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The Role of Olfactory Cues in the Sequential Radiation of a Gall-boring Beetle, Mordellistena convicta

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The role of olfactory cues in the sequential radiation of a gall-boring beetle, *Mordellistena convicta*

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Abstract. 1. Herbivorous insects often have close associations with specific host plants, and their preferences for mating and ovipositing on a specific host-plant species can reproductively isolate populations, facilitating ecological speciation. Volatile emissions from host plants can play a major role in assisting herbivores to locate their natal host plants and thus facilitate assortative mating and host-specific oviposition.

2. The present study investigated the role of host-plant volatiles in host fidelity and oviposition preference of the gall-boring, inquiline beetle, *Mordellistena convicta* LeConte (Coleoptera: Mordellidae), using Y-tube olfactometers. Previous studies suggest that the gall-boring beetle is undergoing sequential host-associated divergence by utilising the resources that are created by the diverging populations of the gall fly, *Eurosta solidaginis* Fitch (Diptera: Tephritidae), which induces galls on the stems of goldenrods including *Solidago altissima* L. (Asteraceae) and *Solidago gigantea* Ait.

3. Our results show that *M. convicta* adults are attracted to galls on their natal host plant, avoid the alternate host galls, and do not respond to volatile emissions from their host-plant stems.

4. These findings suggest that the gall-boring beetles can orient to the volatile chemicals from host galls, and that beetles can use them to identify suitable sites for mating and/or oviposition. Host-associated mating and oviposition likely play a role in the sequential radiation of the gall-boring beetle.

Key words. Gall insects, goldenrod, host races, mordellid beetle, niche construction, olfaction, sequential radiation, sequential speciation, volatiles, Y-tube olfactometer.

Introduction

Species diversity itself may drive the creation of more species (Emerson & Kolm, 2005). In diversifying, organisms may so modify their environment as to create new resources that other organisms can exploit (i.e. ecosystem engineering, Jones *et al*., 1994; Odling-Smee *et al*., 2003; Wright & Jones, 2006) thus providing the opportunity for those organisms to diversify in their turn (Jones *et al*., 1997). Plant-insect interactions make excellent model systems for studying diversification and the effects of ecosystem engineering, because the adaptive radiation of herbivorous insects on new host plants can lead to the subsequent diversification of other organisms that depend on the resources that the primary herbivore creates (i.e. sequential radiation; Abrahamson *et al*., 2001, 2003; Abrahamson & Blair, 2008).

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Behavioural (Eubanks *et al*., 2003), ecological and genetic evidence (Blair *et al*., 2005) suggest that the gall-boring, inquiline beetle *Mordellistena convicta* LeConte is undergoing sequential radiation through adaptation to the galls of two species of goldenrods, *Solidago altissima* L. and *Solidago gigantea* Ait., which are induced by host races of the ecosystem-engineering gall fly *Eurosta solidaginis* Fitch (Diptera: Tephritidae). The beetles from the two host-associated populations are somewhat genetically different according to an allozyme analysis ($F_{ST} = 0.02$), differ in mass, are attacked by different parasitoids (Blair *et al*., 2005), and emerge allochronically from their host galls (Eubanks *et al*., 2003). Mating experiments in the absence of host plants found evidence of assortative mating: given a choice of mates, 79% of mating *S. altissima* beetles and 85% of mating *S. gigantea* beetles mated within their host race (Eubanks *et al*., 2003). In addition, Eubanks *et al*. (2003) found that the beetles exhibited host-specific eclosion, producing surviving offspring only from the natal host, leaving open the question of whether the

host-specific eclosion was caused by female oviposition preference for the natal host or host-specific larval mortality. Neither of these experiments investigated the classic mechanisms for reproductive isolation in host-associated diversification: mating and oviposition on the natal host (Bush, 1969; Tauber & Tauber, 1989).

