Nutrition as a facilitator of host-race formation: The role of food quality in the shift of a stem-boring beetle to a gall host

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Nutrition as a facilitator of host-race formation: the shift of a stem-boring beetle to a gall host

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Abstract. 1. The importance of host-race formation to herbivorous insect diversity depends on the likelihood that successful populations can be established on a new plant host. A previously unexplored ecological aid to success on a novel host is better nutritional quality. The role of nutrition was examined in the shift of the stem-boring beetle Mordellistena convicta to fly-induced galls on goldenrod and the establishment there of a genetically distinct gall host race.

2. First, larvae of the host race inhabiting stems of Solidago gigantea were transplanted into stems and galls of greenhouse-grown S. gigantea plants. At the end of larval development, the mean mass of larvae transplanted to galls was significantly greater than the mass of larvae transplanted to stems, indicating a likely nutritional benefit during the shift. This advantage was slightly but significantly diminished when the gall-inducing fly feeding at the centre of the gall died early in the season. Additionally, there was a suggestion of a trade-off in the increased mortality of smaller beetle larvae transplanted into galls.

3. In a companion experiment, S. gigantea gall-race beetle larvae were likewise transplanted to S. gigantea stems and galls. Besides the expected greater mass in galls, the larvae also exhibited adaptations to the gall nutritional environment: larger inherent size, altered tunnelling behaviour, and no diminution of mass pursuant to gall-inducer mortality.

4. In a third line of inquiry, chemical analyses of field-collected S. gigantea plants revealed higher levels of mineral elements important to insect nutrition in galls as compared with stems.

Key words. Ecological speciation, Eurosta solidaginis, host shift, host-race formation, insect nutrition, Mordellistena convicta, Solidago gigantea.

Introduction

Evidence accumulates that host-race formation through ecologically based selection is an important source of diversity in herbivorous insects (Berlocher & Feder, 2002; Drès & Mallet, 2002; Stireman et al., 2005; Abrahamson & Blair, 2007). The actual prevalence of host-race formation, and thus its importance to insect radiation, is a function of, among other factors, the ease of establishing a viable population on a new host. A novel host presents ecological challenges such as low digestibility of the new plant tissue, lack of familiar feeding stimulants, novel plant phenology, unfamiliar plant defences or increased competition (Denno et al., 1990; Abrahamson & Weis, 1997; Gratton & Welter, 1999; Gassman et al., 2006).

For a host shift to be successful, such costs need to be offset by ecological benefits. Two benefits are well established: novel hosts have been shown to offer an escape from competition (Feder et al., 1995; Messina, 2004; Smith et al., 2007) or from natural enemies (Denno et al., 1990; Brown et al., 1995; Gratton & Welter, 1999; Ishihara & Ohgushi, 2008). Because hosts differ in elemental chemistry, another possible benefit
is superior nutrition on the new host which could promote the establishment of a population and its subsequent adaptation, leading to the formation of a new host race. Given the ubiquity of nutritional differences between plants, such an advantage could be a common facilitator of insect radiation and might suggest a fairly high frequency of successful host shifts.

As a model system for studying this role for nutrition, we examined the formation of a host race of the tumbling flower beetle *Mordellistena convicta* LeConte (Coleoptera: Mordellidae) which shifted from boring stems in several Asteraceae to boring fly-induced galls on the goldenrods *Solidago gigantea* Ait (Asteraceae) and *S. altissima* L. Like shifting to a new host plant, shifting to a new host organ presents ecological challenges (Cook et al., 2002; Joy & Crespi, 2007). In this system, the goldenrod galls induced by the fly *Eurosta solidaginis* Fitch (Diptera: Tephritidae) differ from stems in shape, density, phenology, and chemistry (Abrahamson & McCrea, 1986; Weis et al., 1989; Abrahamson et al., 1991; Abrahamson & Weis, 1997; Mapes & Davies, 2001a,b). In contrast to these challenges, there are indications that *E. solidaginis* galls provide superior insect nutrition (Abrahamson & McCrea, 1986; Diamond et al., 2008). Furthermore, the beetles of the gall host race regularly consume the gall-inducing fly, which could have been an added nutritional benefit during the shift (Uhler, 1961; Abrahamson et al., 1989; Blair et al., 2005; Dixon et al., 2009).

To examine the role of nutrition in the formation of a new host race, we investigated five questions: (i) do galls differ from stems in the nutrients important to insects; (ii) during the host shift, would larvae of the stem-beetle population that shifted to galls have attained greater mass in galls than in stems; (iii) would consumption of the gall-inducing fly have added a further nutritional benefit affecting larval mass; (iv) would there have been a trade-off against enhanced nutrition in decreased survival on the novel host; and (v) do the larvae of the gall host race show signs of adaptation to the novel nutritional environment of galls?

