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HOST-ASSOCIATED GENETIC DIFFERENTIATION IN THE GOLDENROD ELLIPTICAL-GALL MOTH, *GNORIMOSCHEMA GALLAESOLIDAGINIS* (LEPIDOPTERA: GELECHIIDAE)

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Abstract.—Careful study of apparently generalist phytophagous insects often reveals that they instead represent complexes of genetically differentiated host races or cryptic species. The goldenrod elliptical-gall moth, *Gnorimoschema gallaesolidaginis*, attacks two goldenrods in the *Solidago canadensis* complex: *S. altissima* and *S. gigantea* (Asteraceae). We tested for host-associated genetic differentiation in *G. gallaesolidaginis* via analysis of variation at 12 allozyme loci among larvae collected at six sites in Iowa, Minnesota, and Nebraska. *Gnorimoschema gallaesolidaginis* from each host are highly polymorphic (3.6–4.7 alleles/locus and expected heterozygosity 0.28–0.38 within site-host combinations). Although there were no fixed differences between larvae from *S. altissima* and *S. gigantea* at any site, these represent well differentiated host forms, with 11 of 12 loci showing significantly different allele frequencies between host-associated collections at one or more sites. Host plant has a larger effect on genetic structure among populations than does location (Wright's $F_{ST} = 0.16$ between host forms vs. $F_{ST} = 0.061$ and 0.026 among *altissima* and *gigantea* populations, respectively). The estimated F_{ST} between host forms suggests that the historical effective rate of gene flow has been low ($N_em \approx 1.3$). Consistent with this historical estimate is the absence of detectable recombinant (hybrid and introgressant between host form) individuals in contemporary populations (none of 431 genotyped individuals). Upper 95% confidence limits for the frequency of recombinant individuals range from 5% to 9%. Host association is tight, but imperfect, with only one likely example of a host mismatch (a larva galling the wrong host species). Our inferences about hybridization and host association are based on new maximum-likelihood methods for estimating frequencies of genealogical classes (in this case, two parental classes, F_1 and F_2 hybrids, and backcrosses) in a population and for assigning individuals to genealogical classes. We describe these new methods in the context of their application to genetic structure in *G. gallaesolidaginis*. Population phenograms are consistent with the origin of the host forms (at least in the midwestern United States) via a single host shift: *altissima* and *gigantea* moth populations form distinct lineages with 100% bootstrap support. Genetic structure in *Gnorimoschema* is of particular interest because another gallmaking insect attacking the same pair of hosts, the tephritid fly *Eurosta solidaginis*, includes a pair of host races with partial reproductive isolation. *Gnorimoschema gallaesolidaginis* and *E. solidaginis* therefore represent the first reported case of parallel host-associated differentiation, that is, differentiation by evolutionarily independent insect lineages across the same pair of host plants.

Key words.—Gallmaker, genetic structure, *Gnorimoschema*, host races, hybridization, *Solidago*, speciation.

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Accounting for the spectacular diversity of phytophagous insects is a longstanding and difficult problem for evolutionary ecologists (Walsh 1864; Ehrlich and Raven 1964; Mitter et al. 1988; Farrell et al. 1992). One piece of the puzzle may be a propensity among such insects for speciation via host-race formation (perhaps even in sympatry; Berlocher and Feder 2002). A few cases of host-race formation in phytophagous insects are well documented, for instance, races of the tephritid flies *Rhagoletis pomonella* (Feder et al. 1998) and *Eurosta solidaginis* (Abrahamson and Weis 1997). However, little is known of the frequency among insect lineages of genetic differentiation (and eventual speciation) via host-race formation. We do not know the likelihood of host-race formation given an opportunity (i.e., given one insect species attacking two hosts, either in allopatry or sympatry) or how that likelihood might depend on the ecology of the insect or the hosts.

A deeper understanding of the evolutionary importance of differentiation following host shifts will require perspective from two complementary approaches. The first is the study of multiple host shifts within a single clade, for instance, recent work extending analysis of the *Rhagoletis* hawthorn-apple host shift to the 35 or so hosts used by the *R. pomonella* species group (Berlocher 2000). The second is the study of host shifts by multiple evolutionarily independent insect lineages on the same host plant pair. We are taking this latter approach by analyzing host-associated genetic variation in the goldenrod elliptical-gall moth, *Gnorimoschema gallaesolidaginis*, which attacks the same pair of hosts as do the well-studied *Eurosta solidaginis* host races (Abrahamson and Weis 1997); *Gnorimoschema* and *Eurosta* together represent the first known case of parallel host-associated differentiation. *Gnorimoschema* and *Eurosta* are phylogenetically distant (in different orders) and have rather different life histories, so the extent to which they have undergone similar differentiation across their common host pair will shed light on the frequency and importance of host shifts as a general process generating biodiversity among phytophagous insects.

Gnorimoschema gallaesolidaginis is a widespread and common herbivore of goldenrods (Leiby 1922; Fontes et al. 1994; Miller 1963, 2000). Most workers have assumed it to be a single species attacking (syntopically) both *Solidago*

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altissima and *S. gigantea*, although a recent study suggests recognition of two host-specialist species (Miller 2000). Our growing appreciation of cryptic genetic differentiation in insects suggests three alternatives: *G. gallaesolidaginis* might be a single oligophagous species, a complex of host races (Diehl and Bush 1984) with partial genetic differentiation but ongoing gene flow, or a complex of morphologically cryptic species between which gene flow has ceased. We used allozyme electrophoresis to assay genetic variation in *G. gallaesolidaginis* collected from *S. altissima* and *S. gigantea* from six populations in Iowa, Minnesota, and Nebraska. We asked four related questions about genetic differentiation in *G. gallaesolidaginis*: (1) whether there is significant host-associated genetic structure at four sites where the gallmakers occur on both hosts, that is, whether *G. gallaesolidaginis* has distinct *altissima* and *gigantea* host forms; (2) whether phylogeographic data are more consistent with origin of the forms via a single host shift followed by dispersal or with repeated local differentiation; (3) whether host-choice mistakes (larvae of the *altissima* form collected from *S. gigantea* or vice versa) occur in natural populations; and (4) whether *altissima* and *gigantea* forms at each site are reproductively isolated or whether there is detectable gene flow between them. In this paper, we use the term ''host form'' to indicate host-associated, genetically differentiated subpopulations, whether there is ongoing gene flow between them (host races) or not (cryptic species).

Our estimates of the frequency of host-choice mistakes and of gene flow between host forms required two kinds of inferences based on multilocus allozyme genotypes of individuals. First, we needed to accurately infer the frequencies (at each site) of individuals belonging to the *altissima* and *gigantea* host forms and to recombinant genealogical classes (F_1 and F_2 hybrids between forms and backcrosses). Second, we needed to infer the most likely origin, with respect to the same candidate classes, of individual insects. We outline new likelihood-based methods for making both kinds of inferences, and we illustrate their application to our *Gnorimoschema* data. These methods provide several advantages over currently available procedures (e.g., Nason and Ellstrand 1993; Rieseberg et al. 1998) for estimating the extent of hybridization and introgression in natural populations.

Materials and Methods

Study Species, Sites, and Collections

Gnorimoschema gallaesolidaginis (Lepidoptera: Gelechiidae) is a specialist gallmaking herbivore of goldenrods (*Solidago* spp.). The species is univoltine (Leiby 1922), with females laying eggs in autumn on senescent goldenrod foliage. In spring, larvae hatched from overwintering eggs attack new shoots, burrowing into the terminal bud and down the stem to initiate a hollow stem gall; larvae pupate in late summer. The major hosts are *S. altissima* L. and *S. gigantea* Ait., two closely related members of the *S. canadensis* complex that are abundant in prairies, old fields, and disturbed habitats across much of temperate North America. Both hosts are rhizomatous perennials, and they often grow together in dense, intermixed stands.

We report here on collections of *G. gallaesolidaginis* from *S. altissima* and *S. gigantea* at six sites in Iowa, Minnesota, and Nebraska. Because our interest was in the possibility of host-associated genetic differentiation, we focus primarily on collections from four sites (Fig. 1) where the two hosts grew as tightly interdigitated clones (i.e., were clearly syntopic) and where *Gnorimoschema* was abundant on both hosts. Bogus Brook (BB, Mille Lacs Co., MN, 45°43′N, 93°37′W) is an old field/prairie restoration managed by the Minnesota Department of Natural Resources (DNR). Crystal Lake (CL, Adams Co., NE, 40°27′N, 98°26′W) is an open area near Crystal Lake State Recreation Area. Cone Marsh (CM, Louisa Co., IA, 41°24′N, 91°24′W) is an old field in Cone Marsh Wildlife Management Area (Iowa DNR). Zimmerman Field (ZF, Johnson Co., IA, 41°45′N, 91°44′W) is an old field in private ownership. At each of these sites, we collected larvae from both *S. altissima* and *S. gigantea* (henceforth, ''*altissima* moths'' and ''*gigantea* moths''). We also include data from two sites where larvae were collected from just one host. Hayden Prairie (HP, Howard Co., IA, 43°27′N, 92°23′W) is a prairie preserve (Iowa DNR); at this site galls were rare on *S. altissima*, so we analyze only *gigantea* moths. Bellevue (BV, Jackson Co., IA, 42°17′N, 90°49′W) is a roadside population of pure *S. altissima*. Our dataset, therefore, includes four sites (BB, CL, CM, and ZF) with both *altissima* and *gigantea* moths, plus one additional site (HP) for *gigantea* moths and one (BV) for *altissima* moths (Fig. 1).

