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Cascading host-associated genetic differentiation in parasitoids of phytophagous insects

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The extraordinary diversity of phytophagous insects may be attributable to their narrow specialization as parasites of plants, with selective tradeoffs associated with alternate host plants driving genetic divergence of host-associated forms via ecological speciation. Most phytophagous insects in turn are attacked by parasitoid insects, which are similarly specialized and may also undergo host-associated differentiation (HAD). A particularly interesting possibility is that HAD by phytophagous insects might lead to HAD in parasitoids, as parasitoids evolve divergent lineages on the new host plant-specific lineages of their phytophagous hosts. We call this process 'cascading host-associated differentiation' (cascading HAD). We tested for cascading HAD in parasitoids of two phytophagous insects, each of which consists of genetically distinct host-associated lineages on the same pair of goldenrods (*Solidago*). Each parasitoid exhibited significant host-associated genetic divergence, and the distribution and patterns of divergence are consistent with divergence in sympatry. Although evidence for cascading HAD is currently limited, our results suggest that it could play an important role in the diversification of parasitoids attacking phytophagous insects. The existence of cryptic host-associated lineages also suggests that the diversity of parasitoids may be vastly underestimated.

Keywords: insect diversification; host race formation; *Copidosoma*; sympatric speciation

1. INTRODUCTION

At least half of all animal species are parasites (Price 1980; Windsor 1998). Explanations for parasite diversity tend to revolve around three related factors: first, their often tight specialization, attributed to their intimate relationships with hosts (Price 1980); second, strong and disruptive selection pressures associated with specialization on different hosts that foster coevolutionary interactions and co-diversification (Maynard Smith 1966; Rice 1987; Futuyma & Moreno 1988; Thompson 1994); and third, reduced competition following specialization, which may allow coexistence of a diversity of ecologically similar parasite lineages (Simpson 1953; Mayr 1976; Price 1980). Genetic divergence among parasite populations driven by specialization on different hosts is a form of ecological speciation (*sensu* Schluter 1996, 2000), in which species divergence is driven by ecological selective pressures (with or without the additional influence of genetic drift or sexual selection). It has been posited that ecological speciation might occur in the absence of geographical barriers to gene flow (Schluter 2001). In parasites, sympatric divergence could be facilitated by host-associated shifts in phenology, mate selection, sensitivity to chemical compounds and other traits (Bush 1975; Poulin & Morand 2000; Berlocher & Feder 2002), encouraging speculation that ecological speciation (in sympatry) could have been a significant factor in promoting extensive diversification among parasitic organisms (de Meeûs *et al.* 1998; Via 2001; Stireman *et al.* 2005). Recent emphasis has been placed on the plant-feeding (phytophagous) insects as a hyperdiverse (Mitter *et al.* 1988) group of parasites in which ecological speciation may be important. In particular, researchers have stressed divergence of host-associated lineages of phytophagous insects (Mopper & Strauss 1998; Abrahamson *et al.* 2001; Via 2001; Funk *et al.* 2002; Stireman *et al.* 2005), including how such divergence may be driven by selection (e.g. phenology, natural enemies, plant chemistry).

The phytophagous insects are attacked by another hyperdiverse assemblage of specialized parasites: the insect parasitoids. Mechanisms of population and species divergence in parasitoids are largely unexplored, and a particularly interesting possibility is that host-associated differentiation (HAD) may cascade across trophic levels if HAD in a herbivore leads in turn to divergence of its parasitoids. In the major parasitoid clades Tachinidae (Diptera) and parasitic Hymenoptera, extensive adaptive radiation is evident: the Tachinidae are among the most speciose dipteran families (*ca* 10 000 described species, Irwin *et al.* 2003), and the hymenopteran parasitoids may account for up to 20% of all insect species (LaSalle & Gauld 1991). Parasitoids exhibit intimate associations with their hosts and most are highly specialized (Godfray 1994). In addition, many parasitoids are extremely sensitive to cues derived from particular herbivore–host plant interactions, relying on these cues to locate hosts (Vet & Dicke 1992; De Moraes *et al.* 1998; Dicke 2000;

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De Moraes & Mescher 2004). These characteristics of parasitoids suggest that host-related selection and genetic divergence could play an important role in their diversification. Cascading HAD, if widespread, could provide an important contribution to the extensive diversification of parasitoid lineages that attack phytophagous insects.

