *Strauzia longipennis* (Diptera: Tephritidae): Two Sympatric Mitochondrial DNA Lineages in Eastern Iowa

HEATHER J. AXEN,[1] JESSICA L. HARRISON,[2] JOHN R. GAMMONS, IAN G. MCNISH, LAURA D. BLYTHE, AND MARTY A. CONDON[3]

Department of Biology, Cornell College, Mount Vernon, IA 52314–1098

Ann. Entomol. Soc. Am. 103(1): 11–19 (2010)

ABSTRACT *Strauzia longipennis* (Wiedemann) (Diptera: Tephritidae) is a notoriously variable species. Seven varieties were once recognized. Three varieties were elevated to species status. The status of the other four varieties, including the synonyms for *S. longipennis*, has been contested. Such taxonomic instability, particularly when associated with variable patterns of host use, suggests that *S. longipennis* may represent a dynamic complex of host-associated populations in the process of divergence. To detect evidence of genetic differentiation indicating genetically distinct sympatric populations of *S. longipennis*, we sequenced a fragment of cytochrome c oxidase subunit I of mitochondrial DNA of *S. longipennis* from two sites (three habitats) in eastern Iowa. At each site, we found two genetically and morphologically distinct sympatric populations. One corresponds to morphological descriptions of *S. longipennis* variety *typica* (Loew). The other corresponds to descriptions of *S. longipennis* variety *vittigera* (Loew). High levels of genetic differentiation between these divergent sympatric populations suggest the populations might represent host races or incipient species.

KEY WORDS host race, mitochondrial DNA capture, incomplete lineage sorting, phytophagous insects

Phytophagous insects are extraordinarily diverse. Estimates suggest that insects that feed on plants represent more than a third of all described species of animals on earth (Strong et al. 1984, Wilson 1992). Ecological specialization on particular plant taxa or plant parts may help explain this phenomenal diversity (Ehrlich and Raven 1964; Mitter et al. 1991; Eber et al. 1999; Nyman et al. 2000; Scheffer and Wiegmann 2000; Funk et al. 2002; Winkler and Mitter 2008; Condon et al., 2008a,b). Shifts in patterns of host use by highly host-specific insects can result in host associated genetic differentiation (Waring et al. 1990, Via 1991, Filchak et al. 2000, Emelianov et al. 2003, Ferrari et al. 2006), which can result in formation of host races that represent key steps in the process of speciation (Drès and Mallet 2002, Stireman et al. 2005). Although studies of host associated genetic differentiation usually focus on populations of insects that use different host *taxa* (Bush 1969, Abrahamson et al. 2001), sympatric populations also can show specificity to different host parts or tissues of a single species of host (Condon and Steck 1997; Joy and Crespi 2007; Condon et al. 2008a,b).

The Tephritidae, an economically important family of true fruit flies, includes classic examples of host races or incipient species associated with different host plant taxa, e.g., *Rhagoletis* (Bush 1969, Berlocher 2000, Feder et al. 2003) and *Eurosta* (Brown et al. 1996, Abrahamson et al. 2001, Craig et al. 2001). This family also includes examples of cryptic species or host races associated with different parts of host plants (Lisowski 1979, 1985; Condon and Steck 1997; Condon et al. 2008a,b). Lisowski (1979, 1985) suggested that larvae of multiple cryptic species of stem-mining tephritids in the genus *Strauzia* might use different portions of the stem tissue of host plants. Both Lisowski (1979, 1985) and Stolzfus (1988) suggested that *Strauzia longipennis* (Wiedemann) includes several cryptic species. However, Foote et al. (1993) concluded that the available data do not support this decision, and did not recognize the cryptic species.

Strauzia longipennis (Wiedemann) (Diptera: Tephritidae), a pest of economically important sunflowers (*Helianthus annuus* L.), is geographically widespread, ranging from Ontario and Manitoba in the north to Florida, Arizona, and California (Foote et al. 1993). It infests at least two other species of *Helianthus*, as well as *Smallanthus uvedalia* (L.) Mackenzie ex Small and *Ageratina altissima* (L.) King & H.E. Robins. Foote et al. (1993) considered *S. longipennis* to be a highly sexually dimorphic and "amazingly variable taxon" that includes morphologically variable

[1] Current address: Department of Biology, University of Vermont, Burlington, VT 05405-0082.

[2] Current address: Department of Anthropology, Indiana University, Bloomington, IN 47405-7100.

[3] Corresponding author, e-mail: mcondon@cornellcollege.edu.

0013-8746/10/0011–0019$04.00/0 © 2010 Entomological Society of America

Table 1. Criteria used by Lisowski (1979, 1985) and Stoltzfus (1988) to elevate *S. longipennis* var. *vittigera* to species rank

	S. longipennis var. *typica*	*S. longipennis* var. *vittigera*
Lisowski (1979[a], 1985[b])		
Hosts[a,b]	*H. laetiflores* *H. tuberosus*	*H. annuus* *H. laetiflores* *H. tuberosus* *H. tuberosus* × *strumosus* *Helianthus* hybrids
Other criteria	Allozymes[a] Morphology[b]: mesonotum not striped	Allozymes[a] Morphology[b]: mesonotum striped
Stoltzfus (1988)		
hosts	*H. annuus*	*H. hirsutus* *H. tuberosus*
	Mesonotum not striped males: F separate, at least posteriorly, sometimes connected in r_{4+5}; no coalesced wing pigmentation	Mesonotum striped males: F pattern variable, but not connected in r_{4+5}; coalesced wing pigmentation common

[a] Lisowski (1979).
[b] Lisowski (1985).

sympatric subgroups that may represent host races. Early morphological work (Loew 1873) recognized seven varieties of *S. longipennis.* Steyskal (1986) elevated three of Loew's varieties (*arculata, intermedia,* and *perfecta*) to species rank and listed the other four varieties (*confluens, longitudinalis, typica,* and *vittigera*) as synonyms of *S. longipennis.* However, he regarded *S. longipennis* variety *longitudinalis* (Loew) as most clearly synonymous with *S. longipennis* and suggested that the other three varieties deserved closer attention (Steyskal 1986, Foote et al. 1993).