If host preference plays a role in the divergence of the host-associated populations of the gall-boring beetle, then they must have a way to locate their host. Because galls are a scarce and intermittent host, the location method would need to operate long range, most likely by detection of airborne odours from the host. Because many insects have the olfactory capacity to readily distinguish among plant species or even parts of plants (Visser, 1986; Bernays & Chapman, 1994; Bruce *et al.*, 2005), the effects of olfactory cues on mating and oviposition become more appreciable if the gall-boring beetles can distinguish between galled and ungalled natal host plants, especially for detecting host galls in dense sympatric fields. Therefore, we tested whether gall beetles preferred the volatile emissions of their host galls on *S. altissima* and *S. gigantea*. We also further tested whether they responded differently to the volatile emissions of ungalled stems and galls of their host plants. If unmated males and females and mated males orient themselves to the volatiles of their natal host galls, galls may be used as rendezvous sites at which beetles meet potential mates because these beetles would have no other reason to be attracted to the plant. If mated females orient to the volatiles of their natal host galls, this may suggest that olfaction is important to identification of appropriate oviposition sites. The motivation to eat the host plants can be ruled out because adult gall-boring beetles, although they are pollen eaters, do not feed on the host goldenrods which flower months later than the beetle breeding season.

Insects may also actively avoid alternate hosts (Forbes *et al.*, 2005). Avoiding non-natal hosts would reduce the risk of hybridization with other host-associated populations and the chance of ovipositing on these plants. Active avoidance may then act as a barrier to gene flow.

Using both mated and unmated gall-boring beetles of both sexes from both hosts, we conducted Y-tube olfactory experiments to test the following four hypotheses: (i) unmated male and female beetles move towards the volatile emissions of their natal host galls presumably to use them as rendezvous sites for mating (host fidelity); (ii) mated female beetles move towards the volatile emissions of their natal host galls presumably for oviposition (oviposition-site preference); (iii) there is a difference between the responses of beetles exposed to galls and those exposed to ungalled stems (gall-stem difference); and (iv) beetles avoid volatile emissions of their alternate hosts (alternate-host avoidance).

Methods

Study system

Two species of goldenrod attacked by the gall-inducing fly are *Solidago altissima* and *S. gigantea*, which are closely related plant species in the *S. canadensis* species complex (Abrahamson & Weis, 1997; Abrahamson *et al.*, 2001, 2003). They are widely distributed throughout much of the continental United States and southern Canada and are sympatric over most of their range. The gall-inducing fly oviposits in the apical bud of its goldenrod host and the developing larva often induces the development of a gall (Uhler, 1951). The gall-boring beetle oviposits on these galls and the larvae consume gall tissue and often the fly larva as they tunnel through the gall (Uhler, 1951; Abrahamson & Weis, 1997; Blair *et al.*, 2010). Mature beetles emerge from the galls in late spring to feed on pollen from multiple plant species, mate and oviposit (Uhler, 1951).

Sampling and preparation of specimens

To obtain gall-inducing flies and gall-boring beetles, galls stimulated by the flies on *S. altissima* and *S. gigantea* stems were collected in December 2009 and March 2010 from Vermont and northern New York, a region where the two host-associated populations of gall-boring beetles occur in sympatry. Beetles from a sympatric area were used because beetles from these populations must be actively segregating themselves, otherwise genetic differences between the two populations would be swamped out as a result of hybridisation (Blair *et al.*, 2005). The galls were collected after the goldenrod stems had senesced and the insects inside the galls had entered winter diapause.

The collected galls were stored at $-20\,^{\circ}$C, a temperature that facilitates survival in freeze-tolerant insects, until April of 2010 when they were removed from storage for rearing. To rear the insects, galls were placed in screen-covered emergence cages in growth chambers at $23\,^{\circ}$C, 80% relative humidity, and LD 15:9 h daily, which mimics the photoperiod at their normal late-spring emergence time. Cages were monitored multiple times daily for emerging insects. Captured beetles were housed individually and kept in growth chambers with the above-described conditions. Although the timing of mating and egg development of the gall-boring beetle is unknown, previous research has indicated that there is approximately a 1-week lag between peak emergence and peak oviposition (Weis & Abrahamson, 1985). Therefore, the beetles were housed for at least 2 days before attempting to mate them (to obtain mated males and females) and at least another day after that before attempting to use them in the Y-tube experiments.

