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HOST-ASSOCIATED GENETIC DIFFERENTIATION IN PHYTOPHAGOUS INSECTS: GENERAL PHENOMENON OR ISOLATED EXCEPTIONS? EVIDENCE FROM A GOLDENROD-INSECT COMMUNITY

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Abstract.—There is growing awareness of the importance of natural selection in driving genetic divergence and speciation, and several of the most apparent cases of this ecological speciation are provided by the existence of genetically distinct host forms in phytophagous insects. Such examples of host-associated differentiation (HAD) have become increasingly documented, and the implications of this phenomenon for the diversification of insects are becoming widely appreciated. However, instances of HAD remain rare relative to insect diversity and are sparsely distributed both ecologically and taxonomically. We sought to assess the frequency of HAD in a model herbivore community by examining genetic divergence in a variety of herbivores that feed on two closely related and broadly sympatric species of goldenrod (*Solidago altissima* and *S. gigantea*). Using mitochondrial DNA and allozyme data, in conjunction with previously published studies, we found that four of nine herbivores exhibited evidence of HAD, including possible host races or cryptic species. Using a range of reasonable substitution rate estimates for cytochrome oxidase I mitochondrial DNA, we found that HAD appears to have proceeded asynchronously across taxa. This pattern, along with the broadly sympatric distribution of host plants and the specialized life histories of the phytophagous insects, is consistent with sympatric divergence in some or all of these taxa. Although further behavioral and ecological study is needed, our survey of HAD in a community of herbivores indicates that ecological (perhaps sympatric) speciation may have been responsible for generating a significant fraction of the extant diversity of phytophagous insects.

Key words.—Community genetics, ecological speciation, host-associated differentiation, plant gall, *Solidago*, specialization, sympatric speciation.

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Ecologists and evolutionary biologists have long sought to understand the ecological processes governing the rate and extent of diversification among Earth's flora and fauna. Of particular interest is high diversity in groups characterized by tight host or microhabitat specialization: for instance, phytophagous insects (Price 1980; Strong et al. 1984; Mitter et al. 1988), some but not all parasites and parasitoids (Wiegmann et al. 1993; Zietara and Lumme 2002; Simková et al. 2004), and lacustrine fishes (McCune 1997; McKinnon and Rundle 2002; Salzburger et al. 2002). Such diversity motivates a renewed focus on genetic diversification associated with ecological specialization (as opposed to drift in allopatry, sexual selection, and the like; Schluter 2000, 2001; Levin 2004). This ecological speciation model stresses divergence and reproductive isolation evolving as a consequence of divergent selection between differing environments (Schluter 2001). Theoretical and empirical evidence is mounting that genetic differentiation and ecological speciation can even proceed in sympatry, under conditions that appear much less restrictive than previously thought (Rice 1987; Kawecki 1996, 1997; Kondrashov et al. 1998; Dieckmann and Doebeli 1999; reviewed in Via 2001; Berlocher and Feder 2002). Ecological speciation, then, offers a potentially important explanation for diversity in clades whose members tend to specialize on different microhabitats, hosts (for parasites), or host plants (for phytophagous insects).

Phytophagous insects represent a particularly interesting potential arena for diversification via ecological specialization. The astonishing diversity of these insects is one of the most conspicuous patterns in Earth's biodiversity (Price 1980; Strong et al. 1984; Mitter et al. 1988), and specialized insect-host interactions have long been suspected of facilitating evolutionary diversification (Walsh 1864, 1867; Ehrlich and Raven 1964; Bush 1969; Berlocher and Feder 2002; Funk et al. 2002). The occurrence of insect host races (conspecific populations exhibiting recently evolved genetic differentiation with respect to host-plant use; Drés and Mallet 2002) suggests that this kind of diversification is ongoing (Wood 1980; Berlocher and Feder 2002; Nosil et al. 2002; Emelianov et al. 2004).

Unfortunately, our understanding of ecologically driven genetic differentiation in phytophagous insects (and other taxa) is hampered in an important way by the nature of the available data. Several individual cases of host-associated differentiation (HAD) have been studied in detail, for instance, the apple-maggot fly, *Rhagoletis pomonella* (Feder 1998), the goldenrod ball-gall fly, *Eurosta solidaginis* (Abrahamson and Weis 1997), and the pea aphid, *Acyrthosiphon pisum* (Via et al. 2000). These cases and others have provided evidence for ecologically mediated divergence in sympatry (for review, see Berlocher and Feder 2002). However, the insects and hosts studied to date represent only a minute fraction of the tremendous diversity of phytophagous insects, have been scattered both taxonomically and ecologically, and have often been chosen (from the many possible study spe-

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cies) for idiosyncratic reasons. As a result, we do not know if HAD is unusual (perhaps occurring only in a few cases that possess exacting prerequisites for host-associated ecological speciation) or is a frequent phenomenon playing a significant role in diversification among phytophagous insects. This question turns as much on the absence of HAD as on its occurrence, and so it is difficult to answer given that negative results may have remained unpublished or buried in papers focused on different questions. Furthermore, we do not know whether HAD often occurs in sympatry, or what ecological or historical factors (such as behavioral, life-history, or taxonomic characteristics of the insects of the host plants) determine its likelihood.

We offer here a novel approach to answering these questions. Rather than studying a single species in detail, we broaden the focus to consider the community of herbivores feeding on a focal set of plants, the goldenrods *Solidago altissima* and *S. gigantea*. These insects have been confronted with a common opportunity for HAD. If HAD is frequent, then we would expect to see ecological and evolutionary divergence, with respect to host plant, in multiple members of the herbivore community. Although our comparative approach does not establish the behavioral and ecological mechanisms of differentiation in any single species, it does provide insight into how common this form of divergence might be and suggests which mechanisms are more likely. Given data for enough insect species, we will ultimately be able to explore ways in which feeding habit, size, dispersal ability, or other traits of phytophagous insects may encourage or discourage host-related genetic differentiation. The data presented here (for nine herbivores representing six insect families) represent the first such analysis for any host-plant (or host, or microhabitat) pair, and illustrate the potential of this approach to yield new conceptual understanding of diversification in phytophagous insects and, ultimately, in other ecologically specialized groups.

The System

The goldenrod species *S. altissima* and *S. gigantea* (Asteraceae) provide an ideal system to assess the frequency and pattern of HAD across a community of herbivores. The species are closely related (both members of the *S. canadensis* complex in *Solidago* subsection Triplinervae) and morphologically similar, but unambiguously separable, differing primarily in pubescence and clonal architecture. The species are currently broadly sympatric over most of the United States and southern Canada (USDA NRCS 2002) and are often syntopic (microsympatric) in distribution, growing in extensive and thoroughly intermixed stands in prairie, meadow, old-field, and wetland habitats (Abrahamson and Weis 1997; J. D. Nason, J. O. Stireman, S. B. Heard, pers. obs.) These two goldenrods are host to more than 100 species of endo- and exophytic phytophagous insects (Maddox and Root 1990; Root and Cappuccino 1992; Fontes et al. 1994), many of which appear to be shared among them (Stireman, Nason, Heard, pers. obs.). Though some fraction of this total represents species known to be polyphagous or widely oligophagous, there are also many specialized taxa that could conceivably show host-associated genetic differentiation. The third codistributed species of the *S. canadensis* complex, *S. canadensis*, is much less common and less commonly attacked. (Because we have not yet exhaustively collected herbivores from *S. canadensis* and other members of subsection Triplinervae, our estimates of host-associated lineage diversity among herbivores of the group constitute lower bounds.)

Detailed study of the gallmaking fly *Eurosta solidaginis* (Tephritidae) has revealed the existence of ecological and genetic differentiation with respect to host-plant use (Waring et al. 1990; Brown et al. 1996; Abrahamson and Weis 1997; Itami et al. 1998; Smith et al. 2002). Hybridization, behavioral, and ecological studies of *E. solidaginis* all support the hypothesis that differentiation has occurred sympatrically and is due to ecological selective forces of host plants (Craig et al. 1997; Itami et al. 1998) and natural enemies (Brown et al. 1995). Subsequently, Nason et al. (2002) showed significant HAD in allozyme frequencies in another stem-galler of *S. altissima* and *S. gigantea*, the moth *Gnorimoschema gallaesolidaginis* (Gelechiidae). However, they were unable to date the origin of the host-associated forms or to conclude whether they represented well-developed host races or young cryptic species. More recently, behavioral and allozyme data have indicated that an omnivore in this system, the beetle *Mordellistena convicta* (Mordellidae), which feeds internally on plant stems but also acts as a facultative predator of *E. solidaginis*, is behaviorally (Eubanks et al. 2003) and genetically (Blair et al. 2005), but not morphologically, differentiated on these same two *Solidago* species. While none of these studies attempted to take a comparative perspective on host-related divergence in the system, that these three insects exhibit similar host-related differentiation raises the possibility that HAD could be widespread and play an important role in herbivore speciation. However, they still represent only a few percent of the *Solidago* insect community and do not sample many feeding guilds, and so it is not yet clear if the three species studied so far are exceptional cases or whether HAD is common or even ubiquitous among *Solidago* herbivores.