We tested for the existence of cascading HAD in two unrelated parasitoid species. Each parasitoid attacks a herbivore that itself consists of two host-associated lineages that have differentiated on closely related and sympatric plant hosts (Nason *et al.* 2002; Stireman *et al.* 2005). We predicted that, if cascading HAD is present, we should observe significant genetic differentiation associated with host plant use at sites of host plant sympatry and across the ranges of these parasitoid species. We find that each parasitoid exhibits morphologically cryptic genetic divergence consistent with cascading HAD: genetically distinct forms attacking the two host-specialist forms (races or cryptic species; Stireman *et al.* 2005) of their phytophagous insect hosts. To our knowledge, this is the first demonstration of cascading HAD in parasitoids. We argue that such cryptic divergence could be common in insect parasitoids, playing an important role in their diversification and, through a mechanism of positive feedback, diversification of their phytophagous insect hosts as well.

2. STUDY ORGANISMS

The goldenrod gallmakers *Rhopalomyia solidaginis* (Diptera: Cecidomyiidae) and *Gnorimoschema gallaesolidaginis* (Lepidoptera: Gelechiidae) are each composed of genetically differentiated lineages on the closely related and broadly sympatric host plants *Solidago altissima* and *Solidago gigantea* (Asteraceae) (McEvoy 1988; Miller 2000; Nason *et al.* 2002; Stireman *et al.* 2005). In each case, little, if any, morphological distinction exists between the host forms, which were previously thought to represent single species (Gagné 1989; Miller 2000; Nason *et al.* 2002; Stireman *et al.* 2005). Each gallmaker is attacked by a suite of wasp parasitoids. We tested for cascading HAD in one common parasitoid of each gallmaker: *Platygaster variabilis* Ashmead (Platygastridae, attacking *Rhopalomyia*) and *Copidosoma gelechiae* Howard (Encyrtidae, attacking *Gnorimoschema*). Both are polyembryonic (many clonal offspring develop from a single egg), attack their hosts in the egg stage (Leiby 1922), destroy the host larva in its final stadium, and emerge after pupating in the host remains in late summer or early fall. No other hosts are known for *P. variabilis*, but *C. gelechiae* is recorded from at least one other host (*Gnorimoschema salinaris*; Patterson 1915).

3. MATERIAL AND METHODS

(a) *Collections*

To obtain *Platygaster*, we collected *Rhopalomyia* galls from *S. altissima* and *S. gigantea* across the upper Midwestern USA (Iowa, Minnesota, Nebraska, South Dakota), including four sites with sympatric collections from both host plants. For *Copidosoma*, pilot data suggested HAD was subtle, so we concentrated our efforts on three geographically distant sites where we could obtain large sympatric samples of *Gnorimoschema* galls from intermixed stands of *S. altissima* and *S. gigantea*: Fredericton (New Brunswick, Canada), Toronto (Ontario, Canada) and Milaca (Minnesota, USA). At each site, we collected galls in areas ranging from *ca* 1 to 5 ha, dispersing sampling across available plants to maximize genetic diversity and reduce the likelihood of collecting closely related parasitoid individuals. Parasitoids were flash-frozen in liquid nitrogen (for allozyme analysis) or stored in 95% ethanol (for mitochondrial DNA analysis). *Platygaster* specimens were identified by Matt McGown (Florida State Collection of Arthropods) and *C. gelechiae* by one of us (S. B. Heard). Voucher specimens of both hosts and parasitoids have been deposited in the Iowa State University Insect Collection. We refer to parasitoids attacking gallmakers on the two *Solidago* species as *altissima* and *gigantea* individuals or forms.