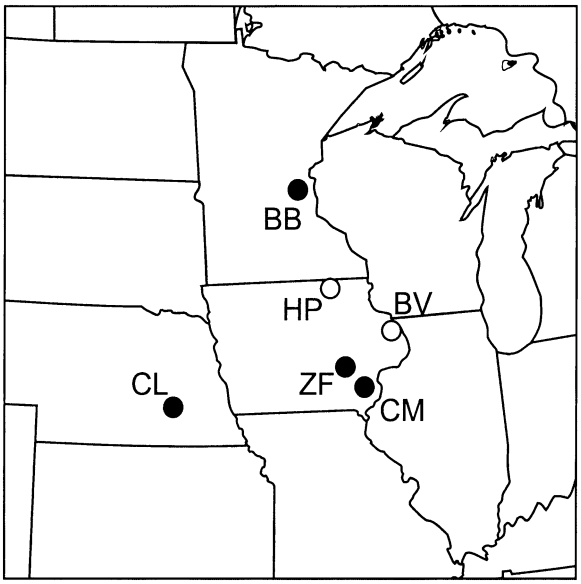

FIG. 1. Locations of *Gnorimoschema* collection sites. Solid symbols represent sites with collections from both *Solidago altissima* and *S. gigantea* hosts; open symbols are sites with collections from one host only. BB, Bogus Brook; BV, Bellevue; CL, Crystal Lake; CM, Cone Marsh; HP, Hayden Prairie; and ZF, Zimmerman Field. See text for site descriptions.

Collections were made during June and July 1999 and 2000. All galls from a particular site were collected on the same day. To minimize collection of multiple larvae from sibling groups, we did not collect galls from stems closer than 2 m apart. We collected galled stems, sorted them by species, and held the cut stems in water for up to 48 h before removing *Gnorimoschema* larvae. Larval mortality during

this procedure was very low. We preserved living larvae by flash freezing in liquid nitrogen and stored them at −80°C for later allozyme analysis. We collected about 50 larvae from each host at each site, except for 96 larvae from *S. gigantea* at Cone Marsh. Representative goldenrod specimens were preserved as herbarium vouchers (deposited in the Ada Hayden Herbarium at Iowa State University).

Allozyme Analysis

Total protein extracts were obtained by grinding larvae at −4°C with 40 μL of Wendel and Parks's (1982) extraction buffer modified to exclude polyvinylpyrrolidone (PVP). This buffer, designed for plant material, is superior to simpler Tris-HCl/mercaptoethanol buffers for many herbivorous insects (J. Nason, pers. obs.). Extracts were absorbed onto Whatman (Ann Arbor, MI) chromatography paper wicks, placed in chilled 96-well plates, and stored at −80°C until electrophoresis. Twelve enzymes were variable in at least one population and exhibited expected subunit structures and patterns of expression: aconitate hydratase (*Acoh*, EC 4.2.1.3), adenylate kinase (*Ak*, EC 2.7.4.3), aspartate aminotransferase (*Aat*, EC 2.6.1.1), fluorescent esterase (*Fe*, 3.1.1.-), glucose-6-phosphate isomerase (*Gpi*, EC 5.3.1.9), glycerol-3-phosphate dehydrogenase (*G3pdh*, EC 1.1.1.8), D-2-hydroxy-acid dehydrogenase (*Hadh*, EC 1.1.99.6), isocitrate dehydrogenase (*Idh*, EC 1.1.1.42), leucine aminopeptidase (*Lap*, EC 3.4.11.1), malate dehydrogenase (*Mdh*, EC 1.1.1.37), phosphoglucomutase (*Pgm*, EC 5.4.2.2), and triose-phosphate isomerase (*Tpi*, EC 5.3.1.1). Enzymes were resolved using 10% starch gels and a 0.04 M morpholine-citrate buffer system adjusted to pH 6.1 (Murphy et al. 1996). Staining protocols for individual loci followed Soltis et al. (1983), except for *Hadh*, which followed Murphy et al. (1996). These loci do not represent an exhaustive search for polymorphism in *G. gallaesolidaginis*.

Estimating Genetic Variation

We summarized levels of genetic variation for each *altissima* and *gigantea* moth population using the proportion of polymorphic loci (*P*, where a polymorphic locus has the frequency of the most common allele <0.99), the number of alleles per locus (*A*), and the expected heterozygosity (H_e) calculated using Levene's (1949) adjustment for finite sample size. Population-level estimates were obtained for *altissima* and *gigantea* moths by averaging over loci, whereas host-level estimates for each host form were obtained by pooling allele frequencies across sites before averaging over loci. Standard errors for all estimates were calculated over loci. For each measure, we used *t*-tests to assess differences between *altissima* and *gigantea* moths at the sympatric-population and host levels. We tested genotype frequencies against Hardy-Weinberg expectations using likelihood-ratio *G*-tests (Sokal and Rohlf 1995) after pooling genotypes with expected frequencies less than three.

Testing for Genetic Differentiation of Host Forms within Sites

We tested for heterogeneity of allele frequencies at each locus between sympatric *altissima* and *gigantea* moth populations with likelihood-ratio *G*-tests (Sokal and Rohlf 1995) using the program PopGene (Yeh and Boyle 1997). A global test for all loci was obtained using Fisher's combined probability test (Sokal and Rohlf 1995). We interpreted rejection of homogeneity, at one locus or globally, as support for the existence of barriers to gene exchange between host forms at a given site.

Estimating Population-Level Phylogeny

We examined phylogenetic relationships among *Gnorimoschema* populations using Nei's genetic distances (Nei 1972) calculated from allozyme frequencies. Population phenograms were constructed from distance matrices by neighbor-joining. An alternative analysis using Cavalli-Sforza's chord measure of genetic distance (Cavalli-Sforza and Edwards 1967) gave congruent results. We estimated bootstrap support for individual nodes from 1000 bootstrap resamplings of the original allozyme frequency data. All procedures were implemented using programs in PHYLIP (Felsenstein 1997).

Hierarchical Analysis of Population Genetic Structure

We calculated Wright's *F*-statistics (F_{IT}, F_{ST}, and F_{IS}) with respect to a three-level hierarchy, suggested by the population-level phylogeny, in which local host-associated subpopulations are nested within *altissima*-moth and *gigantea*-moth groups. All six sites are included in this model, which examines the effect of host species on observed patterns of allele frequency variation. We also conducted separate analyses for *altissima* populations only and for *gigantea* populations only. All *F*-statistics were estimated by Cockerham-Weir ANOVA methods (Weir and Cockerham 1984; Weir 1996) using the program GDA (Lewis and Zaykin 1999), with significance determined by bootstrapping over loci.

Estimating Historical Rates of Gene Flow

We estimated rates of historical gene flow between sympatric moth populations on different hosts and among allopatric moth populations on the same host, using Wright's (1951) expression for historical migration rate: $N_em = (0.25)[(1/F_{ST}) - 1]$. Here, N_em is the average effective number of migrants entering each population to breed in each generation; for populations on different hosts, migrants enter a population to breed via hybridization. Estimates of gene flow from Wright's expression are only crude guidelines, because the underlying infinite-island model makes many assumptions that may not be met in natural populations (Whitlock and McCauley 1999). Therefore, we focus on relative rates of gene flow (in particular, comparing gene flow between host forms in sympatry to gene flow among spatially separated populations on a single host) and do not attempt to interpret the absolute magnitude of N_em.

Estimating Contemporary Rates of Gene Flow and Host Mismatching

To complement our historical estimates, we sought independent estimates of contemporary gene flow based on the frequency of recombinant genotypes in modern populations.

We also sought to quantify the strength of host association in natural populations by estimating the rate of host mismatching—by which we mean attack by one host form on the incorrect host (i.e., attack on *S. altissima* by a larva of the *gigantea* host form or vice versa). Frequent host mismatches could weaken disruptive selection for host specialization, and if assortative mating depends on larval host experience (e.g., via host-influenced timing of emergence) they could also lead to breakdowns in reproductive isolation.

