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The geographic mosaic of co-evolution and the natural enemies of *Eurosta solidaginis*

Michael D. Dixon[1,2], Timothy P. Craig[2] and Joanne K. Itami[2]

[1]*Conservation Biology Graduate Program, University of Minnesota Twin Cities, St. Paul, Minnesota and* [2]*Department of Biology, University of Minnesota Duluth, Duluth, Minnesota, USA*

ABSTRACT

Hypothesis: Natural enemies of a host insect that has diverged across a major biome boundary will display better survival and higher fitness on hosts from their natal biome.

Background: *Eurosta solidaginis* (Diptera: Tephritidae) forms galls on the goldenrod, *Solidago altissima. Solidago altissima* has two subspecies, one in the Great Plains and one throughout the eastern United States. *Eurosta solidaginis* has formed two host races, one on the prairie and one on the forest subspecies of *S. altissima*. These host races differ in gall size, allozyme frequencies, and morphology. An inquiline beetle, *Mordellistena convicta*, and a parasitoid wasp, *Eurytoma gigantea*, are specialist enemies of the larval stage of *E. solidaginis* and they also consume gall tissue. Both consume plant tissue in the gall as well. *Mordellistena convicta* is much more abundant in the prairie than in the forest.

Methods: We measured the abundance and mass of the parasitoid and the beetle in laboratory choice experiments, common gardens, and reciprocal transplant gardens to test for evidence of local adaptation to galls from their natal biomes.

Results: The parasitoid *Eurytoma gigantea* showed evidence of local adaptation consistent with the geographic mosaic of co-evolution hypothesis. The inquiline *M. convicta* did not show evidence of local adaptation, as both prairie and forest populations performed better on prairie galls. However, the population density of *M. convicta* is strongly influenced by interactions with other species that have diversified as the result of the geographic mosaic of co-evolution.

Keywords: co-evolution, oviposition preference, *Solidago altissima*, tritrophic interaction.

INTRODUCTION

Co-evolution is a reciprocal adaptation among interacting organisms (Thompson, 2005), and it is one of the major forces that organize biodiversity because it can link the genomes of interacting species (Thompson *et al.*, 2002). It may also be one of the major forces creating biodiversity, because diversifying co-evolutionary selection for local adaptation can result in the genetic differentiation of populations or speciation (Thompson, 2005). The

Correspondence: M.D. Dixon, Conservation Biology Graduate Program, University of Minnesota Twin Cities, St. Paul, MN 55108, USA. e-mail: dixon091@umn.edu

co-evolutionary process is initiated by the local adaptation of species, and studies of the pattern of local adaptation among interacting species can reveal how adaptive differences among populations evolve (Thompson, 2005). The differences in evolutionary outcomes among interacting species in different areas can produce a geographic mosaic of co-evolution (Thompson, 2005).

In this study, we tested the hypothesis that there is local adaptation of populations of an inquiline beetle, *Mordellistena convicta*, and a parasitoid, *Eurytoma gigantea*, due to the geographic mosaic of co-evolution. Local adaptation occurs when there is improved fitness of each deme in its own habitat, and it occurs due to ongoing natural selection to adapt to environmental differences among locations (Kawecki and Ebert, 2004). One member of an interaction will usually show greater local adaptation than the other, but this does not necessarily mean that adaptation is not reciprocal. The co-evolutionary process requires reciprocal, not symmetrical, selection (Greischar and Koskella, 2007). Parasites are likely to be more locally adapted than their hosts because the fitness consequences of the interaction are usually stronger for parasites than hosts (Greischar and Koskella, 2007), and because the potential for rapid evolution is higher in parasites due to their large populations sizes, short generation times, and higher migration rates compared with their hosts (Price, 1980; Ebert, 1994; Gandon and Michalakis, 2002). Therefore, local adaptation of parasites is considered to be good evidence of co-evolution (Laine, 2005).

Geographical variation in *Solidago altissima* and its insect community

The possibility that the insect community on *Solidago altissima* may be becoming locally adapted due to diversifying co-evolutionary selection is currently being investigated at the boundary between the forest and tall grass prairie biomes of Minnesota (Craig, 2007; Craig *et al.*, 2007). There is a rapid shift in vegetation from grassland to forest cover across this boundary, primarily due to a steep gradient in growing season, temperature, precipitation, and soil characteristics between the two biomes in Minnesota (Tester, 1995). This prairie/forest boundary has existed across the state since the end of the Wisconsin glaciations, and the climatic gradient was likely even steeper in the past (Jacobson and Grimm, 1986).

Solidago altissima is a perennial herb that is widely distributed across the United States. It supports an extensive insect community of herbivores and their natural enemies (for a detailed review, see Abrahamson and Weis, 1997). *Solidago altissima* is divided into two subspecies in Minnesota: *S. a. altissima* in the forest biome and *S. a. gilvocanescens* in the prairie biome of Minnesota and westward across the Great Plains (Semple and Cook, 2006). The two subspecies differ morphologically: *S. a. gilvocanescens* has a greater number of stems per ramet and shorter stems than *S. a. altissima*, and these traits have a genetic component as they are conserved when the plants are grown in a common garden (Craig, 2007). The two subspecies also differ in their sensitivity to water (J. Grochowski and J.R. Etterson, personal communication), indicating that the subspecies may have diverged in response to selection for adaptation to their differing abiotic environments.