(b) *Molecular methods*

(i) *MtDNA amplification*

We extracted insect DNA using PUREGENE DNA extraction kits (Gentra Systems, Inc., Minneapolis, MN) and amplified 450–800 bp of cytochrome oxidase I (*COI*). DNA was amplified in 50 µl PCR reactions containing 5 µl genomic DNA, 5 µl (10×) PCR buffer (Invitrogen), 5 µl (10 mM) dNTP solution, 2.5–3.75 (50 mM) $MgCl_2$, 2.5 µl of forward and reverse primers (5 pmol μl^{-1}) and dH_2O to 50 µl. The primers (C1j1751 and C1N2191 for *Platygaster* and 'Pat' and UEA7 for *Copidosoma*) were taken from Simon *et al.* (1994; first three) and Lunt *et al.* (1996). PCR conditions were: initial denaturing at 94 °C for 2 min, then 35 cycles of 94 °C for 30–45 s, 47–52 °C for 45–60 s, 72 °C for 1 min, and a final 72 °C extension period of 4 min. Sequencing of double-stranded PCR products was carried out on an automated ABI 377 Prism sequencer at the Iowa State University DNA sequencing facility, using ABI Prism Big Dye 3.1 and standard procedures. Sequences were examined with reference to chromatograms and initially aligned using AutoAssembler, with further manual alignment and sequence manipulation using MacClade (Maddison & Maddison 2000). Preliminary analyses revealed no mtDNA sequence variation among *Copidosoma* individuals from the sampled region (seven individuals collected from both hosts and from Minnesota to New Brunswick exhibited a single haplotype for 600 bp of COI; GenBank accession no. DQ267636), and so we do not report mtDNA sequence analysis for that species. We obtained mtDNA sequence data for 30 *P. variabilis* broods (16 from *Rhopalomyia* galls on *S. altissima* and 14 from *S. gigantea*; GenBank accession nos DQ267637–DQ267668).

(ii) *Allozyme methods*

Because *C. gelechiae* is polyembryonic, each allozyme genotype was assessed based on a (clonal) brood of individuals from a single *G. solidaginis* caterpillar. Broods were extracted in a plant extraction buffer (Nason *et al.* 2002). We resolved nine polymorphic enzyme loci: aconitate hydratase (ACOH, EC 4.2.1.3); 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); malate dehydrogenase (MDH, EC 1.1.1.37); phosphoglucomutase (PGM, EC 5.4.2.2); hexokinase (HK, E.C. 2.7.1.1); and lactate dehydrogenase (LDH, EC 1.1.1.27). Allozymes were run in 12% starch (Starch Art Corp.) gels and stained following Soltis *et al.* (1983), except G3PDH and HADH from Murphy *et al.*

(1996). ACOH, HK and PGM were resolved in buffer system 11 of Soltis *et al.* (1983), while the remaining enzymes were resolved in a pH 6 morpholine–citrate buffer (Murphy *et al.* 1996). Banding patterns for each enzyme exhibited expected subunit structures and patterns of expression. These loci do not represent an exhaustive search for polymorphism in *Copidosoma*. Because *COI* sequence variation was informative for *Platygaster*, we did not pursue allozyme analysis for that species.

(c) *Phylogenetic and population genetic analyses*

Phylogenetic reconstruction of *P. variabilis* populations from mtDNA sequence data was conducted with maximum likelihood (ML) and Bayesian methods using PAUP 4.10 (Swofford 2001) and MrBayes 3.0b4 (Ronquist & Huelsenbeck 2003), respectively. An unidentified *Platygaster* species reared from *Rhopalomyia lobata* on *Euthamia graminifolia* (a close relative of *Solidago*) was used to root the tree. The model of sequence evolution for ML analyses was selected using Modeltest (Posada & Crandall 1998), with likelihoods of successively more complex models calculated on an initial neighbour-joining tree (Bio NJ, HKY distances, rate variation with gamma = 0.5). We selected a K81uf model (Kimura 1981) with parameters (in PAUP format): Nst = 6, rmat = (1.0000 9.3172 2.9684 2.9684 9.3172), pinvar = 0, rates = gamma, shape = 0.1986. ML analyses consisted of 50 replicate heuristic searches with TBR branch swapping. Bayesian analysis began with equiprobable priors and a character partition according to codon position. The analysis was run for 1 000 000 generations (sampled every 1000 generations) with eight heated chains and a burn-in of 10 000 generations. Likelihoods levelled off after *ca* 5000 generations. The proportion of trees from the posterior distribution containing a particular node was used to assess support for host-associated clades (i.e. posterior probability). Net divergence between host-associated clades was calculated using Mega 2.0 (Kumar *et al.* 2001). Within-clade average pairwise distances and confidence intervals were estimated using 1000 bootstrap replicates in Arlequin (Schneider *et al.* 2000).