Rhizomes of *S. altissima* and *S. gigantea*, which are both clonal, were collected from northwestern Vermont during October 2009 and overwintered in a cool greenhouse. During the last week of March 2010, the rhizomes were cut into 5-cm long pieces, planted in pots, and allowed to grow in a warm greenhouse. Galls were induced on the goldenrod ramets growing in the greenhouse using gall flies that were collected from the same set of galls from which beetles emerged. Flies were released into cages containing their potted host plants and allowed to mate and oviposit there. The plants that developed galls were used in the gall experiments. Some plants were not exposed to flies and were used in the ungalled stem experiments.

Mating procedure

The experiments used both mated and unmated beetles. To obtain mated beetles, beetles emerging from the galls of the same species of goldenrod were placed in Petri dishes in groups of three, 2–3 days after emergence. Groups of three were used to increase a chance of having both male and female in a mating group because it is not possible to determine the sex of the gall beetles by external observation. Gall beetles were observed for at least an hour or until they mated; beetles that were observed to mate were used in the mated-beetle experiments and those observed not to mate were used in the unmated experiments. After all the experiments were finished, the sex of each beetle was determined post-mortem by applying pressure to abdomens of the specimens to evert their genitalia under a dissecting microscope.

Apparatus

Y-tube olfactometers were used to examine the gall beetles' ability to detect volatile cues, and their preferences for them. The design of the olfactometers followed that of Sabelis and Van de Baan (1983) and Tooker *et al*. (2005). A 2-cm inner-diameter glass Y-shaped tube was connected at its stem to a vacuum pump, which pulled air through the tube. Each Y-tube arm was connected to a rotameter which allowed for the fine adjustment of air flow through each side of the apparatus (100 ml per min through each arm). Teflon™ tubing connected each of the rotameters to a Teflon™ bag. The bags were used to contain either the volatile-emitting sample or an odourless control (empty glass vial).

All volatile-emitting samples came from intact plants rather than cut stems to ensure that none of the volatile emissions were associated with plant-wound responses. The bags were not sealed across the top or bottom so they could be slipped on over the top of the plant and moved down to the height where galls or corresponding stems were present. Once positioned at the appropriate height, the bags were secured around the stems of the plants with twist ties. In the gall trials, as little stem and leaf material as possible was captured in the bagged areas. This separated the response to volatiles emitted by the gall and stem of the plant.

During a trial, the vacuum pump pulled air through an activated charcoal filter to remove any ambient organic volatiles. Then, the air passed through a flask with water to rehydrate the air inside the Y-tube apparatus. After passing through the water, the air stream was split into two paths which met again in the Y-shaped glass tube carrying air that had been exposed to either the experimental treatment or a blank control. Subsequently, the air flowed down the stem of the Y tube, passing the beetle subject before being pulled into the vacuum pump. All materials used in the construction of the olfactometer were inert to prevent confounding effects of volatile emissions from the apparatus itself. Elevation (15°) and fluorescent light (2.3 μE^{-1} m^2 s^{-1} from a 4100 K low-flicker-rate T-8 bulb) were used to entice gall beetles to make a decision in the experiment, as has been done in similar studies (Ginzel & Hanks, 2005; Voss *et al*., 2009).

Experimental procedure

Using the Y-tube olfactometers, the gall beetles' responses to volatile emissions from their natal and alternate host-plant stems and galls were tested. All trials were carried out in a photographic dark room so that the only source of light during testing was the T-8 fluorescent light in front of the Y tube. The plants were not visible to the beetles so that orientation to visual cues was ruled out. A single beetle was loaded into the stem of the Y tube and then the system was sealed and the vacuum pump turned on. A beetle was observed for 10 min or until it moved more than 2 cm up one of the arms of the Y tube. Ten minutes was chosen as the observation time because our preliminary study showed that most of the beetles that did not make a decision in 10 min were unlikely to make a decision in any acceptable amount of time. Any trial where the beetle failed to move past either of the Y-tube arm decision lines after 10 min was recorded as a no decision. The experimental stimulus side of the Y tube was alternated after every 5 trials of the experiment and the plant was changed every 10 trials. These two measures were taken to control for any directional preferences of the beetles in the Y tube and for variation in volatile chemicals among host plants.