These two problems share a solution, because both depend on our ability to identify the genealogical origin of a larva or set of larvae based on multilocus allozyme genotype(s). That is, imagine that each larva could be assigned to one of six genealogical classes: purebred *altissima* moths (P_1), purebred *gigantea* moths (P_2), first (F_1) or second (F_2) generation hybrids between forms, or F_1 backcrosses to either parental form (BP_1, BP_2). For this model of population structure, a mistake in assortative mating would be inferred for each larva identified as an F_1; second generation recombination and potential gene flow between host forms would be inferred for larvae assigned to F_2 or backcross classes; and a host mismatch would be inferred if a larva sampled from *S. altissima* is assigned to the purebred *gigantea* class (or vice versa).

Given a number of diagnostic loci, such inferences are straightforward upon inspection of larval genotypes. However, closely related taxa (such as host races and sibling species) often are not fixed for alternative alleles (e.g., *Eurosta*, Waring et al. 1990; *Rhagoletis*, Feder et al. 1988; *Zeiraphera*, Emelianov et al. 1995; *Gnorimoschema*, see Appendix), necessitating the use of likelihood-based techniques to relate genotypes to genealogical classes. Likelihood methods provide a flexible framework for inferring parentage (Roeder et al. 1989; Smouse and Meagher 1994), identifying immigrants (Rannala and Mountain 1997; Cornuet et al. 1999), and assigning individuals to source populations (Smouse et al. 1990; Paetkau et al. 1995; Davies et al. 1999; Pritchard et al. 2000) under a variety of population structures.

New statistical methods for genealogical inference

We develop here two complementary methods for genealogical inference, given a sample of individuals (with known multilocus genotypes) and a set of candidate genealogical classes to which those individuals might belong. The first provides a population-level, maximum-likelihood estimate of the frequency of each genealogical class and its variance. The second uses a genotype's likelihood of arising in each of the six genealogical classes to assign individuals to these classes and provides estimates of statistical power for correct classification (software available from J. Nason upon request). In each case, we restrict our attention to the set of candidate genealogical classes suggested above: $C = \{P_1, P_2, F_1, F_2, BP_1, BP_2\}$. Recombinant and introgressant types from successive generations are possible, but because the number of possible hybrid types increases exponentially, their enumeration becomes problematic. Although ignoring third and further generation recombinant events could lead one to underestimate the frequency of recombination (because repeated backcrosses will most likely be misidentified as parentals), for our *Gnorimoschema* data additional analysis using a model permitting repeated backcrossing did not change any results.

Both our genealogical-frequency and individual-assignment methods represent advances over currently available techniques for genealogical analysis from multilocus genotypes. Our estimation procedures are distinguished from two conceptually related methods for inferring hybridization on the basis of the genetic information used and the parameters estimated. The multinomial method of Nason and Ellstrand (1993; see also Nason et al. 1992; Epifanio and Philipp 1997) estimates the same parameters (class frequencies and individual likelihoods) but uses information only from parental-form-specific alleles, excluding potentially informative differences in the frequencies of shared alleles. Rieseberg et al.'s (1998) procedure, in contrast, uses both form-specific and shared alleles, but estimates a hybrid index measuring the proportions of alleles in individual genotypes arising from the two parental forms, rather than assigning individual genotypes to genealogical classes. This Bayesian formulation does not update prior with posterior values, however, resulting in a bias toward an equal contribution of forms that could lead one to overinterpret a sample individual's degree of hybridity.

Population-level frequencies of host mismatching and hybridization

We assume that allele frequencies for a set of L loci (in linkage equilibrium) in the P_1 and P_2 populations are accurately estimated from population samples. Expected genotypic frequencies can then be calculated for each defined genealogical class. Let g_i denote the multilocus genotype of the ith sampled individual ($i = 1 \ldots N$) and let $T(g_i \mid C_j)$ be the transmission probability of g_i occurring in C_j, the jth genealogical class ($j = P_1 \ldots BP_2$). Because loci are assumed independent, the probability of a multilocus genotype is the product of probabilities across individual loci. Because F_{IS}-estimates were not significantly different from zero for any population (see Results), we modeled these probabilities assuming random union of gametes as appropriate for each class. A Bayesian estimate of the probability density of an individual's membership in a specific class C_{j*}, given its genotype g_i, is

$$\Pr(C_{j*} \mid g_i) = \frac{T(g_i \mid C_{j*})\Pr(C_{j*})}{\sum_j T(g_i \mid C_j)\Pr(C_j)}, \quad (1)$$

where $\Pr(C_j)$, the prior probability of class j, is an estimate of the frequency of class j in the sample population. Posterior estimates of these probabilities are obtained by summing over individuals:

$$\Pr(C_{j*})' = \frac{\sum_i \Pr(C_{j*} \mid g_i)}{N}. \quad (2)$$

Initially the priors are unknown and are assumed uniform and uninformative (i.e., equal for all genealogical classes); this under- and overassigns individuals to high and low probability classes, respectively. Next, we use the EM algorithm (Weir 1996) to find unbiased, maximum-likelihood solutions for $\Pr(C_j)$ by: (1) assigning initial priors $\Pr(C_j)$ and finding

posterior values $\Pr(C_j)'$; (2) using the posterior values as updated priors; and (3) repeating this process until the prior and posterior probabilities converge. Relying on standard likelihood theory, variances for these estimates were obtained by inverting the expected value of the information matrix (for an example see Roeder et al. 1989).

Individual-level tests of host mismatching and hybridization

An approach complementary to the population-level estimation of genealogical class frequencies is the assignment of individuals to classes based on their particular multilocus genotypes. Previous studies making such assignments have taken a variety of approaches. For example, some researchers (e.g., Paetkau et al. 1995) have simply assigned each individual to the class in which its genotype has the highest probability, without testing the significance of differences in probabilities between classes. Others have developed likelihood-ratio statistics to explicitly test assignment to one class versus another but only for the simplest case of two classes (e.g., nonimmigrant vs. immigrant; Rannala and Mountain 1997). Our method is based on assessment of the relative likelihoods of individual multilocus genotypes under two or more alternative assignments. This method uses Monte Carlo simulations to develop test criteria for assignment of genotypes to specific classes and to estimate the power of these tests. Preliminary analyses of our data indicated hybridization and host mismatching to be uncommon in *G. gallaesolidaginis*, leading us to focus on testing the null hypothesis that individuals collected from *S. altissima* and *S. gigantea* are members of the expected parental classes (P_1 and P_2, respectively). In the event that the null hypothesis is rejected for an individual moth, we evaluate the relative fits of alternative hypotheses (first generation hybridization, F_1, and backcrossing, BP_1 and BP_2) to the genetic data.

Consider an individual moth collected from *S. altissima*: The null hypothesis is that it belongs to the P_1 class (purebred *altissima* form). To test this hypothesis, we begin by simulating genotypic data for 10,000 P_1 individuals based on allele frequencies from *S. altissima* associated larvae at the appropriate collection site. The relative likelihood of a genotype arising in the P_1 class as opposed to the alternative classes P_2, F_1, BP_1, or BP_2 is measured by ratio of the genotype's likelihoods under the constrained model (P_1 only) and the unconstrained model (P_1, P_2, F_1, BP_1, or BP_2): $\Lambda = T(g_i \mid C_{P_1})/\Sigma_j\, T(g_i \mid C_j)$ for $j = P_1, P_2, F_1, BP_1, BP_2$. In practice we use the log likelihood-ratio, $\ln(\Lambda) = \ln[T(g_i \mid C_{P_1})] - \ln[\Sigma_j\, T(g_i \mid C_j)]$, expecting that $\ln(\Lambda)$ will be increasingly negative as the fit of the constrained model (the null hypothesis, that the individual is P_1) becomes increasingly poor. Using significance criterion α, the critical value of the one tailed test to discriminate non-P_1 from P_1 genotypes is the $\alpha \times 10{,}000$th lowest value of the distribution of $\ln(\Lambda)$ for the simulated P_1 genotypes. Because we evaluate the origin of each collected larva (with n larvae per site), we adjust our significance criterion by the Dunn-Sidák method (Sokal and Rohlf 1995) to control experimentwise (= sitewise) error rate: For experimentwise error rate α, we use testwise $\alpha' = 1 - (1 - \alpha)^{1/n}$. (Throughout this paper, we adopt $\alpha = 0.05$.) If an individual's likelihood ratio statistic, $\ln(\Lambda)$, is less than the critical value, we reject the null hypothesis (P_1 origin) in favor of the alternative (the individual is non-P_1). In this event, the individual is removed from the calculation of sample allele frequencies and the entire test procedure repeated for that population. For larvae collected from *S. gigantea*, the likelihood-ratio statistic is $\ln(\Lambda) = \ln[T(g_i \mid C_{P_2})] - \ln[\Sigma_j\, T(g_i \mid C_j)]$, and we use 10,000 simulated P_2 genotypes for each site to determine the critical value to discriminate non-P_2 from P_2 genotypes.