**Materials and methods**

To investigate nutritional conditions during *M. convicta’s* host shift, we simulated the shift by transferring early instar stem-beetle larvae, the host race phylogenetically and behaviorally closer to the stem-boring ancestral population (Blair et al., 2005), from goldenrod stems to *E. solidaginis*-induced galls of greenhouse-grown goldenrod plants. Stem-boring beetles were also transferred to goldenrod stems as controls. In a companion experiment, gall-host-race larvae were transferred to stems and galls to gauge the role of nutrition in the adaptation of the gall host race. During the summers of 2004 and 2006, we conducted the experiments separately in the same greenhouse. The larvae fed from early in their development through their period of exponential growth. After the end of the larval feeding period, we removed them from the greenhouse plants and recorded their survival and final mass. Conducting the experiment on greenhouse plants removed environmental factors such as parasitism, inter- and intra-specific competition, and edaphic conditions. Additionally, to test whether gall nutritional advantages existed in field conditions, we analysed field-collected goldenrod stems and galls for nutrients important to insects.

**The study system**

*Mordellistena convicta* is a complex of at least six morphocryptic species, further subdivided by host races, which feed as larvae inside stems of multiple species in the Asteraceae, including several goldenrods (Ford & Jackman, 1996; Blair et al., 2005). The species and host races are distinguished by allozyme differences. One *M. convicta* morphocryptic species (species 2) has shifted from stems to fly-induced galls on two closely related goldenrod hosts (Blair et al., 2005). Altogether, three host races of species 2 have been identified: a single stem-boring host race which attacks three species of *Solidago* and an *Aster*, and two gall host races which attack only *E. solidaginis* galls, one race on *Solidago gigantea* and the other on *S. altissima* (Eubanks et al., 2003; Blair et al., 2005). The three host races are sympatric in the northern U.S. and southern Canada, the area where galls occur on both *S. gigantea* and *S. altissima*. Phylogenetic analysis indicates that the two gall-boring host races are much more closely related to one another than to the stem host race (Blair et al., 2005). There was one shift from stem to gall, but the plant of origin of the shift is not known nor is it known which of the two closely-related goldenrod galls was the site of the shift (Blair et al., 2005).

The gall-inducer *E. solidaginis* oviposits into the apical buds of the goldenrod in mid-spring. The fly larva burrows to the base of the apical meristem and stimulates the swelling of the stem around it, forming a distinctive ball gall where it feeds in a chamber at the gall’s centre. Analysis of *E. solidaginis* galls on *S. altissima* shows that this central chamber is the site of richer tissue than the rest of the gall (Abrahamson & McCrea, 1986). In the autumn, the larva excavates an exit tunnel out to the gall epidermis and then diapausess in the central chamber of the gall throughout the winter, pupating and eclosing as an adult the following spring (Uhler, 1951; Abrahamson & Weis, 1997).

Female *M. convicta* species 2 oviposit on stems or galls in the late spring (Ping, 1915; Blair et al., 2005). After hatching on the stem or gall surface, larvae burrow in and feed in their respective hosts throughout the summer and into the autumn, experiencing most of their growth in September (Stinner & Abrahamson, 1979, where the beetle is referred to as *M. unicolor*). Beetle larvae in galls are likely to encounter and consume the gall-inducing fly and have been an important source of fly mortality year after year, especially in *S. gigantea* (Uhler, 1961; Brown et al., 1995 where the beetle is referred to as *M. unicolor*, C. P. Blair and W. G. Abrahamson, pers. obs.). Beetle larvae complete their growth prior to overwintering in the plants, where they pupate and emerge as adults the following spring.
Stem-host-race transplant experiment: the simulated host shift

We simulated the host shift using *S. gigantea*, the major host of species 2 (Blair et al., 2005). We transplanted stem-boring beetles from *S. gigantea* stems to galls on the same plant species. Such a within-species shift, besides being the most likely scenario for the actual shift, focuses on the contrast between stem and gall tissue, excluding between-plant differences.

In April 2006 in a greenhouse, we propagated *S. gigantea*, which is clonal, from 5-cm lengths of underground stem (rhizome). The rhizomes had been generated from seven genotypes, or genets, originally collected in April 2004 from the Upper and Lower Lakes Wildlife Management Area (ULLWMA) in New York state (N44°37′, W75°14′), a site within the area where galls occur on *S. gigantea*. We used a seven-genet sample to take into account the genetic variation within the area where galls occur on *S. gigantea* potted stems in ProMixBX™ greenhouse tables, but ramets within a triad were kept together until post-mortem allozyme electrophoresis at the conclusion of the experiment.