Three types of experiment were used to test the gall beetles' reaction to volatiles from natal-host and alternate-host galls and from ungalled plants: (i) one-gall tests with volatiles from a natal-host or an alternate-host gall drawn down one arm of the Y tube and air from the odourless control drawn down the other; (ii) one-stem tests with volatiles from an ungalled plant of the natal or alternate host versus the odourless control, and (iii) a two-gall test with both arms of the Y tube containing volatiles, one from the natal-host gall and one from the alternate-host gall. Both *S. altissima* and *S. gigantea* beetles were tested in the one-gall tests. Only *S. gigantea* beetles, which eclosed in greater numbers, were available for the one-stem and two-gall tests. All of the experiments were carried out with beetles that were 3–25 days old. The mean age of gall beetles tested was 13.4 days with ±0.23 SE. The numbers of beetles that were included in each test are given in Table 1.

Statistical analysis

A chi-square test was used to check for an inherent arm bias in the Y tube using Predictive Analytics Software (PASW), version 18.0 (SPSS Inc., Chicago, IL) Using the same program, a binary logistic regression was carried out for each host-associated population in each test type: four one-gall tests (*S. altissima* natal host and alternate host, *S. gigantea* natal host and alternate host); two one-stem tests (*S. gigantea* natal and alternate ungalled host plant); and one *S. gigantea* two-gall test – all in all, a regression on each of seven independent sets of data. These regressions tested for the effects of variables that were not controlled in the experiments. Multiple potential predictive variables were considered in each regression and varied depending on the type of experiment (Table 2). If there were no effect of volatile chemicals on the preference of the beetle, it would have an equal chance of choosing either arm so any deviations were compared with the null hypothesis of

Table 1. Numbers of beetles in each experiment.

Type of experiment	Beetle's natal host	Individual experiments	N	Moved towards stimulus	Moved away from stimulus	Did not respond
One-gall	*S. altissima*	Natal host gall	86	51	25	10
		Alternate host gall	80	19	57	4
	S. gigantea	Natal host gall	124	84	36	4
		Alternate host gall	133	43	83	7
One stem	*S. gigantea*	Host ungalled stem	64	33	27	4
		Alternate ungalled stem	67	27	33	7
Two galls	*S. gigantea*	Both galls	61	39*	21†	1

*Moved towards natal host gall.
†Moved towards alternate host gall.

Table 2. Predictive variables considered in each experimental condition. The list of predictive variables is provided below the table.

Stimulus	Predictive variables considered
One gall	1, 2, 5, 6, 7, 8
One ungalled stem	1, 2, 5, 7, 9
Two galls	1, 3, 4, 5, 7, 9, 10

1. Field site from which the gall beetle came. 2. Individual plant used in test. 3. *Solidago altissima* plant used. 4. *S. gigantea* plant used. 5. Age of the gall beetle. 6. Mating status of gall beetle. 7. Sex of gall beetle. 8. All interactions between age, mating status, and sex of gall beetle. 9. Interaction between age and sex of gall beetle.10. Interaction between the two plants used.

0.5. Instances where the gall beetle did not cross either of the decision lines during the experiment were not used in this analysis but are reported. A manual backward elimination was carried out for each of the regressions until the best model was determined for each. Akaike's information criterion (AIC) was used to assess the relative likelihood of models.

Results

Experimental validity and sample sizes

A chi-square test for Y-tube arm bias showed that the gall beetles displayed no significant preference for one arm or the other (52.0% right vs. 48.0% left), χ^2 (1, 552) = 1.13, $P = 0.228$. A total of 615 gall beetles were tested across all experiments and of these, 578 made decisions. There were also 25 beetles that made a decision but could not be sexed that were excluded from the analyses.