In this study we examine patterns of genetic divergence in nine common phytophagous insect species (or species complexes) that attack sympatric populations of *S. altissima* and *S. gigantea*. We report new genetic data and analyses for seven of these, including two stem-galling moths (*G. gallaesolidaginis*, Gelechiidae, and *Epiblema scudderiana*, Tortricidae), two gall-making midges (*Asteromyia carbonifera* and *Rhopalomyia solidaginis*/*capitata*, a complex treated variously as one or two species, both Cecidomyiidae), a gall-making fly (*Procecidochares atra*, Tephritidae) and two leaf chewing beetles (*Trirhabda convergens* and *T. virgata*, Chrysomelidae). Our own analyses are complemented by published behavioral and genetic studies of two additional species, the gall-making tephritid fly *E. solidaginis* (e.g., Waring et al. 1990; Brown et al. 1996; Itami et al. 1998) and the stem-boring beetle *M. convicta* (Eubanks et al. 2003; Blair et al. 2005). With the comparative perspective allowed by this survey, we assess the frequency of HAD in this community and the relative likelihood of sympatric versus allopatric modes of divergence. Our results also shed some preliminary light on characteristics of phytophagous insects that may favor or discourage host-associated differentiation

TABLE 1. Cytochrome oxidase I mitochondrial DNA primers used in this study (F, forward; R, reverse).

Taxon	Name	Sequence (5′-3′)	Reference
Gnorimoschema	C1J-2183 (F)	CAACATTTATTTTGATTTTTTGG	Simon et al. (1994)
Trirhabda spp., *Procecidochares*	TL2-N3014 (R)	TCCAATGCACATAATCTGCCATATTA	Simon et al. (1994)
Gnorimoschema	TL2-N3013 (R)	TCCATTACATATAATCTGCCATATTAG	Landry et al. (1999)
Rhopalomyia, *Asteromyia*	LCO1490 (F)	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
Rhopalomyia, *Asteromyia*	HCO2198 (R)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
Epiblema	CIJ-1859E (F)	GGAACAGGATGAACAGTTTACCCCCC	Simon et al. (1994)
Epiblema	C1N-2329 (F)	ACTGTAAATATATGATGAGCTCA	Simon et al. (1994)
Trirhabda spp., *Procecidochares*	UEA7 (F)	TACAGTTGGAATAGACGTTGATAC	Lunt et al. (1996)
Gnorimoschema	CI-V-2195 (F)	TTGATTTTTTGGTCATCCAGAAGT	Simon et al. (1994)

and on probable ecological mechanisms for host-race formation.

MATERIAL AND METHODS

Collections

We collected phytophagous insects of seven species (*A. carbonifera*, *E. scudderiana*, *G. gallaesolidaginis*, *P. atra*, *R. solidaginis*/*capitata*, *T. convergens*, and *T. virgata*) from *S. altissima* and *S. gigantea* from across neighboring midwestern states (primarily Iowa, Minnesota, Nebraska, and South Dakota), and the Canadian provinces of Ontario, New Brunswick, and Alberta. For *G. gallaesolidaginis*, we also collected samples from much of the eastern United States and Canada, the southern Midwest (to Texas), and as far west as Wyoming and Kansas. We targeted these species due to their widespread abundance, apparent specificity, and apparency in the field; with the exception of *Gnorimoschema* (Nason et al. 2002) we had no a priori knowledge of host-associated genetic structure (or its lack) in any of them. Collecting was conducted by locating appropriate prairie, meadow, or wetland habitats that contained both *Solidago* species and haphazardly collecting galls or exophytic insects of the focal taxa in areas ranging from about 0.5 to 10 ha. We dispersed our sampling across the available individual plants to minimize the possibility of collecting multiple samples from the same plant genet. Gall-making insects were either flash-frozen alive in liquid nitrogen or placed in plastic containers and reared. Exophytic species were always flash-frozen soon after collection. Insect species were identified from reared adults and gall morphology using the keys of Hogue (1970), McEvoy (1988), Gagné (1989), Foote et al. (1993), and Miller (2000). Voucher specimens of host plants and adult insects have been deposited in the Iowa State University Ada Hayden Herbarium and the Iowa State University Insect Collection, respectively. Hereafter we refer to insects from each host as ''*altissima*'' and ''*gigantea*'' individuals or forms. We also collected herbivores as they occurred in occasional populations of *S. canadensis* and one population of *S. leavenworthii* (a southeastern member of the *S. canadensis* complex). All collection localities are provided in Appendix 1 available online.

Mitochondrial DNA Methods

We extracted insect mitochondrial DNA (mtDNA) using Puregene DNA extraction kits (Gentra Systems Inc., Minneapolis, MN) and amplified 450–800 bp of cytochrome oxidase I (COI) for each species. DNA was amplified in polymerase chain reactions (PCRs) containing 5 μl genomic DNA, 5 μl (10x) PCR buffer (Invitrogen, Carlsbad, CA), 5 μl (10mM) DNTP solution, 2.5–3.75 (50 mM) $MgCl_2$, 2.5 μl of forward and reverse primers (5 pmol/μl), and dH_2O to 50 μl. Primers were primarily taken from Simon et al. (1994; see Table 1). PCR conditions were as follows: initial denaturing at 94°C for 2 min, 35 cycles of 94°C for 30–45 sec, 47–52°C for 45–60 sec, 72°C for 1 min, and a final 72°C extension period of 4 min. Sequencing reactions of double-stranded DNA were carried out using ABI Prism Big Dye 3.1 using standard procedures and run on an automated ABI model 77 Prism sequencer (Applied Biosystems, Inc., Foster City, CA, at the Iowa State University Office of Biotechnology DNA Sequencing and Synthesis Facility. Sequences were initially aligned using AutoAssembler (Applied Biosystems), with further manual alignment and sequence manipulation conducted using MacClade (Maddison and Maddison 2000). Genbank accession numbers of sequences are provided in Appendix 1 available online only at http://dx.doi.org/10.1554/05-222.1.s1.

For each species, net sequence divergence between populations from alternate hosts (D_a; Nei 1987) was calculated using Mega 2.1 (Kumar et al. 2001) with standard errors estimated by 1000 bootstrap replicates. The fraction of total sequence variation explained by host species (Φ_{ST}) was quantified by hierarchical AMOVA for all taxa using Arlequin 2.000 (Schneider et al. 2000). These analyses included rough groupings into geographic region (e.g., northern plains, eastern Canada) as a level in addition to host plant to obtain more accurate estimates of HAD. To test whether apparent differences in the genetic diversity of host-associated forms were significant, we compared the observed difference in average pairwise distances between the two clades to a distribution of differences generated by randomizing individuals with respect to host (500 replicates).

To supplement AMOVA analyses, we estimated haplotype networks or phylogenetic trees for our sampled populations. We estimated haplotype networks, using TCS1.13 (Clement et al. 2000) for *Epiblema* and *Procecidochares* because they exhibited low levels of sequence variation. For other taxa, we estimated phylogenetic trees using neighbor-joining (NJ) and maximum likelihood (ML) techniques.