Our ability to make accurate assignments depends on the power of the tests to discriminate one genealogical class from another. That is, in addition to detected cases of host mismatching and of hybridization/introgression, some cases may go undetected. The detected and undetected fractions of such cases represent the power ($1 - \beta$) and Type II error (β) of the tests, respectively, and can be estimated via Monte Carlo simulation. Given the low frequency of hybridization events detected, we focused on determining the power of tests discriminating parental forms from each other and from the F_1, BP_1, and BP_2 classes. Because the greatest genetic differentiation will exist between parental forms, with less differentiation between parentals and F_1 hybrids and less again between parentals and backcross classes, we would expect greater power to detect host mismatching than hybridization or backcross events. In each case, to estimate power we calculate $\ln(\Lambda) = \ln[T(g_i \mid C_{P_1})] - \ln(\Sigma_j\, T[g_i \mid C_j)]$ or $\ln(\Lambda) = \ln[T(g_i \mid C_{P_2})] - \ln[\Sigma_j\, T(g_i \mid C_j)]$ depending on which parental form represents the null hypothesis. For each test, the power is the fraction of $\ln(\Lambda)$ values for the 10,000 simulated genotypes of the alternative class that fall beyond the critical value of the distribution for the null class. As an illustration, Figure 2 shows the determination of critical values for tests of P_1 versus P_2 and versus F_1 assignments for one of our study sites (BB).

In cases where the null hypothesis (membership in the expected parental class) is rejected, further analysis is required to assign the individual to one of the alternative classes. For instance, imagine a larva collected from *S. altissima* for which we reject the null hypothesis (P_1 membership). We first determine log likelihood-ratios for that individual's genotype arising in the P_2, F_1, BP_1, or BP_2 classes, based on 10,000 simulated genotypes for each class. Each ratio compares the likelihood under assignment to one class versus the unconstrained likelihood; for instance, for F_1 the likelihood-ratio is $\ln(\Lambda) = \ln[T(g_i \mid C_{F_1})] - \ln[\Sigma_j\, T(g_i \mid C_j)]$. For the individual in question, we ask whether each $\ln(\Lambda)$ value falls within the 95% confidence limits of the simulated distribution of $\ln(\Lambda)$ for the appropriate class. If only one class meets this criterion, the individual is assigned to this class. If two or more classes remain, we test the most likely class as the constrained, null hypothesis pairwise against each of the other classes in turn (with each unconstrained, alternative hypothesis consisting of the most likely and the alternative class). For example, if only the F_1 and BP_1 classes remain for an individual, and its log likelihood is higher for the F_1 class, then the test statistic would be $\ln(\Lambda) = \ln[T(g_i \mid C_{F_1})] - \ln[T(g_i \mid C_{F_1}) + T(g_i \mid C_{BP_1})]$, with an expected distribution generated via simulation for 10,000 F_1 and 10,000 BP_1 genotypes. The BP_1 class is rejected and F_1 class accepted as population of origin if $\ln(\Lambda)$ for the test individual is greater

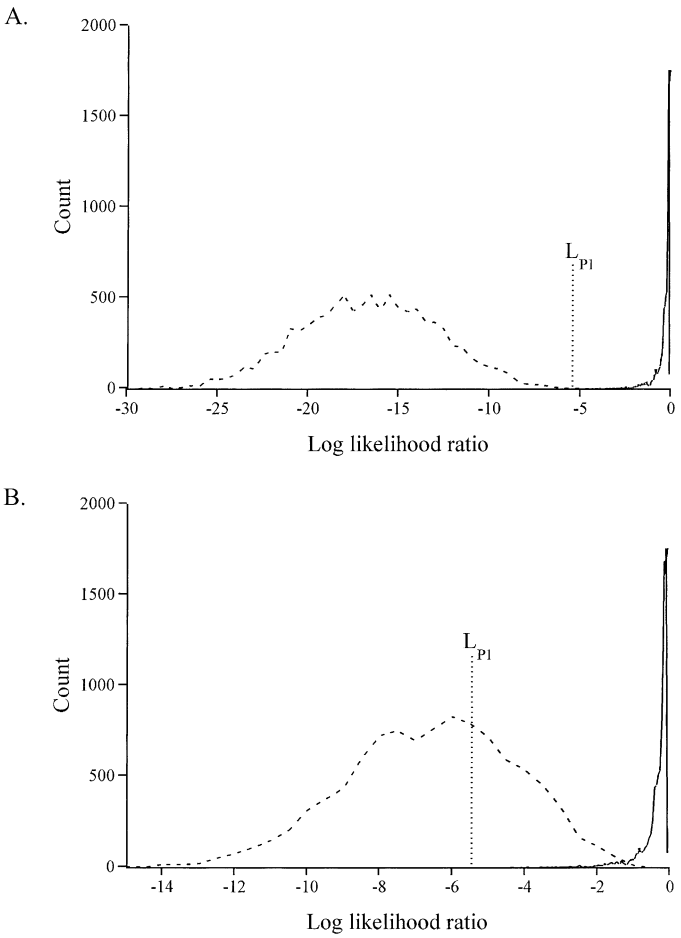

FIG. 2. Visualization of the power of the genetic data to discriminate between genealogical classes. Both figures show the simulated distributions of log likelihood-ratio statistics calculated for different classes under the null hypothesis that individuals are P_1 moths: (A) 10,000 *altissima* (P_1, solid line) and 10,000 *gigantea* (P_2, dashed line) moths; (B) 10,000 *altissima* (P_1, solid line) and 10,000 F_1 hybrid (dashed line) moths. L_{P_1} is the lower critical value of the likelihood-ratio test statistic for P_1 genotypes calculated using an experimentwise error rate (one-tailed test). The power to discriminate P_2 from P_1 moths (A) and F_1 from P_1 moths (B) is determined by the proportion of the distribution for P_2 or F_1 genotypes falling below this critical value.

than the $\alpha \times$ 10,000th highest value for the simulated BP_1 genotypes and within the $(\alpha/2) \times$ 10,000th highest and lowest values for the simulated F_1 genotypes. Otherwise, the assignment is ambiguous: We accept both genealogical classes as potential source populations. Because these tests are applied only to individuals for which the original host form has been rejected, they use an individual testwise significance level of $\alpha = 0.05$.

RESULTS

Genetic Variation in Gnorimoschema gallaesolidaginis

Both *altissima* and *gigantea* moths were highly polymorphic (Table 1). In each population, at least 10 of the 12 loci assayed were variable, and both allelic diversity (range among populations, 3.6–4.7 alleles/locus) and heterozygosity (0.28–0.38) were high. None of these measures differed significantly between any pair of sympatric *altissima* and *gigantea* moth populations, nor did population means or host-level estimates differ between host forms.

For 11 of our 12 loci, genotype frequencies within individual *altissima* and *gigantea* moth populations did not differ from Hardy-Weinberg equilibrium. The *Tpi* locus, in contrast, was significantly out of Hardy-Weinberg equilibrium in three *altissima* and one *gigantea* moth populations. In each of these populations, allelic diversity was low and the frequency of the common allele was greater than 0.92; nonetheless, one or two individuals were homozygous for a low frequency allele (such genotypes had expected frequencies less than 0.002). Inspection of full multilocus genotypes for these individuals did not indicate excess homozygosity at other marker loci.

Genetic Differentiation between Sympatric Populations

Although there were no fixed, diagnostic differences between larvae galling sympatric *S. altissima* and *S. gigantea*, there were substantial differences in allele frequencies as well as low frequency, form-specific alleles at a number of loci (Appendix). Allele frequency profiles differed significantly between *altissima* and *gigantea* moths for a number of loci, indicating *G. gallaesolidaginis* to be genetically differentiated with respect to host species (Table 2). The number of significant frequency differences for each sympatric comparison is well in excess of the number ($\approx$0.5) expected due to Type I error at $\alpha = 0.05$. Accordingly, global tests over all loci were significant for each sympatric pair (df = 24, all $P <$ 0.001). Only one locus (*G3pdh*) failed to exhibit allele frequency heterogeneity between any pair of sympatric populations. Two other loci, *Pgm1* and *Tpi*, were significant for only one population pair; the remaining nine loci each were significant in three or four (of four) tests.