Attraction to natal host galls

Both host-associated populations of gall beetles displayed a preference for moving towards the emissions of their natal host galls [*S. altissima*: *Wald* χ^2 (1, 71) = 8.95, $P < 0.001$; *S. gigantea* beetles: *Wald* χ^2 (1, 111) = 17.74, $P < 0.001$, Fig. 1]. The best model took no predictive variables into effect, including the sex and mating status of the gall beetles. The finding that there was no effect of mating status or sex means that we could not distinguish the support for the first (host
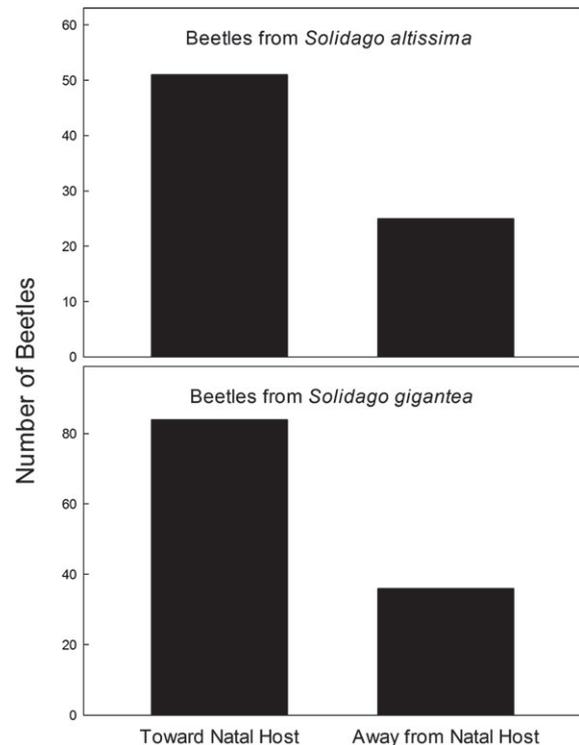

Fig. 1. Decisions of gall beetles when exposed to their natal host galls. The bars show the number of gall beetles that made each decision from the (top) *Solidago altissima* ($P < 0.001$) and (bottom) *S. gigantea* ($P < 0.001$) host-associated populations.

fidelity) and second hypotheses (oviposition preference) as the preferences of mated females are not significantly different from those of unmated males or females or mated males.

Avoidance of alternate host galls

Significantly more gall beetles moved away from the volatile emissions of the alternate host gall and up the control arm of the Y tube, supporting the alternate-host avoidance hypothesis. This was true both for beetles emerging from *S. altissima* galls, *Wald* χ^2 (1, 72) = 13.00, $P < 0.001$, and *S. gigantea* galls, *Wald* χ^2 (1, 122) = 10.78, $P < 0.001$ (Fig. 2). The best model for the beetles emerging from *S. gigantea* galls
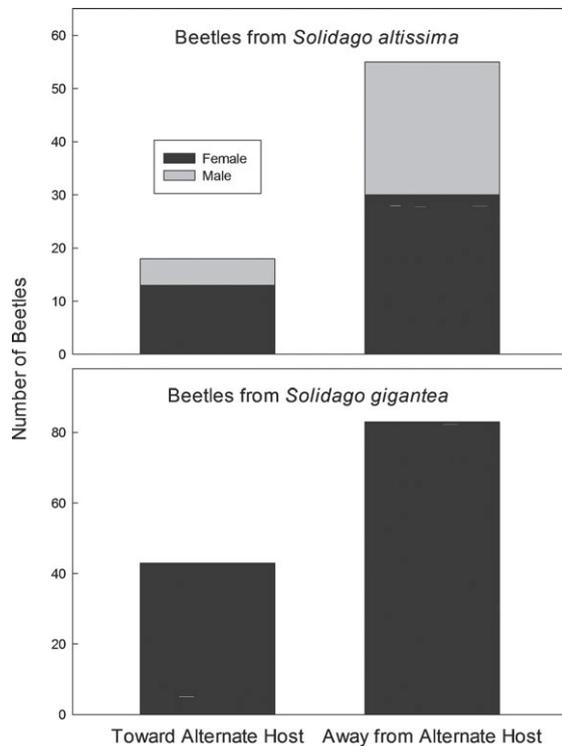
Fig. 2. Decisions of gall beetles when exposed to the alternate host galls. The bars show the number of beetles that made each decision from the (top) *Solidago altissima* ($p < 0.001$) and (bottom) *S. gigantea* ($P < 0.001$) host-associated populations.

