

Sequential radiation of unrelated organisms: the gall fly *Eurosta solidaginis* and the tumbling flower beetle *Mordellistena convicta*

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Abstract

Host shifts and the formation of insect-host races are likely common processes in the speciation of herbivorous insects. The interactions of goldenrods *Solidago* (Compositae), the gall fly *Eurosta solidaginis* (Diptera: Tephritidae) and the beetle *Mordellistena convicta* (Coleoptera: Mordellidae) provide behavioural, ecological and genetic evidence of host races that may represent incipient species forming via sympatric speciation. We summarize evidence for *Eurosta* host races and show that *M. convicta* has radiated from goldenrod stems to *Eurosta* galls to form host-part races and, having exploited the galler's host shift, has begun to differentiate into host races within galls. Thus, host-race formation has occurred in two interacting, but unrelated organisms representing two trophic levels, resulting in 'sequential radiation' (escalation of biodiversity up the trophic system). Distributions of host races and their behavioural isolating mechanisms suggest sympatric differentiation. Such differentiation suggests host-race formation and subsequent speciation may be an important source of biodiversity.

Introduction

A number of studies have established the importance of host plants to the differentiation and diversity of herbivorous insects (Dethier, 1954; Hutchinson, 1959; Ehrlich & Raven, 1964; Strong *et al.*, 1984; Mitter *et al.*, 1988, 1991; Farrell, 1998). Moreover, herbivorous insects have been effectively used to examine the details of the process of speciation and have contributed markedly to the debate surrounding sympatric speciation (Bush, 1975, 1994; Waring *et al.*, 1990; Craig *et al.*, 1993, 1997, 2001; Brown *et al.*, 1995, 1996; Emelianov *et al.*, 1995; Feder *et al.*, 1995, 1999; Berlocher, 1998, 1999). Nevertheless, considerable controversy continues to surround the roles of host-plant specificity and host shifts in the origin of host races and subsequent species (Berlocher, 1998; Barraclough & Vogler, 2000).

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Bush (1969, 1975, 1994) argued that host shifts to novel host-plant species by host-plant-specific herbivores could give rise to divergence of the host-associated populations even when those populations occur sympatrically. Under this scenario, the initial host shift to a closely related or chemically similar host-plant species is followed by changes in the herbivore's mating and/or oviposition preferences, which result in at least partial reproductive isolation of the host-associated populations. Reduced gene flow enables selection, drift and mutation to generate additional differences between the host-associated populations and in due course facilitate sufficient differentiation between the populations that they are unable to interbreed and thus become separate species. Several examples of host shifts and subsequent host-race formation have been documented including the tephritid flies *Rhagoletis* (Bush & Smith, 1998; Feder, 1998) and *Eurosta* (Waring *et al.*, 1990; Craig *et al.*, 1993, 2001), the membracid treehopper *Enchenopa* (Tilmon *et al.*, 1998; Wood *et al.*, 1999), the gelechiid moth *Gnorimoschema gallaesolidaginis* (Nason *et al.*, 2002) and

the agromyzid leaf miner *Phytomyza* (Scheffer & Wiegmann, 2000).

Herbivorous insects with high degrees of host-plant specificity and fidelity are considered to be the most likely to undergo such host-race formation (Abrahamson & Weis, 1997; Feder, 1998), because strict host fidelity links the selection of host plant to mate choice and oviposition preference. Such linkages can produce reproductive isolation, which in turn enables differentiation in the separate host races. Our research on the host-plant specific gall-inducer *Eurosta solidaginis* (Fitch) documenting the existence of host races on *Solidago altissima* L. and *S. gigantea* Aiton (e.g., Waring *et al.*, 1990; Craig *et al.*, 1993, 1994, 1997, 1999, 2000, 2001; Brown *et al.*, 1995, 1996) caused us to wonder whether the natural enemies of this gall inducer have undergone parallel evolutionary changes in response to the herbivore's differentiation. In this report we synthesize our current studies, which examine whether the natural enemies of *Eurosta* have themselves differentiated in response to the gall inducer's formation of host races (Cronin & Abrahamson, 2001; Eubanks *et al.*, 2003; G. Blair, W. Abrahamson & J. Jackman, unpublished data). Current evidence indicates that the parasitoid wasp *Eurytoma gigantea* has not diverged in parallel with *Eurosta*'s host-plant shift (Cronin & Abrahamson, 2001). Wasps reared from galls on *S. altissima* and *S. gigantea* showed no allochronic emergence, no assortative mating and female *E. gigantea* did not prefer to oviposit on galls of their natal host plants. However, our findings for the stem-boring and gall-boring inquiline and facultatively predaceous beetle *Mordellistena convicta* LeConte suggest that it has formed host races that are associated with the host races of the gall inducer (Abrahamson *et al.*, 2001; Eubanks *et al.*, 2003; Blair *et al.*, unpublished data). We are unaware of any previous work that examines such 'sequential radiation' in which the differentiation of an herbivore influences the differentiation of a second unrelated natural enemy, with the result of an escalation of biodiversity up the trophic system.

