# DIVERGENCE OF *EUROSTA SOLIDAGINIS* IN RESPONSE TO HOST PLANT VARIATION AND NATURAL ENEMIES

**Timothy P. Craig[1,2] and Joanne K. Itami[1]**
[1]*Department of Biology, University of Minnesota Duluth, Duluth, Minnesota 55812*
  [2]*E-mail: tcraig@d.umn.edu*

We tested the hypothesis that forest and prairie populations of the gall-inducing fly, *Eurosta solidaginis*, have diverged in response to variation in selection by its host plant *Solidago altissima*, and its natural enemies. A reciprocal cross infection design experiment demonstrated that fly populations from the prairie and forest biomes had higher survival on local biome plants compared to foreign biome host plants. Flies from each biome also had an oviposition preference for their local plants. Each fly population induced galls of the size and shape found in their local biome on host plants from both biomes indicating a genetic basis to the differences in gall morphology. *Solidago altissima* from the prairie and forest biomes retained significant morphological differences in the common garden indicating that they are genetically differentiated, possibly at the subspecies level. The populations are partially reproductively isolated as a result of a combination of prezygotic isolation due to host-associated assortative mating, and postzygotic isolation due to low hybrid survival. We conclude that *E. solidaginis* is undergoing diversifying selection to adapt to differences between prairie and forest habitats.

KEY WORDS: Adaptation, behavior, plant–insect interaction, selection—natural, speciation, reproductive isolation.

Genetic diversification of communities of herbivorous insects and their natural enemies in response to habitat variation is potentially one of the major forces generating biological diversity at the species level and below (Price et al. 1980; Thompson, 2005). This diversification can include ecological speciation that occurs when divergent natural selection between niches and environments produces reproductive isolation (Schluter 2000, 2001). Support for the ecological speciation hypothesis has come from evidence of sympatric speciation in herbivorous insects where strong divergent selection following a host shift leads to the evolution of reproduction isolation (Berlocher and Feder 2002; Drès and Mallet 2002). In this article, we tested the hypothesis that selection on an herbivorous insect to adapt to different habitats can lead to divergence without a host shift or geographic isolation.

The gall-inducing fly *Eurosta solidaginis* (Diptera: Tephritidae) is a member of the community of insects on goldenrod, *Solidago* sp. that has become a model system for the study of host-associated differentiation (HAD) of herbivorous insects (Stireman et al. 2005) and their natural enemies (Funk et al. 2002; Abrahamson et al. 2003; Eubanks et al. 2003; Stireman et al. 2006). *Eurosta solidaginis* has formed host races on the sympatric species of goldenrod, *Solidago altissima* and *S. gigantea*, in the forest biome of North America (Waring et al. 1990; Craig et al. 1993, 1997, 2001, 2007b; Brown et al. 1996; Itami et al. 1998; Stireman et al. 2005). Herbivorous insects in addition to *E. solidaginis* showing HAD on sympatric populations of *S. altissima* and *S. gigantea*, include a gall-forming moth, *Gnorimoschema gallaesolidaginis* (Nason et al. 2002), the gall-inducing midge, and *Rhopalomyia solidaginis* (Stireman et al. 2005). Another gall-inducing midge *Dasineura folliculi* (Dorchin et al. 2009) has shown HAD on *S. gigantea* and *S. rugosa*. Natural enemies of herbivores on *S. altissima* and *S. gigantea* also show HAD including the inquiline *Mordellistena convicta* that attacks *E. solidaginis,* (Abrahamson et al. 2003, Eubanks et al. 2003) the parasitoid of *R. solidaginis,*

*Platygaster variabilis* (Stireman et al. 2006), and the parasitoid of *G. gallaesolidaginis, Copidosoma gelechiae* (Stireman et al. 2006).

We tested the hypothesis that there has been adaptive divergence of *E. solidaginis* in response to differences in host plants and natural enemies in parapatric populations on *S. altissima* in prairie and forest habitats. Population genetic models have shown that such strong divergent selection for local habitat adaptation in allopatrically or parapatrically distributed populations can produce genetic differentiation and speciation much more readily than in sympatry (Coyne and Orr 2004), but there has been a dearth of tests for adaptive genetic divergence of parapatric populations of herbivorous insects in response to habitat differences.

*Solidago altissima* is a perennial herb that forms large clones through rhizomatous spread. It is found from the east coast to the west coast in northern USA and southern Canada, and in the central USA, its range extends south into Texas, USA (Abrahamson and Weis 1997). Ploidy level varies in the *S. altissima* group (Semple 1985; Halverson et al. 2008a; J. K. Itami and K. Johnson, unpubl. data) and the ploidy variation impacts herbivore attack levels (Halverson et al. 2008b). Two subspecies of *S. altissima* have been recognized: *S. altissima altissima* that occurs only in the forest biome and *S. altissima gilvocanescens* that is found only in the prairie biome (Semple and Cook 2006). The designation of species and subspecies in the genus *Solidago* is controversial, and these putative subspecies can be difficult to differentiate (Semple and Cook 2006). Recognizing the uncertainty of these designations, we will refer to the *S. altissima* populations in the two biomes as "prairie" and "forest" plants in this study.

*Eurosta solidaginis* populations on *S. altissima* in the forest and in the prairie biome in Minnesota differ in several traits, including wing patterns (Ming 1989), allozyme frequencies (Itami et al. 1998), and gall size and shape (Craig 2007; Craig et al. 2007a). Some of these traits shift abruptly at the prairie-forest border, and some form clines. Ming (1989) classified fly populations as eastern and western subspecies based on differences in the hyaline area on the wing. The western population has a hyaline area forming a complete bar across the wing, and in the eastern population the hyaline area is separated into two spots by a pigmented region. In contrast to Ming, we have found that the wing patterns follow the distribution of prairie and forest habitats with the complete hyaline bar pattern on flies in the prairie and the two wing spot wing pattern on flies in the forest with intermediates found as the biome border is crossed (T. P. Craig et al., unpubl. data, Brown and Cooper (2006) using a multivariate analysis of wing patterns found that there was a gradation of wing patterns across Iowa, just south of our study area. Prairie populations and forest populations have different allozyme frequencies with an intermediate cluster in the border area (Itami et al. 1998; J. K. Itami et al., unpubl. data). In contrast to wing patterns and allozymes, gall morphology shows a sharp shift at the biome border with galls in the prairie being larger in diameter and more spherical than those in the forest (Craig 2007; Craig et al. 2007a; T. P. Craig et al., unpubl. data). Because of the uncertain status of the prairie and forest populations as species, subspecies, or geographic races we will refer to them as "prairie flies" and "forest flies" based on the biome from which they were collected.

The life history and ecology of *E. solidaginis* has been described in detail by Abrahamson and Weis (1997). *Eurosta solidaginis* oviposits into the bundle of unfolding goldenrod leaves, and onto the terminal meristem from mid-May to mid-June in Minnesota (Craig et al. 1993). The feeding action of first-instar larvae as they burrow into the stem induces gall formation. Larvae continue to feed on gall tissue as the gall grows, and the gall reaches its maximum size by early August.

*Eurosta solidaginis* is attacked by natural enemies that exert selection on gall morphology. *Eurytoma gigantea* (Hymenoptera: Eurytomidae) is a parasitoid that oviposits through the gall wall into the *E. solidaginis* larva in the central chamber, where the wasp larva consumes the host larva and the gall tissue of the inner chamber (Uhler 1951). This parasitoid causes higher mortality rates on larvae in small galls because it has a short ovipositor that prevents it from attacking larvae in larger galls (Weis et al. 1989; Craig et al. 2007a). *Mordellistena convicta* (Coleoptera: Mordellidae) is an inquiline whose larvae burrow into a gall feeding on parenchymal tissue (Ping 1915), and it causes *E. solidaginis* mortality in about 70% of galls in which it feeds. Like the parasitoid it also causes higher mortality in smaller galls (Craig et al. 2007a). In the winter, gall inhabitants are preyed upon by black-capped chickadees, *Poecile atricapillus*, (Passeriformes: Paridae) and downy woodpeckers, *Piscoides pubesens* (Piciformes: Picidae), which cause higher mortality in large galls (Weis et al. 1992; Craig et al. 2007a).