NJ trees employing Tamura-Nei distances (Tamura and Nei 1993) were initially reconstructed for *Gnorimoschema*, *Rhopalomyia*, *Asteromyia*, and *Trirhabda*. Modeltest 3.06 (Posada and Crandall 1998) was used to select and parameterize ML models on the basis of likelihood-ratio tests. Substitution

models used in ML were: K81uf + I + G for *Gnorimoschema* (in PAUP format: base=0.314 0.134 0.130 Nst=6, Rmat=1.000 3.220 0.574 0.574 3.220, rates=gamma, shape=1.76, Ncat=4, Pinvar=0.729; Kimura 1981), K81uf + G for *Rhopalomyia* (base=0.345 0.131 0.136; Nst=6, Rmat=1.0000 7.1439 1.8664 1.8664 7.1439, rates=gamma, shape=0.1960), K81uf + G for *Asteromyia* (base=0.3514, 0.1180, 0.1208; Nst=6, Rmat=1.0000, 7.7248, 2.6496, 2.6496, 7.7248, rates=gamma, shape=0.0056), and HKY for *Trirhabda* spp. (base=0.4102, 0.1271, 0.1555, Nst=2, TRatio=4.8965, rates=equal; Hasegawa et al. 1985). ML searches were conducted using from five to 20 random sequence addition replicates and PAUP options: steepest descent=yes, swap=tbr, rearrlimit=6000, limitperrep=yes). Both NJ and ML analyses were conducted in PAUP*4b10 (Swofford 1998). Branches important for discerning host associated groups were evaluated by performing 1000 NJ bootstrap replicates using distance measures based on ML models. We rooted the ML trees using sequences from the following outgroup taxa: for *Gnorimoschema*, *G. baccharisella* (but using *G. salinaris* instead yields very similar results); for *Rhopalomyia*, *R. fusiformis* and *R. lobata*; for *Asteromyia*, *A. euthamiae*; and for *Trirhabda virgata* and *T. convergens*, *T. canadensis* and *Galerucella lineola*. These taxa represent the taxonomically most closely related groups for which we could obtain adequate sequence data, but we cannot be certain that these represent sister groups to the taxa of interest, as this would require extensive genus-level phylogenetic analyses for each taxon. This uncertainty, however, does not weaken our confidence that each of our ingroup taxa (be it a generalist or a pair of host-specialist forms) represents a monophyletic group (see Results).

The suitability of assuming clocklike evolution was assessed by comparing likelihood scores of trees obtained from ML searches with and without the assumption of a molecular clock. These scores were compared using likelihood-ratio tests to determine whether the null hypothesis of a molecular clock could be rejected (i.e., 2[ln L(clock)—ln L(no clock)], distributed as χ^2 with df = no. taxa − 2). Divergence and gene flow between host-associated forms were estimated using a Markov chain Monte Carlo approach in MDIV (Nielsen and Wakeley 2001) under a finite sites HKY model (Hasegawa et al. 1985). MDIV analyses employed the following parameters: cycles: 2,000,000, burn-in: 500,000, Max T: 100 for *Rhopalomyia*, 20 for *Gnorimoschema*, and five for all others, Max M: 10–25 depending on the species. For analyses involving *E. solidaginis* we used the COI mtDNA sequence data reported in Brown et al. (1996).

Allozyme Methods

General methodology for analysis of allozyme variation in *Epiblema*, *Procecidochares*, *Rhopalomyia*, and *T. virgata* followed Nason et al. (2002). All four species were extracted in the same plant extraction buffer and abbreviations for enzyme names follow from Murphy et al. (1996). For *Epiblema* we resolved the following 14 polymorphic loci: *Acoh, Ak, Aat-A, Aat-B, Est* (fluorescent), *G6pdh, Gpi, G3pdh, Hadh, Idh, Mdh, Mdhp, Pgm*, and *Pgdh.* For *Procecidochares* we resolved 10 polymorphic loci: *Gpi, Idh, Ldh, Mdh-A, Mdh-B, Mdhp-A, Mdhp-B, Pgm, Pgdh*, and *Tpi*. For *Rhopalomyia* we resolved eight polymorphic loci: *Acoh-A, Acoh-B, Gpi, Hadh, Idh, Mdhp, Pgdh*, and *Pep* (Leu-Ala). For *Epiblema*, *Procecidochares*, and *Rhopalomyia*, enzymes were resolved using a 0.04 M morpholine-citrate buffer system adjusted to pH 7 (Murphy et al. 1996). For *T. virgata*, the following 11 polymorphic loci were assayed: *Ak, Cap-A, Cap-B, Gpi, Hadh, Idh*, and *Pgdh* were resolved in gel/electrode buffer system 11 of Soltis et al. (1983), and *Aat, Est* (fluorescent), *Hk*, and *Mpi* were resolved in buffer system 8 of Soltis et al. (1983). Allozymes were run in 12% starch (Starch Art Corp, Smithville, TX) gels and staining procedures for individual loci followed Soltis et al. (1983) with the exception of *G3pdh, Hadh, Ldh, Mpi*, and *Pep* from Murphy et al. (1996). Banding patterns for each of the enzyme loci indicated above exhibited expected subunit structures and patterns of expression. These loci do not represent an exhaustive search for polymorphism in these species.

Allozyme genetic differentiation between sympatric populations of each species occurring on the two host plants was assessed by testing the hypothesis that genotypic distributions are identical between populations. This hypothesis was tested using genotypic tests of population differentiation implemented by the program GENEPOP (Raymond and Rousset 1995). This test provides estimates of a combined *P*-value for test results assuming loci to be statistically independent, as well as estimates of *P*-values of log-likelihood-based exact tests for individual loci. The fraction of total genetic variance explained by host was also assessed by AMOVA in Arlequin 2.000 (Schneider et al. 2000). These tests of allozyme differentiation were applied to sympatric, host-associated collections of each species. We assayed two such sympatric populations for *Epiblema* (central Minnesota and Toronto, Ontario), two for *Procecidochares atra* (both Iowa), three for *Rhopalomyia* (all from Iowa), and one each for *T. virgata* (central Iowa) and *T. convergens* (Edberg, Alberta). In each case we assayed allozyme loci for at least 24 individuals (24–96) per host per population, except in *Rhopalomyia* where allozymic differentiation was particularly strong and smaller sample sizes were sufficient (sample size per population: N_{alt} = 14, 16, 6, N_{gig} = 46, 29, 6)

Interpreting the Genetic Data

Our criterion for identifying HAD is straightforward (but designed to be conservative). We base our conclusions about the occurrence of HAD primarily on results from AMOVA (sequence Φ_{ST} and allozyme F_{ST}; Table 2). Starting with collections of an insect from sympatric populations of both hosts at a number of geographically distant sites, HAD is indicated if there is significant genetic heterogeneity (AMOVA Φ_{ST} or F_{ST}) between host-associated groups of individuals at one or more sites. If our analyses fail to reveal such heterogeneity, then we can neither confirm nor reject the possibility of HAD. In particular, such data are not inconsistent with host-related divergence in a very early stage, at which adaptive loci show differentiation by host but ongoing gene flow is still capable of suppressing divergence at the neutral loci we surveyed.

Phylogenetic reconstructions are not well suited to statis-

TABLE 2. Host-associated divergence and genetic diversity in *Solidago* herbivores. Net percent divergence between and genetic diversity within host-associated populations are indicated for mitochondrial sequence data (COI mtDNA), and AMOVA Φ_{ST} and F_{ST} values are indicated for DNA and allozyme data respectively (*N* is the number of individuals sampled for mtDNA from *altissima*, *gigantea*; bold values indicate $P < 0.01$, dashes indicate no data or not calculated).

Herbivore species	Feeding niche	*N*	bp	Net mtDNA divergence[1]	Within-host mtDNA distance (mean ± SE)		Genetic variance due to host plant[2]		Host races?
					altissima	*gigantea*	Allozyme (F_{ST})	DNA (Φ_{ST})	
Eurosta solidaginis[3]	gall	8,10	297	0.11 ± 0.052	0.53 ± 0.20	0.05 ± 0.05	**0.055**	0.116	yes
Gnorimoschema gallaesolidaginis	gall	52,35	652	1.87 ± 0.48	1.63 ± 0.31	0.73 ± 0.18	**0.159**[4]	**0.544**	yes
Rhopalomyia solidaginis/capitata	gall	19,15	618	10.17 ± 2.0	1.96 ± 0.48	0.16 ± 0.10	**0.883**	**0.853**	yes
Asteromyia carbonifera	gall	20,23	642	0.00 ± 0.001	1.85 ± 0.18	0.74 ± 0.17	—	0.014	no?[5]
Procecidochares atra	gall	8,12	609	0.00 ± 0.001	—	—	0	0	no
Epiblema scudderiana	gall/borer	26,15	454–700	0.00 ± 0.000	—	—	0	0	no
Mordellistena convicta	borer/predator	—	—	—	—	—	**0.019**[6]	—	yes
Trirhabda convergens	leaf-chewer	6,6	634	0.22 ± 0.092	—	—	0	0	no
Trirhabda virgata	leaf-chewer	6,9	634	0.11 ± 0.053	—	—	0.019	0.164	no

[1] Net distances are based on maximum likelihood models used in tree searches ± bootstrapped SE.
[2] All nonsignificant negative F_{ST} and Φ_{ST} values are collapsed to zero.
[3] Original data taken from Brown et al. (1996) and Itami et al. (1998).
[4] Four sympatric sites (Nason et al. 2002).
[5] Fine-scale genetic structure exists, and may or may not be related to host-plant use.
[6] Allozyme data from Blair et al. (2005).

tical testing of HAD but are included because they allow visualization of our sequence data, and because, when HAD is indicated by AMOVA, they may allow inferences about the history of diversification (e.g., the relative ages of host-form pairs, or the direction of host shifting). A single origin of the derived host form is indicated if all populations from one host are more closely related to each other than they are to any population of the alternate host form. The topology of the phylogenetic reconstruction is expected to depend on the age of the host-form pair in question. Early in differentiation, we expect to see no mtDNA divergence and thus no apparent phylogenetic structure; after more time we would expect the ancestral host form to be paraphyletic with respect to the monophyletic derived host form; and eventually coalescence should lead to reciprocal monophyly of the two forms. More complicated scenarios are possible, however, in which multiple host lineages have formed independently in different geographic areas or in which host-associated lineages exist in some regions but not others. Our inferences are dependent on the assumption that when host associated lineages are indicated, they are sister groups. Current morphological and ecological evidence supports this (below), but we cannot completely rule out the existence of additional cryptic lineages on other host plants.