In this synthesis paper, we briefly summarize the evidence for *Eurosta* host races and then offer findings that show the natural enemy *M. convicta* has radiated from goldenrod stems to *Eurosta* galls to form host-part races and, having exploited the gall inducer's host shift, has begun to further differentiate by forming host races within galls.

Biology of *E. solidaginis*

The univoltine North American native *E. solidaginis* (Diptera: Tephritidae) commonly stimulates galls on *S. altissima* and *S. gigantea*, both members of the *S. canadensis* (Asteraceae) species complex (Abrahamson & Weis, 1997; Abrahamson *et al.*, 2001). Both host plants broadly overlap in their distributions through eastern and central North America, from southern Canada to

Florida and Texas, and frequently co-occur in old fields, disturbed areas and roadsides. Although *E. solidaginis* attacks *S. altissima* throughout the host plant's range, this gall inducer infests *S. gigantea* only in southern Canada and the northern tier of the USA (Abrahamson *et al.*, 1989, 1994, 2001; Abrahamson & Weis, 1997).

Adult gall flies emerge, mate and oviposit into the apical buds of their host plants in May in the mid-Atlantic region of the USA. As adult gall flies do not feed and predation on dispersing adults is common (Cronin *et al.*, 2001), they are short-lived with adult phases that vary from hours to perhaps 10 days. Galls begin to appear approximately 3 weeks after oviposition in response to larval stimulus and galls reach their maximum diameter by mid-July. Larvae reach their peak mass in late September and subsequently overwinter within their galls as diapausing third instars (Abrahamson & Weis, 1997).

Host races in *E. solidaginis*

The sympatric *E. solidaginis* populations that infest *S. altissima* and *S. gigantea* in the northern USA and southern Canada are distinct host races that are reproductively isolated as a result of allochronic emergence, assortative mating and strong oviposition preferences for their natal host-plant species (Craig *et al.*, 1993, 1994). For example, '*gigantea*' gall flies emerge on average 10–13 days earlier than '*altissima*' gall flies. Because gall flies strongly associate with their natal host-plant species, they exhibit effective assortative mating in the presence of host plants and adult females demonstrate a clear preference to oviposit in their natal host-plant species. Furthermore, hybridization studies show that hybrids between the host races as well as backcross progeny from crosses between F1 hybrids and '*altissima*' or '*gigantea*' gall flies perform poorly (i.e. are less able to induce galls on their host plant) relative to '*altissima*' and '*gigantea*' gall flies (Craig *et al.*, 1997, 2001). Consequently, disruptive selection is operating that maintains the reproductive isolation of host races.

Allozyme and mtDNA studies (Waring *et al.*, 1990; Brown *et al.*, 1996; Itami *et al.*, 1997; Craig *et al.*, 2001) offer unambiguous evidence of the genetic differentiation of the '*altissima*' and '*gigantea*' attacking gall flies. The patterns of clade formation and the greater heterozygosity of loci in '*altissima*' gall flies indicate that *S. altissima* is the ancestral host plant and that the host shift took place in the north-eastern USA (Waring *et al.*, 1990; Brown *et al.*, 1996).