Ramets were organised into triads consisting of two galled ramets and one ungalled ramet randomly chosen from the same genet. The ungalled ramets had not been exposed to gall flies. The purpose of the 2:1 gall to stem ratio was to provide a sufficient gall sample to compare cases in which *M. convicta* consumed the gall-inducing *E. solidaginis* larvae and those in which they did not. The triads were randomly distributed across greenhouse tables, but ramets within a triad were kept together to provide similar microhabitats for each treatment. In total, we used 303 ramets as hosts for the experimentally transferred beetle larvae. The high number of ramets was necessary to ensure a large enough sample of species 2 beetles because other *M. convicta* species also inhabit *S. gigantea* stems, and the morphocryptic larvae could not be distinguished as to species until post-mortem allozyme electrophoresis at the conclusion of the experiment.

As *M. convicta* females will not oviposit on the ‘wrong’ host, we transplanted larvae. We extracted the larvae from the stems of *S. gigantea* collected from four fields located near Lewisburg, PA (Montour Preserve N41°6′ W76°40′, Bucknell University Natural Area N41°1′ W76°45′, Valley Township N40°59′ W76°41′, and Snyder County Prison N40°34′ W76°53′). We used Pennsylvania stem-beetle larvae because beetles in this area have no exposure to the galls of *E. solidaginis* on *S. gigantea*, which occur about 3° north of Pennsylvania near the Canadian border (Waring et al., 1990; Abrahamson & Weis, 1997). Thus, their final larval masses represent the effects of a novel gall treatment. To the degree that the beetles are adapted to their local *S. gigantea* plants, the experiment might also represent effects of a novel plant treatment.

Upon extracting a larva from a field ramet, we determined its mass to the nearest 0.001 mg on a Mettler UMX2 microbalance and transferred it to a greenhouse gall or stem within a randomly assigned trial, 1 larva per plant. To execute the transfer, we removed epidermal stem and gall tissues with a cork borer (size 1) until white pith was exposed. We inserted trimmed pipet tips into the cavities and gently deposited one larva head-first into the exposed pith. Transfers occurred between 15 August and 13 September 2006; the starting date was determined by the date at which larvae were large enough to be located inside the field plants and handled without injury (about 0.1 mg). We recorded the date of entry as the day a larva completely burrowed into the tissues and was no longer visible. We measured stem diameter, gall length, and two perpendicular gall diameters after all transplants were finished and well after ramets had attained maximum size.

At the end of the larval feeding season, we moved the senescent ramets outdoors to provide larvae with the cooler ambient temperatures found in nature and which lower their metabolism for overwintering. We measured the heights of ramets from soil surface to stem apex and extracted larvae from the plant tissues from 7 to 28 November. The extractions occurred in the same order as the transfers to minimise any effect of time in treatment. We recorded the area of the plant where the larva was located and determined larval survival upon extraction. As larvae did not exit the plants, those whose remains could not be found after a thorough search of the ramet were assumed to have died soon after transfer, when they were tiny, rendering their shriveled remains imperceptible. We determined the final larval masses of successfully extracted *M. convicta*, and stored the larvae at −80 °C for allozyme electrophoresis to determine their species.

In the galled ramets, we also noted the presence of the gall-inducer *E. solidaginis*. If a fly larva was missing or dead, its death was characterised as early or late by the absence or presence, respectively, of the exit tunnel from the central chamber to the gall epidermis that the flies excavate at the end of the feeding season. Therefore, *E. solidaginis* larvae in galls without an exit tunnel died earlier than those in galls with an exit tunnel. Fly larvae who died so early in gall formation that the gall developed no central chamber, were presumed to have died before the beetle was transferred and thus not to have succumbed to beetle predation. Flies who died after the formation of a central chamber were more than likely killed by the beetle, as all other natural enemies were excluded from the experiment, although it is possible that some of those flies died from other causes (Uhler, 1961; Abrahamson et al., 1989).

Gall-host-race transfer experiment

In the same greenhouse in summer 2004, using identical methods except as noted below, we transferred gall-host-race
beetles to 90 galls and 45 stems of the same seven genotypes of *S. gigantea*. This experiment needed only 135 ramets because all *M. convicta* larvae found in galls are species 2. We extracted the gall host-race larvae from galls collected in July 2004 from ULLWMA and nearby sites in the area where *S. gigantea* galls occur. The transfers took place between 3 August and 3 September 2004, starting, as in the stem experiment, when larvae were large enough to be handled. During the following February, larvae were extracted from the experimental plants, which had been kept outside since the end of the feeding season, and their mass was determined on a Cahn model 4700 electrobalance to the nearest 0.001 mg. The extraction later in the non-feeding cold-weather period probably means that the larval masses were lower and mortality higher than they would have been if larvae had been extracted in November.