Craig et al. (2007a) demonstrated that the natural enemy community exerts divergent selection on gall morphology in the prairie and forest biomes, and that there are differences in gall morphology between the biomes. In the eastern Minnesota forest populations a combination of selective bird predation on *E. solidaginis* larvae in large galls and parasitoid and inquiline induced mortality on larvae in small galls produces stabilizing selection for an intermediate gall size (Craig 2007; Craig et al. 2007a). The lack of bird predation on larvae in large galls in the prairie combined with parasitoid attack and high rates of inquiline mortality on larvae in small galls produces directional selection for larger, more spherical galls in the prairie than in the forest (Craig 2007; Craig et al. 2007a). The morphology of *E. solidaginis* galls is determined by the interaction of fly genotype, plant genotype, and the environment (Weis and Abrahamson 1986). To demonstrate that the fly populations are locally adapted to their natural enemies, it is necessary to show that the differences between prairie

and forest galls are due to genetic differences between the fly populations.

## HYPOTHESES TESTED

We tested three hypotheses about local adaptation in *E. solidaginis*. First, that the *E. solidaginis* fly populations performed better on *S. altissima* from their local biome than on *S. altissima* from the foreign biome. Second, that flies have a preference for plants from their local biome. Third, that there is a genetic basis to the different gall morphology induced by prairie and forest flies that protected them from local natural enemies. We also tested the hypotheses that there was pre- and postzygotic reproductive isolation between the prairie and forest *E. solidaginis*.

# Methods

To test for adaptation in *E. solidaginis* in prairie and forest environments to its host plant and natural enemies, we used a reciprocal cross infection design. Reciprocal cross infection designs measure all possible interactions of host and parasite populations in a common garden or a laboratory setting and they have been extensively used to test the local adaptation hypothesis in a variety of interactions (Parker 1985; Ebert 1994; Laine 2005). In a host–parasite interaction, the local adaptation of the parasite is the result of the outcome of a host genotype × parasite genotype × environment interaction. In reciprocal cross infection experiments, the environment is held constant so that the impact of the host genotype × parasite genotype interaction can be measured.

Two different methods of evaluating the host genotype × parasite genotype interaction for local adaptation in the interaction have been proposed (Gandon 1998; Kawecki and Ebert 2004; Greischar and Koskella 2008). The local versus foreign criterion for local adaptation is that the local population performs better within its own habitat than a foreign population and the home versus away criterion is that a local population performs better in its local habitat than in a foreign habitat. Kawecki and Ebert (2004) argue that the local versus foreign comparison is the most useful, but it is often useful to apply both criteria and compare the results (Nuismer and Gandon 2008). The experiments in this study allowed both criteria to be applied.

## LOCAL ADAPTATION TO HOST PLANTS AND NATURAL ENEMIES

We established a common garden in Duluth, Minnesota USA at the University of Minnesota Duluth Research and Field Studies Center, to conduct the reciprocal cross infection experiments to test local adaptation hypotheses. We collected *S. altissima* rhizomes from six sites paired by latitude from north, central, and southern Minnesota (Fig. 2). In May 2002, we transplanted rhizomes from the prairie north site and the forest central site into the garden. In 2003, we transplanted rhizomes collected from all six sites. Rhizomes were cut into equal size sections and propagated in 18.9 L pots. In 2002, a potting mixture of two parts local soil, two parts compost, and one part perlite was used, and in 2003 Promix$^{®}$ potting mixture was used. Plants were given supplemental water as needed. Generally, a single rhizome yielded a single stem (ramet) during the first year, but some rhizomes produced multiple stems. Each rhizome produced from one to more than 10 new rhizomes each year that developed into new stems the following year, so that in the second and third years that a potted plant was used a single genet had produced multiple stems per pot. Each stem generally has a single terminal bud, which is the oviposition site of *E. solidaginis*.

### Reciprocal cross infection experiments

We tested for local adaptation among fly populations in reciprocal cross infection experiments in the common garden in 2002 and 2004 to test predictions of the local adaptation hypothesis. We tested the prediction that the local fly populations would have higher fitness than the foreign fly population on the local *S. altissima* population (from the same biome), and that they would have an oviposition preference for local host plants. We also tested the prediction that each fly population would induce galls of the size and shape found in their own biome. Based on extensive previous studies, we made the assumption that gall morphology determines susceptibility to natural enemies (Abrahamson and Weis 1997; Weis et al. 1992; Craig et al. 2007a).

*2002 experiment*—We randomly assigned 480 plants to 24 fine nylon mesh cages ($1 \times 1 \times 1.5$ m$^3$). Each cage contained 10 pots of plants from the prairie north site and 10 from the forest central site. Plants were randomly assigned to positions within the cage. Flies used in this experiment were collected in late October 2001 from areas within 5 km of the prairie north and forest central sites. Galls were stored in a freezer at $-5°$C through the winter. Flies from each site were reared separately in mesh bags in the spring in an incubator with the temperature and day length cycle set to the mean outdoor conditions.

Oviposition preference is defined as nonrandom oviposition on plant resources offered sequentially or simultaneously (Singer 1986; Craig et al. 1989). To measure oviposition preference, we counted the number of ovipunctures on each plant. Ovipunctures are marks left on the bud when the female inserts her ovipositor (Abrahamson and Weis 1997), and there is a strong positive correlation between the number of ovipunctures and the number of eggs oviposited (Craig et al. 1997, 1999). A single mated female was placed in each of the 24 cages described above. Every 24 h the ovipunctures were counted, and plant height was measured. Females that had not ovipunctured any plants within 24 h were replaced with a new fly. Prairie flies ovipunctured plants in all 12

of the replicates, but forest flies ovipunctured plants in only 10 of the replicates, and so the remaining two replicates were excluded from the rest of the experiment. The experiments were conducted from 13 June to 27 June 2002.

An additional five mated females from the same host population that had ovipunctured the plants in the host preference experiment were released in each cage containing 20 plants on 28 June 2002. Ten replicate cages were completed using flies from the forest population and 12 from the prairie population. Caged flies will repeatedly ovipuncture buds until they are so damaged that the *E. solidaginis* cannot survive (Craig et al. 1999). To prevent this over-attack, we counted ovipunctures every 4 daylight hours, and plants with 10 or more ovipunctures per bud were removed from the experiment and placed in cages without flies to prevent further oviposition. The removal of preferred plants had the effect of increasing ovipunctures on the initially nonpreferred plants. If fewer than five flies were observed in the cage during the ovipuncture census new flies were released to bring the total back to five. We counted the total number of ovipunctures that the plant had received when it was removed from the cage. On 6 July, all plants were placed in cages without flies to protect them from oviposition by wild flies. Cages were removed on 15 July when all wild flies had finished oviposition.

Galls were collected from the experimental plants in mid-October 2002, and placed in individual mesh bags for each plant, and gall inhabitants were reared in June 2003. We excluded bird predation on larvae by collecting galls in October because predation primarily occurs during the winter (Abrahamson and Weis 1997).

We measured *E. solidaginis* performance as the total of *E. solidaginis* plus their natural enemies (*E. gigantea*, and *M. convicta* if they had consumed *E. solidaginis*) that survived to emergence. We used larval survival as an indicator of plant effects on mortality because it included all *E. solidaginis* that had survived from oviposition to the late larval stage when they could be attacked by their natural enemies. We therefore excluded *M. convicta* that were found in the gall, but did not interact with *E. solidaginis*. We also recorded mortality due to natural enemies to determine whether this subsequent mortality created differences in survival rates.

We measured gall size and shape, from galls in the common garden and from 150 galls haphazardly collected at the north prairie and central forest sites where we had collected rhizomes in October 2002. Gall diameter and length were measured with dial calipers. Gall shape was derived from the ratio of gall diameter to length, with more spherical galls having a ratio closer to 1.0.

*2004 experiment*—To test the local adaptation hypotheses on plants and flies from a wider range of sites from each biome we again measured oviposition preference, offspring performance, and gall morphology in a reciprocal cross infection experiment in 2004. In spring 2004, three potted plants from each of the six populations were placed in a randomized design in 10 cages so that each cage contained 18 potted plants for a total of 180 pots in the experiment. All of the plants were potted in 2003 and so they contained multiple ramets. Five of the cages were randomly assigned to receive prairie flies and five were randomly assigned to receive forest flies.