The survivorship of the derived '*gigantea*' host race is enhanced in at least some populations by reduced natural-enemy attack and diminished competition. A survey of 23 New England old fields that included sites with sympatric '*altissima*' and '*gigantea*' gall fly populations found appreciably higher survival of '*gigantea*' attacking gall flies compared with '*altissima*' gall flies

(36% vs. 21%, respectively) primarily due to the reduced attack by the parasitoid wasp *Eurytoma obtusiventris* and downy woodpeckers on the 'gigantea' host race (Abrahamson *et al.*, 1994; Brown *et al.*, 1995). Behavioural studies with *E. obtusiventris* suggest that this early attacking parasitoid uses host-plant cues to locate its prey (Brown *et al.*, 1995). Thus, the gall inducer's shift to *S. gigantea* effectively reduces the incidence of parasitism by *E. obtusiventris* (Brown *et al.*, 1995). Furthermore, because of higher levels of gall-inducer attack on *S. altissima* and the lack of an oviposition deterrent in female gall flies, competition among larvae on the ancestral host *S. altissima* is likely more severe than on *S. gigantea* (Abrahamson *et al.*, 1994; Hess *et al.*, 1996; Craig *et al.*, 2000).

Biology of *M. convicta*

Mordellistena convicta [Coleoptera: Mordellidae; identified by J. Jackman, Texas A&M University, College Station, TX, USA; this taxon has been variously referred to as *M. unicolor*, *M. nigricans* and *M. aspersa* (Harrington, 1895; Ping, 1915; Milne, 1940; Liljebblad, 1945; Uhler, 1951)] is a purported generalist herbivore that bores in the stems of host plants from two tribes of the Compositae: Astereae (*Aster vimineus*, *A. pilosus*, *Euthamia graminifolia*, *S. altissima*, *S. gigantea*, *S. juncea* and *S. rugosa*) and Heliantheae (*Ambrosia artemisiifolia* and *Xanthium strumarium*) (Cappuccino, 1992; Ford & Jackman, 1996; Blair *et al.*, unpublished data). This native North American beetle is also known to feed on the parenchyma of *E. solidaginis* galls and, although it can complete development by feeding on plant tissue alone, to act as a facultative predator on the gall inducer (Uhler, 1951; Abrahamson & Weis, 1997). Furthermore, *M. convicta* occurs in *Eurosta* galls on both *S. altissima* and *S. gigantea*, and is an important source of mortality to the gall fly (Harrington, 1895; Uhler, 1961; Abrahamson *et al.*, 1989; Brown *et al.*, 1995; Sumerford & Abrahamson, 1995; Abrahamson & Weis, 1997). Series of specimens that emerged from various host plants and host-plant parts show no morphological differentiation (identification by J. Jackman) and, as a consequence, *M. convicta* has been thought of as a polyphagous species (Ford & Jackman, 1996). However, our genetic, ecological and behavioural work with *M. convicta* suggests that this taxon is composed of a complex of cryptic species as well as numerous host races.

Results

Genetic differentiation in *M. convicta*

The purported generalist *M. convicta* is composed of at least six reproductively isolated multilocus enzyme genotypes (cryptic species) based on fixed differences among alleles of the host-associated beetle populations

(Blair *et al.*, unpublished data). Furthermore, some of these cryptic species are further subdivided into host races, which are associated with particular host plants or host-plant parts. An allozyme survey identified five variables and consistently scorable enzymes for *M. convicta* including phosphoglucosmutase (PGM) (EC 5.4.2.2) and isocitrate dehydrogenase (IDH) (EC 1.1.1.42) on amine-citrate (pH 6.1), and aspartate aminotransferase (AAT) (EC 2.6.1.1), malate dehydrogenase (MDH) (EC 1.1.1.37) and adenylate kinase (AK) (EC 2.7.4.3) on Tris-Maleate-EDTA (pH 7.4) (Murphy *et al.*, 1996). These allozyme data were analysed using PHYLIP (Felsenstein, 1993) and Biosys-2 (Swofford & Selander, 1991) including the LINKDIS module (Black & Krafur, 1985). Here, we report genetic evidence that populations of cryptic species 2, which is found in the stems and galls of *S. gigantea* and *S. altissima*, have genetically differentiated into host races in stems vs. galls. We found significant allele frequency differences ($\chi^2 = 506$, d.f. = 1, $P < 0.0001$) at the MDH locus between populations of stems and galls (Table 1); however, there were no differences at any of the five loci examined between populations inhabiting galls of *S. altissima* and *S. gigantea*.