We assessed relatedness among *Copidosoma* populations (across hosts and sites) using allozyme allele frequencies. For each site, we used GenePop (Raymond & Rousset 1995) to perform tests of genic (allelic) differentiation, relative to collection from *S. altissima* versus *S. gigantea*, for each allozyme locus, and a genotypic test for all loci pooled. We used our allozyme data to assess the likely number of HAD events. If HAD occurred once, then genetic variation among populations should be better explained by host plant than by geography. Alternatively, if HAD occurred repeatedly in different regions, then geography should explain genetic variation better. We tested these hypotheses by examining two hierarchical AMOVA models: one with host plant nested within site, and one with site nested within host plant. These analyses, along with estimates of pairwise F_{st} values between host forms (with significance estimates based on 1013 permutations) were conducted in Arlequin 2.000 (Schneider *et al.* 2000). We also constructed an unrooted neighbour-joining tree of *Copidosoma* populations, based on Cavalli-Sforza distances calculated from allele frequency data (Cavalli-Sforza & Edwards 1967), using Phylip (Felsenstein 2004). Neighbour-joining bootstrap support (1000 replicates) of nodes was assessed using the BootSeq module of Phylip.

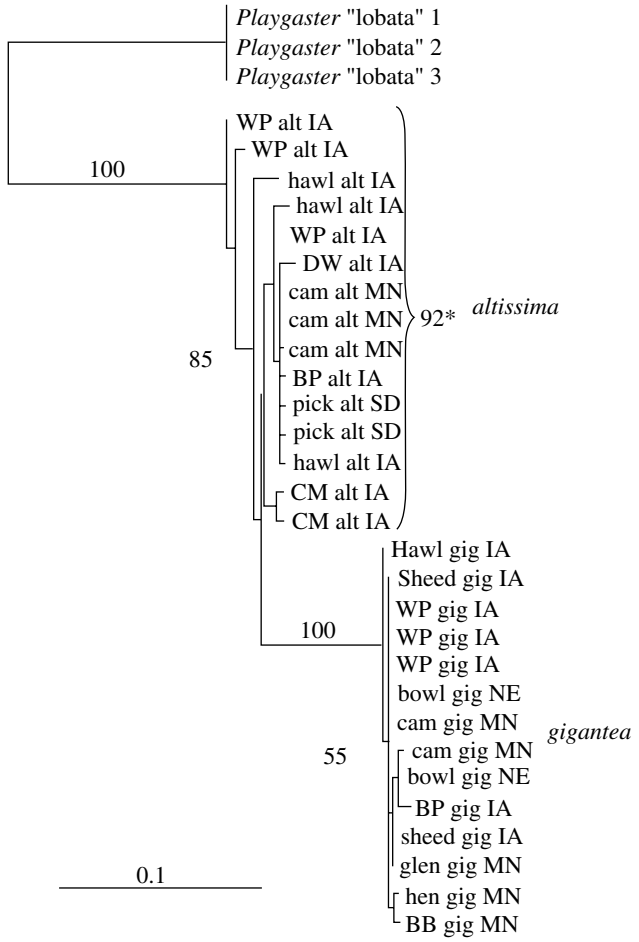

Figure 1. The ML tree of highest likelihood ($-\ln L = 1121.78$) for midwestern populations of *Platygaster variabilis* parasitoids of the gall midge *Rhopalomyia solidaginis*. Numbers above or to the side of clades indicate NJ bootstrap support. Numbers in parentheses indicate the percentage of trees containing the indicated clade that were sampled in the Bayesian phylogenetic analysis (see text). *Note that 92% of NJ bootstrap replicates indicated a monophyletic *altissma* clade despite the paraphyly inferred by ML and Bayesian methods.

4. RESULTS

(a) Platygaster variabilis

Our ML analyses of *P. variabilis* resulted in a single tree of highest likelihood ($-\ln L = 1121.78$). Both ML and Bayesian trees support a strong division between *P. variabilis* reared from *Rhopalomyia* on *S. gigantea* and *S. altissima* regardless of geographical affinity (figure 1). Strong support for the *gigantea* clade is indicated by high percentages of neighbour-joining bootstraps and of trees sampled from Bayesian posterior distributions (100 and 99%, respectively). It is unclear whether the *altissma* and *gigantea* clades are sister, or whether the *gigantea* clade arose from within the *altissima* clade. The ML tree of highest likelihood and the summary of Bayesian trees suggest paraphyly of the *altissima* clade with respect to the *gigantea* clade (although with less than 50% support in Bayesian analysis), whereas bootstraps based on neighbour-joining recover a monophyletic *altissima* clade in 92% of replicates (figure 1). Divergence between the clades is quite strong (mean ± s.e., 5.53 ± 1.03% (uncorrected), 8.31 ± 2.2% (corrected)) despite an apparent lack