Population-Level Phylogeny

Population phenograms constructed from genetic distances support the conclusion that *G. gallaesolidaginis* is well differentiated genetically with respect to host species. The neighbor-joining tree clusters *altissima* and *gigantea* moths into two distinct lineages with 100% bootstrap support (Fig. 3). Our decision to root the tree at the *altissima-gigantea* node was based on its 100% bootstrap support and on other evidence for genetic differentiation described above. All other nodes had bootstrap values less than 50%, indicating little confidence in relationships among populations within host forms. Omission of the *Tpi* locus (which was not in Hardy-Weinberg equilibrium) had no effect on these conclusions.

Hierarchical Population Genetic Structure

F_{ST} corresponding to the correlation of genes within host forms ($F_{ST} = 0.159$) was substantial and significant (Table 3). In analyses conducted separately by host, F_{ST} among populations was significantly greater than zero for *altissima* moths ($F_{ST} = 0.061$) and for *gigantea* moths ($F_{ST} = 0.026$) and these estimates are not significantly different from each other.

TABLE 1. Genetic variation over 12 allozyme loci for *Solidago altissima* and *S. gigantea* moth populations calculated at the per site, population, and host levels. Standard errors in parentheses. *P*, fraction polymorphic loci; *A*, mean alleles per locus; H_e, effective heterozygosity. Study site abbreviations are defined in the text. None of the paired differences at the sympatric site, population, or host levels were significant.

Moth host sp. (Site)	*N*	*P*	*A*	H_e
S. altissima (BB)	48	0.833 (0.108)	3.58 (0.53)	0.257 (0.072)
S. gigantea (BB)	48	1.000	4.42 (0.36)	0.323 (0.063)
S. altissima (CL)	48	0.917 (0.080)	3.58 (0.51)	0.365 (0.074)
S. gigantea (CL)	48	0.917 (0.080)	3.58 (0.45)	0.315 (0.059)
S. altissima (CM)	48	0.917 (0.080)	4.00 (0.49)	0.375 (0.074)
S. gigantea (CM)	96	0.833 (0.108)	4.67 (0.60)	0.345 (0.075)
S. altissima (ZF)	48	1.000	4.08 (0.54)	0.368 (0.073)
S. gigantea (ZF)	47	0.917 (0.080)	3.75 (0.51)	0.281 (0.067)
S. altissima (BV)	48	0.917 (0.080)	3.75 (0.46)	0.374 (0.075)
S. gigantea (HP)	46	0.833 (0.108)	3.83 (0.63)	0.328 (0.076)
Population means				
S. altissima	48	0.912 (0.035)	3.80 (0.23)	0.348 (0.033)
S. gigantea	57	0.900 (0.038)	4.05 (0.23)	0.318 (0.030)
Host-level estimates				
S. altissima	240	0.917 (0.080)	5.50 (0.47)	0.365 (0.075)
S. gigantea	285	0.917 (0.080)	5.75 (0.52)	0.336 (0.072)

Although genotype frequencies were consistent with Hardy-Weinberg proportions within individual populations, the correlation of genes within individuals within populations was significant when taken over all populations ($F_{IS} = 0.043$). This result is largely attributable to *altissima* ($F_{IS} = 0.087$) rather than *gigantea* moths ($F_{IS} = 0.006$; Table 3). However, these host-associated F_{IS}-values are not significantly different from one another.

Historical Rates of Gene Flow

Estimates (from F_{ST}) of historical effective migration rates (N_em) among populations within host forms were 3.85 and 9.37 for *altissima* and *gigantea* moths, respectively, whereas the migration rate between host forms was 1.32. Using confidence limits estimated for F_{ST}, only the difference in N_em-values for *gigantea* moths and between host forms was significant.

TABLE 2. Single locus and global probabilities associated with tests of genetic differentiation between sympatric host-associated forms of *Gnorimoschema*. Study site abbreviations are defined in the text.

Locus	Sympatric study sites			
	BB	CL	CM	ZF
Aat	0.001	0.012	0.005	0.003
Acoh1	0.000	0.000	0.000	0.000
Ak	0.234	0.000	0.000	0.000
Fe1	0.049	0.219	0.020	0.006
G3pdh	0.247	1.000	0.222	0.097
Gpi	0.000	0.000	0.013	0.111
Hadh	0.000	0.000	0.000	0.000
Idh2	0.005	0.000	0.000	0.001
Lap2	0.000	0.000	0.000	0.000
Mdh1	0.005	0.004	0.000	0.000
Pgm1	0.113	0.199	0.067	0.000
Tpi	0.122	0.021	0.367	0.055
Global probability	<0.001	<0.001	<0.001	<0.001

Contemporary Rates of Gene Flow and Host Mismatching

Analyses of contemporary gene flow and host mismatching were generally consistent with historical, F_{ST}-based estimates of gene flow. Our results show both host mismatches and gene flow between host forms to be rare in natural populations of *G. gallaesolidaginis*.

Population-level frequencies of host mismatching and hybridization

Estimates of contemporary frequencies of recombination between host forms suggest gene flow to be rare. For 431 *altissima* and *gigantea* moths genotyped from our four sympatric sites, the estimated frequencies of F_1, F_2, BP_1, and BP_2 classes each were zero. Moreover, for three sites (BB, CL, and ZF) there was no evidence of host mismatching: For

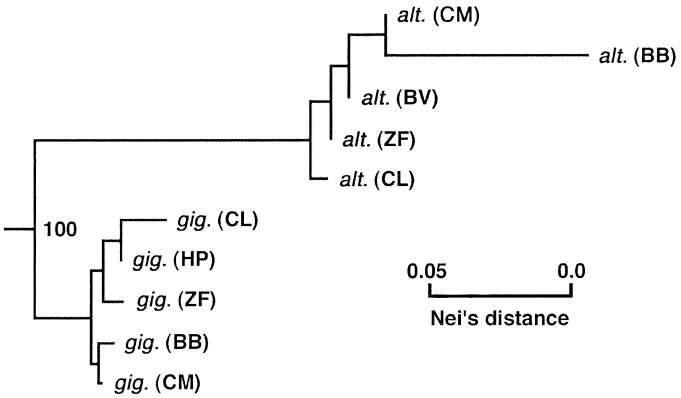

FIG. 3. Estimated population phylogeny constructed using neighbor-joining and Nei's (1972) genetic distance. Moth populations collected from *Solidago altissima* (*alt.*) and *S. gigantea* (*gig.*) host plants form distinct lineages with 100% bootstrap support between them. Bootstrap support for other nodes in the tree was less than 50%.

TABLE 3. Estimates of Wright's *F*-statistics for populations for a three-level hierarchy (top row, with subpopulations nested with *Solidago altissima* and *S. gigantea* groupings, as suggested by the population phylogeny) and for two-level hierarchies with respect to each host plant. The 95% bootstrap confidence intervals are given in parentheses.

Grouping	Wright's *F*-statistics		
	F_{IS}	F_{IT}	F_{ST}
Hosts	0.043 (0.027–0.065)	0.230 (0.134–0.318)	0.159 (0.066–0.244)
altissima moths	0.087 (0.046–0.135)	0.143 (0.093–0.193)	0.061 (0.034–0.088)
gigantea moths	0.006 (−0.039–0.051)	0.032 (−0.017–0.076)	0.026 (0.010–0.043)