Eurosta solidaginis (Diptera: Tephritidae) forms galls on *Solidago* sp. (Asteraceae). The fly emerges in mid-May to mid-June and oviposits on the terminal bud of the goldenrod plant. The larva hatches and burrows into the stem where it induces the formation of a gall in which it feeds until it enters diapause in the fall. *Eurosta solidaginis* emerges as an adult the following spring (Abrahamson and Weis, 1997).

Eurosta solidaginis has formed host races on *S. altissima* and the closely related *S. gigantea* (Craig *et al.*, 1993, 1997, 2001). The *E. solidaginis* host race on *S. altissima* in the prairie and forest populations may also be undergoing genetic divergence as the flies on either side of the prairie/forest border differ in a number of characters, including wing patterns (T.P. Craig, M. Eskelson and J.K. Itami, unpublished data) and allozyme frequencies (Itami *et al.*, 1998). Galls in the prairie on *S. a. gilvocanescens* are larger and rounder than those in the forest on *S. a. altissima* (Craig, 2007; Craig *et al.*, 2007). Gall morphology is under genetic control of the fly because both prairie and forest populations induce their characteristic gall morphologies on either host subspecies (T.P. Craig and J.K. Itami, unpublished data). Finally, prairie and forest fly populations have higher rates of gall formation and larval survival on the fly's local host plant subspecies (T.P. Craig and J.K. Itami, unpublished data). In addition, populations of *E. solidaginis* on *S. altissima* west of Michigan have a unique mtDNA haplotype that is not found in eastern populations (Brown *et al.*, 1996).

Eurytoma gigantea emerges 3–4 weeks after the emergence of *E. solidaginis*. The female oviposits through the wall of the gall into the *E. solidaginis* larva, and successful attack by *E. gigantea* is limited because the parasitoid ovipositor is too short to reach all larvae (Weis *et al.*, 1989). The *E. gigantea* larva consumes the fly larva and sometimes gall tissue as it usurps the gall as its home and food source until it emerges the following year (Weis *et al.*, 1989).

Mordellistena convicta is part of a cryptic species complex of which one species is a goldenrod gall specialist (Abrahamson *et al.*, 2003; Blair *et al.*, 2005). Assortative mating and higher oviposition success have been observed, which suggests host race formation on *S. altissima* and *S. gigantea* (Abrahamson *et al.*, 2001; Eubanks *et al.*, 2003). In our study area, the beetles emerge about 4 weeks after *E. solidaginis* (M.D. Dixon, personal observation). Females oviposit on the surface of galls, and when larvae hatch they tunnel into the parenchyma of the gall (Ping, 1915). The larvae can complete development by consuming only plant tissue, but it often preys upon the larval host as well (Uhler, 1961). The beetle kills *E. solidaginis* more frequently in small galls, probably as a result of its random burrowing feeding strategy, resulting in more frequent encounters with the larval hosts in these galls (Craig *et al.*, 2007).

Mordellistena convicta and *E. gigantea* cause disproportionately high mortality on larvae in small galls, while birds have higher predation rates on larvae in larger galls in the forest biome (Weis *et al.*, 1992; Craig *et al.*, 2007), producing stabilizing selection on *E. solidaginis* gall size. The lack of trees in the prairie results in low populations of tree-dwelling birds. In the absence of selection by birds against larger galls, *E. solidaginis* evolved to induce *Solidago* to produce larger galls in the prairie biome (Craig, 2007; Craig *et al.*, 2007). *Eurytoma gigantea* has significantly longer ovipositors in the prairie than in the forest, and this may be the result of adaptive genetic differentiation of the populations (Craig *et al.*, 2007). However, ovipositor length is correlated with gall size, so the differences in ovipositors may also have an environmental component (Weis *et al.*, 1989).

In this study, we tested the hypothesis that prairie and forest populations of *M. convicta* and *E. gigantea* have diverged in response to selection for local adaptation to their hosts. Galls are plant tissues, but their characteristics are determined by an interaction of the fly genotype, the plant genotype, and the environment (Weis and Abrahamson, 1986), so if the beetle populations are locally adapted it could be due to adaptation to each of these factors or interactions among these factors. Higher beetle density on a host may be due to a preference for that host, higher survival on that host, or a combination of both.

We tested the hypothesis that the beetles from the prairie and forest biomes prefer to lay their eggs on, and that they are physiologically adapted to feeding on, local galls from their natal biome. Preference trials were performed to determine if the beetles recognize and preferentially oviposit on their natal host plants. Larval performance was assessed in common garden and reciprocal transplant experiments on prairie and forest plants. We also tested the hypothesis that *E. gigantea* had higher densities on galls from their local biome in a reciprocal transplant experiment.