A maximum-likelihood tree of *M. convicta* cryptic species 2, based on a calculation of genetic distance by a Brownian motion model of genetic drift (Felsenstein, 1981), was rooted with the most closely related cryptic species 1, which occurs in stems of *S. altissima*, *S. gigantea* and *Euthamia graminifolia*. All gall beetles clustered in one closely related group, regardless of their host-plant association or geography and all stem beetles formed a second closely related group (Fig. 1). The direction of host shift has likely been from stems to galls given the fact that most *Mordellistena* are stem borers, suggesting that this is the ancestral condition. Furthermore, the maximum-likelihood tree places the species 2 stem beetles as ancestral to gall beetles.

We used amplified fragment length polymorphism (AFLP) procedures to further investigate the possibility of genetic differentiation between gall beetles. An analysis of 93 markers in two primer sets using PHYLIP (Felsenstein, 1993) found no difference ($F_{st} = 0.0056 \pm 0.0059$) between 13 beetles inhabiting galls on *S. gigantea* and 12 beetles in galls of *S. altissima* (S. Morehead, A. Whipple, M. Russell & W. Abrahamson, unpublished data). We conclude that if there is genetic differentiation between gall beetles, the differentiation is very slight because neither allozymes nor AFLPs could detect significant genetic difference between gall-dwelling beetles on *S. altissima* and *S. gigantea*.

Allochrony

Emergence studies performed in 2 years found evidence of allochronic adult emergence between stem and gall-dwelling beetles as well as between gall beetles emerging from *S. gigantea* and *S. altissima*. On average, stem-beetle

Table 1 Allele (electromorph) frequencies for five enzyme loci assayed in cryptic species 2 of *Mordellistena convicta* associated with stems and galls of *Solidago altissima* and *S. gigantea*.

Enzyme locus	Allele	Galls			Stems		
		<i>S. altissima</i>		<i>S. gigantea</i>	<i>S. altissima</i>		<i>S. gigantea</i>
		N.E. (n = 95)	PA (n = 123)	N.E. (n = 255)	N.E. (n = 6)	PA (n = 4)	N.E. (n = 26)
MDH	67	–	–	0.014	0.500	0.625	0.630
	75	0.058	0.004	0.025	0.083	–	0.043
	100	0.942	0.963	0.961	0.417	0.375	0.326
	133	–	0.033	–	–	–	–
AK	76	–	0.009	–	0.083	–	–
	100	0.972	0.978	0.973	0.917	1.000	1.000
	124	0.028	0.013	0.027	–	–	–
AAT	40	–	0.008	–	–	–	–
	70	0.026	0.008	0.029	–	–	0.019
	100	0.974	0.971	0.971	1.000	1.000	0.981
	130	–	0.008	–	–	–	–
	140	–	0.004	–	–	–	–
PGM	74	–	0.004	–	–	–	–
	79	–	0.004	–	–	–	–
	84	–	0.008	–	–	–	0.019
	89	–	0.004	–	–	–	–
	95	–	–	–	0.083	–	–
	100	0.637	0.597	0.734	0.583	0.875	0.769
	108	0.011	–	–	–	–	–
	116	0.353	0.323	0.266	0.333	0.125	0.212
	121	–	0.020	–	–	–	–
	126	–	0.032	–	–	–	–
IDH	50	–	–	0.002	–	–	–
	56	–	–	0.006	–	–	–
	63	–	–	0.006	–	–	–
	75	–	0.004	–	–	–	–
	100	0.958	0.980	0.948	1.000	1.000	1.000
	138	0.042	0.016	0.018	–	–	–
	175	–	–	0.020	–	–	–

N.E., New England (New Hampshire and Vermont); PA, Pennsylvania; n, sample size. Data are organized according to host-plant species, host-plant organ attacked and geographic location. Allele frequencies at MDH differ markedly between stem and gall beetles. Enzyme loci used were AAT, AK, IDH, MDH and PGM.