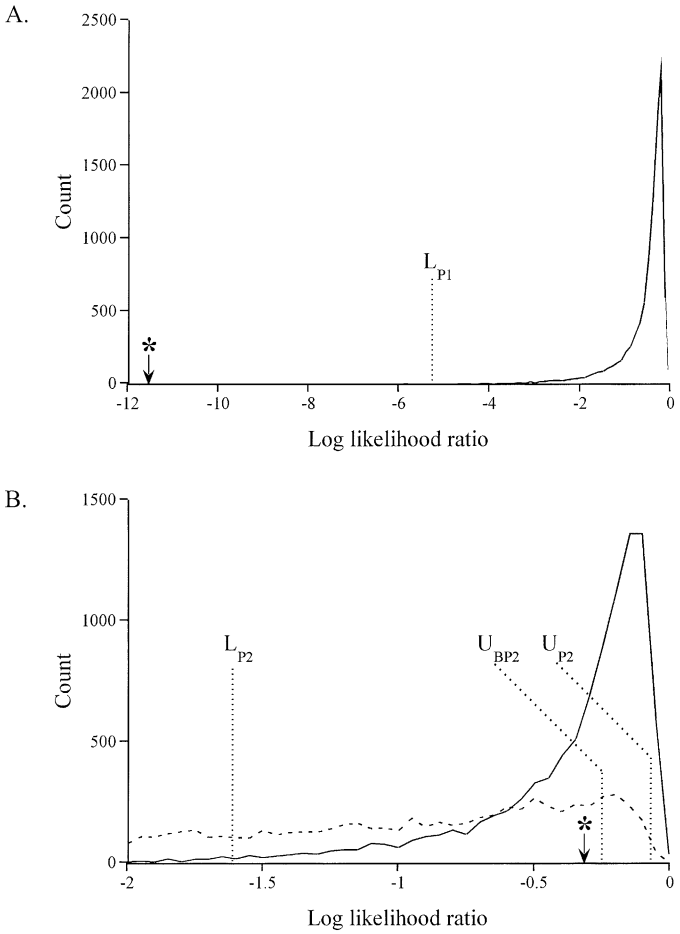

FIG. 4. Individual assignment tests for the one *Gnorimoschema gallaesolidaginis* individual inferred not to belong to its expected parental class. (A) Evidence for rejection of membership in the expected parental class (P_1). Solid line: expected distribution of the log likelihood-ratio test statistic for simulated P_1 (*altissima*) genotypes. L_{P_1} indicates the critical value of the distribution (one-tailed test, experimentwise $\alpha = 0.05$). Asterisk indicates the log likelihood-ratio for the test individual, leading us to reject membership in P_1. Similar tests reject membership in the F_1 and BP_1 ($P_1 \times F_1$) classes but not P_2 and BP_2 ($P_2 \times F_1$). (B) Solid and dashed lines: expected distributions of the log likelihood-ratio test statistics for simulated P_2 (*gigantea*) and BP_2 genotypes, respectively. U_{P_2} and L_{P_2} are upper and lower critical values for P_2 genotypes (two-tailed test), and U_{BP_2} is the upper critical value for BP_2 genotypes (one-tailed test). Asterisk indicates the log likelihood-ratio for the test individual, from which we cannot reject membership in either class (P_2 or BP_2).

larvae collected from *S. altissima* and *S. gigantea* the estimated frequencies of the expected parental classes (P_1 and P_2, respectively) were one. For our typical sample size of 48 individuals/population, an upper 95% binomial confidence limit on the aggregate frequency of classes other than the expected parental class is 6.1%. For CM, there was no evidence of mismatching for larvae from *S. gigantea*. However, for larvae from *S. altissima* the estimated frequencies of the P_1 and P_2 classes were 0.979 (95% confidence limits 0.938–1.000) and 0.021 (0.000–0.062), respectively, suggesting that one of 48 larvae was in fact a *gigantea* moth (a host mismatch).

Individual-level tests of host mismatching and hybridization

Consistent with the population-level analysis, individual assignment tests revealed only one case of 431 sampled insects with significant evidence for a host mismatch or hybridization event. As expected, this was for one individual collected from *S. altissima* at site CM: We rejected the hypothesis that this individual belonged to the expected parental class ($P < 0.0001$, Fig. 4). The rarity with which we rejected membership of individuals in the expected parental classes was not due to limited power to discriminate *altissima* and *gigantea* forms; this power exceeded 0.95 even after adopting a stringent testwise α' to control experimentwise error rate for each study site (Table 4).

What is the origin of the one individual (collected from *S. altissima* at CM) clearly not belonging to the expected parental class? The log likelihoods of this individual's genotype arising in genealogical classes P_1, P_2, F_1, BP_1, and BP_2 are −17.9, −6.7, −10.2, −12.1, and −7.7, respectively. Having rejected the hypothesis that this individual is P_1, we conducted tests of assignment to each of the remaining genealogical classes. Likelihood-ratio tests rejected assignment to F_1 ($P = 0.008$) and BP_1 ($P = 0.001$) classes but not assignment to P_2 ($P = 0.45$) or BP_2 ($P = 0.27$). We then tested the constrained hypothesis of P_2 membership versus the unconstrained hypothesis (P_2 or BP_2). For this final test, the individual's likelihood-ratio statistic was below the critical value of the one-tailed test for simulated BP_2 genotypes ($P = 0.11$) and within the critical values of the two-tailed test for simulated P_2 genotypes ($P = 0.44$); therefore, neither class can be rejected as a potential source for this individual (Fig. 4). However, we note that backcross hybridization first requires the formation of F_1 hybrids, for which significant evidence was not found in any of our study populations. Furthermore, assignment to BP_2 would mean postulating a host mismatch (because the individual was collected from *S. altissima*, not *S. gigantea*) in addition to hybridization. The

TABLE 4. Statistical power for assignment tests of individuals to genealogical classes. The null hypothesis for each test is that the individual belongs to the parental class corresponding to the host on which it was collected (P_1 for individuals collected from *Solidago altissima*; P_2 for those from *S. gigantea*). Entries in rows labeled "*i* vs. *j*" give statistical power ($1 - \beta$, for each collecting site) for identifying an individual of class *i* against the null hypothesis of membership in class *j*. For instance, entries for F_1 versus P_1 are probabilities of correctly rejecting P_1 membership for an individual with an F_1 hybrid genotype. Study site abbreviations are defined in the text.

Test	Site	Power (experimentwise α)[1]
P_2 vs. P_1	BB	0.999
	CL	0.994
	CM	0.954
	ZF	0.950
F_1 vs. P_1	BB	0.620
	CL	0.710
	CM	0.436
	ZF	0.333
BP_1 vs. P_1	BB	0.113
	CL	0.215
	CM	0.088
	ZF	0.070
BP_2 vs. P_1	BB	0.907
	CL	0.903
	CM	0.755
	ZF	0.692
P_1 vs. P_2	BB	1.000
	CL	0.993
	CM	0.979
	ZF	0.961
F_1 vs. P_2	BB	0.950
	CL	0.744
	CM	0.558
	ZF	0.457
BP_1 vs. P_2	BB	0.988
	CL	0.923
	CM	0.841
	ZF	0.787
BP_2 vs. P_2	BB	0.408
	CL	0.250
	CM	0.148
	ZF	0.096

[1] Experimentwise α: corrected for tests done on multiple larvae at a site to achieve sitewise $\alpha = 0.05$; see text for details.

most parsimonious interpretation in this particular case is assignment to the P_2 class, a conclusion consistent with the class frequency analysis for this population. Taken together, these arguments strongly suggest a case of host mismatching rather than backcross hybridization.

Among the remaining 430 larvae, individual assignment tests identified no significant evidence of F_1 or first generation backcross hybridization between *altissima* and *gigantea* host forms. However, interpretation of this result hinges on an assessment of our power to identify recombinant individuals. For F_1 hybrids, using experimentwise $\alpha = 0.05$, the power of the test discriminating F_1 from P_1 and P_2 membership ($1 - \beta_{F_1}$) ranged from 0.33 to 0.71 (mean = 0.52) and 0.46 to 0.95 (mean = 0.68), respectively, across populations (Table 4). Given these power values, how many undetected F_1s could be consistent with the absence of detected F_1s? For any host form at any site, the number of detected (x) and undetected ($N_{F_1} - x$) F_1 individuals will be binomially distributed with $P(x) = C_x^{N_{F_1}}(1 - \beta_{F_1})^x(\beta_{F_1})^{N_{F_1}-x}$. An upper 95% confidence limit on the number of undetected F_1s in a sample without detected F_1s ($x = 0$) is $\max(N_{F_1}) = \ln(0.05)/\ln(\beta_{F_1})$. For moths collected from *S. altissima*, $\max(N_{F_1})$ averaged across sites is 4.5, suggesting hybridization at a rate of no more than 9%. For moths from *S. gigantea*, average $\max(N_{F_1})$ is 2.9, suggesting a hybridization rate of no more than 5%. Our power to identify backcross individuals (discriminating BP_1 from P_1 and BP_2 from P_2 genotypes) was lower, ranging from 0.070 to 0.215 (mean = 0.121) and 0.096 to 0.408 (mean = 0.225), respectively, across sites (Table 4). Based on these power calculations, the frequencies of BP_1 and BP_2 hybridization could be as high as 58% and 29%, respectively. However, such high backcross frequencies are unlikely in the absence of substantial F_1 hybridization, a possibility ruled out by both population- and individual-level analyses above.

DISCUSSION

Differentiation and Taxonomic Status of the altissima and gigantea Host Forms

Gnorimoschema attacking *S. altissima* and *S. gigantea* have long been treated taxonomically as a single oligophagous entity under the name *G. gallaesolidaginis*. Our data unequivocally reject this hypothesis: The host forms show consistent and significant allele-frequency differences in all four of our sympatric populations (Table 2). Furthermore, there are significant differences between host forms in the phenology of gall initiation and adult emergence (S. Heard, unpubl. data; see Miller 2000).