METHODS

Field collections

Galls were collected in Minnesota and North Dakota (Fig. 1) for use in the laboratory and field experiments and to assess beetle and parasitoid abundance in the wild. Sites were classified as prairie or forest based on a map of the original vegetation of Minnesota (Marschner, 1974), although the current vegetation may differ because of extensive modification to it since European settlement. Collections took place in the spring after the galls had over-wintered in the field. The 2004 cohort consisted of eight prairie and eight forest sites, and the 2005 cohort consisted of 11 forest and 11 prairie sites. Galls were collected

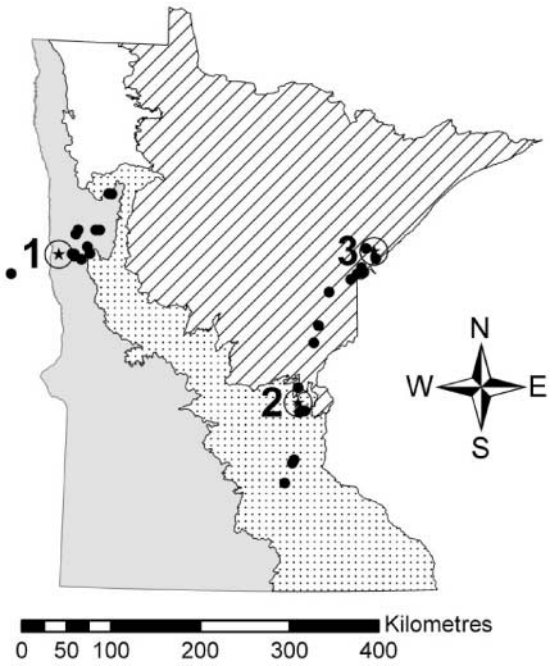

Fig. 1. The major biomes of Minnesota, with boreal forest depicted in a hatched pattern, eastern hardwood forest stippled, and prairie in grey. The white indicates aspen parklands, which were not sampled in this study. Gall collection locations are marked as dots and experiment sites are marked with numbered stars. Location 1 is the prairie transplant garden, location 2 is the forest transplant garden, and location 3 is the common garden.

from many of the same sites in both years, but because many northern Minnesota forest sites had low *M. convicta* density in 2005, new forest sites in central Minnesota were used in 2006.

Galls were held in a cold room at 4°C until all collections had been made. They were then placed in mesh emergence bags in an environmental chamber with temperatures and day/night cycles set to match mean outdoor conditions. After *E. solidaginis* had finished emerging, a subset of galls was frozen for later dissection and the remainder transferred to plastic bags for parasitoid and inquiline collection. Daily emergence of *M. convicta* was recorded and the beetles were placed into plastic holding cages where they were supplied with water and bee pollen until they were used in experiments.

Gall dissections

A subset of 100 galls from each site in the 2004 cohort and 80 galls from each site in the 2005 cohort were saved for laboratory dissection. The length and diameter of the gall was recorded. The cause of *E. solidaginis* mortality was scored using the following criteria: mortality due to *M. convicta*, mortality due to *E. gigantea*, early larval death, and late larval death. The number of *M. convicta* larvae present and the presence or absence of its parasitoids were also recorded. *Mordellistena convicta* larvae were dried in an oven with desiccant at 45°C for 48 h and their dry weights were recorded. The effect of biome, site nested within biome, and various interaction terms on the number of beetles per gall were analysed. We also tested whether the same factors, with the addition of whether or not the *E. solidaginis* was killed by the beetle, had a significant effect on mean larval mass. Both analyses were performed using the general linear model (GLM) procedure in MINITAB 14. A binary logistic regression was performed to determine the significance of biome and gall diameter on the presence or absence of *E. gigantea* in wild-collected galls.

Oviposition preference

Mordellistena convicta oviposition must be observed directly because they do not leave scars or other evidence of oviposition (Eubanks *et al.*, 2003). It is not possible to sex *M. convicta* without destructive dissection of the insect. Forest and prairie beetles were thus held in separate holding chambers until nearly all beetles had emerged to ensure both sexes were represented in the population and to allow mating. The beetles mate readily in the laboratory (Ping, 1915).

To determine whether *M. convicta* preferentially oviposited on galls from its native biome, a single freshly cut galled stem of both *S. altissima gilvocanescens* and *S. a. altissima* was placed in separate cylindrical plastic observation chambers. Ten beetles from the entire population in the holding cages were placed in each observation chamber (there is less than a 1 in 1000 chance that there will not be a female in a group of 10 randomly chosen beetles). Individual beetle locations were scored every 10 min for 2 h into the following categories: ovipositing on a gall, on a gall but not ovipositing, on a non-gall plant part such as stem or leaf, or elsewhere on the cage. A beetle was considered to begin a new bout of oviposition only if it had left the gall and returned. Twenty replicates of this experiment were performed.

An analysis of the effects of treatment number and biome, and diameter as a covariate, on number of ovipositions on each plant and the mean number of minutes a beetle spent on each plant was performed using analysis of variance (ANOVA) and analysis of covariance (ANCOVA) as parts of the GLM procedure of MINITAB.

Field experiments

Local adaptation of populations of species can be assessed using common garden, reciprocal cross infection, and reciprocal transplant experiments (Kawecki and Ebert, 2004), and we used all of these approaches to study local adaptation in *Mordellistena convicta*. In common garden studies, a number of populations of a species is grown in a single environment (Lively, 1989; Lively and Jokela, 1996; Laine, 2005) so that multiple host populations can be evaluated for their susceptibility to a local parasite population (Stiling and Rossi, 1996). In reciprocal cross infection experiments, all possible combinations of hosts and parasite populations are evaluated in a common environment so that the fitness of parasites on local and foreign hosts can be measured (Burdon *et al.*, 2002; Thrall *et al.*, 2002; Lively *et al.*, 2004; Laine, 2005). In a reciprocal transplant experiment, hosts and parasite populations are evaluated in both local and foreign environments. In both common garden and reciprocal cross infection experiments, genetic differences among parasites in the ability to perform on hosts from different areas can be measured. However, such experiments do not permit the evaluation of the effect of variation of the environment or the host genotype × environment interactions on the local adaptation of parasite populations. Insect genotype × host genotype × environment interactions create the geographic mosaic of co-evolution (Thompson, 2005), and these interactions can only be measured in reciprocal transplant experiments (Nuismer and Gandon, 2008).