Following Steyskal's suggestion, Stoltzfus (1988) studied *Strauzia* but disagreed with the conclusions of Steyskal (1986). Stoltzfus (1988) recognized *S. longipennis* variety *typica* (not variety *longitudinalis*) as a synonym of *S. longipennis,* and raised variety *longitudinalis* and *vittigera* to full species rank. Stoltzfus considered each (including *S. longipennis* variety *typica*) to be highly host-specific. Lisowski (1985) also raised *S. longipennis* variety *vittigera* to species status (Table 1). Although both authors used host records and morphology to justify their decisions to elevate variety *vittigera* to species rank, assessment of host records and morphology differ between the authors (Table 1). Consequently, reconciling information from the two authors is difficult.

Lisowski (1979, 1985), who worked primarily in Illinois, evaluated evidence from extensive host records, morphology, and 16 allozyme loci. He recognized seven reproductively isolated lineages of *Strauzia,* some of which correspond to varieties of *S. longipennis.* Although Lisowski (1985) did not find fixed morphological characters, he used genetic data and host records to propose that the lineage corresponding to descriptions of variety *vittigera* be elevated to species status. His diagnosis of this species includes specimens reared mainly from *Helianthus tuberosus* L. ($N = 266$) but also from other species or hybrids of *Helianthus: Helianthus annuus* L. ($N = 7$), *H.* "*tuberosus*-like" ($N = 8$), and *H.* × *laetiflorus* ($N = 8$). Specimens he recognized as variety *typica* were reared only from *H. tuberosus.* Because sympatric populations seemed to be reproductively isolated, but used the same host plant species (*H. tuberosus*), Lisowski (1985) proposed they may be specific to certain areas of the plant (e.g., pith in the upper versus lower parts of the stem).

Independently, but without genetic data, Stoltzfus (1988) also elevated variety *vittigera* to species rank. He based his decision on his observations of flies on hosts in Virginia, Ohio, and Iowa, and on his evaluation of specimens of *vittigera* from geographically widespread localities (Ontario west to Montana, and south to Virginia and Arizona). Specimens he included in *typica* were collected from Ontario to Manitoba, south to Kansas and North Carolina. Flies Stoltzfus includes in variety *vittigera* were reared from *H. tuberosus* and *Helianthus hirsutus* Rafinesque but not *H. annuus.* Specimens Stoltzfus (1988) recognized as variety *typica* were reared only from *H. annuus* but not *H. tuberosus* (Table 1). Thus, the host records associated with specimens considered to be variety *typica* differ between Stoltzfus (1988), who associates the species only with *H. annuus,* and Lisowski (1979, 1985) who does not record *H. annuus* as a host. Despite disagreement over hosts of variety *typica,* both authors argue that variety *vittigera,* which uses *H. tuberosus* as a host, should be elevated to species status.

Morphological descriptions of collections recognized as variety *vittigera* differ between Lisowski and Stoltzfus (Table 1). Unlike Lisowski (1985), who found no sexual dimorphism in variety *vittigera,* Stoltzfus (1988) found considerable variation in the wing pattern of males reared from *H. tuberosus.* Like variety *typica* (as treated by Foote et al. 1993, but not by Stoltzfus) (see Table 1), males of variety *vittigera* are polymorphic for a wing pattern in which pigment seems to be coalesced into a single dark brown stripe, quite unlike the yellowish "F-shaped" pattern characteristic of females and some males. Given such morphological variation, Foote et al. (1993) (p. 374) "conclude that the additional taxa recognized as full species by Stoltzfus are not well delimited on solely morphological grounds. Rather than question their validity, we suggest that studies involving biochemistry, genetics . and additional field work are needed to establish the true status of the taxa . . . ".

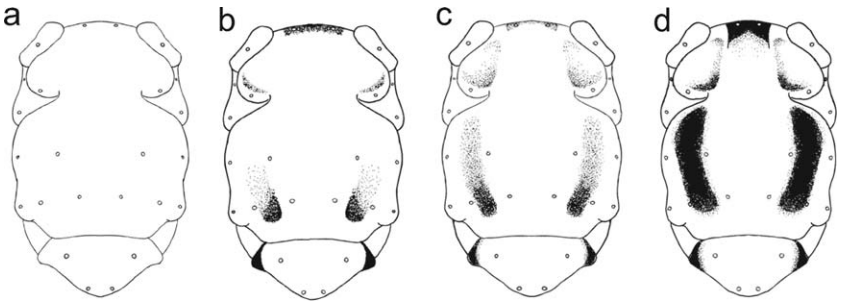

Fig. 1. Thorax pigmentation patterns associated with different lineages: (a) no markings, typical of *S. perfecta*; (b) faint markings, typical of *S. longipennis* variety *typica*; (c) intermediate markings; and (d) dark markings, typical of *S. longipennis* variety *vittigera*.

Intrigued by the puzzle presented by these highly sexually dimorphic and morphologically variable flies, we undertook a preliminary molecular and morphological study of *Strauzia* to determine whether *S. longipennis* includes genetically distinct sympatric groups that might represent host races or cryptic species. We report results from analysis of mitochondrial cytochrome c oxidase subunit I (COI) sequence and morphology (wing and thorax) of individuals of *S. longipennis* and *Strauzia perfecta* (Loew) swept from plants growing in three sites in eastern Iowa.