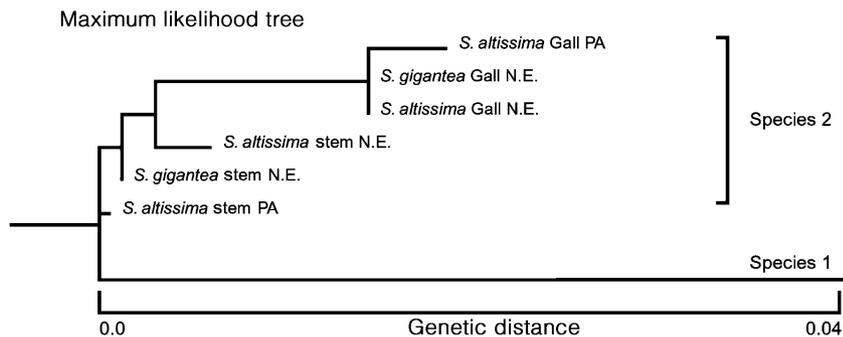


Fig. 1 Maximum-likelihood tree of populations of cryptic species 2 within *Mordellistena convicta* based on a PHYLIP 3.57c calculation of genetic distance by a Brownian motion model of genetic drift (Felsenstein, 1981, 1993). The maximum-likelihood tree is an unrooted tree that has been rooted by designation of *M. convicta* cryptic species 1 as outgroup. Distances on the tree represent amount of expected change. N.E., New England; PA, Pennsylvania.

adults emerged 9.3 and 11.2 days earlier than gall-beetle adults (Welch's $t' = 26.1$, d.f. = 352.8, $P < 0.001$ and Student's $t = 3.6$, d.f. = 28, $P = 0.001$ for 1996 and 1997

Pennsylvania populations, respectively). Furthermore, gall beetles from *S. gigantea* emerged 1.1 days earlier than gall beetles from *S. altissima* ($t = 2.7$, d.f. = 799,

$P = 0.007$). Furthermore, these allochronic differences in gall beetles are consistent across a range of temperatures (Eubanks *et al.*, 2003). However, these results should be viewed with great caution because we suspect that such allochrony is not biologically important for these beetles. Beetles from different natal origins have many opportunities to meet and mate as a consequence of their adult longevity (personal observations; Jackman & Nelson, 1995). In addition, their polyphagous adult habit of visiting numerous species of flowering plants to feed on pollen (S. Morehead, C. Blair, N. Ivanick & W. Abrahamson, unpublished data) likely enhances encounters between beetles of different origin. Finally, field studies (Weis & Abrahamson, 1985; Jackman & Nelson, 1995) and our experience with *M. convicta* in the laboratory suggest that females have a lengthy period of reproductive receptivity.

Reproductive isolation of *S. altissima* and *S. gigantea* gall beetles via mate choice

No-choice and choice mating experiments performed with beetles from galls of both *S. altissima* and *S. gigantea* showed that gall beetles assortatively mate according to natal host (Eubanks *et al.*, 2003). In no-choice experiments, gall beetles showed significant preference to mate with beetles from the same host-plant source. For example, 31% of the 70 beetles tested from *S. altissima* galls mated with other *S. altissima* gall beetles and 44% of the 86 beetles tested from *S. gigantea* galls mated with other *S. gigantea* gall beetles. But when 58 beetles were tested with prospective mates from the other host-plant source, only 17% mated. Similarly, choice-mating experiments also found assortative mating according to host-plant source (Eubanks *et al.*, 2003). Gall beetles ($n = 38$) from *S. altissima* mated with other 'altissima' gall beetles 79% of the time but with 'gigantea' gall beetles only 21% of the time. Gall beetles ($n = 62$) from *S. gigantea* demonstrated even stronger preference with 85% mating with other 'gigantea' gall beetles but only 15% mating with 'altissima' gall beetles. We conclude that gall beetles have well developed assortative mating in spite of the lack of detectable evidence for genetic differentiation of gall beetles from *S. altissima* vs. *S. gigantea*.

Reproductive isolation of gall beetles via oviposition choice and offspring survival

No-choice and choice oviposition and offspring survival experiments performed with gall beetles from *S. altissima* and *S. gigantea* indicated that either adult female gall beetles have a strong preference to oviposit into their natal host-plant species and/or that offspring differentially survive in their natal host-plant species (Eubanks *et al.*, 2003). The contribution of oviposition preference vs. offspring survival is not easy to separate for this beetle

because we have no reliable means to score oviposition preference. Oviposition scars are not visible and oviposition behaviour in this tiny insect is difficult to observe. All goldenrod stems used in these no-choice and choice trials bore a three to four-week-old, rapidly developing gall.