Common garden/reciprocal cross infection experiments

Local adaptation of the beetles to plants from their natal biome was assessed in a common garden in Duluth, Minnesota, USA at the University of Minnesota Duluth Research and Field Studies Center. *Solidago altissima* plants were grown from rhizomes collected in the field at three prairie sites in western Minnesota (Hawley, Timber Lake, and Morris) and three forest sites in eastern and east-central Minnesota (Faribault, Duluth, and Carlos Avery Wildlife Management Area), then separated into prairie and forest cages. At the beginning of June, *E. solidaginis* were released into the cage with plants from their natal biome so that flies formed galls only on plants from their own biome. Four prairie and four forest goldenrod plants that had successfully produced galls were then randomly assigned to individual experimental cages. Ten beetles were introduced into each of the 20 experimental cages. The experiment was conducted again in 2006 using the same methods except that plants were repotted in new soil, the number of goldenrod rhizomes per pot was standardized, and the garden was placed on a drip irrigation system. At the end of the experiments, the galls were collected, measured, and dissected. The result of the interaction between *E. solidaginis* and *Mordellistena* was recorded (beetle and fly present, beetle present in a gall where the fly had died early, or beetle killed the fly larva). The live mass of the beetle larvae was recorded. The effects that cage, plant biome, gall diameter (as a covariate), and the interaction between biome and gall diameter had on the number of beetles per gall

and consumption or survival of *E. solidaginis* were tested with ANOVA and ANCOVA using the GLM procedure in MINITAB.

Reciprocal transplant experiment

In the reciprocal transplant experiment, *Eurosta* galls on their local *S. altissima* plants were reciprocally transplanted between forest and prairie sites. This allowed us to test the hypothesis that the *E. solidaginis* populations were locally adapted to their natural enemy populations since they were exposed to natural enemies in their local environments. Local adaptation by *E. solidaginis* would be indicated if there were lower rates of mortality from natural enemies on the local (from that biome) *E. solidaginis* than on the foreign (from the other biome) *E. solidaginis*. Conversely, if there were higher natural enemy mortality on the local than foreign *E. solidaginis*, it would indicate local adaptation by the natural enemies.

The prairie site was located at the Minnesota State University Moorhead's Regional Science Center – Buffalo River Site, located 24 km east of Moorhead, Minnesota, USA. The forest site was located at the Cedar Creek Natural History Area, located 56 km north of St. Paul, Minnesota, USA.

Solidago altissima plants were grown and galls initiated by *E. solidaginis* as described above in the common garden experiments at the University of Minnesota Duluth Research and Field Studies Center. Twenty plant genotypes from three prairie sites and 20 genotypes from three forest sites were placed in each location in a block design of six plants, with one plant from each site randomly located within each block. The gardens were planted the last week of June, concurrent with the beginning of *M. convicta* emergence. Plants were watered once during the first week after planting to offset any transplant stress. When buds began to form, floral heads were covered with a sheath of Reemay® cloth to prevent cross-pollination with native plants.

Galls were collected in mid-October after senescence. They were measured and dissected, and the mordellid larvae were weighed according to the method described for the field collections. ANOVA and ANCOVA were performed using the GLM procedure in MINITAB to assess the effects of such factors as garden location, plant origin, block, and gall diameter in influencing beetle mass. Binary logistic regression was used to predict beetle presence or absence based on garden location, gall diameter, and plant biome.

RESULTS

Field survey

We dissected 1588 galls in 2005 and 1383 galls in 2006 that had been collected from the field. *Mordellistena convicta* were abundant at all 16 prairie sites (Table 1) and were absent in 6 of 12 forest sites. There were significantly more beetles per gall at prairie sites than at forest sites in both 2005 ($F_{1,1571} = 6.30$, $P = 0.013$) and 2006 ($F_{1,1365} = 11.94$, $P = 0.001$). The number of beetle larvae was positively correlated with gall diameter in 2005 ($F_{1,1571} = 31.17$, $P < 0.001$) and 2006 ($F_{1,1365} = 50.97$, $P < 0.001$). The number of beetles per gall differed among sites from the same biome in 2005 ($F_{14,1571} = 11.63$, $P < 0.001$) and 2006 ($F_{15,1365} = 8.20$, $P < 0.001$). The effect of gall diameter varied with biome in both 2005 ($F_{1,1571} = 31.32$, $P < 0.001$) and 2006 ($F_{1,1365} = 42.34$, $P < 0.001$) because larger prairie galls