Table 1. Individual genic and global genotypic tests of significant isolation relative to host plant (*S. altissima* or *S. gigantea*) for allozyme loci assayed for *Copidosoma gelechiae* in three sympatric populations. (N_{alt} and N_{gig} indicate the number of individuals sampled relative to host for each population. Values are *p*-values (bold indicates significance at $p=0.05$) and n.s. indicates $p>0.1$.)

locus	Milaca (Minnesota, USA)	Toronto (Ontario, Can.)	Fredericton (New Bruns., Can.)
N_{alt}/N_{gig}	71/66	88/82	44/58
ACOH	**0.000**	n.s.	**0.027**
G3PDH	n.s.	**0.012**	n.s.
HADH3	**0.014**	n.s.	n.s.
HK	—	n.s.	n.s.
IDH	**0.000**	n.s.	0.075
LDH	n.s.	**0.007**	n.s.
MDH	**0.001**	**0.001**	n.s.
PGI	n.s.	0.083	**0.001**
PGM	n.s.	n.s.	0.076
overall (genotypic)	**0.000** ($\chi^2_{16}=52.3$)	**0.001** ($\chi^2_{14}=36.2$)	**0.033** ($\chi^2_{18}=30.5$)
F_{st}	0.0139	0.0510	0.0015

of distinguishing morphological characters (M. MacGown, personal communication). Within-race genetic diversity was higher for the *altissima* clade than the *gigantea* clade (average uncorrected pairwise divergence (±s.e.) 1.24±0.164 and 0.194±0.039%, respectively; $p<0.05$), paralleling patterns of genetic diversity in the *Rhopalomyia* host (Stireman *et al.* 2005).

(b) Copidosoma gelechiae

For *Copidosoma*, each sympatric population pair exhibited significant host-associated genetic isolation at multiple allozyme loci (table 1). Estimates of F_{st} between host-associated population pairs were generally low, but varied more than an order of magnitude across sites (0.002–0.05). Despite significant host-associated structure at each site, in the neighbour-joining tree *C. gelechiae* populations grouped according to geography rather than host plant (figure 2; although bootstrap support for these clusters was only moderate (60–73%)). This pattern of host-associated genetic structure nested within geographical structure is supported by AMOVA analyses: the model with host nested within geography indicates significant genetic structure at both levels and explains more total variance, while the reverse model results in negative F_{st} values for between-host comparisons (table 2). Furthermore, the three population pairs differed in the allozyme loci that exhibited significant HAD (table 1). Together, these analyses suggest that HAD may have occurred independently in multiple geographical regions.

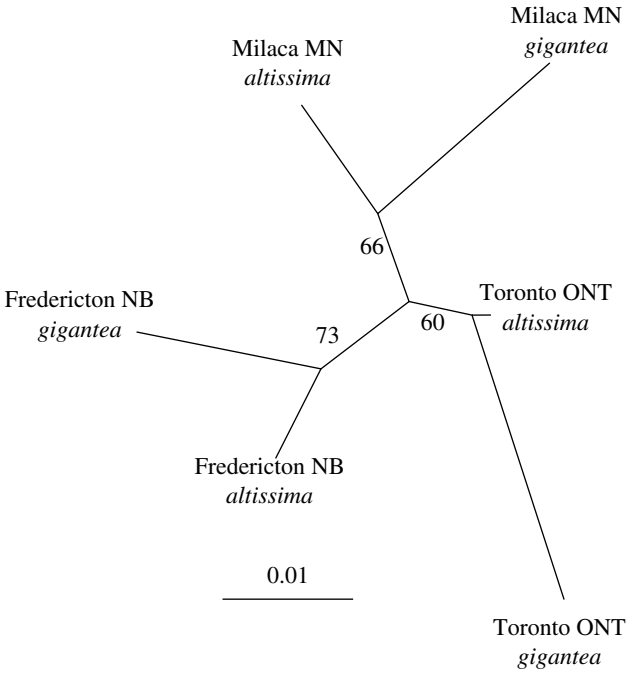

Figure 2. An unrooted neighbour-joining tree of *Copidosoma gelechiae* populations based on Cavalli-Sforza distances calculated from allozyme data (see text). Numbers at the nodes indicate bootstrap support. Note that despite significant evidence of host-associated genetic differentiation, all populations cluster according to geographical site.