included no other predictive variables. Two predictive variables remained during the analysis of beetles emerging from *S. altissima* galls. The sex of the beetle was significant [*Wald* χ^2 (1, 72) = 6.901, $P = 0.009$], with males being more likely to avoid the alternate host gall than females (Fig. 2). The interaction between age and sex was also significant, with older females being more likely to avoid the alternate host gall at the same rate as males [*Wald* χ^2 (1, 42) = 6.524, $P = 0.011$]. There was no significant trend among males with regard to age [*Wald* χ^2 (1, 29) = 0.184, $P = 0.184$].

Lack of preference for ungalled stems

Solidago gigantea gall beetles displayed no significant attraction to the emissions of their ungalled host plant [*Wald* χ^2 (1, 56) = 0.437, $P = 0.508$], supporting the gall-stem difference hypothesis. The best model took no predictive variables into account. The *S. gigantea* beetles displayed a similar reaction to the volatile emissions of *S. altissima*: no significant trends were found, *Wald* χ^2 (1, 58) = 0.827, $P = 0.363$. The best model excluded all predictive variables.

Choice between two galls

The two-gall experiment, in which *S. gigantea* gall beetles were exposed to the volatile emissions of a natal *S. gigantea*
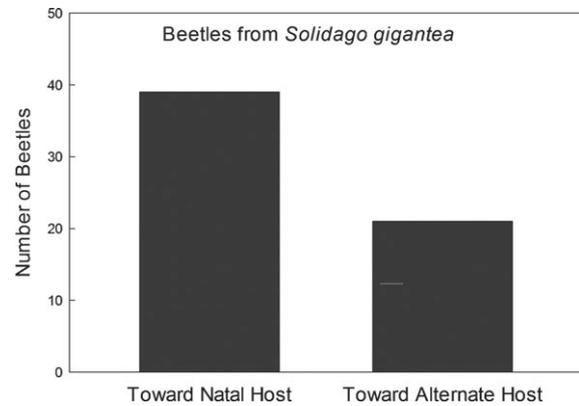

Fig. 3. Decisions of gall beetles from *Solidago gigantea* galls when simultaneously exposed to the galls of both host plants ($P = 0.007$).

gall and a gall of *S. altissima*, found that these gall beetles showed a preference for moving towards the volatile cues of their natal host and away from the cues of the alternate host, *Wald* χ^2 (1, 56) = 7.363, $P = 0.007$ (Fig. 3). There were no significant predictive variables in the best-fitting model.

Discussion

Attraction to natal host galls

Our results show that gall beetles can sense and react to the volatile emissions from their natal host gall. There were no differences between mated and unmated gall beetles in any of the experimental conditions, and males and females displayed the same basic patterns across conditions. This suggests that (i) gall beetles use the volatile emissions from host galls to locate their host plant for finding mates and (ii) mated males retain a preference for host galls to find further mating opportunities. Because mated females' preference for their natal host galls was not any stronger than that of unmated females and also because females may mate multiple times, the current data precluded us from concluding that females are attracted to galls for the purpose of oviposition.

The gall beetles were attracted to the volatile emissions of their natal host galls regardless of their sex or mating status, suggesting that gall beetles are attracted to the volatile chemical cues of their host plants for pairing up with mates and possibly for oviposition as well as the two appear to be operating via the same mechanism. It is most parsimonious to think that a single change in preference drives host fidelity and host-specific oviposition (Bush, 1969).

Avoidance of alternate host galls

The gall beetles tended to avoid the volatile emissions of the alternate host galls, consistent with our hypothesis. This avoidance of the alternate host galls may reduce mixed matings and oviposition mistakes (Forbes *et al.*, 2005, 2009). Such avoidance combined with attraction to the natal host could lead

to the spatial segregation of beetles at a microhabitat level. As a consequence, gall beetles seeking mates will mostly segregate by host plant.