Table 1. Number of *M. convicta* larvae per gall at field collection sites

Site #	Biome	Mean larvae per gall	Standard deviation
22	Forest	0.0000	0.0000
26	Forest	0.0000	0.0000
29	Forest	0.0000	0.0000
74	Forest	0.0000	0.0000
124	Forest	0.0000	0.0000
125	Forest	0.0000	0.0000
27	Forest	0.0100	0.1000
137	Forest	0.0227	0.1499
28	Forest	0.0253	0.1581
126	Forest	0.0331	0.1795
128	Forest	0.1084	0.3128
130	Forest	0.1111	0.3536
23	Prairie	0.2000	0.4924
119	Prairie	0.2813	0.5171
139	Prairie	0.3500	0.6183
127	Prairie	0.3636	0.5968
24	Prairie	0.4278	0.6256
120	Prairie	0.4286	0.7733
121	Prairie	0.4309	0.7542
4	Prairie	0.5833	0.8293
141	Prairie	0.6296	0.7322
140	Prairie	0.7125	1.1495
133	Prairie	0.8537	1.0076
134	Prairie	0.9250	0.9649
135	Prairie	0.9359	0.9444
122	Prairie	0.9506	0.9862
123	Prairie	1.0100	1.2034
136	Prairie	1.1266	1.1915

Note: Sample size was 80–100 galls per site per year. See Appendix 1 for locality information.

contained more beetles than any other galls. The biome in which the galls were collected was not a significant predictor of beetle mass in either year, but beetle mass was positively related to gall diameter in 2006 ($F_{1,321} = 13.18$, $P < 0.001$). Sites within the same biome differed in mean beetle mass in both 2005 ($F_{7,232} = 2.20$, $P = 0.035$) and 2006 ($F_{13,321} = 6.64$, $P < 0.001$). Predation on the host fly did not result in a higher mean beetle mass in the field collections.

Larval parasitism was slightly higher by *Eurytoma gigantea* in galls collected from forest sites (mean ± standard error = 0.11441 ± 0.00857) than from prairie sites (0.10308 ± 0.00763), and this difference was statistically significant in binary logistic regression ($Z = 3.01$, $P = 0.003$, odds ratio = 5.56). Their abundance was negatively correlated with gall diameter ($Z = -6.55$, $P < 0.001$, odds ratio = 0.86), and the effect of size was related to source biome ($Z_{\text{interaction}} = -2.20$, $P = 0.028$, odds ratio = 0.93).

Laboratory oviposition preference

We only tested the oviposition preference for prairie and forest plants by prairie beetles due to low emergence of wild *M. convicta* from field-collected galls from forest sites in 2005. An unfortunate failure of climate control in the laboratory resulted in the loss of our *M. convicta* colony after the initial 20 replicates, so these results must be considered preliminary due to small sample size. The number of ovipositions was not significantly related to gall diameter, shape, or source biome. There was no significant difference between the time beetles spent on prairie and forest plants.

Common garden/reciprocal cross infection experiments

Few beetles emerged from the 2004 cohort of forest galls, so the common garden experiment in 2005 was conducted solely with prairie beetles. Due to these differences in the execution of the experiment, the data for the 2 years is presented separately.

2005 trial

Significantly more prairie *M. convicta* larvae were found in galls with larger diameters ($F_{1,365} = 88.25$, $P < 0.001$). The mean number of surviving *M. convicta* larvae did not differ between prairie or forest galls based on biome of plant origin alone, but the effect of gall diameter differed between plant biomes ($F_{1,365} = 11.93$, $P = 0.001$) because more beetle larvae were found per gall (mean ± standard deviation = 0.709 ± 1.536) in the larger prairie galls than in forest galls (0.222 ± 0.501).

Mean larval mass of the prairie *M. convicta* was greater in prairie galls ($F_{1,119} = 11.12$, $P = 0.001$) (Fig. 2). The mean larval mass was positively correlated with gall diameter ($F_{1,119} = 5.83$, $P = 0.017$). The mean mass of a *M. convicta* larva from a gall in which a beetle larva had eaten the *E. solidaginis* was higher than in those galls in which the *E. solidaginis* survived ($F_{1,119} = 4.69$, $P = 0.032$) (Fig. 3).

2006 trial

Forest *M. convicta* larval survival was higher ($F_{1,101} = 5.75$, $P = 0.018$) in prairie galls (mean ± standard deviation = 0.709 ± 0.684 beetles per gall) than in forest galls (0.2364 ± 0.508 beetles per gall). In contrast, prairie *M. convicta* larval survival did not differ significantly ($F_{1,202} = 0.56$, $P = 0.453$) between prairie (mean ± standard deviation = 0.353 ± 0.684 beetles per gall) and forest galls (0.249 ± 0.610 beetles per gall) in the 2006 trial. The number of beetle larvae increased significantly with gall diameter in both prairie and forest beetles (prairie $F_{1,202} = 14.83$, $P < 0.001$, forest $F_{1,101} = 9.92$, $P = 0.002$).

Both prairie ($F_{1,29} = 10.21$, $P = 0.003$) and forest ($F_{1,19} = 5.00$, $P = 0.038$) *M. convicta* larvae were larger in galls on prairie plants than those on forest plants (Fig. 2). Gall diameter was not a significant predictor of beetle mass in either population. The mean mass of forest *M. convicta* larvae from galls in which the gall maker had been killed was significantly larger than from those in which the host had survived ($F_{1,19} = 7.05$, $P = 0.016$) (Fig. 3). A similar pattern was found in prairie beetles, although the difference in mean mass was not statistically significant in 2006 ($F_{1,29} = 3.02$, $P = 0.093$) (Fig. 3).