Materials and Methods

Collection Procedure and Study Sites. We collected *Strauzia* (N = 102) at two sites (three habitats) in eastern Iowa: 1) a disturbed area at a railroad crossing in Mount Vernon (site RR, 41° 55.784 N, 91° 25.415 W), 2) a cultivated garden at Indian Creek Nature Center (site 1-IC, 41° 57.981 N, 91° 34.475 W), and 3) a restored prairie at Indian Creek Nature Center (site 2-IC, 41° 57.292 N, 91° 34.733 W). At these sites, putative *Strauzia* hosts (e.g., *H. tuberosus*, *Helianthus strumosus* L., and *Ambrosia trifida* L.) were common, but *H. annuus* was absent (or extremely rare). At each site we searched for flies, attempted to capture all the flies we saw, and recorded site of capture. Captured specimens were frozen on dry ice and stored in 90% ethanol at −80°C. Vouchers of the plants from which we captured *Strauzia* were collected at the conclusion of fieldwork. Plants were determined by Paul Christiansen, an expert on prairie plants of Iowa (Christiansen and Muller 1999), and were compared with specimens held at United States National Herbarium. Vouchers of plants are stored in the herbarium of Cornell College. Vouchers of *Strauzia* are currently held at Cornell College for future studies and will be deposited in the National Museum of Natural History.

Sampling Protocol. We used the key from Stoltzfus (1988) to sort our specimens (N = 102) because the key in Foote et al. (1993) does not distinguish *S. longipennis* variety *typica* from variety *vittigera*. Following the Stoltzfus (1988) key, we assigned flies with no thorax markings to *S. perfecta* (Fig. 1a), those with pale notal patterns and no stripes (Fig. 1b) to variety *typica*, and those with definite notal stripes (Fig. 1d) to variety *vittigera*. We sorted specimens into four groups that characterized the morphological variation we observed in our collections: *S. perfecta* (N = 19), *S. longipennis* variety *typica* (N = 15), variety *vittigera* (N = 43), and "intermediates" (N = 25). Flies considered intermediates (Fig. 1c) either showed mesonotal markings that were both lighter than the definite stripes of variety *vittigera* and longer than the markings associated with variety *typica*, or had wing patterns that differed from descriptions in the key in Stoltzfus (1988) (Table 1).

For molecular analysis we chose 36 individuals: 25 individuals that could be assigned unambiguously (using the Stoltzfus key) to distinct groups: *S. perfecta* (N = 7: 2♀ 5♂), variety *typica* (N = 8: 1♀ 7♂), variety *vittigera* (N = 10: 3♀ 7♂), and 11 specimens (4♀ 7♂), with intermediate phenotypes. Specimens were chosen haphazardly with respect to site (*Appendix* 1).

Molecular Protocol. Whole genomic DNA was extracted from the left mid- and hind legs of each specimen using the DNeasy tissue kit (QIAGEN, Valencia, CA) following manufacturer's instructions. We used primers CO-1460-F (5′-TACAATCTATCGCCTAAACTTCAGCC-3′) and CO-3014-R (5′-TCCATTGCACTAATCTGCCATATTA-3′) to amplify 1,527 bp of the COI. Polymerase chain reaction (PCR) products were cleaned using the QIAquick PCR purification kit (QIAGEN) following manufacturer's instructions and sent to be sequenced by Macrogen (Seoul, Korea). For sequencing reactions, we used the same external primers that were used for the PCR amplifications. Consensus sequences for each individual were formed using Sequencher 4.1.4 software (Gene Codes, Ann Arbor, MI) and edited to 1,377 bp. The useful portion of each sequence-read was ≈800–900 bp; the consensus sequence for each specimen was based on a combination of one bidirectionally confirmed (overlapping) sequence region (300–400 bp) and two unconfirmed (nonoverlapping) regions (≈1,000 bp, combined). The sequences were very clean and easily read, and conspecific sequences corroborated each other; thus, we chose to use longer sequences rather than shorter fully overlapping sequences. The alignment of individual consensus sequences was accomplished first by using ClustalW in MEGA version 3.1 (Kumar et al. 2004) and then aligned by eye. No indels were detected in the alignment.