**Morphocryptic species identification**

As species 2 larvae are morphologically identical to other members of the cryptic species complex, it was not possible to control for their identity when they were transferred. Therefore, once larvae were extracted, we determined their species using horizontal starch-gel electrophoresis employing two enzymes, *Idh* (1.1.1.42) and *Aat* (2.6.1.1) on Amine-Citrate (Morpholine), pH 6.1 (Murphy et al., 1996). Both enzymes are reliable polymorphic discriminators between *M. convicta* morphocryptic species that occur in *S. gigantea* (Blair et al., 2005).

**Chemical analysis**

We collected 55 paired samples of *S. gigantea* stems and galls from ULLWMA on 31 July 2006 when field plants were fully grown and just beginning to flower, and galls were fully grown and just beginning to flower, and galls were full sized. Both the gall and stem member of a pair were collected from the same genet, thereby controlling for the effect of plant genotype in the analysis. The samples were dried at 70 °C to a constant mass and ground in a Wiley mill to pass a 20-mesh sieve. Pennsylvania State University Agricultural Analytical Services Laboratory conducted Inductively Coupled Plasma (ICP) Spectroscopy after microwave digestion to determine the concentrations of N, P, K, Ca, Mg, S, Mn, Fe, Cu, B, Al, Zn, and Na. We performed paired $t$-tests on concentrations of each of these elements, natural-log-transformed to improve normality.

**Statistical analysis**

At the end of the two transfer experiments, we assessed the effect of nutrition through comparison of larval mass in the gall and stem treatments. Survival rates and the effect of fly death on larval mass were also compared between treatments.

We used analysis of covariance (General Linear Model, SPSS 15.0, SPSS Inc., Chicago, Illinois) to examine the effect of treatment on the fresh mass of larvae (final mass). The general form of the model included larval final mass as the response variable, treatment as a fixed factor, plant genet as a random factor, and larval fresh mass at the time of transfer (initial mass) and time spent in the treatment as covariates. We calculated time as the total number of days between complete entry into experimental tissue and the date of extraction. To meet the assumptions of ANCOVA, we square-root-transformed stem-host-race initial and final masses and log-transformed gall-host-race initial mass. In addition, we compared the final masses of beetle larvae in both transfer experiments using independent sample $t$-tests.

We used ANCOVA to discern the effect of the death of the gall-inducing fly on the final mass of the species 2 stem and gall host races in the gall treatment. The model included final larval mass as the response variable, early gall-fly death as a fixed factor, genet as a random factor, and gall volume, initial larval mass, and time in treatment as covariates. We used one-way analysis of variance (ANOVA) post-hoc to compare the final masses of beetle larvae in galls where the fly had died early, late, or not at all. Additionally, we used an independent samples $t$-test in galls where the fly had died early to distinguish the effect on beetle larval mass of fly death during gall formation versus fly death during the early beetle predation period. Additionally, we used an independent samples $t$-test to compare the mean mass of larvae transferred to galls where the fly died early with the mean mass of larvae transferred to stems.

We used a Generalised Linear Model (GZLM, SPSS 15.0) to estimate the effect of treatment on beetle survival. Models were formed with survival as the binary dependent variable, treatment and plant genet as factors, and initial mass (transformed as in the general linear model) as a covariate. We examined survival within each treatment by the same process. Models for beetles in gall treatments included gall-fly death and plant genet, and the covariates gall volume and initial mass. Survival models for stem treatment included plant genet and the covariates initial mass, stem length, and stem width.

**Results**

**Stem-host-race transfer experiment**

The gall treatment produced species 2 stem-host-race beetles of 30% greater final mass than those in the stem treatment, even though 91% of the species 2 survivors of the gall treatment tunneled out of the gall into the stem ($F_{1,15} = 75.72, P < 0.001$; Fig. 1a). Mean final larval mass was higher in galls in every plant genet. Of the 239 successfully extracted stem larvae, 90.8% were identified as species 2 according to their allozyme banding patterns. Larvae not identified as species 2 were excluded from analysis of final mass.

The fate of the gall-inducing fly influenced the final larval mass of species 2 stem-host-race beetles in the gall treatment. If the fly died before excavating an exit tunnel, beetle final mass was significantly negatively affected ($F_{1,133} = 7.68, P = 0.006$; Fig. 2a) When the fly died early, the mean final mass
of beetle larvae was significantly less than when the fly died after excavating an exit tunnel, or was still alive at extraction (**ANOVA**, $F = 4.564$, $P = 0.034$) although it was still 22.5% greater than the final mass attained in stems ($t = 4.632$, d.f. = 122, $P = <0.001$). No significant difference existed between the final mass of beetle larvae in galls where the gall inducer died late or was present at extraction (**ANOVA**, $F = 0.002$, $P = 0.961$). There was also no difference between the final mass of beetles from galls where the fly had died before the beetle was transferred, and galls where the fly had died early but after the beetle was transferred ($t = −0.111$, d.f. = 44, $P = 0.912$). In galls with a successfully extracted live species 2 stem beetle, 32.4% of the flies died early, 14.7% died after excavating an exit tunnel, and 52.9% survived. Of the flies who died early, only nine (6.6% of the total flies) died before the beetle larvae were transferred.