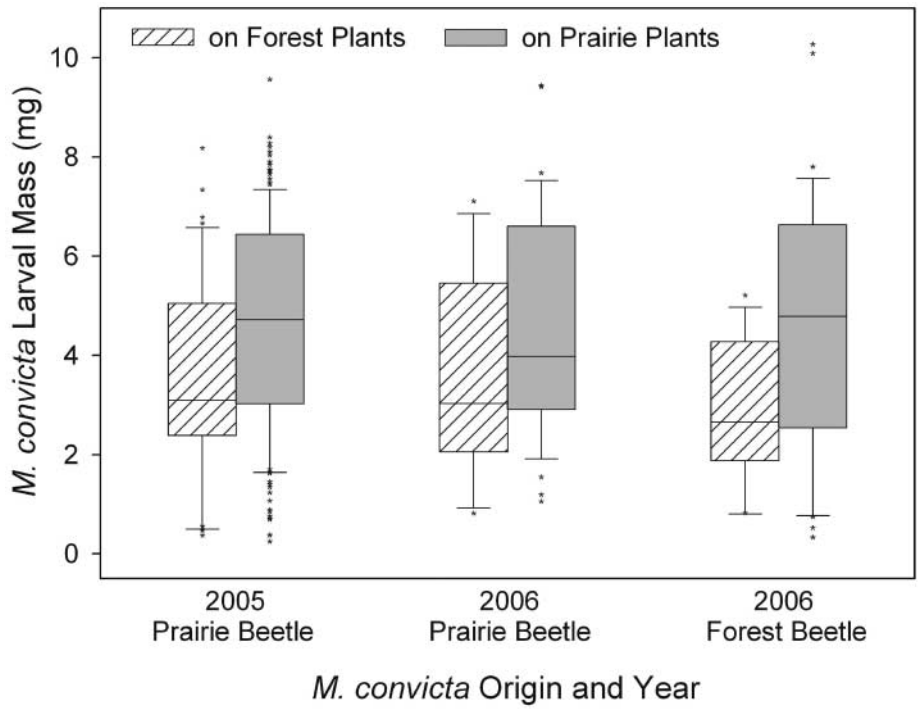

Fig. 2. Median mass of *M. convicta* in a common garden experiment. Adult beetles were provided with a choice of plants on which to oviposit. All beetles were larger when reared in prairie galls.

Reciprocal transplant experiment

In both the Moorhead (prairie) and Cedar Creek (forest) sites, *M. convicta* abundance was positively related to gall diameter ($Z = 6.28$, $P < 0.001$, odds ratio = 1.25). Neither the geographic location of the transplant garden nor the source biome of the galls within each garden was a significant predictor of the number of surviving larvae of *M. convicta*.

Mean mass of the mordellid larvae was not significantly affected by plant biome, gall diameter, or garden location in either of the reciprocal transplant gardens.

Eurytoma gigantea presence was negatively correlated with gall diameter at both sites ($Z = -4.56$, $P < 0.001$, odds ratio = 0.85). The parasitoids from each biome were more frequently found in their local host galls in each biome. *Eurytoma gigantea* larvae were found in 31.6% of galls per plant on prairie plants, but only 16.6% of galls on forest plants at the Moorhead garden ($Z = 2.57$, $P = 0.010$, odds ratio = 2.72). They were found in 18.6% of galls per plant on forest plants, but only 12.9% of galls on prairie plants at the Cedar Creek garden ($Z = -2.25$, $P = 0.024$, odds ratio = 0.42).

DISCUSSION

Our results provide evidence that differences in the abiotic environment can result in a cascade of divergence from plants to herbivores to natural enemies. In this community, local adaptation by *Solidago altissima* to different environments has led to divergence in *Eurosta*

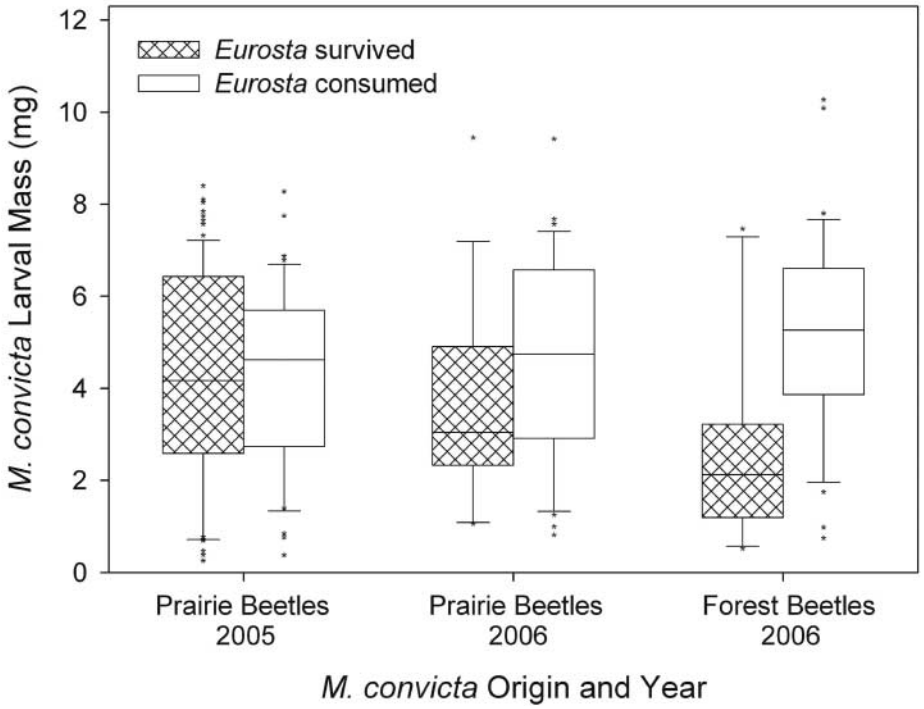

Fig. 3. The effect of predation on *E. solidaginis* by *M. convicta* on beetle performance in 2 years of common garden experiments. The median mass of beetle larva from a gall in which the host had either survived or been killed is displayed.