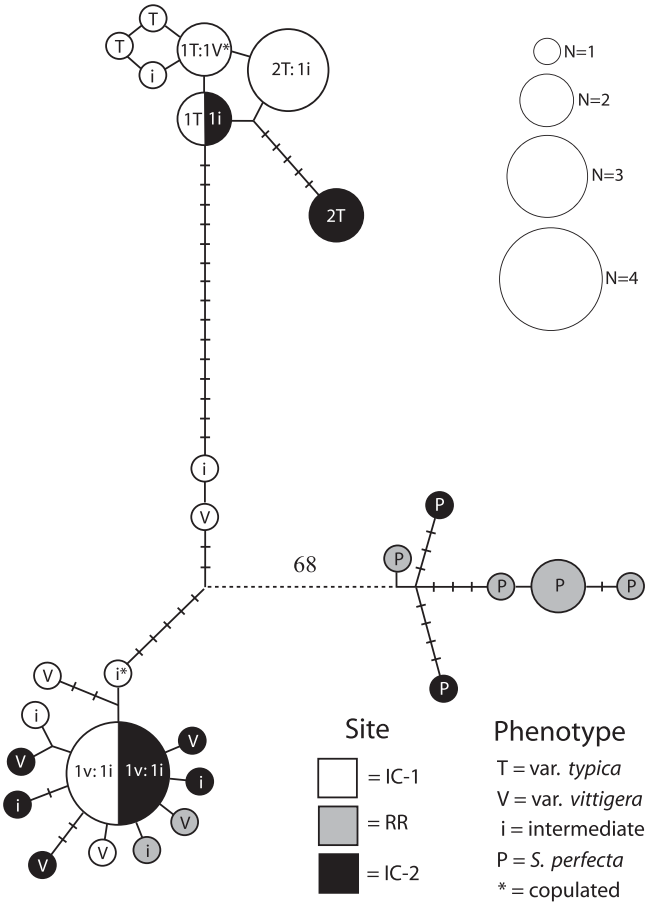

Fig. 2. Haplotype network (drawn by hand from TCS network) showing three distinct haplotype groups. Circles are proportional to the number of individuals within a particular haplotype; lines between haplotypes represent single base pair changes, each additional base pair change is separated by tickmarks. Shading represents sites (see key). Letters represent the flies' phenotypes (see key). For haplotypes represented by more than one individual, the number preceding the letter is the sample size for that phenotype.

Haplotype Network Analysis. A haplotype network was constructed in TCS 1.21 by using the statistical parsimony method with a 95% plausible connection limit (Clement et al. 2000). Secondary analyses were conducted using a 75-step fixed connectivity limit. Gaps were treated as missing data.

Results

Haplotype analysis of 1,377 bp of COI sequences of 36 specimens revealed three distinct haplotype groups (Fig. 2). Because these haplotype groups correspond to morphological groups defined by Stoltzfus, we refer to them by the names of those groups. The haplotype network (Fig. 2) shows that the sample of *S. perfecta* specimens ($N = 7$) includes six haplotypes, variety *typica* ($N = 12$) includes seven haplotypes, and variety *vittigera* ($N = 17$) includes 14 haplotypes. We found ≈4.9% (68-bp) sequence divergence between *S. perfecta* and the *vittigera-typica* complex. Members of the *vittigera-typica* complex differ from each other by at least 16 bp (≈1.2%).

All specimens that keyed out unambiguously as *S. perfecta* ($N = 7$) (Fig. 3) formed one haplotype group (Fig. 2). Most of these flies were captured as adults on giant ragweed (*Ambrosia trifida* L.). Another haplotype group (Fig. 2), which we refer to as the variety *typica* haplotype group, included 12 flies (1♀ 11♂), 11 of which were captured as adults on *H. tuberosus.* The variety *typica* haplotype group included eight flies that keyed out unambiguously as variety *typica,* and three intermediates. Two of the intermediates had variety *typica*-like (unstriped) mesonota and intermediate wing patterns, e.g., St35 (Fig. 4c) and St46 (Fig. 4f), and the third had an intermediate mesonotal pattern (e.g., St37) (Fig. 1c). The variety *typica* haplotype group also included one fly (a male) (Fig. 4b) that keyed out unambiguously as variety *vittigera.* That fly (St48, a male with dorsal stripes and a coalesced wing pattern) was caught in copulo with St49, a female in the variety *vittigera* haplotype group. The third haplotype group, which we call the variety *vittigera* haplotype group ($N = 17$) included nine of 10 flies identified unambiguously as variety *vittigera* (the 10th

Fig. 3. Wings typical of *S. perfecta*. Connection refers to the connection of the apical pigmentation pattern that resembles the letter "F" with other pigmented areas in the wing. (Online figure in color.)

fly—St48—belonged to the *typica* haplotype group but was morphologically assigned to the variety *vittigera* group), and eight flies assigned to the intermediate morphology category. Of those eight intermediates, all four females (St29, St49, St50, and St51) had intermediate mesonotal patterns (Fig. 1c) but normal wings (Fig. 5). The four males had variable intermediate morphologies: one male (St19) had intermediate mesonotal and wing patterns (coalesced variation), two males (St25 and St62) had an intermediate mesonotal pattern and coalesced wings, and one male (St38) had mesonotal stripes and noncoalesced wings (F not connected). Flies in the *vittigera* haplotype group were captured on *H. tuberosus*, *H. strumosus*, and *A. trifida* (*Appendix* 1). Two flies tentatively assigned to the *vittigera* group were outliers, both genetically and morphologically. Those flies differed by 9 bp (St27) and 10 bp (St38) from the common variety *vittigera* haplotype group and by 16 and 17 bp from variety *typica* haplotype group. Both had mesonotal stripes characteristic of the *vittigera* group, but the stripes on St38 were pale. Both specimens also had dark banding across the abdomen, which was not observed in any other specimens.

Distributions of morphological characters clearly differ between males of the variety *typica* and variety *vittigera* clades (Fig. 6). With the exception of the single male found in copulo with a variety *vittigera* female, no males of the variety *typica* haplotype group had coalesced wings or dark mesonotal stripes. In