Flies were reared from galls from six sites from each of the biomes collected from areas roughly along a transect connecting the sites. Cages assigned to receive attack by flies from either the forest or prairie biome received seven randomly chosen females and seven randomly chosen males from that biome. The flies were released on 4 June 2004, and the flies removed and the number of ovipunctures were counted on 7 June 2004. Forest plants in cages with prairie fly treatments received fewer ovipunctures than plants in the other treatments. To equalize levels of oviposition among treatments forest plants from this treatment were placed temporarily in new cages each with seven additional prairie fly females and seven additional males. These plants were checked twice daily for ovipunctures until they had reached a level of attack approximately equal to that of the other treatments. Flies were then removed, the ovipunctures counted, and the plants returned to their original blocks. Galls were collected in October and dissected to determine the fate of the gall occupants. The maximum width and length of each gall was measured with dial calipers.

## POSTZYGOTIC REPRODUCTIVE ISOLATION

The postreproductive isolation hypothesis was tested with a common garden experiment to compare fly survival by prairie fly larvae, forest fly larvae, and hybrid larvae produced by mating prairie and forest flies in 2003 and 2009. We hypothesized that hybrid larvae would have reduced survival compared to the parental populations creating postzygotic isolation between the prairie and forest populations. We also tested the hypothesis that the gall morphology induced by hybrid larvae differed from those induced by prairie and forest fly larvae in a way that could influence their susceptibility to natural enemies.

### *2003 hybrid garden experiment*

We used plants from the prairie north and forest central sites initiated in 2002. We randomly assigned 960 potted plants to 48 different replicates in $1 \times 1 \times 1.5 \, \text{m}^3$ fine nylon mesh cages. Some cages were used for multiple replicates, and in these cases all flies and plants were replaced before a new replicate was initiated. Each cage contained either 20 prairie plants or 20 forest plants, and we initiated 12 replicates of each treatment, although some replicates were not completed because few plants received ovipunctures. We had four mating treatments: forest females mated with forest males (FF) on forest plants, prairie females mated with prairie males (FP) on prairie plants, prairie females mated with forest

males (PF) on forest plants, and forest females mated with prairie males (FP) on prairie plants.

To obtain flies, galls were collected in late October 2002 from areas within 25 km of the prairie north and forest central sites. Flies were released into the cages between 15 June and 1 July. Five mated females were initially released in each cage. We used the same methods described in the 2002 and 2004 experiments to maintain five flies per cage, to survey ovipunctures, and to remove plants with greater than 10 ovipunctures from the cage. Galls were collected from the experimental plants in mid-October 2003, and placed in individual mesh bags for each plant, and gall inhabitants were reared in June 2004. The diameter and length of each gall was measured.

### 2009 hybrid garden experiment

We used plants from all six populations initiated in 2003 which had been propagated and repotted several times in the intervening years. Six treatments consisting of a complete reciprocal cross design were conducted: forest females × forest males (FF) on forest plants, prairie females × forest males (PF) on forest plants, forest females × prairie males (FP) on forest plants, prairie females × prairie males on prairie males (PP) on prairie plants, forest females × prairie males (FP) on prairie plants, prairie females × forest males (PF) on prairie plants. Groups of 10 male and 10 female flies were added to each cage starting on 31 May. Plants were checked for oviposition daily and additional groups of 10 male and 10 female flies were added until the mean number of ovipunctures per stem was greater than 10. Flies were removed when ovipunctures exceeded a mean of 10 per stem. The total number of flies added to individual cages range from 20 to 60. The last flies were added June 13. Ovipunctures per stem were surveyed from June 12 to June 21. Galls were collected October 15, and dissected to determine gall occupants.

### PREZYGOTIC ISOLATION ASSORTATIVE MATING EXPERIMENT 2006

To test the hypothesis that the host-associated fly populations assortatively mated producing prezygotic reproductive isolation we conducted an experiment in which both host-associated populations were placed in cages with both host subspecies. Two 1-m³ cages were constructed from PVC pipe and covered with a fine mesh material. Two pots containing prairie plants and two pots containing forest plants were placed in each cage each day. The location of the plants was randomly assigned. To obtain flies, galls were collected from the 2005 cohort from along both sides of the prairie-forest border in Clay and Becker Counties in Minnesota USA in May 2006, and reared in the laboratory. Experiments were run in outdoor cages on eight sunny days when the weather was favorable for mating in June 2006. Each day of the experiment, 20 males and 20 females of each host-associated population were

placed in each cage to make a total of 80 flies. The origin of the fly could be identified on the basis of whether the hyaline band was continuous across the entire wing (prairie) or whether it was separated into two distinct spots (forest). A small number of flies with intermediate wing patterns, possibly indicating that they were hybrids were not used in the experiment. For each mating, we recorded whether the mating was assortative or nonassortative, and whether mating occurred on prairie plants, forest plants, or the cage. Mating pairs of flies were removed and immediately replaced with virgin flies to maintain an equal ratio of flies from the forest and prairie. Each day all unmated flies were removed from the cage and placed back in the common pool of flies. These flies were combined with newly emerged flies to make up the next day's experimental population. New plants were used each day of the experiment.

### BIOME DIFFERENCES IN PLANT MORPHOLOGY
### 2002 experiment

We measured plant height in the common garden and at the north prairie and central forest sites where we had collected rhizomes in October 2002. Plants at the field sites were haphazardly sampled by choosing a point on the horizon and walking through the field in a straight line measuring every plant within 1 m of either side of the transect (Prairie $n = 23$, Forest $n = 54$).

### 2004 experiment

To compare the plant morphological characteristics among populations in 2004, during the first week of September, we haphazardly selected one ramet from each pot, and measured plant height, stem diameter 5 cm above the base of the plant, the maximum length and width of the leaf 10 nodes below the top leaf on the plant, and the total number of leaves on the plant.

### STATISTICAL ANALYSIS

We used a general linear model in the Minitab® statistical package to analyze *E. solidaginis* larval survival, natural enemy mortality, and gall diameter in the common garden experiments conducted in 2002 and 2004, and in 2004 we added an analysis of gall shape. Plant biome (prairie or forest) and fly biome (prairie or forest) were used as fixed effects, and replicate was a random effect. In 2004, we added site as a fixed effect nested within biome because we had chosen matched paired sites at specific latitudes. We used the number of stems ovipunctured per pot as a covariate to account for variation in oviposition among plants in influencing larval survival. We used the number of larvae as a covariate to account for variation in the number of available hosts among plants in influencing natural enemy mortality. We also used a general linear covariance model to analyze ovipuncture preference in the 2002 and 2004 experiments. Fly biome and plant biome were used as fixed treatment effects, replicate was

used as a random factor nested within fly biome, and plant height was used as a covariate. In 2004, we added site as a fixed effect nested within biome. We analyzed all of the data using both the foreign versus local and the home versus away criteria. Because they produced exactly the same results we report only the foreign versus local analyses. To analyze plant height and gall diameter variation in the field and the common garden in 2002, we used an analysis of variance (ANOVA) where plant subspecies and location (field or common garden) were fixed effects.

We used ANOVA to analyze the 2003 and 2009 reproductive isolation experiments. We measured the differences between the forest flies and hybrids, between forest and prairie flies, and between prairie flies and hybrids in larval survival, gall diameter, and gall shape. We used prairie or forest plants as fixed effects, mating treatment (PP, FF, PF, FP) as a fixed effect nested with treatment, replicate as a random effect nested with treatment, and the number of stems ovipunctured as a covariate. In 2009, we used prairie or forest plants as fixed effects, mating treatment (PP, FF, PF, FP) as a fixed effect nested within treatment, site as a fixed effect nested within treatment, replicate as random effect nested within treatment, and the number of stems ovipunctured as a covariate.

We used chi-square analysis to test for nonrandom mating in the assortative mating experiment.