Given that *altissima* and *gigantea* moths are genetically distinct, are they host races (lineages with partial reproductive isolation as a consequence of adaptation to different hosts; Diehl and Bush 1984) or cryptic species between which gene flow has ceased? Miller (2000) has recently taken the latter interpretation, publishing the name *G. jocelynae* for moths from *S. gigantea*. There are, however, no morphological traits of larvae or adults diagnostic for either form (Miller 2000). Miller's treatment is instead based primarily on differences in color and shape of the bung (a disk of larval silk and plant resins constructed by the larva) sealing the gall's exit hole. However, bung traits are not unambiguously free of host-plant influence. Miller (2000) reported that larvae transferred from between hosts produce bungs typical of their host of origin; however, with small sample sizes (three larvae transferred in each direction) the conclusion that bung differences are primarily a function of insect genotype, and indicate species status, must remain tentative.

Our population genetic data provide independent lines of evidence bearing on the status (host race vs. species) of the *G. gallaesolidaginis* host forms. Such evidence comes first from levels of contemporary recombination between forms and second from the extent of host-associated genetic differentiation.

Our estimates of recombination via hybridization between *G. gallaesolidaginis* host forms suggest substantial reproductive isolation between *altissima* and *gigantea* moths. Whereas historical gene flow measurements based on F_{ST}

provide crude guidelines at best (Whitlock and McCauley 1999), our estimate for gene flow between sympatric host forms ($N_em \approx 1.3$) is in rough accord with the rarity of detected gene flow events in modern populations. Furthermore, these estimates strongly suggest that gene flow between host forms in sympatry is much less than gene flow among populations within forms ($N_em \approx 4$ and $N_em \approx 9$ for *altissima* and *gigantea* moths) over distances of approximately 500 km. Population- and individual-based estimates of hybridization are consistent with these historical estimates: There is no convincing evidence of gene flow via hybridization or backcrossing between *altissima* and *gigantea* moths, suggesting that mistakes in mate choice are uncommon or absent. However, upper 95% confidence limits for the frequencies of F_1 hybrids in our four sympatric populations ranged from 5% to 9%, and our power to discriminate backcross from parental classes (BP_1 vs. P_1 and BP_2 vs. P_2) was less than 40% (Table 4). Thus, pending analysis of many more collections, as well as the development of additional marker loci (both underway), we cannot rule out low levels of gene flow between forms in contemporary populations—levels consistent with estimated rates of hybridization between the *E. solidaginis* host races (2–3%; Abrahamson and Weis 1997) and between the *R. pomonella* races (4–6%; Feder et al. 1998).

Our analyses of genetic structure in *Gnorimoschema* indicate that host associated genetic differentiation is significant, but limited. Population phenograms show *altissima* and *gigantea* moths forming distinct, well-supported lineages (at least in the midwestern United States) consistent with the origin of host forms via a single host shift (Fig. 3). The lack of fixed allelic differences, however, suggests a recent origin of the two forms. Furthermore, genetic differentiation between *altissima* and *gigantea* moths is intermediate between that typically found within insect species and that typical of insect sibling-species pairs. For example, allozyme studies of genetic differentiation within moth species ($n = 20$; Peterson and Denno 1997) reveal F_{ST}-estimates ranging from 0.001 to 0.09 (mean = 0.032). Previously documented host-race pairs reveal similar or somewhat stronger differentiation: for instance, $F_{ST} = 0.012$ between apple and hawthorn races of *R. pomonella* (McPheron et al. 1988), $F_{ST} = 0.055$ between *altissima* and *gigantea* races of *E. solidaginis* (Itami et al. 1998), $F_{ST} = 0.065$ between larch and pine races of *Zeiraphera diniana* (Emelianov et al. 1995), and $F_{ST} = 0.21$ between clover and alfalfa races of the aphid *Acyrthosiphon pisum* (Via 1999). Differentiation between host forms of *G. gallaesolidaginis* ($F_{ST} = 0.16$) falls into the range for host-race pairs but exceeds typical within-species estimates. Finally, for genetic differentiation between sympatric species pairs, the largest dataset available comes from allozyme studies of *Drosophila* ($n = 23$; Coyne and Orr 1997). Nei's distance ($D = 0.07$, Fig. 3) between *G. gallaesoliginis* host forms is comparable to distances among semispecies of *D. athabasca* and *D. paulistorum* ($D = 0.02$–0.17) but substantially lower than for any sympatric species pair ($D = 0.19$–1.17).

In summary, patterns of gene flow and genetic differentiation suggest that the *G. gallaesolidaginis* host forms are either well-established host races or rather young cryptic species (if the latter, then host race formation has already occurred, followed by further differentiation and the completion of reproductive isolation). Like Miller's (2000) bung-structure data, however, our population genetic data do not (yet) come down clearly for or against species status for *altissima* and *gigantea* moths. As a result, we believe taxonomic recognition of distinct *Gnorimoschema* species on *S. altissima* and *S. gigantea* is probably premature.

Host Association, Assortative Mating, and the Maintenance of Differentiation between Host Forms

Our genetic data clearly indicate that sympatric populations of *G. gallaesolidaginis* on the two goldenrod hosts have evolved and now maintain strong genetic differentiation. We can currently say much less about the ecological mechanisms by which this differentiation is maintained. We cannot appeal to microallopatric separation of the host forms, because at our four sympatric sites, ramets of the two host species are separated by only centimeters. In other known phytophagous host-race pairs (e.g., *Eurosta*, Abrahamson and Weis 1997; *Rhagoletis*, Feder et al. 1998; *Enchenopa*, Wood et al. 1999), a common suite of factors often contributes to the maintenance of host association and assortative mating: strong host preference, mating on the host plant, and differences between host races in the timing of adult emergence. We do not yet know if these same factors are at work in *Gnorimoschema*.

Our data establish strong but imperfect host preference in *Gnorimoschema* (one likely host mismatch among 431 genotyped larvae). These data are consistent with a similar rate of mismatching reported by Miller (2000). Preliminary experimental evidence (Miller 2000; S. Heard, unpubl. data) indicates that host-mismatched larvae can sometimes complete development on the wrong host, suggesting that host-dependent larval survivorship may not be a strong enforcer of host affiliation or reproductive isolation. We do not yet know what insect behavior keeps mismatches rare. Interestingly, *Gnorimoschema* differs from other reported host-race pairs in that host preference may be exerted at least in part by larvae rather than adult females: Galls are initiated in spring by larvae that hatch from overwintering eggs and attack new growth unavailable to ovipositing females. Adult host preference during oviposition in autumn might lead to apparent larval preference the following spring because new shoots arise close to old ones (both hosts are perennials sending up new ramets from persistent rhizomes). However, this is unlikely to account for the strong host association we observed, because at our study sites ramets of the two hosts grow so thoroughly intermingled that a hatching larva cannot be assured of having its first encounter with a ramet of the correct host.

Our gene flow data also suggest strong assortative mating: None of 431 genotyped individuals were identified as F_1 hybrids (upper 95% confidence limits 9% and 5% hybrids for collections from *S. altissima* and *gigantea*, respectively). Although we cannot yet unequivocally exclude the possibility of random mating followed by strong postmating barriers to hybridization, this scenario seems unlikely as such barriers are themselves expected to generate strong selection for assortative mating by host form (Dobzhansky 1951; Coyne and Orr 1997). It remains unknown whether adults of the two host forms mate exclusively on their respective host plants

(which would assure assortative mating), or whether individuals display direct preference for mates of their own host form. Assortative mating could also arise as a result of phenological offsets between host forms in the timing of adult emergence. Such offsets exist in *Gnorimoschema* (S. B. Heard, unpubl. data) but do not appear large enough to entirely account for the very high degree of assortative mating implied by our genetic data.

Parallelism in Host Race Formation?

The list of phytophagous insects showing host-associated genetic differentiation is long, and getting longer (e.g., Emelianov et al. 1995; Abrahamson and Weis 1997; Feder et al. 1998; Rossi et al. 1999; Via 1999; Wood et al. 1999). *Gnorimoschema gallaesolidaginis*, however, is of particular interest because its two host species (*S. altissima* and *S. gigantea*) are the same hosts attacked by the well-documented races of the tephritid fly *E. solidaginis* (Abrahamson and Weis 1997). Our results establish *G. gallaesolidaginis* and *E. solidaginis* as the first documented case of parallel host-race formation: host race formation in evolutionarily independent lineages across the same host pair. (The stem-boring and gall-inquiline beetle *Mordellistena convicta* is probably a third parallel; Abrahamson et al. 2002.) If such parallelism proves to be widespread among the diverse herbivore fauna of goldenrods (or among other phytophagous insects), it will supply powerful evidence that host-associated genetic differentiation has been, and continues to be, an important engine of diversification among insects.