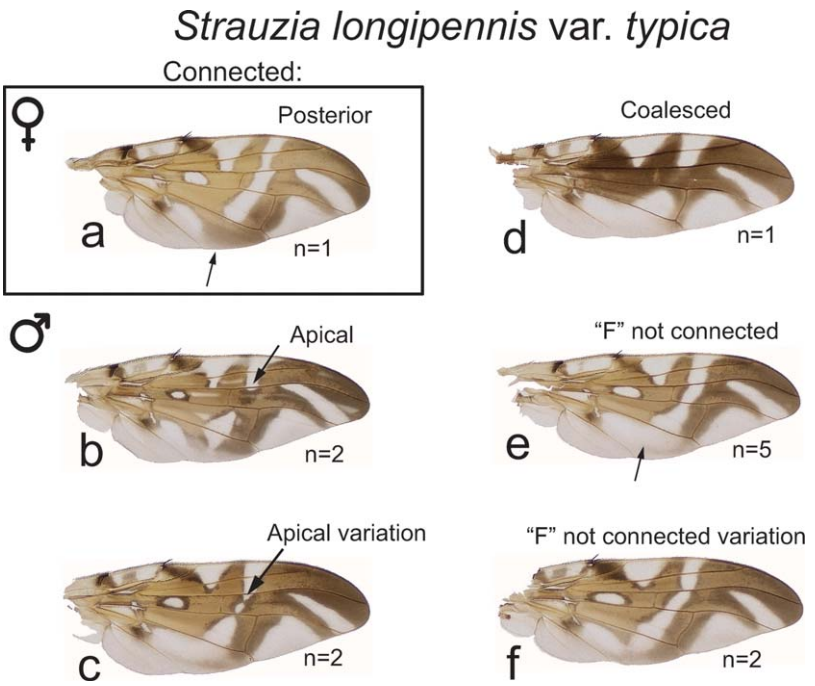

Fig. 4. Wings representing morphological variation within the *S. longipennis* variety *typica* haplotype group. Arrows point to areas where the apical "F" pattern is often fused with other regions of pigmentation. (a) Females. (b–f) Males. (a) In females, the F is fused posteriorly. (b and c) In four males, the F was connected in r_{4+5} (no variety *vittigera* showed this pattern). (d) The single male with a variety *typica* haplotype and a coalesced wing pattern was captured in copulo with a female in the variety *vittigera* haplotype group. We suspect that individual belongs to variety *vittigera* and reflects an mtDNA capture event or incomplete lineage sorting. (e) Unlike females, the F-pattern in most males is not connected posteriorly (see arrow); (f) Occasional variants (male) show some evidence of "intermediate" or weak posterior connection (also see b). (Online figure in color.)

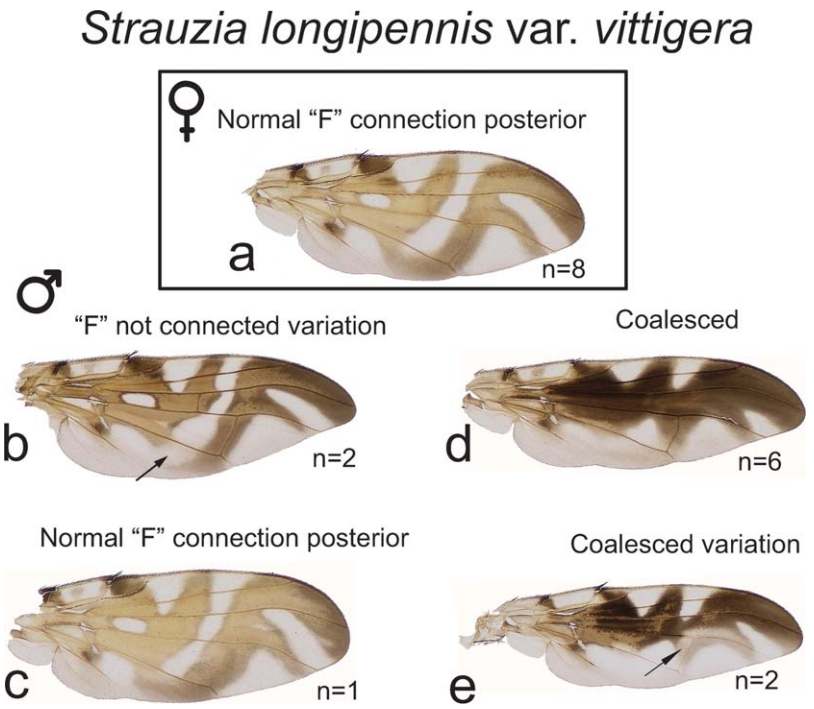

Fig. 5. Wings representing morphological variation within the variety *vittigera* haplotype group. (a) Females. (b–e) Males. (a) In females, the apical pigmented "F" pattern is typically connected posteriorly. In males, the apical pigmented F-pattern can be unconnected (b) or connected posteriorly (c). Unlike the variety *typica* (Fig. 4b and c) no specimens in the variety *vittigera* haplotype group had F connected in r_{4+5}. Coalesced wing patterns are variable, with pattern (d) more common than pattern (e) . (Online figure in color.)

contrast, 70.0% of the males in the variety *vittigera* haplotype group had coalesced wings and dark mesonotal stripes (Fig. 6).

Discussion

Our results are consistent with previous work that highlights both genetic and morphological differences between *S. longipennis* variety *typica* and variety *vittigera.* Mitochondrial sequence divergence (≥1% between variety *typica* and variety *vittigera* haplotype groups), is similar to divergence found between other tephritid host races [e.g., 0.8% divergence between *Eurosta solidaginis* Fitch host races; Brown et al. 1996). Morphological characters of flies in mitochondrial (mt)COI haplotype groups were consistent with host-specific groups described by Stoltzfus (1988). With one exception, we could use his key to sort flies into groups that differed genetically. The sole exception of a male having a variety *vittigera* phenotype and a variety *typica* haplotype may be an example of either incomplete lineage sorting or local mtDNA capture. The former reflects an ancestral polymorphism, and the latter results when mtDNA typical of one species is found in a different species as a consequence of occasional hybridization events (Avise 1994, Shaw 2002). In addition, the occurrence of genetically distinct and sympatric groups of flies associated with *H. tuberosus* is consistent with evidence from allozymes (Lisowski 1979).