No-choice trials with 22 'altissima' gall beetles resulted in beetle emergence from 42% of the *Eurosta* galls on *S. altissima* and no beetle emergence from galls on *S. gigantea*. Similar trials with 24 'gigantea' gall beetles yielded beetle emergence from 50% of the galls on *S. gigantea* but no emergence from galls on *S. altissima*. Likewise choice experiments, in which each trial caged gall beetles from both host-plant sources with three galled stems of *S. altissima* and three galled stems of *S. gigantea*, had a similar result (Eubanks *et al.*, 2003). 'Altissima' gall beetles emerged only from *S. altissima* galls in 58% of the replicate trials but emerged from galls of both host-plant species in only 8% of the replicate trials. No emergence occurred in the remaining 33% of 'altissima' beetle replicates. 'Gigantea' gall beetles emerged only from *S. gigantea* in 42% of the replicates but from galls of *S. altissima* in only 8% of the replicates. In the remaining 50% of the 'gigantea' gall beetle replicates, no emergence was recorded. Thus, the results of both no-choice and choice oviposition trials indicate that either adult female gall beetles have a strong preference to oviposit into their natal host-plant species or that there are appreciable differences in offspring survival according to natal host-plant species.

Selection on stem and gall beetles to maintain the host shift

The rearing of insects from stems and galls from both *S. altissima* and *S. gigantea* found appreciable differences in the emergence of parasitoids of *M. convicta* (Fig. 2). For example, the parasitoid wasp *Tetrastichus ainsliei* never emerged from galls yet had appreciable emergence from stems, which suggests that although this parasitoid frequently strikes stem beetles, it does not attack gall beetles. A species of the parasitoid wasp *Quadrastichus* (identified by G. Melika, Systematic Parasitoid Laboratory, Koszeg, Hungary) appeared primarily in the New England samples and here it emerged much more frequently from galls on *S. gigantea* than galls on *S. altissima*. In Pennsylvania, *Quadrastichus* was present in stems; however, it occurred at zero or near-zero levels. These results suggest that selection due to differential mortality caused by parasitoids may help maintain the beetle's shift from stems to galls and potentially to favour gall beetles in *S. altissima* over beetles in galls on *S. gigantea*.

Additional evidence of selection that may maintain the shift from stems to galls comes from the finding that beetles from galls consistently have greater mass than beetles from stems. For example, a comparison of species

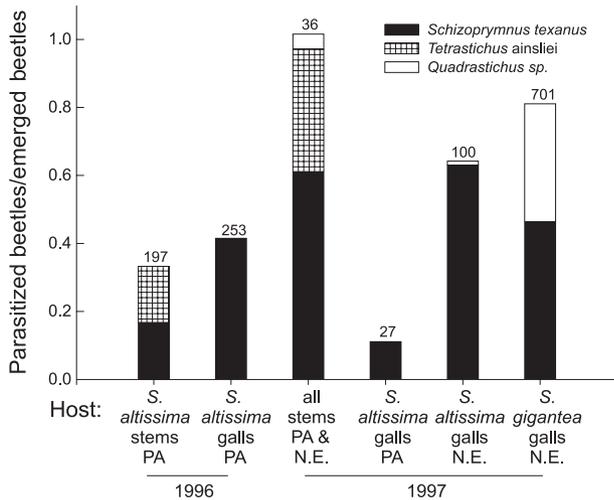


Fig. 2 Relative *Mordellistena convicta* mortality from its three major parasitoids by host plant and location expressed as the ratio of parasitized beetles per live *M. convicta* emerged. The numbers above the bars are live *M. convicta* emergence from the host. Because total beetle mortality is not known, relative mortality estimates were generated by dividing parasitoids emerged from each host by the average number of dead *M. convicta* they represent. *Schizopyrmnus texanus* infests *M. convicta* at one parasitoid per *M. convicta* larva, *Tetrastichus ainsliei* at a mean of 4.7 parasitoids per *M. convicta* larva and *Quadrastichus sp.* at a mean of 5.7 per gall. Because 1997 stem samples were small, all stems were combined for that year. These figures do not include *M. convicta* mortality caused by parasitoids that failed to emerge from the host or mortality from any cause besides parasitism.