5. DISCUSSION

Both *P. variabilis* and *C. gelechiae* exhibit morphologically cryptic genetic differentiation associated with use of the distinct *altissima* and *gigantea* races of their host gallmaking insects. In *Platygaster*, this divergence is relatively deep, indicating the existence of cryptic sibling species. In *Copidosoma*, divergence is subtle, perhaps reflecting initial stages of population divergence, in which significant gene flow still occurs between populations. While other studies have documented HAD in parasitoids (for instance, Morehead *et al.* 2001 for ant-attacking phorids and Aldrich & Zhang 2002 for Hemiptera-attacking tachinids), we believe our data provide the first evidence presented for cascading HAD from herbivores to their parasitoids. That cascading HAD may occur in other insect guilds as well is suggested by recent evidence of host-associated genetic and phenotypic divergence in a facultative predator of *Eurosta* gall flies (*Mordellistena convicta*) in this same *Solidago* system (Eubanks *et al.* 2003; Blair *et al.* 2005).

(a) *Mode of differentiation*

Genetic and ecological evidence suggests the possibility that the phytophagous hosts of *Platygaster* and *Copidosoma* have evolved host-associated lineages in sympatry (Stireman *et al.* 2005). Could divergence of the parasitoids have occurred in sympatry as well? Parasitoids in general are highly specialized and intimately associated with their hosts—life-history traits thought to facilitate sympatric

Table 2. Hierarchical AMOVA models for populations of *Copidosoma gelechiae* with either host plant (of host herbivores) nested within region (model A) or vice versa (model B).

factor	d.f.	SS	variance component	percentage of variation	*p*
model A					
among regions	2	16.89	0.0269	3.59	0.063
between host plants (within regions)	3	7.87	0.0174	2.32	0.000
within host plants	660	465.81	0.7058	94.09	0.000
model B					
between host plants	1	4.25	−0.0037	−0.50	0.717
among regions (within host plants)	4	20.51	0.0409	5.51	0.000
within regions	660	465.81	0.743	94.99	0.000

divergence (Berlocher & Feder 2002). Current geographical barriers to gene flow in *Platygaster* and *Copidosoma* are probably negligible given the broadly sympatric and often syntopic distribution of their hosts (and host's food plants), and given that such small insects often have excellent dispersal capability (e.g. Antolin & Strong 1987).

Unfortunately, inferring modes of divergence from current geographical and genetic data is extremely difficult and often controversial (Losos & Glor 2003). However, two major lines of evidence suggest that sympatric divergence is possible, at least for *Copidosoma*. First, divergence between *altissima* and *gigantea* lineages of *Copidosoma* is much shallower than the corresponding divergence in its host, *G. gallaesolidaginis*. *Gnorimoschema gallaesolidaginis* has strong HAD in both mtDNA (Φ_{st} = 0.544) and allozymes (F_{st} = 0.159), while *C. gelechiae* exhibits little mtDNA variation (J. O. Stireman, unpublished data) and local host-associated allozyme F_{st} values of 0.002–0.05. (For *Platygaster*, evidence for non-concordance is equivocal, with 5.5% net divergence between host forms, versus 6.7% between the *Rhopalomyia* hosts; large bootstrap variances and the likelihood of different rates of molecular evolution in host and parasitoid (Arbogast *et al.* 2002) prevent an inference of relative timing.) Second, allozyme data from our widely spaced (more than 1000 km) study populations suggest that *Copidosoma* has experienced multiple independent occurrences of HAD, whereas *Gnorimoschema* shows evidence of a single event (Stireman *et al.* 2005). These results are inconsistent with the concurrent divergence expected under the simplest vicariant allopatric model, although of course more complicated allopatric models could be invoked in which multiple vicariant events and changing geographical distributions could produce similar genetic patterns. Further behavioural, ecological and genetic work will be needed to establish the mechanisms responsible divergence in *Copidosoma* and *Platygaster* (and such work is underway).