RESULTS

Of the seven herbivores surveyed with new data here, two (*Gnorimoschema* and *Rhopalomyia*) consist of genetically differentiated *altissima* and *gigantea* host forms (in addition, host races are well known for *Eurosta* and recently indicated for *Mordellistena*; Abrahamson and Weis 1997; Abrahamson et al. 2001, 2003; Eubanks et al. 2003; Blair et al. 2005). For the other five species, our genetic data are consistent with a single, host-generalist clade. However, our results are conservative because we cannot rule out HAD on finer geographic scales or occurring without neutral genetic divergence.

Gnorimoschema shows strong evidence for rangewide HAD in both mtDNA and allozyme datasets (1.9% net sequence divergence, allozyme $F_{ST} = 0.16$; Table 2), confirming our previous analysis (Nason et al. 2002) that found significant differentiation across hosts at a few Midwestern sites. Estimates of divergence and migration from MDIV are consistent with relatively recent divergence of host forms and low levels of gene flow (Table 3). Moths of the *altissima* form harbor significantly more genetic variation than do *gigantea* moths ($P < 0.01$; Table 2), suggesting an ancestral association of *G. gallaesolidaginis solidaginis* with *S. altissima* and subsequent colonization of *S. gigantea* as in *Eurosta* (Abrahamson and Weis 1997). Phylogenetic reconstructions of *Gnorimoschema* populations based on mitochondrial COI sequence (Fig. 1) confirm host-associated genetic structure across a large number of populations with sympatric collections of moth larvae from both hosts. This pattern suggests that populations on the derived host share a common origin (as opposed to rampant local host-shifting, which would lead to a tree in which populations clustered by geography). Although bootstrap support for the node subtending the *gigantea* clade is only moderate (75%), both NJ and ML recon-

TABLE 3. Scaled divergence times (T) and migration rates (M) estimated from sequence data using MDIV with 95% credibility intervals given in parentheses. Herbivore species that exhibit evidence of HAD are marked in bold.

Herbivore species	Divergence (T)[1]	Migration (M)
Rhopalomyia solidaginis	7.6 (6.6, 94.8)	0.05 (0.01, 0.54)
Gnorimoschema gallaesolidaginis	0.88 (0.80, 19.44)	0.21 (0.05, 0.768)
Eurosta solidaginis	3.76 (0.14, 4.88)	0.32 (0.44, 19.5)
Epiblema scudderiana	0.01 (0.07, 2.93)	10.8 (1.05, 14.67)
Asteromyia carbonifera	0.25 (0.05, 1.95)	23.25 (3.70, 24.55)
Procecidochares atra	0.186 (0.07, 2.922)	3.63 (0.9, 14.56)
Trirhabda convergens	0.01 (0.08, 4.87)	9.4 (1.08, 9.82)
Trirhabda virgata	0.90 (0.13, 4.87)	2.5 (0.33, 9.74)

[1] Likelihood surfaces were often nearly flat for estimates of T and M, sometimes resulting in maximum likelihood estimates that are not included in the 95% credibility intervals (see text).

structions support a monophyletic clade of *Gnorimoschema* feeding on *S. gigantea*. The *altissima* and *gigantea* forms of *G. solidaginis* are assumed to together constitute a monophyletic clade (i.e., they are either sister or else one is paraphyletic with respect to the other). This combined monophyly is strongly supported by the fact that the two host forms are morphologically indistinguishable, but are together easily diagnosable versus all other species in their species group (Miller 2000). A single collection of *Gnorimoschema* from *S. canadensis* was well nested within the *altissima* clade (data not shown); in most places, however, *S. canadensis* is simply not attacked (S. B. Heard, pers. obs.). A single collection of *Gnorimoschema* from *Solidago leavenworthii* in Florida was nested within *gigantea* genotypes (data not shown).

Rhopalomyia gall midges exhibit even greater genetic divergence between host plants (10.2% net sequence divergence; Table 2). Sympatric host-associated populations also exhibited highly significant allozyme differentiation with fixed allelic differences at some loci, a combined $P < 0.001$ over loci for each population comparison ($\chi^2 = 82.4$ to ∞, df = 12–16), and a between-host F_{ST} of 0.88 ($P < 0.001$). Coalescence analyses (MDIV) support strong divergence between host forms with little or no current migration (Table 3). As in *Gnorimoschema*, the *gigantea* clade exhibits less genetic variation than the *altissima* clade ($P < 0.01$; Table 2). Phylogenetic analysis indicated greater than 90% bootstrap support for host-plant defined clades (Fig. 2a). The two species names (*R. solidaginis* and *R. capitata*) that have been applied to *Rhopalomyia* from the two hosts were considered likely synonyms by Gagné (1989) and both were originally described from ''*Solidago canadensis*.'' However, our results strongly support the separation of these clades into distinct species (*R. solidaginis* on *S. altissima* and *R. capitata* on *S. gigantea*) by McEvoy (1988). *Rhopalomyia solidaginis* and *R. capitata* are presumed to be sister species based on adult and gall morphology and their females are easily separable from all other North American *Rhopalomyia* (McEvoy 1988, pers. comm.; Gagné 1989). We have not exhaustively searched for host-specialist lineages on other *canadensis*-complex goldenrods, although a collection from *S. leavenworthii* nests within *gigantea*-associated *Rhopalomyia*.

AMOVA analyses and phylogenetic reconstructions for five other species (the gall-making midge *A. carbonifera*, the leaf-chewing beetles *T. convergens* and *T. virgata*, the gall-making tortricid moth *E. scudderiana*, and the gall-making tephritid fly *P. atra*) based on mtDNA sequences provided no evidence of HAD (Table 2). MDIV analyses of coalescence indicate that each of these herbivore species are characterized by very low estimates of divergence and high estimates of migration except perhaps *T. virgata* in which slight divergence is hinted at (but not supported in other analyses; Table 3). For *Asteromyia* (Fig. 2b), the lack of host-plant related mitochondrial differentiation extends even to a collection from *S. leavenworthii* and another from *S. fistulosa* (a member of *Solidago* subsection Venosae, and not a close relative of *S. altissima* and *S. gigantea*; Semple et al. 1999). However, there is fine-scale phylogenetic structure within the species that may or may not represent host-related diversification. One small clade (Fig. 2b, clade 1) includes three individuals from *S. gigantea* (from Iowa, South Dakota, and Nebraska, $>$ 800 km apart), and while our sampling is not yet comprehensive enough to rule out occurrence of related individuals on *altissima*, other individuals from these and nearby populations occupy phylogenetically distant positions. A similar case exists for *altissima* individuals from the Black Hills and west-central Iowa (Fig. 2b, clade 2). Finally, there are several highly divergent sequences (e.g., two lineages at base and those labeled ''gig 3 ON Can''; Fig. 2) that are genetically distant from other collections from the same or nearby sites, suggesting the possible existence of more distantly related clades of unknown geographic range and host breadth. Together, these results suggest a complicated relationship between host-plant use, biogeographic history, and ancestral polymorphism. This is also hinted at by evidence from allozymes of genetically differentiated lineages of *A. carbonifera* on *S. altissima* that differ slightly in gall morphology (Crego et al. 1990), and it is possible that HAD has occurred in at least one of the cryptic lineages identified by Crego et al. (1990). For *Trirhabda* (Fig. 3), none of the five ML reconstructions of highest likelihood suggests divergent host-related clades in either *T. virgata* or *T. convergens*. Combined tests of allozyme differentiation between sympatric host-associated populations of *T. virgata* and *T. convergens* were not significant ($\chi^2 = 13.6$, 13.7, df = 12, 18, $P = 0.33$, 0.75, respectively), nor were estimates of F_{ST} (Table 2). *Epiblema* (Fig. 4a) and *Procecidochares* (Fig. 4b) exhibited very little mtDNA variation, and what variation did occur was unrelated to host-plant use (Table 2). Lack of HAD was confirmed by allozyme analyses of two sympatric populations of *Epiblema* (Minnesota and Ontario: $\chi^2 = 7.3$, 13.7,