## Results

### LOCAL ADAPTATION TO HOST PLANTS

*2002 experiment*

In 2002, each local fly population had higher larval survival than the foreign population on their local host plants (Fig. 1A). There were 118 galls formed and 59 individuals survived until emergence: 41 *E. solidaginis*, 18 *E. gigantea*, and one *M. convicta*. Summing these emergence numbers resulted in 60 *E. solidaginis* larvae being used to measure larval survival. Larval survival was significantly influenced by the interaction of fly biome and plant biome (Fig. 1A, Table 1A). The main effects of number of stems ovipunctured, fly biome, plant biome, and replicate were not significant predictors of the larval survival (Table 1A).

*Forest plants*—The forest fly population had significantly higher larval survival on forest plants than the prairie fly population (Fig. 1A, Table 1B). Neither the covariate number of stems ovipunctured nor the replicate was a significant predictor of larval survival (Table 1B).

*Prairie plants*—Prairie flies had significantly higher rates of larval survival on prairie plants than the forest flies (Fig. 1A, Table 1C). Neither the covariate number of stems ovipunctured nor the replicate was a significant predictor of larval survival (Table 1C).
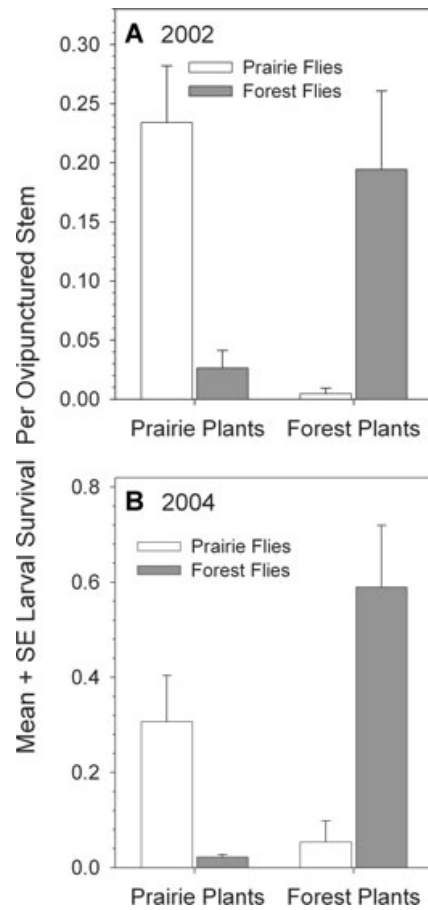


**Figure 1.** Survival of *E. solidaginis* in the common garden experiments to the late larval stage in (A) 2002 and (B) 2004.

**Table 1.** Analysis of variance of *E. solidaginis* larval survival in the 2002 common garden experiment.

| 2002 Source | N | Larval survival | |
| --- | --- | --- | --- |
| | | F | P |
| A. All plant | | | |
| Plant biome | 1 | 1.11 | NS |
| Fly biome | 1 | 0.09 | NS |
| Plant biome×fly biome | 1 | 23.14 | 0.001 |
| Replicate (fly biome) | 20 | 0.84 | NS |
| Number of stems ovipunctured | 1 | 0.86 | NS |
| Error | 417 | | |
| B. Forest plants | | | |
| Fly population | 1 | 10.49 | 0.01 |
| Replicate | 1 | 0.83 | NS |
| Number of stems ovipunctured | 9 | 0.57 | NS |
| Error | 204 | | |
| C. Prairie plants | | | |
| Fly population | 1 | 12.21 | 0.001 |
| Replicate | 1 | 0.87 | NS |
| Number of stems ovipunctured | 11 | 0.47 | NS |
| Error | 192 | | |

**Table 2.** Analysis of variance of *E. solidaginis* larval survival and natural enemy mortality in the 2004 common garden experiment.

| 2004 Source | df | Larval survival | |
| --- | --- | --- | --- |
| | | *F* | *P* |
| A. All plants | | | |
|    Plant biome | 1 | 7.35 | 0.007 |
|    Fly biome | 1 | 5.05 | 0.026 |
|    Plant biome×fly biome | 1 | 19.19 | 0.0001 |
|    Site (plant biome) | 6 | 1.96 | NS |
|    Replicate (fly biome) | 9 | 0.52 | NS |
|    Number of stems ovipunctured | 1 | 12.79 | 0.0001 |
|    Error | 161 | | |
| B. Forest plants | | | |
|    Fly biome | 1 | 17.07 | 0.0001 |
|    Site | 3 | 1.83 | NS |
|    Replicate (fly biome) | 8 | 0.39 | NS |
|    Number of stems ovipunctured | 1 | 10.311 | 0.002 |
|    Error | 73 | | |
| C. Prairie plants | | | |
|    Fly biome | 1 | 4.96 | 0.029 |
|    Site | 3 | 0.50 | NS |
|    Replicate (fly biome) | 8 | 0.67 | NS |
|    Number of stems ovipunctured | 1 | 3.24 | NS |
|    Error | 90 | | |

**Table 3.** Analysis of variance for mean number of ovipunctures per stem per pot in the 2002 common garden preference experiment.

| 2002 Source | df | Adj. mean square | *F* | *P* |
| --- | --- | --- | --- | --- |
| A. All flies | | | | |
|    Fly biome | 1 | 28.77 | 1.26 | NS |
|    Plant biome | 1 | 2.10 | 0.09 | NS |
|    Plant height | 1 | 268.94 | 11.75 | 0.001 |
|    Individual fly (fly biome) | 20 | 178.54 | 7.80 | 0.0001 |
|    Fly biome×plant biome | 1 | 4.53 | 0.20 | NS |
|    Fly biome×plant height | 1 | 2.75 | 0.12 | NS |
|    Plant biome×plant height | 1 | 3.17 | 4.26 | NS |
|    Fly biome×plant biome×plant height | 1 | 116.29 | 5.08 | 0.025 |
|    Error | 398 | 22.88 | | |
| B. Forest flies | | | | |
|    Plant biome | 1 | 10.95 | 10.95 | 0.008 |
|    Plant height | 1 | 59.69 | 1.86 | NS |
|    Individual fly | 9 | 205.87 | 1.29 | NS |
|    Plant biome×individual fly | 9 | 160.93 | 5.03 | 0.0001 |
|    Error | 174 | 24.54 | | |
| C. Prairie flies | | | | |
|    Plant biome | 1 | 12.73 | 1.61 | NS |
|    Plant height | 1 | 147.51 | 18.67 | 0.0001 |
|    Individual fly | 11 | 6.38 | 0.81 | NS |
|    Plant biome×individual fly | 11 | 18.86 | 2.36 | 0.009 |
|    Individual fly×plant height | 11 | 34.25 | 4.33 | 0.0001 |
|    Error | 195 | 7.93 | | |

### 2004 experiment

In 2004, there was again higher larval survival of the local fly populations on local host plants than that of the foreign fly populations (Fig. 1B). A total of 227 galls were formed with 62 *E. solidaginis,* 60 *E. gigantea*, and one *M. convicta* emerging in the experiment. Summing these emergence numbers resulted in 123 *E. solidaginis* larvae being used to measure larval survival. Plant biome significantly affected larval survival with higher survival on forest plants than on prairie plants. Larval survival was not significantly influenced by the site within biomes. Fly biome also affected larval survival with higher survival of forest flies than prairie flies. Larval survival was significantly influenced by the interaction of fly biome and plant biome (Fig. 1B, Table 2A). The covariate number of stems ovipunctured was also a significant predictor of the rates of larval survival. The replicate had no significant effect on fly survival.

   *Forest plants*—Forest flies had a significantly higher rate of larval survival on forest plants than prairie flies (Fig. 1B, Table 2B). The covariate number of stems ovipunctured also significantly influenced the number of surviving larvae. There was no significant variation in fly survival among plants from different sites or among replicates.

   *Prairie plants*—Prairie flies had significantly higher larval survival than forest flies on prairie plants (Fig. 1B, Table 2C). There was no significant variation in fly survival among plants from different sites. Neither the covariate the number of stems ovipunctured nor the replicate had a significant impact on larval survival.

### Oviposition preference 2002

Forest and prairie flies had significantly different oviposition preferences. The number of ovipunctures a stem received was significantly influenced by an interaction among fly biome, plant biome, and plant height (Table 3A). The number of ovipunctures a stem received was also strongly influenced by the main effect of variation among individual flies. Flies had a preference for tall plants, and the covariate plant height was significant. To clarify the meaning of the three-way interaction, we analyzed prairie and forest flies separately.