If ecological selection is involved in divergence of host-associated parasitoid races, what might be the source of this selection? Selection for divergence may be associated with physiological incompatibilities and reduced hybrid fitness in the parasitoid–host insect interaction, analogous to those argued for host-race formation in phytophagous insects (Drés & Mallet 2002). Alternatively, selection may be associated with the two different plant species on which insect hosts are found, such as tradeoffs associated with detecting and locating hosts (and perhaps mates) via plant cues (e.g. Bernays 2001). In the latter case, HAD by parasitoids could be sparked simply by diet expansion of the host insect, without actually requiring genetic or phenotypic differentiation of the host insect relative to plant use. Our current data do not support this scenario, since divergence of the parasitoids examined here is less than (*Copidosoma*) or at least no greater than (*Platygaster*) that of their hosts. However, they also do not reject it, as we have not yet tested for HAD in parasitoids of generalist goldenrod herbivores (e.g. the tortricid moth gallmaker *Epiblema*). Finally, parasitoid divergence might be driven by neither host nor host's host plant alone, but by their *interaction*. For example, parasitoid differentiation might be encouraged by allochronic isolation due to phenological differences in the hosts' development resulting from traits of their host plants.

(b) *Cascading host-associated differentiation and parasitoid diversification*

It is unclear how frequently HAD occurs in phytophagous insects, but our previous analysis of the herbivore community associated with *S. altissima* and *S. gigantea* suggests it may be relatively widespread (Stireman *et al.* 2005). If herbivore HAD frequently cascades to parasitoids, this process could be integral to the extraordinary radiations of parasitoid lineages that attack phytophagous insects (e.g. most Chalcidoidea, Ichneumonoidea, Tachinidae). The results of several studies hint at strong ecological pressures, favouring population divergence in parasitoids. In *Agathis* braconid parasitoids of *Greya* moths, Althoff & Thompson (2001) found localized morphological differentiation (ovipositor length) and patterns of searching behaviour specific to alternate host plants (*Heuchera* spp.) of the *Greya* hosts, although there was no evidence of neutral genetic structure among geographically separated parasitoid populations. Similarly, in a study of *Diaeretiella* braconid wasps attacking cabbage aphids and Russian wheat aphids, Baer *et al.* (2004) detected significant local population divergence and fitness tradeoffs among wasps using different hosts (though they failed to find evidence of host races at a larger scale).

Well-documented examples of host–parasite cospeciation are more widespread in interactions involving parasites of vertebrates (Hafner & Nadler 1988; McCoy *et al.* 2001) and bacterial endosymbionts (Clark *et al.* 2000, Lo *et al.* 2003). The high host specificity that makes

this possible is a trait shared by many parasitoids. However, the cascading HAD that we have identified differs from these interactions in several important respects. First, unlike traditional parasites, parasitoids almost always kill their hosts, preventing vertical transmission; the free-living adult stage that must then locate a new host provides opportunities for transmission among taxa. Second, cascading HAD takes place in a tritrophic context, where the *interaction* between plant and insect may contribute to diversification in the parasitoids. When herbivores expand their host range, parasitoids experience an entirely new adaptive environment that may include novel plant volatile and surface chemicals that might be used in host location, novel plant toxins ingested by hosts, novel plant morphologies and altered insect and plant phenologies. All of these factors could create divergent selection pressures and contribute to HAD.

One particularly interesting implication of this tritrophic perspective is that it may create positive feedback between parasitoid and herbivore diversification. If enemy-free space (*sensu* Jeffries & Lawton 1984) is an important factor in facilitating herbivore HAD (e.g. Brown *et al.* 1995; Berdegue *et al.* 1996), but parasitoids often 'catch up' by forming host races on these plant-specific herbivore lineages, then repeated cycles of shifting and HAD may result in ever increasing diversity of both groups. This provides a potentially powerful and testable explanation for the exceptional diversification of insect taxa possessing phytophagous and parasitoid life histories.

One line of work that challenges our argument for the importance of cascading HAD is Wiegmann *et al.*'s (1993) comparative analysis, which found no overall evidence that the parasitoid habit was associated with increased diversity. However, Wiegmann's result may be due to the inclusion of many parasitoid groups that attack non-phytophagous or host-generalist phytophagous insects. In such cases, the critical interaction between herbivore and host plant is absent or mitigated. The patterns of parasitoid diversity and the potential for HAD we demonstrate here suggest that the impressive radiations of parasitic Hymenoptera and Diptera might arise in part from their parasitism of specialist phytophagous insects. Furthermore, the two cases of morphologically cryptic parasitoid HAD we document represent relatively ancient, well-differentiated and reproductively isolated lineages (*Platygaster*) as well as recent differentiation (*Copidosoma*). That deeply divergent parasitoid lineages can remain morphologically cryptic suggests that parasitoid clades may harbour substantial unappreciated diversity. If so, then insect parasitoids might even surpass the phytophagous insects in the number of genetically and ecologically distinct lineages.

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