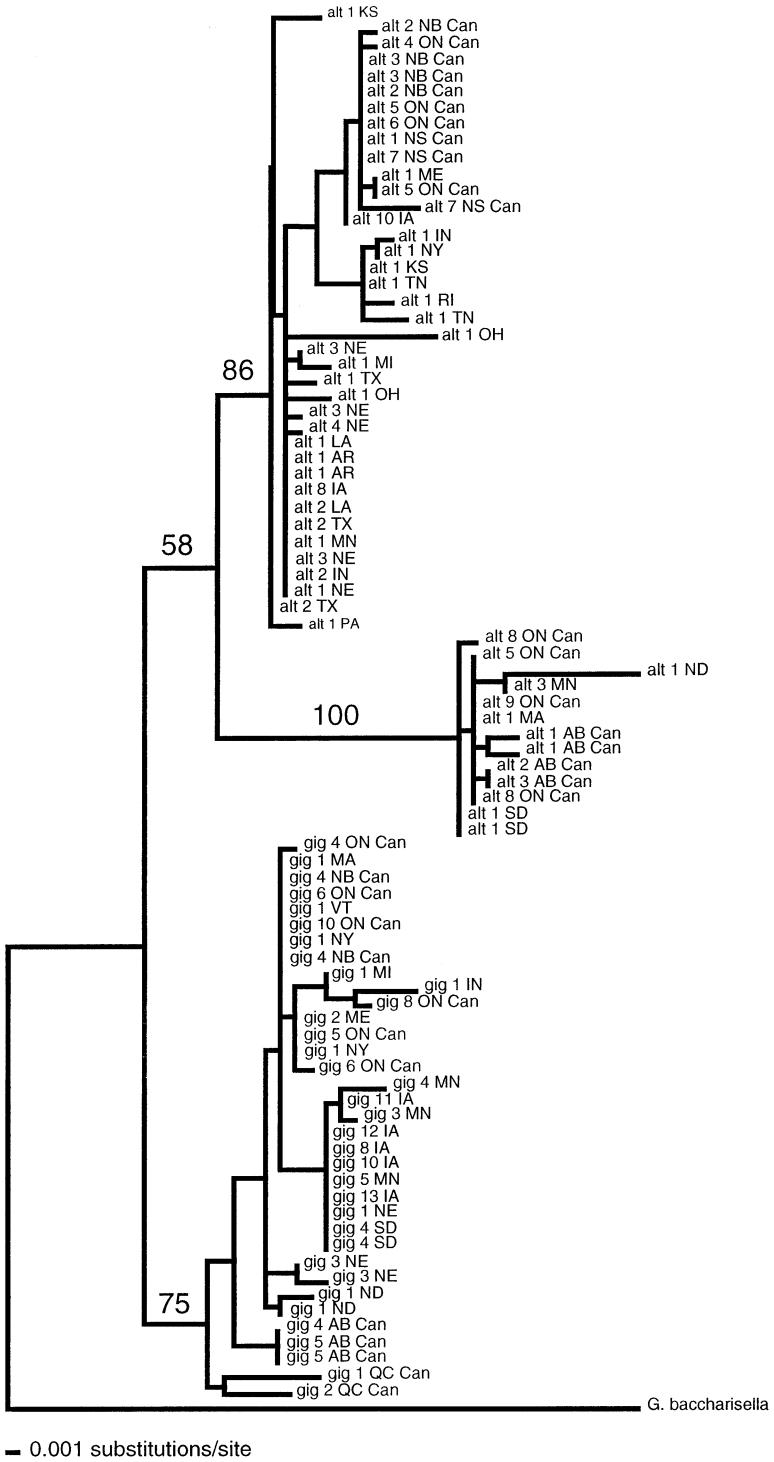

FIG. 1. One of 13 equally likely trees ($-\ln L = 2003.61$) trees inferred by maximum likelihood for *Gnorimoschema*. Reconstructions differ only in fine-scale relationships within host associated clades. The numbers above branches indicate bootstrap (neighbor-joining) support for the subtended clade. The notation ''alt'' indicates a *Solidago altissima* host and ''gig'' indicates a *S. gigantea* host. The U.S. state or Canadian province for each individual is indicated after an integer representing the sampling site within that state/province.

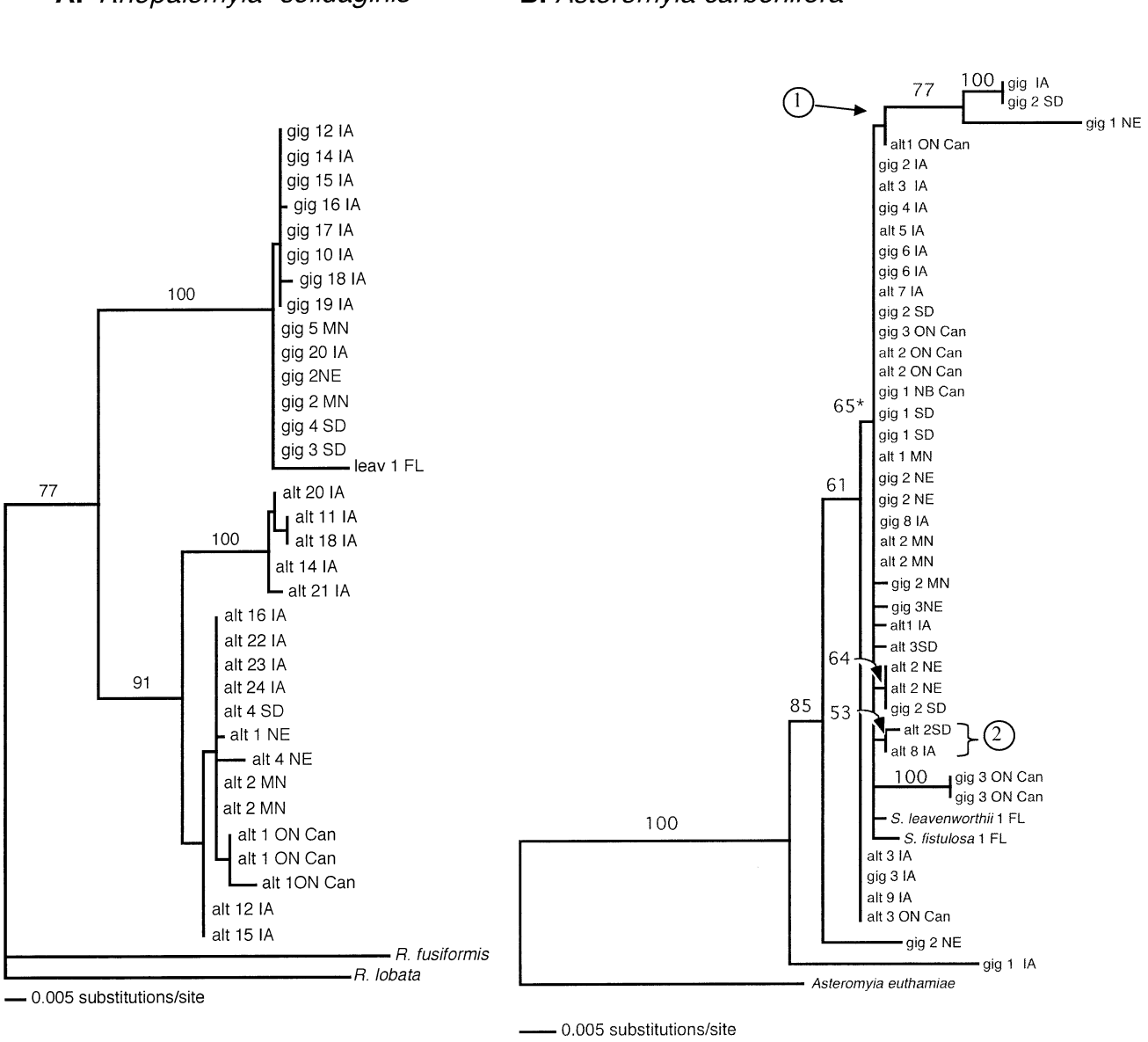

FIG. 2. Maximum likelihood reconstructions for the gall midges (A) *Rhopalomyia* (single most likely tree, −lnL = 1724.41) and (B) *Asteromyia* (one of three trees, −lnL = 1425.06). Numbers above branches indicate bootstrap support. In (B), circled numbers refer to derived clades containing individuals from one or both hosts referred to in the text, and the value marked with an asterisk indicates bootstrap support for the subtended clade not including clade 1. For *Rhopalomyia*, ''leav 1'' indicates an individual sampled from *Solidago leavenworthii*, also a member of the *S. canadensis* complex.

df = 22, 12, P = 1.0, 0.97, respectively; Table 2) and *Procecidochares* (two Iowa sites: χ^2 = 14.87, 8.54, df = 20, 20, P = 0.78, 0.99, respectively; Table 2). Allozyme data for *Asteromyia* are not yet available.