The major determinant of survival for stem-origin beetles was larval mass at the time of transfer to galls (initial mass) because it predicted survival in the gall treatment, although not in the stem treatment (Table 1). The larger the stem-origin beetle at transplantation to a gall, the greater its chance of survival (odds ratio = 4.97). Otherwise, there were no significant predictors of survival in either treatment. All stem-origin larvae, not just species 2, were included in survival analyses as the identities of those that had died could not be determined. Mortality in the stem treatment was 8.9% and in the gall treatment 12.8%.

**Fig. 1.** Effect of feeding site on final fresh mass of *Mordellistena convicta* species 2 larvae. Bars represent mean mass ± SE for (a) stem-host-race beetles and (b) gall-host-race beetles.

**Fig. 2.** Effect of gall-fly larval mortality on the final fresh mass of *Mordellistena convicta* species 2 larvae. Early death occurred before the fly larva was fully grown; late death occurred afterwards. Bars represent mean mass ± SE for (a) stem-host-race beetles and (b) gall-host-race beetles. Different letters over bars within a graph indicate statistical differences among means ($P < 0.05$).
**Gall host-race transfer experiment**

The final mass of gall-host-race beetles transferred to galls was 77.6% larger than the mass of those transferred to stems ($F_{1,92} = 127.45, P = < 0.001$; Fig. 1b). In contrast to the stem beetles, 81% of the surviving gall beetles remained in the gall and 19% tunnelled a short way into the stem. The mean final mass of the gall host race was significantly larger than that of the stem host race in both stems ($t = 3.754$, d.f. = 48.323, $P = < 0.001$) and galls ($t = 16.142$, d.f. = 80.977, $P = < 0.001$).

All successfully extracted gall-host-race larvae were confirmed by allozyme analysis to be species 2. Because no other *M. convicta* species is found in *S. gigantea* galls (Blair *et al*., 2005), all gall-host-race beetles that were not recovered were assumed to have been species 2.

In contrast to the stem host race, early fly absence had no effect on the final mass of the gall host race in the gall treatment ($F_{1,48} = 0.86$, $P = 0.359$; Fig. 2b). There was no significant difference in larval final mass whether the fly died early, late, or not at all (ANOVA, $F = 0.183$, $P = 0.833$). In the gall treatment, fly mortality was more than double that in the stem-host-race experiment: in galls with a successfully extracted live beetle, 57.7% of the flies died early, 19.2% of the flies died late, and 23.1% of the flies survived. As only two (3.8% of total flies) died before beetle transfer, the mean beetle final mass in galls was not compared with the mean final mass in galls where the fly died early, but after beetle transfer.

The best fitting model of gall-host-race survival contained the predictor treatment, which had a significant effect on survival (Table 2). Ironically, gall treatment was a negative factor: a gall-origin beetle had a 2.4% greater risk of death in the gall treatment than in the stem treatment. Mortality in the stem treatment was 15.5% and in the gall treatment 31.5%. In contrast to the stem beetles, gall-origin beetle survival in galls was not significantly affected by initial larval mass.

**Nutritional profile of galls versus stems**

Chemical analyses revealed that several elemental nutrients differed in their concentration by plant organ (Table 3) and that galls contained significantly more N and P, the nutrients typically important to insects, than stems of the same genet.

**Discussion**

The shift of *M. convicta* species 2 from stems to galls presented challenges typical of a move to a novel host. The *E. solidaginis* galls on *S. gigantea* are large round stem swellings requiring novel larval behaviour: tunnelling in circles in order to remain in the galls rather than in a straight line as in stems (Blair *et al*., 2005). Furthermore, the galls have a different tissue density which must affect tunnelling behaviour (Abrahamson & Weis, 1997). Also they appear later than the stems, which might alter oviposition timing. Finally galls induced by the same fly on a closely related goldenrod, *S. altissima*, show a different chemical make-up from stems of the same plant (Abrahamson & McCrea, 1986; Weis *et al*., 1989; Abrahamson *et al*., 1991; Abrahamson & Weis, 1997; Mapes & Davies, 2002a,b). Such novel chemistry can negatively affect larval physiology and require the development of new feeding behaviours (Gross *et al*., 2004; Gassman *et al*., 2006). These initial difficulties of the host shift increase the need for compensating benefits on the new host.