2 stem and gall beetles showed that stem beetles had on average 2.6 times less mass than gall beetles with means of 0.88 ± 0.11 and 2.27 ± 0.02 mg, respectively. Thus, heavier beetles consistently emerge from galls vs. stems probably as a consequence of the availability of better nutrition in gall parenchyma vs. stem, and of the markedly higher amounts of fat and protein available to *Mordellistena* larvae that consume the gall inducer. Such predation on the gall inducer by gall beetles is common as Uhler (1961) found that gall beetles ate the *Eurosta* larva in 73% of the galls in which they were present.

Finally, beetles from galls on New England *S. gigantea* were about 12% heavier than those from galls on New England *S. altissima* (2.30 ± 0.02 vs. 2.06 ± 0.06 mg, respectively; $t = 3.98$, d.f. = 799, $P < 0.001$). This difference may be a consequence of the larger mean diameter of *S. gigantea* galls (Sumerford & Abrahamson, 1995; Abrahamson & Weis, 1997), which may offer more and/or better-quality plant tissue to the developing *Mordellistena* larvae. As a result, selection may act to maintain the host shift from stems to galls because of enhanced larval performance, which could translate into increased adult longevity, vigour and/or fecundity.

Discussion

Diversification of *M. convicta* cryptic species 2 in response to *Eurosta* host races

Our results provide evidence that *M. convicta* species 2 exists as a group of host races associated with specific host plants and host-plant parts. *Mordellistena convicta* has radiated from stems to the galls of *Eurosta* and facultatively preys on the gall inducer. *Eurosta*'s host shift from *S. altissima* to *S. gigantea* has resulted in the formation of gall fly host races, which in turn have provided the opportunity for the subsequent diversification of *M. convicta* by way of exploitation of galls on two host plants. Thus, *M. convicta* has undergone 'sequential radiation' that has produced an escalation of biodiversity up the trophic system. Our studies (Eubanks *et al.*, 2003; Blair *et al.*, unpublished data; Morehead *et al.*, unpublished data) are the first to examine and document that a host shift and subsequent host-race formation by an herbivorous insect may have contributed to the host shift and host-race formation of a second, unrelated species.

Finding host races among the populations of the specialized gall-inducer *E. solidaginis* was no surprise given the apparent connection of ecological specialization to rapid and extensive diversification (Price, 1980; Futuyma & Moreno, 1988). For herbivorous insects, a shift to a novel host plant can represent the initial step in diversification and speciation (Bush, 1975; Futuyma & Moreno, 1988; Thompson, 1996). Shifts in host use by initially monophagous or narrowly oligophagous herbivores are well documented (Carroll & Boyd, 1992; Bush, 1994; Berlocher, 1998). Distinct host races, and ultimately species, may result from such shifts as long as gene flow is reduced sufficiently between the ancestral and derived host-associated populations (Diehl & Bush, 1984; Bush, 1994; Craig *et al.*, 1994; Berlocher, 1998; Cronin & Abrahamson, 2001). For example, gall inducers that attack different host-plant species but show no morphological differences have been assumed to be the same species. Yet such taxa have increasingly been shown to be distinct and specialized species after detailed behavioural and/or genetic tests (Roininen *et al.*, 1993; Kopelke, 2000, 2001; Nyman, 2000).

Our finding that the purported generalist herbivore *M. convicta* exists as a complex of cryptic species, with subsequent subdivision into host races, was a surprise (Blair *et al.*, unpublished data). *Mordellistena convicta* was thought to lack host specificity, and adults disperse away from their larval host plant visiting many food sources during their long reproductive lives. Thus, our findings are distinctive in that the factors that have typically been important in promoting and maintaining reproductive isolation during host-race formation in other insects appear to be relatively unimportant in *M. convicta* (Eubanks *et al.*, 2003).