solidaginis. This, in turn, has had an impact on two of the natural enemies of *E. solidaginis*: *Mordellistena convicta* and *Eurytoma gigantea*.

Geographic differences in *Mordellistena convicta* populations

We found evidence of local adaptation in the parasitoid *E. gigantea* and a lack of evidence of local adaptation in the inquiline *M. convicta*. To our knowledge, this is the first test of local adaptation in insects on a large geographic scale where the geographic mosaic of co-evolution is likely to produce co-evolutionary diversification. Local adaptation in insects has been documented on individual trees or plant clones (Edmunds and Alstad, 1979; Karban, 1989; Hanks and Denno, 1994; Mopper *et al.*, 2000; Egan and Ott, 2007), but these locally adapted insect demes must break up and recombine after the death of their hosts, preventing the maintenance of local genetic differentiation. Other studies have failed to find evidence of local adaptation in insects (Strauss, 1997).

The hypothesis of local adaptation of *M. convicta* was not supported in this study. When provided with a choice, the beetle displayed no significant preference for ovipositing on its local host plant. We found no evidence that beetles are better adapted to their local host plants: all beetle larvae, regardless of their biome of origin, were larger when they were reared on prairie galls. In a variety of insect taxa, including the Coleopterans, female mass is

positively correlated with both the number of ovarioles a female has and her fecundity (Honek, 1993), so the increased mass in larvae in galls on prairie plants indicates that there is higher fitness on that host. This implies that the prairie gall provides higher quality resources for larval growth. This higher resource quality could be due to prairie galls containing a greater quantity of nutrients, having higher nutrient quality, or having fewer secondary compounds that retard larval growth than their forest counterparts. *Solidago altissima* produces a number of secondary compounds (Singh *et al.*, 1998; Tori *et al.*, 1999; Mapes and Davies, 2001a, 2001b), but nothing is known of geographic differences in the expression of these chemicals or of their effects on phytophagous insects.

Effect of *Eurosta* consumption on *Mordellistena convicta* fitness

The increased mass of beetle larvae that consumed *E. solidaginis* larvae compared with those that fed exclusively on plant tissue may indicate that predatory behaviour is adaptive. These results are interesting because past studies have reported conflicting results as to the trophic position of other species in this genus. *Mordellistena splendens* was found to kill but not consume other insects that it encountered in the stem (Stiling and Strong, 1983), while stable isotope analysis indicated that prey items were a significant part of the diet of *M. aethiops* and that species appeared to specifically seek out prey (Tooker and Hanks, 2004).

Mordellistena convicta is a predator of the *E. solidaginis* larva, and we found that it performed better when it consumes the larva than when it does not. However, the larva has not evolved to be a very efficient predator, as it sometimes appeared unable to recognize its prey: galls occasionally contained both living *E. solidaginis* and *M. convicta* larvae in the central chamber. If the *M. convicta* has only recently evolved predatory behaviour, our results indicate that there should be selection for evolution of more efficient host-seeking behaviour.

Sequential 'speciation' in *Eurytoma gigantea*

We found evidence of local adaptation in *E. gigantea* that supports the concept of sequential speciation (Abrahamson *et al.*, 2001), with divergence of the parasitoid following that of the host plant and gall-maker. *Eurytoma gigantea* have longer ovipositors in the prairie than in the forest (Craig *et al.*, 2007), and we found that prairie and forest populations of *E. gigantea* have diverged in their use of host galls. In the reciprocal transplant experiment, we found more *E. gigantea* on the local hosts than on the host from the other biome, and this could be due to local differences in host preference, the ability to survive on local hosts, or both. The wasp may be physiologically better adapted for survival on its local host plant subspecies and fly population, perhaps due to adaptation to differences in host plant or insect chemistry. It could also prefer to oviposit on its local hosts. A local host preference could be genetically based or it could be environmentally induced, as studies of hymenopteran parasitoids have found that larval and early adult experience can influence host choice by female parasitoid wasps (Vet and Groenewold, 1990; Kester and Barbosa, 1991). Whether host preference is heritable or not, it could still result in differentiation between the populations due to their association with one host plant more frequently than the other. Behavioural experiments are needed to determine if the populations differ in host preference, and whether this preference is the result of larval experience or heritable genetic differences.