   *Forest flies*—Forest flies had a highly significant preference for forest plants, but the significant interaction of plant origin and the individual fly indicates that the strength of this preference
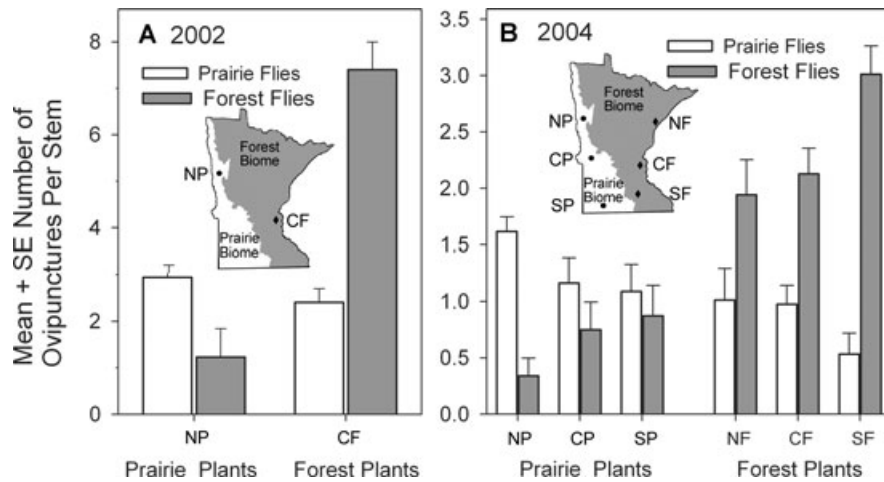
**Figure 2.** Oviposition preference of prairie and forest populations of *E. solidaginis* as measured by the number of ovipunctures per stem in the common garden experiments in (A) 2002, and (B) 2004. Forest (•) and Prairie (•) host plant collection sites are shown on map insets.

was variable among flies (Fig. 2A, Table 3B). The covariate plant height did not have a significant effect on preference.

*Prairie flies*—Prairie flies oviposited more frequently on prairie plants, but this difference was not statistically significant, and the highly significant interaction between individual fly and plant biome indicates that flies differed in their preference among plant populations (Fig. 2A, Table 3C). There was a strong preference for tall plants, but again the significant interaction between the individual fly and plant height indicated that this preference was variable.

### Oviposition preference 2004

In the 2004 experiment, the prairie and forest fly populations again showed strong differences in their preference for forest and prairie plants as indicated by the highly significant fly biome by plant biome interaction (Table 4A, Fig. 2B). Plant height also played a significant role in determining preference as indicated by the significance of the main effect of plant height, and the significant two- and three-way interactions with fly biome and plant biome (Table 4A). Because of the significant interaction of fly and plant biome, we again analyzed the fly populations separately.

*Forest flies*—Forest flies again had a strong preference for forest plants, and they showed a preference among plants from different sites within a biome (Table 4B, Fig. 2B). The number of ovipunctures a stem received was significantly influenced by the plant height, plant biome, and plant population within biome (Table 4B). The number of ovipunctures was positively correlated with plant height, and there was a significant interaction between plant biome and height. Forest flies attacked none or very few

**Table 4.** Analysis of variance for mean number of ovipunctures per stem per pot in the 2004 common garden preference experiment.

| 2004 Source | df | Adj. mean square | *F* | *P* |
|---|---|---|---|---|
| A. All flies | | | | |
| Fly biome | 1 | 1.63 | 0.50 | NS |
| Plant biome | 1 | 52 | 8.08 | 0.005 |
| Plant height | 1 | 2877 | 448.84 | 0.0001 |
| Cage (fly biome) | 8 | 55 | 8.48 | 0.0001 |
| Fly biome × plant biome | 1 | 134 | 20.97 | 0.0001 |
| Plant biome × plant height | 1 | 215 | 33.69 | 0.0001 |
| Fly biome × plant height | 1 | 28 | 4.40 | 0.036 |
| Fly biome × plant biome × plant height | 1 | 638 | 99.52 | 0.0001 |
| Error | 1943 | 19 | | |
| B. Forest flies | | | | |
| Plant biome | 1 | 194 | 26.75 | 0.0001 |
| Plant height | 1 | 1718 | 237.24 | 0.0001 |
| Block | 4 | 44 | 1.53 | NS |
| Site (plant biome) | 1 | 90 | 3.13 | 0.014 |
| Plant biome × height | 1 | 777 | 107.34 | 0.0001 |
| Error | 941 | 7 | | |
| C. Prairie flies | | | | |
| Plant biome | 1 | 13 | 2.39 | NS |
| Plant height | 1 | 1051 | 189.91 | 0.0001 |
| Block | 4 | 92 | 16.66 | 0.0001 |
| Site | 4 | 11 | 2.06 | NS |
| Plant biome × height | 1 | 50 | 9.11 | 0.03 |
| Error | 994 | 5 | | |
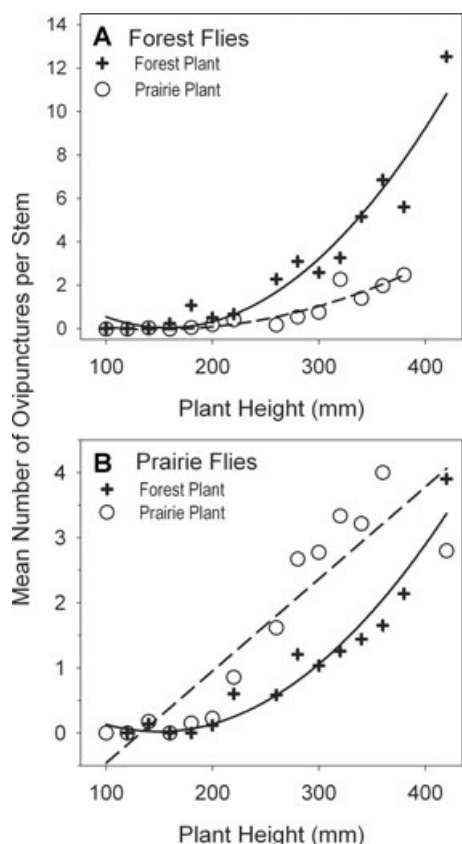
**Figure 3.** The mean number of ovipunctures regressed on host plant height categories on forest and prairie plants for (A) forest flies and (b) prairie flies in 2004.



**Figure 4.** The mean gall diameter of prairie and forest flies on forest and prairie plants in the common garden experiments in 2002 and 2004 and in natural populations in 2002.

plants of either biome shorter than 250 mm but oviposition increased rapidly on tall forest plants over 300 mm ($y = 3.894 - 0.04910x + 0.000156x^2$, $r^2 = 93.3\%$, $P < 0.0001$) whereas oviposition on prairie plants over 300 mm remained low ($y = 0.2544 - 0.00587x + 0.000028x^2$, $r^2 = 85.9\%$, $P < 0.0001$) (Fig. 3A).

*Prairie flies*—Prairie flies oviposited more frequently on prairie plants than on forest plants (Fig. 2B, Table 4C). The number of ovipunctures that a plant received was significantly influenced by plant height, and by the interaction between plant height and plant biome (Table 4C). The number of ovipunctures per stem by prairie flies increased linearly with height on prairie plants ($y = -1.874 + 0.01413x$, $r^2 = 84.3\%$, $P < 0.0001$) but increased more slowly with a curvilinear pattern on the forest plants ($y = 1.040 - 0.01364x + 0.000046x^2$, $r^2 = 94.2\%$, $P < 0.0001$) (Fig. 3B).