The avoidance behaviours observed in this experiment are similar to findings from two other species with multiple host races, *Rhagoletis pomonella* (Forbes *et al.*, 2005) and *Diachasma alloeum* (Forbes *et al.*, 2009). It has been proposed that avoidance behaviours for non-natal hosts can be an isolating mechanism that operates to reduce backcrossing between hybrids and parental host races because hybrids receive conflicting attraction and avoidance signals from both hosts which could cancel one another (Forbes *et al.*, 2005; Feder & Forbes, 2010). As a result, the lack of a response to the chemical emissions of either potential host plant by hybrids decreases their chances of finding a mate, facilitating host-associated differentiation (Linn *et al.*, 2004).

The only treatment in which any of the predictive variables were included was that in which gall beetles emerging from *S. altissima* plants were exposed to volatiles from *S. gigantea* galls. Here, male and older female *S. altissima* beetles were more likely to move away from the volatile emissions of *gigantea* galls than were younger female *S. altissima* beetles. Males may respond to mating cues earlier in their lives than females to gain reproductive advantage over other males (Emlen & Oring, 1977) by arriving at the correct mating site earlier. The decreased response from younger females may indicate that these females are not yet able to mate. It is possible that females may need to feed as adults before they have the resources to produce their eggs. *Solidago altissima* females have less body mass than *S. gigantea* females (Blair *et al.*, 2005) and thus may need to feed longer before mating.

Neutral reactions to ungalled stems

The *S. gigantea* gall beetles' neutral reaction to ungalled stems suggests that the beetles are responding only to the galls, rather than to the plant itself. This finding makes ecological sense as the larvae of gall beetles are adapted to feed on galls, not stems (Blair *et al.*, 2010) and there are far fewer galls than stems in a given field, making long-range tracking via volatiles a useful trait. Cuing in on stem volatiles would not help them find mates or gall oviposition sites because there are many goldenrod fields that lack galls and galled ramets are often clustered in a small patch. The volatiles emitted from the galls and ungalled stems must be different, especially if the gall beetles are cuing in on a wound response emitted by the plants in response to gall formation (Stelinski *et al.*, 2006; Takabayashi *et al.*, 2006). It is unlikely that the gall-boring beetles visually find galls because they begin mating while the first galls are tiny nodules still hidden in the base of the leaf bud (Weis & Abrahamson, 1985; Abrahamson & Weis, 1997). There is evidence that the volatiles emitted by galled and ungalled *S. altissima* plants have different concentrations of salicylic acid (Tooker *et al.*, 2008), a hormone frequently emitted by plants that have been wounded (Bennett & Wallsgrove, 1994; Rani, 2006; Jahangir *et al.*, 2009). This suggests that differentiation of the gall-boring beetle from its stem-boring ancestors (Blair

et al., 2005) may have been as a result of an attraction to these volatiles.

Reproductive isolation

It is difficult to estimate the level of reproductive isolation represented by the results of these tests in conjunction with previously studied assortative mating in these beetles (Eubanks *et al.*, 2003). How these behaviors interact and play out in the field is unknown. If these laboratory findings are a good approximation of long-range search in a goldenrod field, then *S. gigantea* beetles, for instance, would land on the correct gall between 65% of the time (two-gall test) and 70% of the time (one-gall test). Once on the gall, given a choice of beetles of both host races, 85% of *S. gigantea* beetles would mate within their host race (Eubanks *et al.*, 2003). On the correct gall, however, the majority of beetles would be members of the *S. gigantea* host race, given an equal number of beetles of both host races in the field. But relative abundances of beetle host races vary widely from field to field and year to year. Also to be factored in is the suggestion in these results of a mechanism for post-zygotic isolation: the possibility that hybrids will have conflicting attraction-avoidance signals from each plant thereby failing to mate or oviposit (Forbes *et al.*, 2005).