Influence of *Mordellistena convicta* on divergence of the interaction between biomes

The geographic mosaic of co-evolution resulting in local adaptation of species can alter community interactions of species that are not locally adapted. The mordellid beetle has not become locally adapted as the result of diversifying co-evolutionary selection in contrast to the geographic differentiation of *S. altissima*, *E. solidaginis*, and *E. gigantea* into prairie and forest populations. However, *Mordellistena convicta* may be exerting selection for greater differentiation in other species in the interaction. We propose that *M. convicta* has contributed to a feedback cycle that has produced both larger galls and higher *M. convicta* densities in the prairie. If we assume that *E. solidaginis* originated in the forest as proposed by Waring and colleagues (1990), then galls were initially small and *M. convicta* population densities were low. If *Eurosta solidaginis* then colonized the prairie, the absence of bird predation on larvae in large galls would have selected for an increase in gall size. Once the lack of bird predation initiated an increase in gall size, a positive feedback loop would have been set in motion where increasing *M. convicta* densities increased selection for larger galls. This would have occurred because *M. convicta* survives better in large galls but causes higher mortality of *E. solidaginis* in small galls. The beetle has higher survival, larger mass, and greater fecundity in large galls, so that as *E. solidaginis* gall size increased, *M. convicta* population size would have increased. Since *M. convicta* causes higher mortality on *E. solidaginis* larvae in small galls, an increase in *M. convicta* population densities would result in increased selection on *E. solidaginis* to induce large galls. The cycle would continue until both the larger gall size and higher beetle densities found in the prairie were produced. The same logic would apply if *E. solidaginis* had originated in the prairie: when the fly colonized the forest, bird predation would have selected for smaller gall size, initiating a negative feedback cycle of decreasing gall size and decreasing *M. convicta* population densities.

The positive feedback loop is lacking in the forest due to the presence of birds: birds select against large gall size, preventing both the evolution of increased gall size and the resulting increase in mordellid population density. The beetle thus contributes to the differences in evolutionary trajectories of the communities in the prairie and in the forest by contributing to the selection for differences in *E. solidaginis* gall size and indirectly *E. gigantea* ovipositor length. The beetle may not be locally adapted, but if it were absent, it would greatly alter the co-evolutionary dynamics of the other species in each biome.

CONCLUSION

Our results support the hypothesis that the communities of natural enemies and herbivores on *S. altissima* in the prairie and forest are following independent evolutionary trajectories (Craig, 2007), which indicates that there is a geographic mosaic of co-evolution. Differences in the abiotic biome cascade upwards to produce differences in the plant, the gall-maker, *E. solidaginis*, and the parasitoid, *E. gigantea*. The inquiline *M. convicta* has not differentiated in response to differentiation in other members of the community, but variation in its population density and the selection it appears to exert on other members of the community differs dramatically between the forest and the prairie.

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APPENDIX 1: LOCALITY INFORMATION

Site #	Site name/locality	Biome	Latitude	Longitude
4	Felton Prairie Wildlife Management Area (WMA)	Prairie	47°02′59″N	96°26′36″W
20	Rahn Park (S. St. Paul)	Forest	44°47′45″N	93°13′45″W
22	West Duluth	Forest	46°43′19″N	92°11′16″W
23	Zimmerman Prairie	Prairie	47°06′26″N	96°08′43″W
24	Clay County #3	Prairie	46°55′48″N	96°16′40″W
26	Barnum	Forest	46°31′20″N	92°42′00″W
27	Banning Junction	Forest	46°11′00″N	92°51′30″W
28	Hinckley	Forest	46°00′36″N	92°55′31″W
29	Seven Bridges Road (Duluth)	Forest	46°51′18″N	92°00′45″W
33	Carlos Avery WMA #3	Forest	45°19′22″N	93°03′10″W
74	Rice Lake Road (Rice Lake)	Forest	46°57′38″N	92°09′13″W
119	Bejou WMA	Prairie	47°28′40″N	95°58′30″W
120	Mahnomen County Waterfowl Production Area	Prairie	47°28′30″N	95°55′40″W
121	Felton Prairie – Assinboia Skipper Unit	Prairie	47°05′00″N	96°24′20″W
122	Eastern Zimmerman Prairie (Detroit Lakes WMA)	Prairie	47°06′28″N	96°05′18″W
123	Hawley	Prairie	46°52′00″N	96°13′00″W
124	Riverside (Duluth)	Forest	46°42′38″N	92°12′23″W
125	Becks Road 2 (Duluth)	Forest	46°42′14″N	92°17′26″W
126	Morgan Park (Duluth)	Forest	46°46′05″N	92°13′33″W
127	Chaffee	Prairie	46°37′30″N	97°21′30″W
128	Stanley	Forest	45°33′26″N	93°09′07″W
129	Coon Lake	Forest	45°18′24″N	93°07′56″W
130	Seneca Waste Water Treatment Plant (St. Paul)	Forest	44°49′47″N	93°12′17″W
131	Doran Lake	Prairie	46°48′16″N	96°20′21″W
134	Bluestem Prairie #1	Prairie	46°51′19″N	96°28′50″W
135	Bluestem Prairie #2	Prairie	46°51′17″N	96°26′48″W
136	Bluestem Prairie #3	Prairie	46°49′59″N	96°26′45″W
137	New Market #2	Forest	44°35′34″N	93°20′20″W
138	Jay Cooke State Park	Forest	46°38′57″N	92°22′49″W
139	Oak Lake	Prairie	Unknown – volunteer collected	
140	Becker	Prairie	Unknown – volunteer collected	
141	Westbury	Prairie	Unknown – volunteer collected	