## LOCAL ADAPTATION TO NATURAL ENEMIES
### Genetic basis of gall morphology
*2002 common garden experiment*—Galls induced by prairie and forest flies in the common garden had the morphology found in their local biomes. In the 2002 common garden experiment, galls initiated by the prairie flies had significantly larger di-
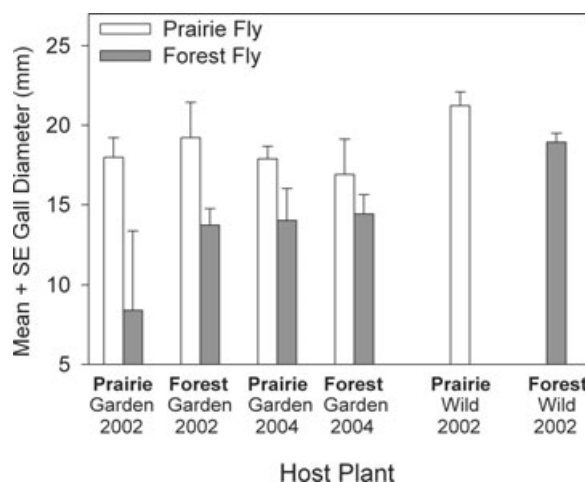
ameters than those initiated by forest flies on plants from both biomes (Fig. 4, Table 5). Gall diameter in the common garden was not significantly influenced by the plant biome, the interaction of fly biome and plant biome, or the block (Table 5). In the field, galls in the prairie were significantly larger than those in the forest ($F_{1144} = 17.50$, $P < 0.0001$, Fig. 4). Galls grown in the common garden had significantly smaller diameters than those from the wild ($F_{1144} = 18.96$, $P < 0.0001$, Fig. 4), but there was no significant interaction between the location where the gall developed (garden or field) and the fly population.

*2004 common garden experiment*—In the 2004 garden, gall diameters on plants from both biomes induced by prairie flies were significantly larger than those induced by forest flies (Fig. 4, Table 5). The plant biome also had a significant effect on gall size (Table 5). The interaction of fly origin and plant biome, site within biome, and the block had no significant effect on gall diameter.

On plants from both biomes, galls induced by prairie flies were significantly more spherical than those induced by forest flies (Fig. 5, Table 5). There were no significant differences in gall shape among sites within a biome, the interaction of plant biome and fly biome.

*2003 common garden hybrid experiment*—Prairie flies and hybrids between prairie and forest flies on plants from both biomes had similar large, spherical galls, but forest flies on forest plants had small, ellipsoid shaped galls (Fig. 6). There were significant differences among treatments in gall size among treatments. There was a significant impact of plant biome on gall shape, and a marginally nonsignificant effect of treatment on gall diameter (Table 6).

*2009 common garden hybrid experiment*—There were no significant differences in gall size or shape among treatments or plant biome in 2009 (Table 6), although the trends in galls size and

**Table 5.** Analysis of variance of gall diameter and gall shape in the 2002 and 2004 common garden local adaptation experiments.

| Source | 2002 df | 2002 gall diameter | | 2004 df | 2004 gall diameter | | 2004 gall shape | |
|---|---|---|---|---|---|---|---|---|
| | | F | P | | F | P | F | P |
| Fly biome | 1 | 10.65 | 0.002 | 1 | 6.93 | 0.009 | 9.80 | 0.002 |
| Plant biome | 1 | 0.01 | NS | 1 | 4.37 | 0.038 | 1.10 | NS |
| Site (plant biome) | NA | NA | NA | 4 | 0.84 | NS | 0.75 | NS |
| Fly biome×plant biome | 1 | 0.01 | NS | 1 | 3.44 | NS | 1.84 | NS |
| Cage (fly biome) | 20 | 1.81 | 0.050 | 9 | 1.01 | NS | 2.17 | 0.027 |
| Error | 45 | | | 149 | | | | |

shape were similar to 2003. Galls were smaller than in other years (mean gall diameter $\pm$ SE = 12.34 $\pm$ 0.34 range 7.35 to 25.24) indicating that environmental conditions, probably a deficiency in water, were not optimal during the period of maximum gall growth.

### POSTZYGOTIC REPRODUCTIVE ISOLATION

In 2003, we found no statistically significant difference in larval survival between the forest biome flies and hybrids of forest and prairie flies, or prairie biome flies and hybrids between forest and prairie flies (Table 7, Fig. 7A). A total of 352 galls were formed with 72 *E. solidaginis*, 87 *E. gigantea*, and 13 *M. convicta* emerging. Summing these emergence numbers resulted in 172 *E. solidaginis* larvae being used to measure larval survival.

In 2009, we found significantly higher survival in the two pure host-associated populations than in the hybrids on both host plants (Table 7, Fig. 7B). There were no significant differences between survival rates on plants from the two biomes, and there were no significant differences among sites nested within each biome. A total of 204 galls were formed with 43 *E. solidaginis*, 52 *E. gigantea*, and three *M. convicta* larvae were dissected out of the galls. Summing these emergence numbers resulted in 98 *E. solidaginis* larvae being used to measure larval survival.

### PREZYGOTIC ISOLATION

The forest and prairie host-associated populations assortatively mated when they mated on the host plant, but not when they

mated on the cage. Chi-square heterogeneity tests indicated that the results from the different cages could be pooled. Of matings on the host plant 46 were assortative and 26 were nonassortative ($\chi^2$ = 5.55, $P < 0.05$), whereas of the matings on the side of the cage 53 were assortative and 52 were nonassortative. When we considered only the assortative matings, we found that matings were significantly more frequent on the host subspecies from which the flies had been reared than on the other host subspecies for both fly populations (forest flies 19 matings on forest
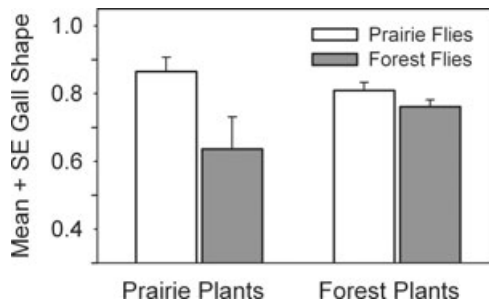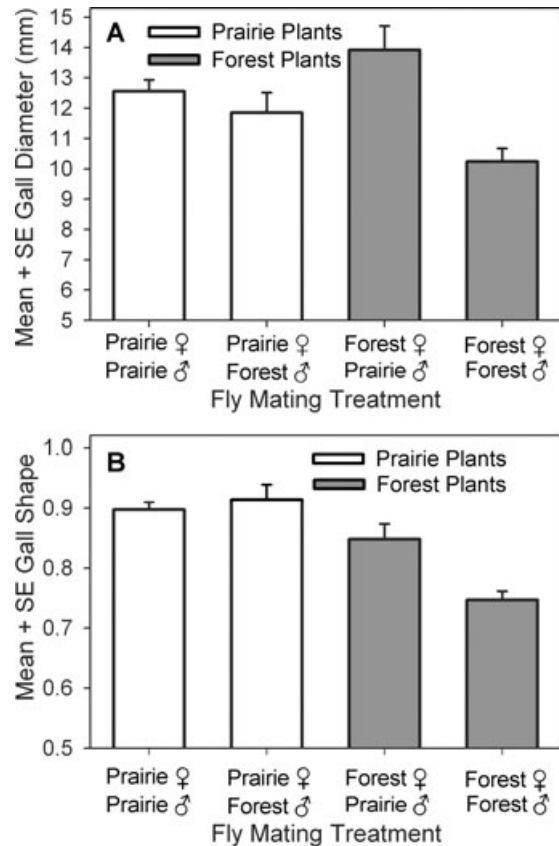




**Figure 6.** Gall diameter and gall shape in the 2003 common garden experiment induced by prairie flies (Prairie Female × Prairie Male), forest flies (Forest Female × Forest Male) and hybrids (Prairie Female × Forest Male and Forest Female × Prairie Male).



**Figure 5.** The mean gall shape of prairie and forest flies on forest and prairie plants in the 2004 common garden experiment.