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**Table 1.** Generalised linear models for predicting survival of *Mordellistena convicta* larvae of the stem-boring host race.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$N$</th>
<th>$P_{model}$</th>
<th>Parameter</th>
<th>Wald $\chi^2$</th>
<th>d.f.</th>
<th>$P_{parameter}$</th>
<th>$b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>303</td>
<td>0.001</td>
<td>Intercept</td>
<td>0.055</td>
<td>1</td>
<td>0.815</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial mass</td>
<td>9.525</td>
<td>1</td>
<td>0.002</td>
<td>2.268 ± 0.735</td>
</tr>
<tr>
<td>Gall</td>
<td>202</td>
<td>0.002</td>
<td>Intercept</td>
<td>0.666</td>
<td>1</td>
<td>0.415</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial mass</td>
<td>10.996</td>
<td>1</td>
<td>0.001</td>
<td>3.053 ± 0.921</td>
</tr>
</tbody>
</table>

Separate model results are shown for the entire data set and for larvae transferred into galls. (None of the measured factors significantly affected survival for larvae transferred into stems, and the model with parameters was not significantly better than the intercept-only model.) The model probability was derived by comparing the fitted model against an intercept-only model using likelihood ratios.

**Table 2.** Generalised linear models for predicting survival of *Mordellistena convicta* larvae of the gall-boring host race.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$N$</th>
<th>$P_{model}$</th>
<th>Parameter</th>
<th>Wald $\chi^2$</th>
<th>d.f.</th>
<th>$P_{parameter}$</th>
<th>$b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>134</td>
<td>0.018</td>
<td>Intercept</td>
<td>28.742</td>
<td>1</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gall treatment</td>
<td>4.889</td>
<td>1</td>
<td>0.027</td>
<td>-1.093 ± 0.494</td>
</tr>
<tr>
<td>Gall</td>
<td>77</td>
<td>0.070</td>
<td>Intercept</td>
<td>5.860</td>
<td>1</td>
<td>0.015</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Initial mass</td>
<td>1.855</td>
<td>1</td>
<td>0.173</td>
<td>0.374 ± 0.275</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fly death</td>
<td>3.802</td>
<td>1</td>
<td>0.051</td>
<td>1.063 ± 0.545</td>
</tr>
</tbody>
</table>

Separate model results are shown for the entire data set and for larvae transferred into galls. (None of the measured factors significantly affected survival for larvae transferred into stems, and the model with parameters was not significantly better than the intercept-only model.) The model probability was derived by comparing the fitted model against an intercept-only model using likelihood ratios.
The facilitating role of nutrition in host-race formation

The greater final masses of *M. convicta* species 2 stem-origin beetles that were transferred to galls, indicate that during the host shift, the galls would have provided a nutritional advantage for the beetle. The advantage held across all seven genets suggests that this contrast is widespread among *S. gigantea* clones.

Greater mass in insects is generally related to greater fitness. Larger larvae become larger adults better able to endure a wide range of environmental stresses, compete effectively, and reproduce successfully, often producing larger or more numerous offspring (Awmack & Leather, 2002; Rhainds & Ho, 2002; Smith, 2002). The fitness benefits derived from enhanced nutrition in the novel environment would be a positive offset against the costs of a host shift, and thereby promote the formation of host races.

This benefit occurred even though 91% of the species 2 stem borers did not remain in the gall. Like the present day stem host race, at the inception of the shift, the stem-origin beetles probably would not have had the circular-tunnelling adaptation necessary to remain in the gall feeding on the rich tissue for the duration of their growth. The final masses recorded here indicate that these beetles would still have enjoyed a considerable weight-gain advantage over the population that remained in stems, simply by feeding in the galls as they tunnelled out to the stems.

Enhanced food quality of galls

Analysis of field-collected stems and galls revealed that *S. gigantea* galls in field conditions indeed have elevated nutritional elements. One of the principal explanations of the adaptive nature of insect galls is the nutrition hypothesis – that galls provide optimal food for the gall inducer through regulation of nutrient levels (Forrest, 1971; Price *et al.*, 1986, 1987; Hartley & Lawton, 1992; Gange & Nice, 1997; Diamond *et al.*, 2008). Depending on the nutritional requirements of the gall inducer, the gall may exhibit increased or decreased levels of particular nutrients, giving galls a different nutritional profile from the rest of the plant (Hartley, 1998). In particular, on *S. altissima*, a very close relative of *S. gigantea*, *E. solidaginis* galls contain an inner nutritive zone surrounding the larva at the centre of the gall (Abrahamson & McCrea, 1986; Weis *et al.*, 1989). In a study of field *S. altissima* collected in winter, this zone contained 1.3 times more N and 1.6 times more P than stems of ungalled ramets, whereas the N and P levels in the outer layers of the gall were similar to those in stem tissue (Abrahamson & McCrea, 1986). Additionally, both the gally larvae and *M. convicta* beetle larvae inhabiting those galls concentrated N and P (Abrahamson & McCrea, 1986, where the beetle is referred to as *M. unicolor*).