Ultimately, we wish to understand whether the parallelism between *Gnorimoschema* and *Eurosta* extends beyond similar patterns of genetic differentiation on the same host species. In particular, does this differentiation reflect similar phylogeographic histories of these groups, and is it driven and maintained by similar ecological mechanisms? For instance, in both *Eurosta* (Abrahamson and Weis 1997) and *Gnorimoschema* (S. Heard, unpubl. data) the *gigantea* host form suffers lower mortality as a result of parasitoid attack. In *Eurosta* this reduced mortality is hypothesized to have eased the shift to the derived host (Abrahamson and Weis 1997). In *Gnorimoschema*, however, we do not yet know which host is ancestral, because our limited geographic sampling has not provided phylogeographic data allowing us to reconstruct polarity of the shift.

Included in the assessment of parallelism will be the question of whether the *Gnorimoschema* host shift (and subsequent differentiation) could have happened in sympatry, as the *Eurosta* shift likely did. Recent theoretical and empirical advances (Via 2001; Berlocher and Feder 2002) suggest that sympatric origins are plausible, but our current data (both phylogeographic and ecological) are insufficient to resolve the issue. Evidence that the *Gnorimoschema* and *Eurosta* parallelism extends to origins in sympatry would add credence to the notion that diversification among phytophagous insects can proceed even without allopatric isolation (Via 1999; Berlocher and Feder 2002). Unfortunately, allopatric and sympatric origins are notoriously difficult to distinguish (Berlocher and Feder 2002), and we cannot yet reject either the sympatric or allopatric origin scenarios for the *G. gallaesolidaginis* host forms. Although much more remains to be done, our emerging picture of genetic structure in *G. gallaesolidaginis* adds support to the notion that diversification following host shifts (whether sympatric or not) has been an important contributor to the genesis of biodiversity in phytophagous insects.

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APPENDIX

Allozyme allele frequencies for 12 enzyme loci assayed in *Gnorimoschema gallaesolidaginis.* Data are organized by study site (see text for abbreviations) and host plant species (*alt, Solidago altissima*; *gig, S. gigantea*).

		Study site									
		BV	BB		CL		CM		HP	ZF	
Enzyme	Allele	*alt*	*alt*	*gig*	*alt*	*gig*	*alt*	*gig*	*gig*	*alt*	*gig*
Aat1	2	0.0208	0.0208	0.0104	0.0208	0.0312	0.0521	0.0474	0.0667	0.0106	0.0319
	4	0.9479	0.9792	0.8750	0.9792	0.9062	0.9167	0.8263	0.8778	0.9787	0.8511
	5	0.0104									
	6	0.0104		0.0938		0.0625	0.0208	0.1263	0.0556	0.0106	0.1170
	8			0.0208			0.0104				
	9	0.0104									
Acoh1	1	0.0521		0.0417		0.0426		0.0625	0.0814	0.0375	0.0319
	2			0.0104				0.0250	0.0233		0.0426
	3	0.0625	0.0208	0.4062	0.0556	0.6064	0.0286	0.3125	0.3837	0.0250	0.3936
	4	0.2708	0.3854	0.1042	0.4000	0.0319	0.5286	0.1250	0.1163	0.4500	0.1064
	5	0.1771	0.0208	0.0938	0.0444	0.1915		0.0750	0.1744	0.0375	0.0426
	6		0.0104	0.0208	0.0556	0.0106		0.0938	0.0349	0.0250	0.0745
	7	0.1667	0.4062	0.3229	0.0778	0.0957	0.1000	0.3000	0.1279	0.2000	0.3085
	8	0.1250	0.0625		0.2556		0.2000		0.0581	0.0875	
	9	0.1458	0.0938		0.1111	0.0213	0.1429	0.0062		0.1375	
Ak	1		0.0312				0.0139			0.0217	
	2	0.6277	0.9375		0.4787	0.0208	0.5972	0.0389	0.0227	0.4674	0.0638
	3	0.3511	0.0208	0.8021	0.5213	0.8958	0.3750	0.7889	0.7841	0.4783	0.8723
	4			0.0417							
	5	0.0213	0.0104	0.1354		0.0833	0.0139	0.1556	0.1705	0.0326	0.0532
	7							0.0111			0.0106
	8			0.0208				0.0056	0.0227		
Fe1	1			0.0208							
	2			0.0312	0.0625	0.0745	0.0104	0.0895	0.0667		0.0435
	3	0.0104	0.0312			0.0319				0.0417	
	4	0.9479	0.9271	0.9062	0.9271	0.8830	0.9792	0.8895	0.9000	0.9583	0.9457
	5	0.0417	0.0312	0.0312				0.0053			0.0109
	6		0.0104	0.0104	0.0104	0.0106	0.0104	0.0158	0.0333		
G3pdh	2			0.0104				0.0052			
	4			0.0104						0.0208	
	6	1.0000	1.0000	0.9792	1.0000	1.0000	0.9896	0.9948	1.0000	0.9792	1.0000
	8						0.0104				
Hadh	1			0.0312		0.0217		0.0333	0.0814		
	2			0.0104					0.0116	0.0106	
	3	0.0106		0.3646	0.0312	0.3804	0.0333	0.3389	0.2209		0.3478
	4									0.0426	
	5	0.4149	0.1667	0.5312	0.4688	0.4348	0.4556	0.5389	0.4651	0.2766	0.6196
	6							0.0056	0.0465		
	7	0.4894	0.7604	0.0625	0.4792	0.1630	0.4222	0.0833	0.1628	0.6383	0.0326
	8	0.0532			0.0208		0.0556		0.0116	0.0319	
	9	0.0319	0.0729				0.0333				
Idh2	1							0.0104	0.0109		
	2		0.0104	0.0208	0.0426	0.0106	0.0106	0.0156			
	4		0.9062	0.8646	0.6809	0.8617	0.7447	0.8542	0.8478	0.8462	0.9468
	5		0.0208			0.0532		0.0104	0.0543	0.0128	0.0106
	6		0.0104	0.1042	0.0213	0.0745	0.0213	0.0990	0.0761		0.0426
	7		0.0521	0.0104	0.2553		0.2234	0.0104	0.0109	0.1410	
Lap2	2							0.0053			
	3						0.0213	0.0053			0.0116
	4		0.0729	0.4896		0.6383	0.0106	0.4947	0.5909	0.0286	0.4302
	5	0.4896	0.8333	0.5000	0.4239	0.3617	0.5957	0.4043	0.3977	0.5143	0.5349
	6	0.4792	0.0938	0.0104	0.5435		0.3191	0.0319		0.4286	0.0233
	7	0.0312			0.0217		0.0106	0.0532	0.0114	0.0286	
	8				0.0109		0.0426	0.0053			
Mdh1	4	0.0938	0.0208		0.0851	0.0104	0.2083			0.1383	
	5		0.0104	0.0208		0.0417		0.0104	0.0217		0.0319
	6	0.9062	0.9583	0.9375	0.9149	0.9375	0.7917	0.9896	0.9783	0.8191	0.9574
	7			0.0104		0.0104					
	8		0.0104	0.0312						0.0426	0.0106

APPENDIX. Continued.

		Study site									
		BV	BB		CL		CM		HP	ZF	
Enzyme	Allele	*alt*	*alt*	*gig*	*alt*	*gig*	*alt*	*gig*	*gig*	*alt*	*gig*
Gpi1	1		0.0333	0.0319		0.1042	0.0104	0.0474	0.0111	0.0208	0.0465
	2	0.6957	0.3000	0.7979	0.6458	0.8333	0.6042	0.7579	0.8000	0.6979	0.7558
	3	0.0652	0.4556	0.0106	0.0417		0.1354	0.0632	0.1222	0.1354	0.0349
	4	0.1848	0.0889	0.1170	0.2500	0.0417	0.1354	0.0579	0.0667	0.0729	0.0814
	5	0.0109	0.1111	0.0213	0.0208	0.0208	0.0833	0.0474		0.0312	0.0349
	6	0.0435	0.0111	0.0213	0.0208		0.0312	0.0263		0.0208	0.0465
	8									0.0208	
	9				0.0208						
Pgm1	1	0.2065	0.1771	0.1354	0.1458	0.2500	0.2283	0.1596	0.1512	0.2308	0.0532
	2				0.0417	0.0208	0.0217	0.0106	0.0349		0.0319
	3	0.6087	0.6979	0.6875	0.5833	0.5938	0.5652	0.5745	0.6744	0.5000	0.7872
	5	0.1304	0.1250	0.1250	0.2083	0.1146	0.0870	0.2074	0.1163	0.1410	0.0745
	6	0.0543		0.0312	0.0208	0.0208	0.0652	0.0319	0.0233	0.1026	0.0213
	8			0.0208			0.0326	0.0160		0.0256	0.0319
Tpi	2	0.0208		0.0208						0.0278	0.0106
	3	0.9271	1.0000	0.9688	0.9479	0.8542	1.0000	0.9948	1.0000	0.9306	0.9894
	5				0.0104						
	6	0.0521		0.0104	0.0417	0.1458		0.0052		0.0417	