DISCUSSION

Repeated and Parallel Host-Race Formation in the Goldenrod Insect Community

The results presented in this paper, along with previous work on the tephritid fly *E. solidaginis* (Waring et al. 1990; Itami et al. 1998; Smith et al. 2002) and a recent study of the beetle *M. convicta* (Blair et al. 2005) show that at least four taxa of insect herbivores attacking sympatric populations of *S. altissima* and *S. gigantea* (*E. solidaginis*, *G. gallaesolidaginis*, *M. convicta*, and *R. solidaginis*/*capitata*) have evolved pairs of specialist host forms or species on these two host plants. Importantly, in the three species subject to mtDNA analyses (*Eurosta*, *Gnorimoschema*, and *Rhopalomyia*) there is evidence for parallelism in at least the direction of host shifting across cases: in each species, the *gigantea* clade exhibits less genetic variation than the *altissima* clade, suggesting that *S. altissima* is the ancestral host plant. The four species differ in the extent to which differentiation has progressed, with, for instance, much less gene flow between host forms in *Gnorimoschema* (Nason et al. 2002) than in

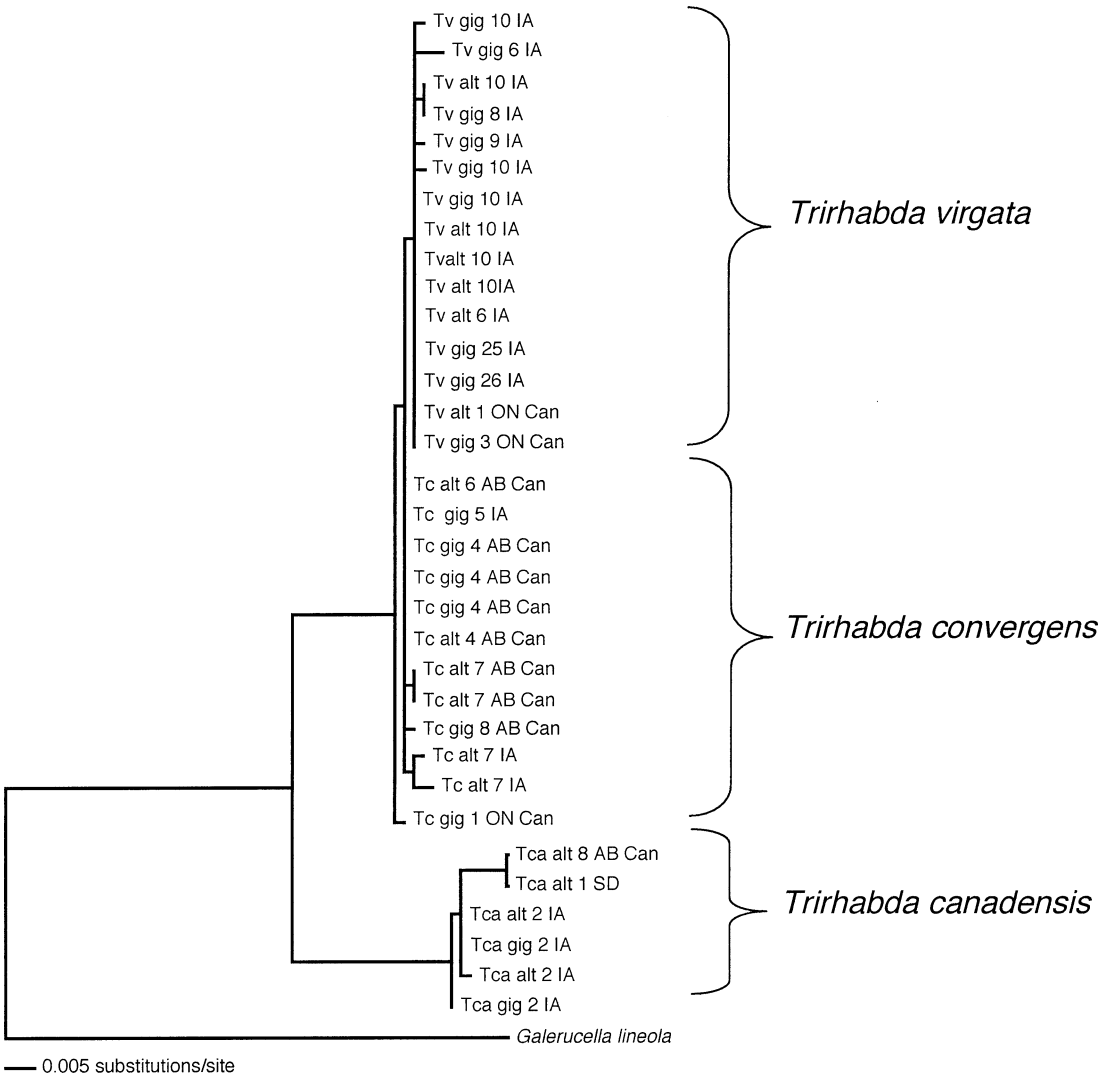

FIG. 3. One of five equally likely maximum likelihood reconstructions for the leaf beetles *Trirhabda convergens* and *T. virgata* ($-\ln L = 1422.24$), with *T. canadensis* and *Galerucella lineola* as outgroups.

Eurosta (Abrahamson and Weis 1997). In part, this likely reflects differences in the ages of the host-associated pairs (see below), but future studies of ecological factors affecting the rate of host-race formation once it has begun would likely be rewarding. Again, however, despite strong morphological support for a close relationship between members of each pair of host related lineages, we cannot be certain that they are indeed sister taxa without exhaustive sampling with respect to geography and host-plant.

For five other species, preliminary genetic analyses show no clear evidence of host-related genetic divergence. In each of these species (*A. carbonifera*, *E. scudderiana*, *P. atra*, *T. convergens*, and *T. virgata*), at least some individuals collected from different host plants shared synapomorphic substitutions and/or entire haplotypes, which suggests at least superficially that these taxa have not experienced widespread HAD. This lack of mtDNA divergence, along with the absence of allozymic differentiation documented for all species except *Asteromyia*, suggests that each species exists as a single generalist form feeding on either host. There are, however, several caveats with respect to these negative results. First, for several species there was little mtDNA sequence variation within and between the populations that we sampled (Table 2). As a result, the mtDNA data provided relatively little power to detect host-related differentiation, if it exists (although for *Epiblema*, *T. convergens*, *T. virgata*, and *Procecidochares*, variable and therefore more powerful allozyme markers also failed to reveal host-associated divergence). Second, lineage sorting could still be incomplete if host races have formed in these taxa relatively recently (e.g., $< 2N$ generations), making it possible that *altissima* and *gigantea* forms could share apparently derived haplotypes in spite of complete reproductive isolation. Finally, failure to detect HAD at the continental scale of our mtDNA analyses does not rule out the possibility of differentiation having taken place at a local scale, within populations at particular geographic locales. Expanded sampling and the analysis of more variable markers are both currently underway. As a result of