Our field-collected *S. gigantea* galls likewise contained significantly higher concentrations of several elemental nutrients, including N and P. The contrast between stems and galls was greater in our summer-collected *S. gigantea* galls than in the winter-collected *S. altissima* – 1.5 times more N and 1.9 times more P than stem tissue, even though the N and P levels represented a mixture of the central nutritive zone tissue and the presumably more stem-like outer layers of the gall. These nutrient levels represent the plant in full summer production while beetle larvae are present and about to enter their most intense period of growth (Stinner & Abrahamson, 1979). The nutrient contrasts are likely even greater between stems and the gall inner nutritive zone.

Because *M. convicta* larvae concentrate N and P (Abrahamson & McCrea, 1986), as do many insect herbivores, the elevation of these nutrients in the gall is probably an indicator of food quality for this insect (Elser *et al.*, 2000; Huberty & Denno, 2006). Both elements are essential to larval growth, and their limitation is an abiding problem for most insects: N is a component of amino acids and proteins, and P is necessary for protein production. There is an extensive literature demonstrating their importance to every area of growth, survival, and reproduction of insect herbivores (Abrahamson & Weis, 1987;
The effect of fly death on gall nutritional levels

Gall nutrition during the host shift would have been influenced by the fate of the gall-inducing fly. Although the gall inducer could be a rich source of nutrition for a developing beetle larva (Dixon et al., 2009), our findings indicate that larvae of the *M. convicta* stem host race do not benefit from the death of *E. solidaginis*. On the contrary, if the gall inducer died early, then the final mass of a stem-origin larva was reduced (although not to the level of a stem-origin beetle transferred back to a stem).

This reduced mass suggests that the nutritive quality of galls declines after the death of the gall inducer. A test of the gall nutrition hypothesis on *S. gigantea* using a lepidopteran stem borer, likewise showed that greater larval mass resulted late in the season or not at all (Diamond et al., 2008). During gall induction and early development, *E. solidaginis* serves as a source of growth hormones, stimulating the surrounding plant cells to divide and proliferate (Mapes & Davies, 2001a,b). This inner core of gall tissue is richest in nutrients, presumably because it is directly affected by the gall inducer. The negative effect of early gall-inducer death on stem-host-race beetles as well as on the lepidopteran stem borer indicates that, for galls to maintain their highest nutrient levels, *E. solidaginis* is necessary throughout the season (Diamond et al., 2008).

Furthermore, two comparisons of beetle mass suggest that, during the shift, any nutritional benefit from consumption of the gall inducer would have done little or nothing to ameliorate the effects of gall tissue nutritive decline. First of all, when the fly died early, there was no difference in mean mass between stem-host-race larvae who very likely ate the fly larva and those who definitely did not eat it, because they entered the gall after the fly had died. Second, late in the season, there was no difference in mean mass between stem-host-race larvae who very likely ate the fly and larvae from galls where the fly survived. Although these comparisons are not decisive, they do suggest that, compared with gall tissue quality, fly predation is not important to beetle mass.

Therefore, during the host shift, *M. convicta* probably would have been best served by feeding on the nutrient-rich gall tissue that is preserved by *E. solidaginis* activity and refraining from fly predation early in the season. Although only beetles feeding in a gall where the fly had died late or not at all would have gained the highest nutritional benefit, all beetles would in any case have been better fed in galls than in stems.

The survival cost of the host shift

High mortality on the novel host is not unexpected (Mulatu et al., 2004; Keeler & Chew, 2008) and there was a trend to higher mortality of stem-origin beetles in galls. However, gall treatment did not significantly predict mortality. Instead the cost of the host shift was suggested by the fact that, unlike the larvae who died in stems, those that died in the galls were significantly smaller at transfer than the survivors. This fact has implications for the host shift because, in accordance with the classic host-shift model, the most likely way for this shift to have occurred was through a mutation in the mother’s host preference for oviposition, a shift to laying eggs on galls rather than stems (Blair et al., 2005). At the time of the shift, larvae emerging from eggs laid on galls by the females with the new host preference would have been very tiny and as such, may have had similar mortality rates.

On the other hand, it is possible that the mortality rate of small larvae has few implications for the host shift. As would not have happened during the shift, stem-host-race larvae transferred to galls in the experiment had previously fed on stem tissues. It is possible that the smaller larvae could not make an effective transition between food sources, as, in some insects, prior larval feeding experience can affect subsequent nutrient utilisation and sensitivity to deterrents (Renwick et al., 2001; Panzuto et al., 2002).

The stem-beetle results exemplify the complicated tradeoff that probably occurred in the host shift. Galls provided *M. convicta* with an escape from a parasitoid that causes heavy mortality in stems (Blair et al., 2005). This gall advantage would have combined with improved nutrition, especially in galls where the gall inducer did not die early, to weigh against the possible increase in larval mortality from feeding in the gall.