Although we did not rear flies, differences in sex ratios of captured flies associated with potential hosts suggest differences in the biology of these flies. Sex ratios of flies identified morphologically as *S. perfecta* (6♀ 13♂) are similar to sex ratios of flies identified morphologically as variety *vittigera* (20♀ 44♂). Both were collected on plants recorded as hosts (Lisowski 1985, Stoltzfus 1988, Steyskal 1986, Foote et al. 1993). In contrast, the sex ratio of flies corresponding to the variety *typica* haplotype group differed significantly from both *S. perfecta* ($\chi^2 = 3.84$, $P < 0.05$) and variety *vittigera* ($\chi^2 = 4.49$, $P < 0.05$) and was distinctly more male biased (1♀ 16♂). Adults in the variety *typica* group were collected from the surfaces of *H. tuberosus* and *A. trifida,* neither of which was considered to be a host by Stoltzfus (1988). At our study sites, *H. annuus* was either absent or very rare. According to Stoltzfus (1988), *H. annuus* is the only host plant for variety *typica.* If so, and if females of variety *typica* are found mainly on their host, that could account for our failure to collect more than one female of variety *typica.*

Our mtCOI data support previous conclusions that variety *typica* and variety *vittigera* are divergent lineages (Lisowski 1979, 1985; Stoltzfus 1988). These lineages may represent host-races of flies that may be specific to different host taxa and/or different parts of the same host taxon. Drès and Mallet (2002) defined host-races as "genetically differentiated, sympatric populations of parasites that use different hosts, and between which there is

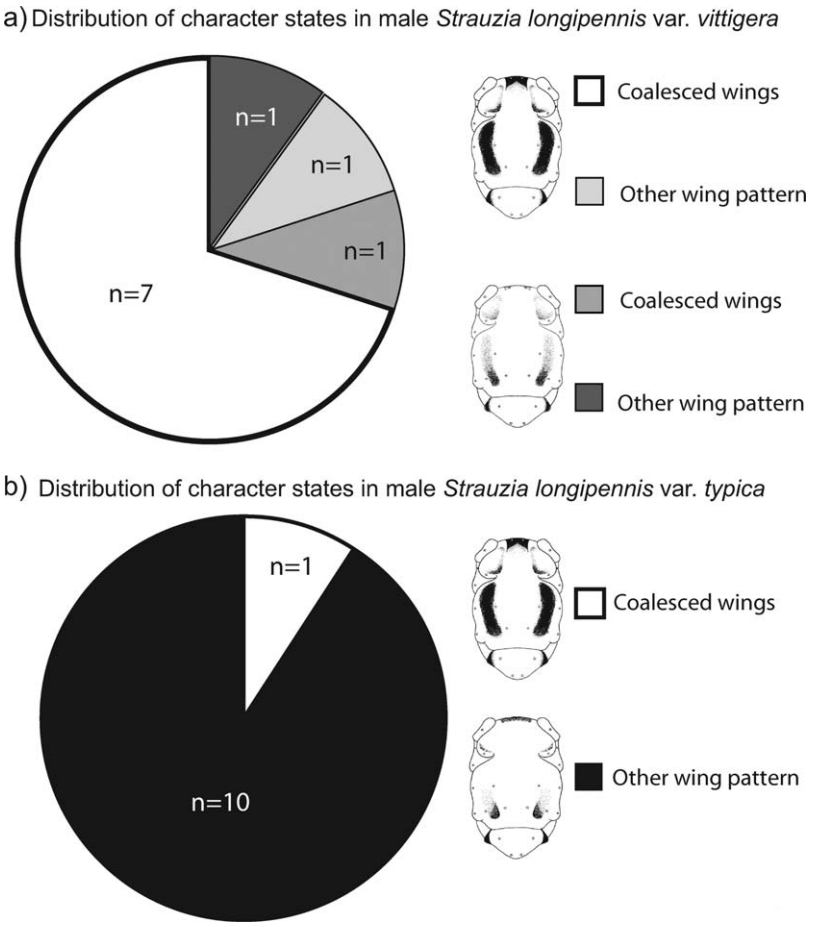

Fig. 6. Distributions of character states among males. Shading indicates samples with particular thorax and wing patterns. (a) *S. longipennis* variety *vittigera* haplotypes (N = 10 males). Thorax patterns range from dark mesonotal stripes (Fig. 1d) to pale mesonotal stripes (Fig. 1c). Most males in the variety *vittigera* clade had dark mesonotal stripes and coalesced wings. Coalesced wings include all "coalesced" variants shown in Fig. 5. (b) variety *typica* haplotypes (N = 11 males). Most males had faint short posterior mesonotal marks (Fig. 1b). Only one male in the variety *typica* haplotype group had dark mesonotal stripes (Fig. 1d) and coalesced wings. That male (*vittigera* phenotype, *typica* mtDNA), which was collected in copulo with a variety *vittigera* female, may represent evidence of either incomplete lineage sorting (Avise 1994) or mtDNA capture (Shaw 2002).

appreciable gene flow." We discovered a copulating pair of flies representing two haplotype groups. However, the two individuals were similar morphologically (both had dark markings on the scutum, characteristic of variety *vittigera*). The male's haplotype belonged to the variety *typica* haplotype group, but his phenotypic traits were characteristic of variety *vittigera.* The mitochondria of this individual could be evidence either of ancestral mtDNA polymorphism or mtDNA capture via recent hybridization (Avise 1994, Shaw 2002). Future analysis of nuclear genes should allow us to distinguish between those two hypotheses.