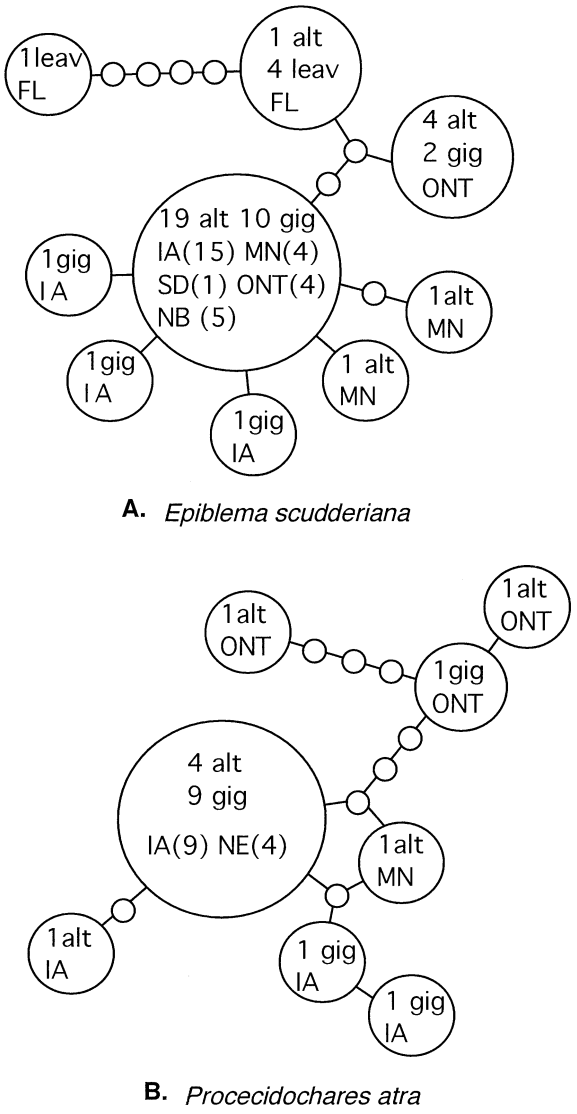

FIG. 4. Haplotype networks for (A) the stem-galling moth *Epiblema* and (B) the rosette-gall forming fruit fly *Procecidochares*. Numbers within circles indicate the number of individuals sampled that possess the haplotype by host plant and/or locality. For *Epiblema*, ''leav'' indicates larvae sampled from *Solidago leavenworthii* in Florida, also a member of the *S. canadensis* complex.

these caveats, our assessment of the frequency of HAD (4/9, 44%) is a conservative one, although even 44% is an impressive figure.

Effects of Insect Life History on Genetic Divergence

Can the occurrence (or lack) of HAD be related to life-history characteristics of phytophagous insects? Answers to such questions will require the synthesis of genetic and ecological data from many insect species, and our survey of nine herbivores (while the broadest available for any host-plant pair) is not yet broad enough to provide powerful statistical hypothesis tests. Nonetheless, our results provide a glimpse of the power of our comparative approach for tackling questions about ecological facilitators of diversification.

One plausible hypothesis is that HAD might be more likely in gallmakers, with their highly intimate relationships with their hosts, than in other feeding guilds. Interactions between gall-makers and plants are intimate because the insects must interact with plant biochemistry to produce both the gall structure and the proliferating plant tissues upon which they feed. This intimacy is reflected in the higher mortality of endophytic insects attributed to plant defenses (Cornell et al. 1998) and by the extreme host-specificity of most gall-makers (Gagné 1989; Dreger-Jauffret and Shorthouse 1992). At first glance, the results of our genetic surveys seem to provide little support for the intimacy hypothesis, as frequencies of HAD are not very different between guilds: three of six gall-making herbivores show clear HAD (Table 2), as does one of three insects that do not make galls. However, the pattern of occurrence of HAD within gall-makers is intriguingly consistent with the intimacy hypothesis, because for each of the three gall-makers apparently without HAD, there are reasons to suspect less intimate associations with the host plants than found for many gall-makers. *Epiblema scudderiana* belongs to a genus made up primarily of stem borers (Miller 1987), and makes only a rudimentary gall from which it extends its boring into adjacent stem pith (Stireman, Nason, Heard, pers. obs.). *Procecidochares atra* also forms a relatively simple gall that is not completely enclosed, with larvae feeding on meristematic tissue cloaked in a sheath of tightly imbricate leaves. Finally, *A. carbonifera* may be secluded from its plant host because its galls are lined with a symbiotic fungus that may modulate interactions between plant and insect (Weis 1982).

Among insects in our survey that do not make galls, only the stem borer *Mordellistena* exhibits HAD (Eubanks et al. 2003; Blair et al. 2005). The external leaf-chewing *Trirhabda* species (which are reported as herbivores of several additional *Solidago* species as well as species in *Aster*; Messina and Root 1980; Messina 1982; Blatt et al. 1999) do not exhibit evidence of HAD, consistent with their relatively less intimate associations with the host plant. We are currently expanding our genetic surveys to include more species, both gall-making and of other feeding guilds, to achieve statistical power in comparative analyses of the sort outlined here.

Dating Divergences and Origins in Allopatry versus Sympatry

Recent discussions of ecological speciation via HAD have emphasized the possibility that it may operate without geographic isolation, that is, in sympatry (Bush 1994; Dieckmann and Doebeli 1999; Berlocher and Feder 2002). Currently, *S. altissima* and *S. gigantea* are broadly sympatric across North America and in most locations are syntopic in local populations, and this raises the possibility that insect taxa exhibiting HAD on these plant taxa may have diverged in sympatry (as has been argued forcefully for *Eurosta*; Craig et al. 1993, 1997; Abrahamson and Weis 1997). However, such inferences are notoriously difficult to test (e.g., Berlocher and Feder 2002; Losos and Glor 2003), and the complex glacial history of North American floras means that present-day sympatry does not rule out one or more periods of allopatry over the history of the taxa.

Our comparative approach to the *Solidago* herbivore sys-

tem provides a novel source of evidence relevant to the question of sympatry versus allopatry. Because at least four insects have experienced HAD, we can ask whether across insect species, episodes of divergence appear to coincide in time. The answer can help distinguish among five hypothetical scenarios for parallel HAD: (1) insects diverged synchronously via cospeciation with host plant; (2) insects diverged synchronously during a single episode of host allopatry; (3) insects diverged asynchronously during a prolonged period of host allopatry; (4) insects diverged asynchronously during multiple episodes of host allopatry; and (5) insects diverged asynchronously in sympatry. A finding of synchronous origins would support the allopatric hypothesis (because sympatric speciation does not require a biogeographic or historical trigger for differentiation) as well as the prolonged allopatry and multiple episodes allopatry hypotheses. In contrast, a finding of asynchrony would reject the cospeciation and single episode allopatry hypotheses, but would be consistent with the remaining scenarios. A further possibility, given the apparently ancient division in *Rhopalomyia*, is that herbivore divergence occurred on the ancestral host plant (or some alternate host plants) and predated divergence of *S. altissima* and *S. gigantea*, which were later colonized in parallel by these related herbivore lineages. This scenario is difficult to evaluate, but given the likely sister-group relationship between these *Rhopalomyia* and their absence on other *Solidago* species (McEvoy 1988; Gagné 1989), it seems unlikely.

In our survey, insects showing host-associated divergence display a wide range of (net) mtDNA genetic divergences (Table 2), from 0.11% in *Eurosta* to 10% in *Rhopalomyia* (no mtDNA data are available for *Mordellistena*). The bootstrapped 95% confidence intervals around these divergences do not overlap, and at first glance, this may appear sufficient evidence to conclude that divergences are indeed staggered. However, extrapolating from significant differences in sequence divergence to differences in the relative timing of divergence requires two additional steps. First, the sequence in question must be shown to evolve in a clocklike fashion (or data must be corrected for violations of clocklike evolution). For *Eurosta*, *Gnorimoschema*, and *Rhopalomyia* the data are consistent with clocklike behavior (likelihood-ratio tests; *Eurosta*: $\chi^2 = 4.88$, df = 17, $P = 0.9$ [data from Brown et al. 1996]; *Gnorimoschema*: $\chi^2 = 80.5$, df = 86, $P > 0.1$; *Rhopalomyia*: $\chi^2 = 38.24$, df = 34, $P > 0.1$). (We note that, although we are taking advantage of clocklike evolution, our inferences depend only on relative timing of divergences, not on absolute dates.) Second, net sequence divergence must be interpreted in light of possible variation in rates of mtDNA evolution across taxa. That rates of mtDNA evolution are variable in insects is supported by a survey of published rate estimates (Appendix 2 available online only at http://dx.doi.org/10.1554/05-222.1.s2), with estimates varying almost 10-fold from 0.6% per million years (Prüsser and Mossakowski 1998), to nearly 5% per million years (Venanzetti et al. 1993). However, these rate estimates may be distorted by artifacts of substitutional saturation. When estimates of rates of mtDNA sequence evolution from published studies are plotted against their calibration times, the estimated rate depends strongly on the calibration age (Fig. 5). This is likely

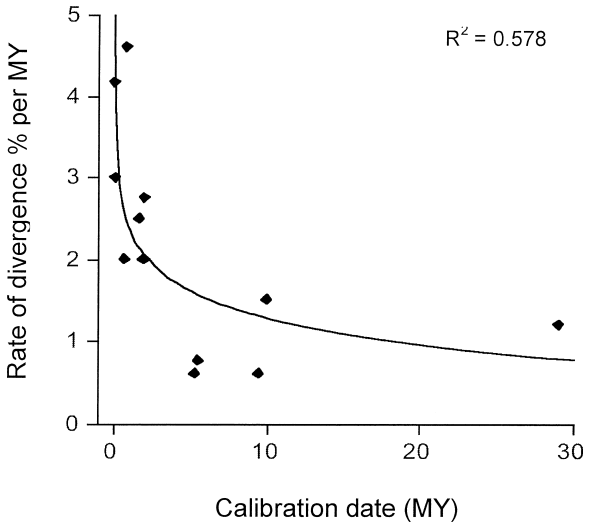

FIG. 5. Estimated rates of molecular evolution in percent divergence per million years versus calibration dates used in their estimation for studies listed in Appendix 2 (online only supplement; $r^2 = 0.578$, $N = 13$, $F_{1,11} = 15.05$, $P = 0.0026$). For studies containing multiple calibration dates a median estimate was calculated. The regression equation is $y = -1.083\log(x) + 2.367$.