For example, allochronic emergence and host-facilitated mating are crucial sources in the reproductive isolation of host races within *R. pomonella* and *E. solidaginis* (Craig *et al.*, 1993; Bush, 1994). In contrast, *M. convicta* shows differences in emergence phenology that are likely insufficient to reproductively isolate even stem and gall beetles, let alone the gall beetle host races, given the longevity and vagility of adults (Abrahamson *et al.*, 2001; Eubanks *et al.*, 2003; Blair *et al.*, unpublished data). However, this is not to suggest that the later emergence of gall beetles compared with stem beetles is not adaptive. 'Altissima' gall beetles emerge in Pennsylvania just as galls are becoming visible on *S. altissima* (Weis & Abrahamson, 1985) suggesting that the timing of emergence is synchronized with the availability of hosts. Furthermore, these beetles do not feed on their larval host plants as adults (goldenrods are not flowering when adult beetles are actively feeding and mating), but instead feed at a wide variety of pollen-producing flowers. The habit of feeding at many pollen sources coupled with their adult longevity facilitates encounters among adult beetles from different host plants or host-plant organ origins. Consequently, host association may be relatively unimportant to *Mordellistena's* assortative mating.

Mordellistena convicta is similar to these other insect taxa with host races in that natural-enemy attack may be assisting host-race formation. Natural-enemy attack on herbivores can differ among ancestral and derived host plants and can assist host-race formation when a derived host provides reduced enemy attack or even enemy-free space (Jeffries & Lawton, 1984; Abrahamson *et al.*, 1994; Brown *et al.*, 1995; Feder, 1995). Stem beetles of *M. convicta* exclusively experience mortality from *T. ainsliei*, which could be an important selective difference between stem and gall beetles given the rates of *T. ainsliei* attack. Similar differential natural-enemy attack occurs among gall beetles. Although beetles in *S. gigantea* galls are heavily attacked by a species of *Quadrastichus*, this natural enemy rarely attacks beetles in *S. altissima* galls creating enemy-reduced space in *Eurosta* galls on the latter host plant.

In summary, the patterns of genetic and behavioural differentiation among beetle populations of cryptic species 2 suggest that the split between gall beetles on *S. gigantea* and *S. altissima* is more recent than the split between stem and gall beetles. Furthermore, the acquisition of assortative mating and assortative oviposition and/or differential offspring survival apparently has preceded any development of detectable genetic differences between 'gigantea' and 'altissima' gall beetles. Thus, our data suggest a host shift from stems to galls within species 2 with differentiation aided by selection for later emergence time, greater mass made possible by superior nutrition, and survival on the new host enhanced by escape from *T. ainsliei*. We do not yet know if *Eurosta* galls on *S. gigantea* and *S. altissima* were simultaneously

colonized by stem beetles of cryptic species 2 or if the stem beetle's shift to galls was initially into the gall on only one host-plant species with a subsequent shift to the alternate host plant.

Did stem and gall beetles differentiate in sympatry?

Whether the stem and gall host races originated in sympatry or allopatry is important to our understanding of the processes that may have led to the present-day patterns of differentiation. The most parsimonious explanation of the present-day traits of these host races is one of sympatric differentiation. For example, gall beetles will virtually always occur sympatrically with stem beetles because galls cannot exist without stems and stem beetles are very frequent and abundant. Furthermore, it is likely that these host plants have occurred sympatrically in permanently open marginal habitats since well before European colonization transformed the North American landscape (Marks, 1983).

Our findings imply that diversification can occur if assortative mating and oviposition preference and/or offspring survival create reproductive isolation that could lead to sympatric speciation, even in an organism whose adult apparently lacks strong host-plant fidelity for both mating and feeding. Furthermore, the diminished mortality as a consequence of reduced attack by natural enemies on gall beetle populations relative to stem populations may provide selection that would maintain the host shift (Abrahamson *et al.*, 2001). A key point arising from our studies of both *Eurosta* and *Mordellistena* is that behaviour can limit gene flow and may facilitate the genetic differentiation of populations in sympatry (Abrahamson *et al.*, 2001). Indeed, the existence of multiple levels of differentiation, which range from fixed differences indicative of cryptic species to no genetic differentiation between behaviourally isolated gall-dwelling populations, is congruent with the expectations of host-plant related sympatric speciation.

The behavioural, ecological and genetic attributes of stem and gall populations of *M. convicta* are such that geographic isolation is not a prerequisite to their differentiation. Indeed, it is difficult to construct allopatric circumstances for the differentiation of the stem and gall host races. To require geographic isolation in order to generate the observed differentiation of *M. convicta* is a less parsimonious explanation than is sympatric speciation (Abrahamson *et al.*, 2001).

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