Gall beetle attraction to their natal hosts in the Y-tube tests seemed not quite as strong as long-range host location by host races of the parasitoid wasp *Diachasma alloeum* who locate their tephritid fly hosts by following volatiles emitted by the fly's host plant (Forbes *et al.*, 2009). Their performance in a Y-tube olfactometer showed what seems to be greater attraction to host volatiles: 81% to 92% as opposed to 67% and 70% in *S. altissima* and *S. gigantea* beetles. Likewise, the pepper weevil (*Anthonomus eugenii*) was attracted to its three hosts in Y-tube tests with a range of about 70–100% (Addesso & McAuslane, 2009). On the other hand, only 59% of females of the tomato host race of the spider mite *Tetranychus urticae* responded positively to host volatiles in a Y tube (Gotoh *et al.*, 1993).

But these Y-tube results are only part of the reproductive isolation picture. They relate to a long-range search, only one of the possible behaviours involved in host-plant location and acceptance (Visser, 1986; Bernays & Chapman, 1994). The abovementioned *T. urticae* females, for instance, showed an increase to 81% attraction to their host when offered a portion of tomato leaf versus a leaf portion from the alternate host (Gotoh *et al.*, 1993). Once on a gall, beetles may further refine their host choice by short-range chemical, tactile, and visual cues. The host galls, for instance, have markedly different surfaces, *S. gigantea* galls being glabrous and *S. altissima* trichomatous (Abrahamson & Weis, 1997). Furthermore, host location is only one of the host-related adaptations to the selective environment of different hosts. Reproductive isolation in host races comprises many other elements besides host location, such as pleiotropy, sexual selection, divergent pheromones, or reduced hybrid survival (Via, 2001; Drès & Mallet, 2002). It is not known how many other isolating mechanisms, both pre- and post-zygotic, or how many countervailing forces might exist in these organisms. What the attraction and avoidance mechanisms found here

indicate is that there is some reproductive isolation associated with long-range host search in gall beetles.

Sequential speciation and ecosystem engineers

To date, most of the research on sequential radiation has focused on parasitoids of herbivorous insects (Abrahamson *et al.*, 2003; Abrahamson & Blair, 2008; Feder & Forbes, 2010). Our system is unique in that the gall-boring beetle is an inquiline rather than a parasitoid. However, niche exploiters of ecosystem engineers (e.g. the gall-inducing fly), whether parasitoids or inquilines, seem to follow the same basic ecological and evolutionary trends. Both groups use volatile chemicals emitted by plants to locate their hosts and both seem to be strongly associated with the host plant (Forbes *et al.*, 2009).

In each of the plant-insect systems involving sequential radiation that has been examined (Crespi & Abbot, 1999; Abrahamson *et al.*, 2003; Abrahamson & Blair, 2008; Feder & Forbes, 2010), the sequentially radiating organism poses an appreciable threat to the organism it is evolutionarily tracking. However, this does not necessarily need to be the case as niche exploiters are not necessarily obligate predators of the ecosystem engineers. The gall-boring beetle frequently does eat the gall-inducing fly, but does not need to do so in order to survive (Blair *et al.*, 2005). In some cases, the dependent niche exploiter leaves its ecosystem engineer alone, a situation that may allow the engineer to flourish and to open up more resources for subsequent generations. Morris *et al.* (2000) discovered an example of such an organism that exploits the habitat of the *Acacia* galls. *Advenathrips inquilinus* is a species of thrips that is a true inquiline which does not prey on its gall-inducing ecosystem engineer, although it does share the same food source. In other words, the organisms sequentially radiating may also be herbivores, as is the gall beetle, suggesting that instead of biodiversity increasing solely up the trophic ladder as previously proposed (Abrahamson *et al.*, 2003), sequential radiation may be able to account for increasing biodiversity at a single trophic level.

Although diversity cascading up the trophic ladder explains a lot of the diversity observed among insects, diversity cascading sideways across a single rung of the trophic ladder has the potential to explain even more. Each horizontal step also opens up resources for specialist natural enemies. Just as *M. convicta* has different parasitoids than *E. solidaginis*, each niche exploiter that is undergoing sequential radiation across the trophic ladder can provide additional niches for natural enemies. The result can be increasing biodiversity throughout the entire trophic system. Biodiversity seems to have a huge potential to open up new niches that allow for the creation of more biodiversity.

Acknowledgements

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