**Table 6.** Analysis of variance of gall diameter and gall shape in the 2003 and 2009 hybrid experiments. Fly treatments were prairie fly, or forest fly or hybrids between forest and prairie fly populations as explained in the text.

| Year of hybrid experiment | 2003 | | | | | 2009 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | df | Gall size | | Gall shape | | df | Gall size | | Gall shape | |
| | | F | P | F | P | | F | P | F | P |
| Factor | | | | | | | | | | |
| Plant origin | 1 | 0.12 | 0.727 | 5.62 | 0.021 | 1 | 0.02 | 0.888 | 2.10 | 0.160 |
| Fly treatment (biome) | 2 | 3.37 | 0.047 | 2.48 | 0.093 | 4 | 0.68 | 0.614 | 0.40 | 0.810 |
| Site (plant biome) | NA | NA | NA | NA | NA | 4 | 0.77 | 0.543 | 1.29 | 0.275 |
| Replicate (plant biome treatment) | 16 | 3.23 | 0.001 | 1.43 | 0.127 | 12 | 1.63 | 0.088 | 1.11 | 0.352 |
| Error | 332 | | | | | 190 | | | | |

*S. altissima*, and five on prairie *S. altissima*, prairie flies 17 matings on prairie *S. altissima* five on forest *S. altissima* ($\chi^2$ two-way contingency table = 14.653, df = 1, $P < 0.0001$). If mating had been assortative, but independent of the host plant, we would have expected no significant difference in the frequency of matings on natal and nonnatal hosts.

### BIOME DIFFERENCES IN PLANT MORPHOLOGY

A genetic basis to the morphological variation in host plants traits found in the field was indicated by the retention of these differences in the common garden. In 2002, the plants from the central forest site were significantly taller than those from the prairie north site in both the field and in the common garden (Fig. 8A). We found that plant height at the end of the growing season was significantly influenced by plant biome ($F_{1303} = 273.75$, $P < 0.0001$), but not the site, that is whether the plant was growing in the experimental garden or in the field. Plant height was also significantly influenced by the interaction between site and plant biome ($F_{1303} = 6.21$, $P < 0.01$).

In the 2004, common garden experiment the prairie and forest *S. altissima* populations again showed significant morphological differences. A multivariate analysis of variance (MANOVA) on the characters of the six plant populations showed that there was highly significant variation between prairie and forest populations (Wilk's = 0.74507, $F_{5161} = 11.017$, $P < 0.0001$), among the populations nested within the prairie and forest biomes (Wilk's = 0.72493, $F_{20534} = 2.724$, $P < 0.0001$) and among blocks (Wilk's = 0.5392, $F_{45723} = 2.421$, $P < 0.0001$). Univariate analysis showed that forest plants were significantly taller, had larger stem diameters, and greater leaf lengths than prairie plants (Table 8, Figs. 8B, C, and E). The sites nested within the biomes differed significantly in height, total numbers of leaves, leaf length, and width in a complex pattern that did not show a consistent trend with latitude (Table 8, Figs. 8B, D–F).

## Discussion

Differences in host plants and natural enemies in prairie and forest habitats have led to the genetic differentiation and partial reproductive isolation of populations of *E. solidaginis* on *S. altissima* in the two biomes.

### LOCAL ADAPTATION TO HOST PLANTS

*Eurosta solidaginis* fly populations from the prairie and forest biomes had higher larval survival on their local host plants, meeting the local versus foreign criteria for local adaptation of Kawecki and Ebert (2004). We found no difference in survival on plants from different sites within each biome indicating that populations were adapted to forest and prairie habitats and not to local sites within the biomes. Prairie and forest flies both had an oviposition preference for their local host plants where their offspring fitness is higher. Both populations had an imperfect oviposition preference for their local hosts, and this may occur due to gene flow between populations that mix alleles for oviposition preference, or because there is weak selection for discriminating against oviposition on a novel host that they do not normally encounter (Uesugi 2008).

**Table 7.** Analysis of variance of larval survival in the 2003 and 2009 hybrid experiment. Fly treatments were prairie fly, or forest fly or hybrids between forest and prairie fly populations as explained in the text.

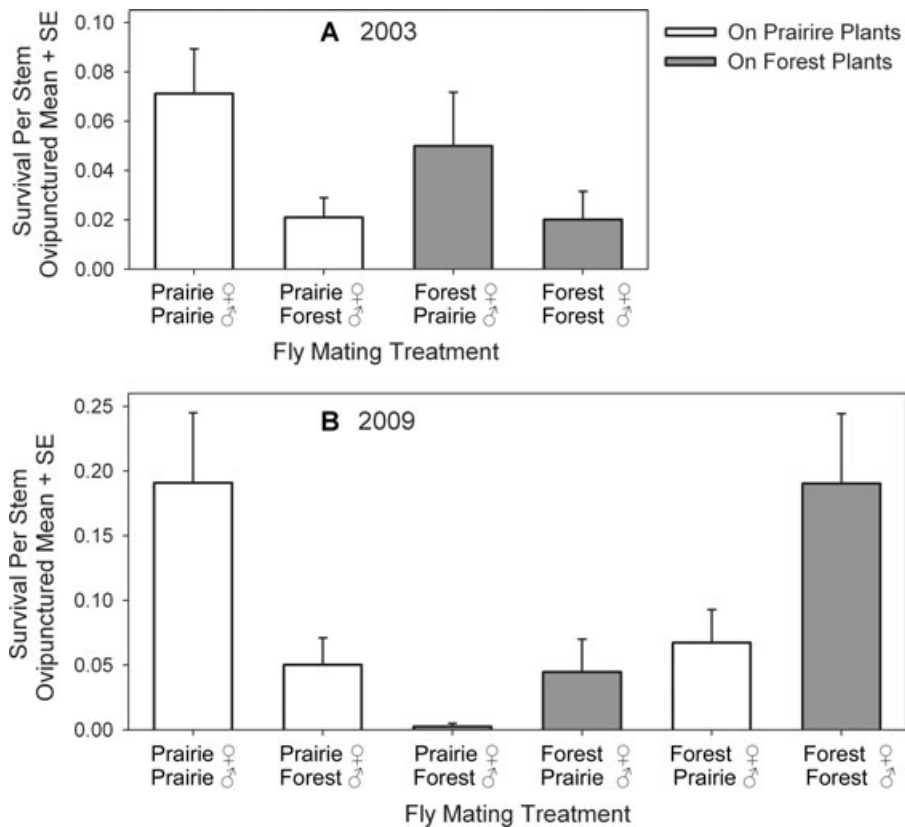| Factor | 2003 | | | 2009 | | |
|---|---|---|---|---|---|---|
| | df | F | P | df | F | P |
| Total stems OP | 1 | 2.00 | 0.158 | 1 | 20.95 | 0.0001 |
| Plant origin | 1 | 1.48 | 0.226 | 1 | 1.11 | 0.311 |
| Fly treatment (biome) | 2 | 0.49 | 0.612 | 4 | 4.32 | 0.022 |
| Site (plant biome) | NA | NA | NA | 4 | 1.12 | 0.349 |
| Replicate (plant biome treatment) | 40 | 1.36 | 0.070 | 12 | 1.50 | 0.123 |
| Error | 455 | | | 338 | | |

**Figure 7.** Survival of flies in the hybrid fly common garden experiments in (A) 2003 survival until the late larval stage in the 2003 common garden experiment by prairie flies (Prairie Female × Prairie Male) on prairie plants, forest flies (Forest Female × Forest Male) on forest plants, and hybrids (Prairie Female × Forest Male) on prairie plants and (Forest Female × Prairie Male) on forest plants. (B) 2009 survival until the late larval stage in the 2009 common garden experiment by prairie flies (Prairie female × Prairie Male), forest flies (Forest Female × Forest Male), and hybrids (Prairie Female × Forest Male) and (Forest Female × Prairie Male) on prairie and forest plants.

Genetic differentiation among *S. altissima* from the two biomes was indicated by the maintenance of their morphological differentiation and their differences in susceptibility to attack by *E. solidaginis* populations in a common garden. Plants also differed significantly among sites within biomes in some morphological characteristics, but not in their susceptibility to attack by the fly populations. Although we did not test the hypothesis, these morphological differences among plants could be due to local adaptation to their environments including divergent selection by *E. solidaginis*. Experiments have shown that prairie and forest plant populations differ in their sensitivity to water with prairie plants performing poorly when given supplemental water (J. Growchowski and J. Etterson, unpubl. data). Local adaptation of plants to their abiotic environment has been widely documented (Linhart and Grant 1996; Joshi et al. 2001), but this would be the first example of local adaptation in plants being correlated with local adaptation of their herbivores.

Differences among *S. altissima* ploidy levels have been demonstrated to influence *E. solidaginis* performance (Halverson et al. 2008b). Both forest and prairie populations *S. altissima*

populations vary in their ploidy level (J. K. Itami and K. Johnson, unpubl. data), and variation in the frequency of ploidy levels between and within biomes may be one of the factors producing the geographic mosaic of plant variation to which flies must adapt.