Nutrition-related adaptations in the gall host race

The nutritional environment of a novel host leads to behavioural and physiological changes in larval ability to use the new food source (Joshi & Thompson, 1995; Guedes et al., 2003; Messina, 2004; Fricke & Arnqvist, 2007; Keeler & Chew, 2008). The *M. convicta* gall host race exhibited several adaptations to feeding in the gall. As has been described for other insects (Ikonen et al., 2003; Gassmann, 2006), the gall host race retained the ability to digest, and to develop on, the ancestral stem host, but they had much larger final masses on their natal gall host.

Indeed, feeding in galls appears to have resulted in a size change for species 2 beetles. Size changes have been recorded as an adaptation to larval feeding in a new host (Messina, 2004; Fricke & Arnqvist, 2007). The *M. convicta* shift to galls has produced a larger beetle: field-collected gall-beetle adults are three times larger than the stem race (Blair et al., 2005). In the stem treatment, gall beetles had 25% larger final masses than the stem beetles in spite of being removed in the winter after they would presumably have lost more mass.

Furthermore, gall-origin beetles exhibited a behavioural adaptation to feeding in galls: most of them remained in the gall, whereas most of the stem-origin beetles ate their way into the stem. Remaining in the gall involved tunnelling in a curved path rather than in the relatively linear path that characterises stem tunnelling. Behavioural adaptations are common in host shifts (Gassmann et al., 2006), and a tunnelling adaptation has
been recorded in another beetle larva (Messina, 2004). Such an adaptation allows the gall host race to maximise their exposure to the highly nutritious gall tissue.

The most remarkable trait in the gall host race, and the one most definitely involving nutrition, was the ability to gain large final mass in the galls where the gall-inducing fly had perished relatively early in the season. In contrast to the cases of the stem-host-race beetles and the stem-boring caterpillar (Diamond et al., 2008), the early death of the gall fly had no effect on gall-host-race final mass. This is a vital adaptation for the S. gigantea gall host race where field-collected S. gigantea galls year after year show severe fly mortality from beetles, at some sites approaching 100% (Abrahamson et al., 1989; Brown, 1995; C. P. Blair and W. G. Abrahamson, pers. obs.).

Our experiments suggest that the gall host race has developed a complex adaptation – inflicting increased mortality on the flies but experiencing no ill effects. There was far greater early fly mortality in galls containing a gall-origin beetle than in galls containing a larva of the stem host race (58% vs 32%) and far greater overall fly mortality (77% vs 47%). In fact, fly mortality in galls that contained a gall-origin beetle was close to the 14-year average of 73% fly mortality found in field-collected S. altissima galls that contained a beetle (Uhler, 1961). The gall-origin beetles’ infliction of high fly mortality can be accounted for simply by the fact that they remain in the gall, and thus are more likely to come across the central chamber; once there, they virtually always eat the fly larva (Uhler, 1961).

Our results do not hint at what behavioural or physiological changes enabled beetles of the gall host race to avoid the ill effects of early fly death. The potential of this adaptation, however, is perhaps seen in a study of M. convicta in S. altissima galls in the Midwestern U.S. (Dixon et al., 2009). In two out of three groups of gall beetles in a common garden experiment, larval mean mass was significantly higher in galls in which the fly had been killed. Although the effect was not found in the field, these results suggest that Midwestern S. altissima gall beetles have taken this adaptation one step further, going beyond neutralising the effect of fly death as seen here in S. gigantea gall beetles.

On the other hand, the gall host race continued to pay a survival price for the host shift with a higher mortality rate in galls than in the ancestral stems. A high mortality rate in a novel host may linger in the derived population after other adaptations have taken place (Ikonen et al., 2003; Gross et al., 2004). Gall-host-race mortality does not seem to be a result of problems of small larvae as exhibited by the stem beetles, because it was not affected by initial larval mass. If such elevated mortality occurs year after year, then enhanced food quality would be a vital compensation.

Although the gall host race appeared to incur a greater survival cost in both galls and stems than did the stem-origin beetles, this comparison cannot be made based on these results, as beetles of the gall host race were removed from the experimental plants in February rather than November. Since winter is a bottleneck season for these insects, the gall beetles undoubtedly had more chances than the stem beetles to die.

In summary, nutrition appears to have been a positive factor in the shift of M. convicta morphocryptic species 2 from stems to galls. Furthermore, in the time since the shift, the population on the derived host has responded to the novel nutritional environment by developing specific adaptations that maintain and enhance the nutritional advantage. Our results indicate that the nutritional profile of the novel host can be an important factor in facilitating a host shift and in driving adaptations in the new host race. Given the prevalence of nutritional differences between plants and between plant organs, food quality may be an important factor in the speciation of herbivorous insects. Host-race formation may often be easier than it looks.

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References


Nutrition facilitates host shift


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