Acknowledgments

For help with identification, we thank Paul Christiansen (*Helianthus*) and Allen Norrbom (*Strauzia*). We thank Nor Meyer and Matt Lewis for technical support, and the Indian Creek Nature Center for generously allowing us to use their preserve as a study site. We are grateful to Allen Norrbom and Sonja Scheffer for helpful comments on the manuscript and to Stewart Berlocher for providing a copy of the Lisowski M.S. thesis and Ph.D. dissertation. Funding for this research was provided by the Becky and Al Pulk Fellowships (to H.J.A., J.L.H., and I.G.M.), the National Science Foundation (NSF DEB 0330845, to M.A.C.), student-faculty development grants from Cornell College (to M.A.C., J.R.G., and L.D.B.), and the Beta Beta Beta honors society (to I.G.M. and L.D.B.).

References Cited

Abrahamson, W. G., M. D. Eubanks, C. P. Blair, and A. V. Whipple. 2001. Gall flies, inquilines, and goldenrods: a model for host-race formation and sympatric speciation. Am. Zool. 41: 928–938.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman & Hall, New York.

Berlocher, S. H. 2000. Radiation and divergence in the *Rhagoletis pomonella* species group: inferences from allozymes. Evolution 54: 543–557.

Brown, J. M., W. G. Abrahamson, and P. A. Way. 1996. Mitochondrial DNA phylogeography of host races of the goldenrod ball gallmaker, *Eurosta solidaginis* (Diptera: Tephritidae). Evolution 50: 777–786.

Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). Evolution 23: 237–251.

Christiansen, P., and M. Muller. 1999. An illustrated guide to Iowa prairie plants. University of Iowa Press, Iowa City, IA.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657–1660.

Condon, M. A., and J. G. Steck. 1997. Evolution of host use in fruit flies of the genus Blepharoneura (Diptera: Tephritidae): cryptic species on sexually dimorphic host plants. Biol. J. Linn. Soc. 60: 443–466.

Condon, M. A., D. C. Adams, D. Bann, K. Flaherty, J. Gammons, J. Johnson, M. L. Lewis, S. Marsteller, S. J. Scheffer, F. Serna, and S. Swensen. 2008a. Uncovering tropical diversity: six sympatric cryptic species of *Blepharoneura* (Diptera: Tephritidae) in flowers of *Gurania spinulosa* (Cucurbitaceae) in eastern Ecuador. Biol. J. Linn. Soc. 93: 779–797.

Condon, M., S. J. Scheffer, M. L. Lewis, and S. M. Swensen. 2008b. Hidden Neotropical diversity: greater than the sum of its parts. Science (Wash., D.C.) 320: 928–931.

Craig, T. P., J. D. Horner, and J. K. Itami. 2001. Genetics, experience, and host-plant preference in *Eurosta solidaginis:* implications for host shifts and speciation. Evolution 55: 773–782.

Drès, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Philos. Trans. R. Soc. Lond. B Biol. Sci. 357: 471–492.

Eber, S., S. Knowll, and R. Brandl. 1999. Endophagous insects and structural niches on plants: ecology and evolutionary consequences. Ecol. Entomol. 24: 292–299.

Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. Evolution 18: 586–608.

Emelianov, I., F. Simpson, P. Narang, and J. Mallet. 2003. Host choice promotes reproductive isolation between host races of the larch budmoth *Zeiraphera diniana.* J. Evol. Biol. 16: 208–218.

Feder, J. L., S. H. Berlocher, J. B. Roethele, H. Dambroski, J. J. Smith, W. L. Perry, V. Gavrilovic, K. E. Filchak, J. Rull, and M. Aluja. 2003. Allopatric genetic origins for sympatric host-plant shifts and race formation in *Rhagoletis.* Proc. Natl. Acad. Sci. U.S.A. 100: 10314–10319.

Ferrari, J., H.C.J. Godfray, A. S. Faulconbridge, K. Prior, and S. Via. 2006. Population differentiation and genetic variation in host choice among pea aphids from eight host plant genera. Evolution 60: 1574–1584.

Filchak, K. E., J. B. Roethele, and J. L. Feder. 2000. Natural selection and sympatric divergence in the apple maggot *Rhagoletis pomonella.* Nature 407: 739–742.

Foote, R. H., F. L. Blanc, and A. Norrbom. 1993. Handbook of the fruit flies (Diptera; Tephritidae) of America North of Mexico. Comstock Publishing Associates, Cornell University Press, Ithaca, NY.

Funk, D. J., K. E. Filchack, and J. L. Feder. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. Genetica 116: 251–267.

Joy, J., and B. Crespi. 2007. Adaptive radiation of gall-inducing insects on a single host plant. Evolution 61: 784–795.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 5: 150–163.

Lisowski, E. 1979. Biochemical systematics of the genus *Strauzia* (Diptera: Tephritidae). M.S. thesis, University of Illinois, Urbana-Champaign.

Lisowski, E. 1985. Taxonomy and biology of *Strauzia* (Diptera: Tephritidae) in Illinois. Ph.D. dissertation, University of Illinois, Urbana-Champaign, IL.

Mitter, C., B. Farrell, and D. J. Futuyma. 1991. Phylogenetic studies of insect-plant interactions: insights into the genesis of diversity. Trends Ecol. Evol. 6: 290–293.

Nyman, T., A. Widmer, and H. Roininen. 2000. Evolution of gall morphology and host-plant relationships in willow-feeding sawflies (Hymenoptera: Tenthredinidae). Evolution 54: 526–533.

Scheffer, S. J., and B. M. Wiegmann. 2000. Molecular phylogenetics of the holly leafminers (Diptera: Agromyzidae: *Phytomyza*): species limits, speciation, and dietary specialization. Mol. Phylogenet. Evol. 17: 244–255.

Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: what DNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc. Natl. Acad. Sci. U.S.A. 99: 16122–16127.