a consequence of divergence estimates that do not take into account mutational saturation (Arbogast et al. 2002). Moreover, it suggests that saturation occurs much more quickly than expected merely on the basis of codon degeneracy. COI rate estimates with calibration times between 0.01 and 10 million years are most relevant to interpretation of our results, and these suggest a range of 1.5–2.8% divergence per million years (Appendix 2 available online). Plotting uncorrected net divergences and confidence intervals estimated for our *Solidago* herbivores against this range of rates produces a set of plausible intervals around the divergence estimates of haplotypes associated with alternate hosts for each species (Fig. 6). These intervals do not overlap (i.e., given our assumptions concerning the range in substitution rates, the origins of the three pairs of host forms were almost certainly asynchronous). Asynchronous divergence is also supported by the widely varying F_{ST}-estimates among taxa that parallel patterns of net sequence divergence (Table 2) and by widely separated ML estimates of divergence times estimated by MDIV. These analyses make several simplifying assumptions including approximately equal effective population sizes (of females) in ancestral and descendent populations and similar rates of gene flow for mtDNA across species, both of which can influence divergence estimates of mtDNA (Arbogast et al. 2002). However, given the extent of variation in mtDNA divergence among taxa, we believe that population size and migration are unlikely sole explanations for our results, and that differences in the time of phylogenetic splitting are, at the least, important contributing factors generating the pattern.

Although Monte Carlo variances of T (scaled divergence times) from MDIV are notoriously large and credibility intervals broad (Nielsen and Wakeley 2001), ML estimates of T for *Rhopalomya* and *Gnorimoschema* are widely different (7.6 vs. 0.88 respectively; Table 3). The likelihood surface

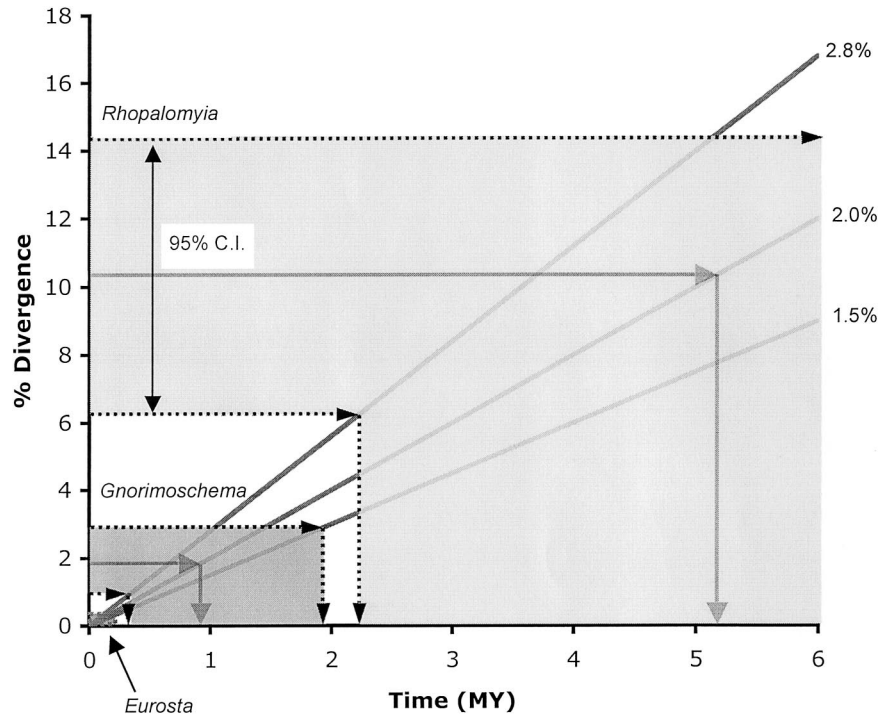

FIG. 6. Molecular divergence against time (million years, MY), illustrating the relative divergences (cytochrome oxidase I) of host-plant associated clades of *Gnorimoschema*, *Rhopalomyia*, and *Eurosta*. Estimates of net divergence between host associated clades, along with 95% confidence intervals (gray, see text), are mapped onto three rates of evolution for the gene cytochrome oxidase I spanning the range of published rate estimates for divergences less than 10 million years old. It can be seen along the x-axis that there is no overlap in confidence intervals between these taxa regardless of the rate of molecular evolution used.

for *Eurosta* was extremely shallow, resulting in a rather high estimate of divergence, though credibility intervals and migration rates suggest shallower divergence between host forms than in *Gnorimoschema*. Estimates of migration ($2Nm$) parallel these findings (0.04, 0.16, and >0.88 for *Rhopalomyia*, *Gnorimoschema*, and *Eurosta*, respectively; Table 3).

The apparently asynchronous origins of the three host-form pairs allow us to reject two of our five scenarios for parallel HAD. Asynchrony is inconsistent with origins via cospeciation with the goldenrod hosts and is also inconsistent with common origins during a single (short) episode of host allopatry. From timing data alone, we cannot reject any of the three remaining scenarios: asynchronous origins during longstanding allopatry, multiple episodes of allopatry, or origins in sympatry. The hypothesis of longstanding allopatry seems unlikely, as it would require *S. altissima* and *S. gigantea* to have been allopatric for most of their history, establishing sympatry only very recently (more recently than the origins of the *Eurosta* host races). As the two goldenrods have very similar habitat requirements and are excellent dispersers, such a history would be surprising even given range movements associated with glaciations. Multiple episodes of allopatry (at least three would be required) are conceivable, perhaps in glacial refuges associated with different glacial maxima, although this scenario is distinctly unparsimonious. The sympatric scenario is currently more difficult to evaluate, as the status of sympatric divergence models remains controversial (Berlocher and Feder 2002). *Eurosta*'s HAD most likely occurred in sympatry or parapatry (Abrahamson and Weis 1997); like *Eurosta*, both *Gnorimoschema* and *Rhopalomyia* possess some of the ecological attributes that have been associated with sympatric divergence (Diehl and Bush 1984; Rice 1987). For instance, both species exhibit at least some degree of allochrony in host-plant attack and adult emergence (*Gnorimoschema*, Seehawer 2002; *Rhopalomyia*, J. O. Stireman, unpubl. data), and *Rhopalomyia*, like *Eurosta*, mates on or near the host plant (Spence 1969; J. O. Stireman, pers. obs.). None of this, however, can definitively establish sympatric origins for the host-form pairs, and so further ecological work is underway.

Conclusions

In summary, evidence of host-associated genetic divergence and its implied role for ecological speciation is now available for four (*E. solidaginis*, *G. gallaesolidaginis*, *R. solidaginis*/*capitata*, and *M. convicta*) members of the herbivore community feeding on the goldenrods *S. altissima* and *S. gigantea*. This represents 44% of the nine species that have been examined. For the other species (*A. carbonifera*, *E. scudderiana*, *P. atra*, and the two *Trirhabda* species) the genetic evidence suggests a lack of host-associated genetic divergence at a broad scale, although we cannot rule out cryptic divergence (e.g., at adaptive loci only). Of course, we do not expect these figures to extrapolate directly to the entire 100+ species that comprise the herbivore community on these goldenrods, because we have not yet tested for HAD in broadly polyphagous members of the community (such as phloem-

feeding Hemiptera/Homoptera, generalist Lepidoptera, or Orthoptera) that we expect to lack differentiation. Furthermore, because our study is the first to our knowledge to consider HAD at the community level, we cannot yet know whether the *Solidago* system is typical of other host-plant pairs. Still, our results, along with previous behavioral and ecological studies of some of the taxa considered here, provide evidence that host-related divergence and subsequent speciation has likely been a significant mode of speciation in phytophagous insects. In turn, this argues for an important role for ecological (and possibly sympatric) mechanisms of speciation in the diversification of the earth's most speciose group of terrestrial organisms. A full evaluation of the importance of HAD in the diversification of this and other diverse parasitic groups will require continued detailed behavioral, ecological, and genetic study of specific interactions along with broader comparative analyses such as ours in additional systems.

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