Steyskal, G. C. 1986. Taxonomy of the adults of the genus *Strauzia* Robineau-Desvoidy (Diptera: Tephritidae). Insecta Mundi 1: 101–117.

Stireman, J. O., III, J. D. Nason, and S. B. Heard. 2005. Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. Evolution 59: 2573–2587.

Stoltzfus, W. B. 1988. The taxonomy and biology of *Strauzia* (Diptera: Tephritidae. J. Iowa Acad. Sci. 95: 117–126.

Strong, D. R., J. H. Lawton, and R. Southwood. 1984. Insects on plants: community patterns and mechanisms. Harvard University Press, Cambridge, MA.

Via, S. 1991. The genetic structure of host plant adaptation in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. Evolution 45: 827–852.

Waring, G. L., W. G. Abrahamson, and D. Howard. 1990. Genetic differentiation among host-associated populations of the gall-maker *Eurosta solidaginis* (Diptera: Tephritidae). Evolution 44: 1648–1655.

Wilson, E. O. 1992. The diversity of life. Harvard University Press, Cambridge, MA.

Winkler, I. S., and C. Mitter. 2008. The phylogenetic dimension of insect-plant interactions: a review of recent evidence, pp. 240–263. *In* K. J. Tilmon [ed.], Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. University of California Press, Berkeley, CA.

Received 17 June 2009; accepted 13 October 2009.

Appendix 1. Specimens used for molecular analysis

Specimen ID	Sex specimen	Initial morphological assessment	Haplotype group	Collection site	Adult location (plant species)	GenBank accession
st19	M	intermediate	var. *vittigera*	1-IC	*Helianthus strumosus*	EU736157
st25	M	intermediate	*vittigera*	RR	*Ambrosia. trifida*	EU736145
st29	F	intermediate	*vittigera*	1-IC	*H. tuberosus*	EU736143
st35	M	intermediate	*typica*	1-IC	*H. tuberosus*	EU736178
st37	M	intermediate	*typica*	1-IC	*H. tuberosus*	EU736174
st38	M	intermediate	*vittigera*	1-IC	*H. tuberosus*	EU736161
st49	F	intermediate	*vittigera*	1-IC	*H. tuberosus*	EU736159
st46	M	intermediate	*typica*	2-IC	*H. tuberosus*	EU736171
st50	F	intermediate	*vittigera*	2-IC	*H. tuberosus*	EU736151
st51	F	intermediate	*vittigera*	2-IC	*A. trifida*	EU736154
st62	M	intermediate	*vittigera*	2-IC	*A. trifida*	EU736152
st33	M	*typica*	*typica*	1-IC	*H. tuberosus*	EU736177
st34	M	*typica*	*typica*	1-IC	*H. tuberosus*	EU736142
st36	M	*typica*	*typica*	1-IC	*H. tuberosus*	EU736179
st41	M	*typica*	*typica*	1-IC	*H. tuberosus*	EU736175
st42	M	*typica*	*typica*	1-IC	*H. tuberosus*	EU736176
st43	F	*typica*	*typica*	1-IC	*H. tuberosus*	EU736172
st48	**M**	*vittigera*	*typica*	**1-IC**	*H. tuberosus*	**EU736173**
st44	M	*typica*	*typica*	2-IC	*H. tuberosus*	EU736169
st45	M	*typica*	*typica*	2-IC	*A. trifida*	EU736170
st17	M	*vittigera*	*vittigera*	1-IC	*H. tuberosus*	EU736148
st21	F	*vittigera*	*vittigera*	1-IC	*H. tuberosus*	EU736150
st23	M	*vittigera*	*vittigera*	1-IC	*H. tuberosus*	EU736158
st27	M	*vittigera*	*vittigera*	1-IC	*H. tuberosus*	EU736160
st53	M	*vittigera*	*vittigera*	2-IC	*A. trifida*	EU736147
st54	F	*vittigera*	*vittigera*	2-IC	*H. tuberosus*	EU736155
st55	M	*vittigera*	*vittigera*	2-IC	*H. tuberosus*	EU736144
st56	M	*vittigera*	*vittigera*	2-IC	*H. tuberosus*	EU736146
st64	F	*vittigera*	*vittigera*	RR	*A. trifida*	EU736156
st15	M	*perfecta*	*perfecta*	2-IC	*A. trifida*	EU736166
st5	F	*perfecta*	*perfecta*	2-IC	*A. trifida*	EU736167
st1	F	*perfecta*	*perfecta*	RR	Unidentified grass	EU736165
st8	M	*perfecta*	*perfecta*	RR	Unidentified grass	EU736164
st10	M	*perfecta*	*perfecta*	RR	*H. tuberosus*	EU736162
st32	M	*perfecta*	*perfecta*	RR	*A. trifida*	EU736163
st39	M	*perfecta*	*perfecta*	RR	*A. trifida*	EU736168

Characters used for morphological assessment are described in the text. Specimens identified as var. *typica* correspond to descriptions of *Strauzia longipennis* var. *typica;* those identified as *vittigera* correspond to descriptions of *Strauzia vittigera* (Loew) (Stoltzfus 1988). Haplotype group assignment as in Fig. 2. Collection sites at Indian Creek Nature Center: 1-IC = site 1-IC, 41° 57.981 N, 91° 34.475 W; 2-IC, 41° 57.292 N, 91° 34.733 W. Additional specimens were collected along railroad tracks outside Mount Vernon, Iowa: site RR, 41° 55.784 N, 91° 25.415 W. Adult location: specimens were swept from the surfaces of plants. The specimen of var. *vittigera* with the mtDNA haplotype of var. *typica* is highlighted in bold.