## LOCAL ADAPTATION TO NATURAL ENEMIES

Gall morphology in the prairie and forest fly populations is under strong diversifying selection by natural enemies (Craig et al. 2007a), and our results indicate that prairie and forest flies have genetic differences in the gall morphologies that they induce. Our results are in agreement with the conclusion of Weis and Abrahamson (1986) that *E. solidaginis* gall morphology is the result of the interaction of the insect genotype, plant genotype, and the environment. Prairie and forest flies induced galls in the garden with morphologies similar to those in the field indicating a genetic basis to the differences in gall morphology. The prairie flies produced significantly larger more spherical galls and forest flies produced smaller more ovoid galls on both populations of host plants in all years except 2009. The local gall morphology provides better protection against local natural enemies than the
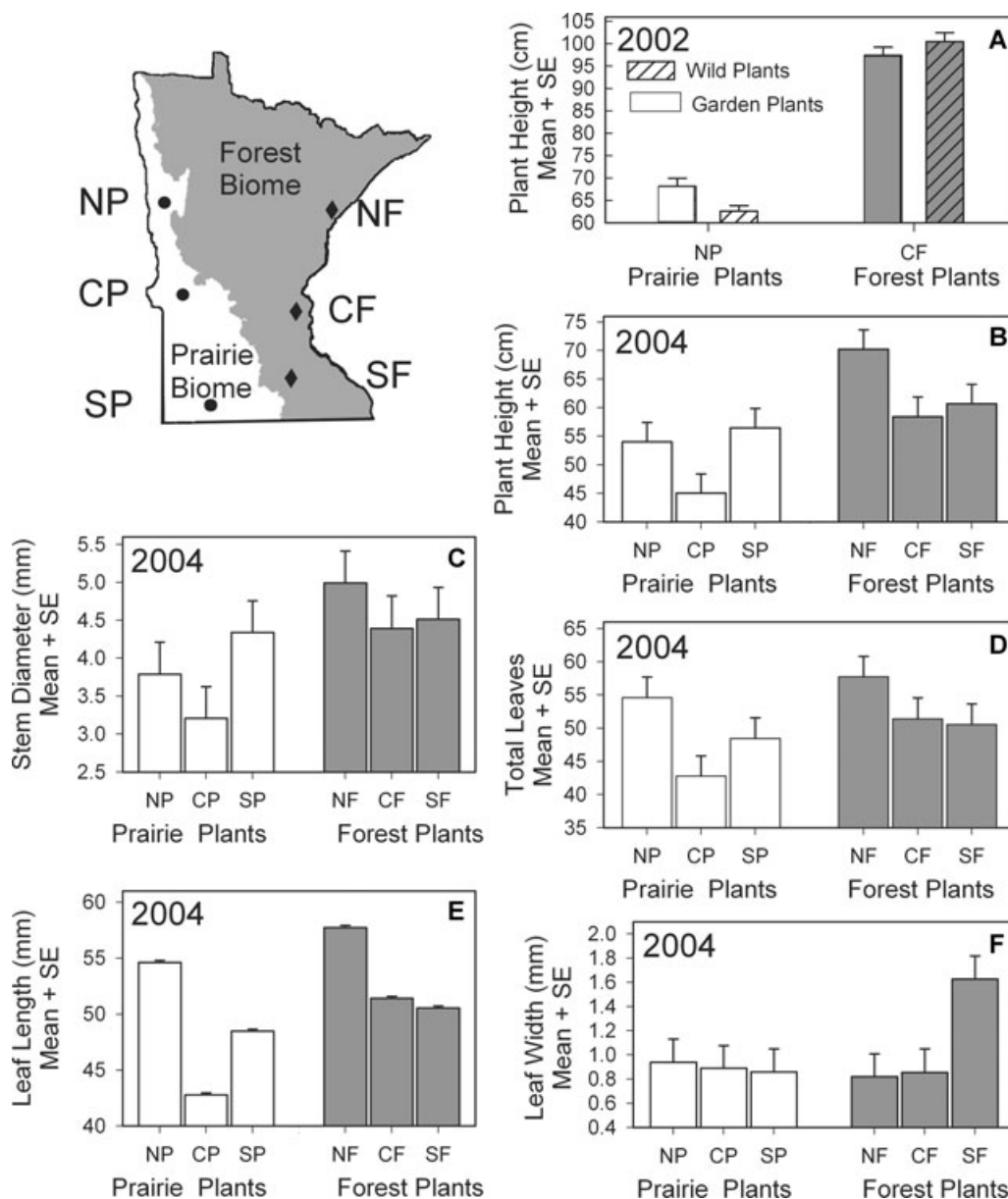
**Figure 8.** (A) Comparison of plant height of prairie and forest populations in 2002 in the wild and in the common garden experiment. (B-F) The morphological characteristics of S. altissima plants from six populations grown in the 2004 common garden experiment. In 2004, six populations were used, matched for latitude, north, N, central, C, and south, S from the prairie (P) and from the forest (F). Forest (♦) and Prairie (•) host plant collection sites are shown on map insets.

foreign gall morphology (Craig et al. 2007a) again supporting the local adaptation hypothesis (Kawecki and Ebert 2004). An autosomal inheritance of gall characteristics is also indicated as having either a male or a female parent from the prairie resulted in galls with a prairie morphology.

Gall morphology was also influenced by plant biome. Host plant biome had a significant effect on gall diameters in 2004, and the interaction of plant biome with fly biome in the 2002 experiment had a significant effect on gall diameter. Gall shape was also significantly influenced by plant biome in 2004.

Strong environmental influences on gall morphology were demonstrated by the consistently smaller size of galls in the garden than in the field. The small gall size resulted in a higher proportion of parasitism by *E. gigantea* than has been reported in the field. Although the distribution of gall sizes found in the garden were well within the ranges typical in the field, there was a greater frequency of galls in the smaller size classes in the garden. The small gall size in the garden does not appear to be due to reduced plant vigor as plants grew at least as vigorously in pots as in the field in all years except in 2009 (this article, T. P. Craig and

**Table 8.** Univariate analysis of *S. altissima* traits from six populations of plants from the prairie and forest biome in the 2004 common garden experiment.

| Plant trait | Biome | | Site within biome | |
|---|---|---|---|---|
| | $F_{1,165}$ | $P$ | $F_{4,165}$ | $P$ |
| Plant height | 16.52 | 0.0001 | 3.29 | 0.013 |
| Stem diameter | 6.16 | 0.0140 | 1.20 | 0.315 |
| Total leaves | 3.31 | 0.0710 | 2.65 | 0.035 |
| Leaf length | 45.70 | 0.0001 | 5.98 | 0.0001 |
| Leaf width | 1.73 | 0.1900 | 2.87 | 0.025 |

J. K. Itami, unpubl. data). The small galls in 2009 were probably due to under-watering plants during the late summer. Although this did not reduce fly survival compared to other years, it did produce visible signs of reduced plant and gall growth on some plants before the watering rate was increased.

### REPRODUCTIVE ISOLATION

There is evidence of partial reproductive isolation between prairie and forest fly populations due to both pre- and postzygotic reproductive isolation. Reduced hybrid survival in 2009 compared to the pure fly populations indicated postzygotic isolation. The data also suggest that the environment has a strong influence on the relative fitness of hybrids and pure forest or prairie flies. Oviposition in the garden plots was unusually late in 2003 due to cold, rainy weather. Horner et al. (1999) has shown *E. solidaginis* has a narrow time window for gall induction, the unusual environment may have created an oviposition period that was a poor match with this window reducing pure prairie or forest fly survival and minimizing the differences with hybrid survival.

Hybrids fitness can be reduced by either intrinsic or extrinsic isolation (Coyne and Orr 2004). Intrinsic isolation occurs because hybrids have poor viability due to genetically based developmental problems (Coyne and Orr 2004). Hybrids may also have reduced hybrid fitness due to extrinsic isolation. In extrinsic isolation, populations have normal development but have low fitness because their intermediate genotypes are not well adapted to available ecological niches (Coyne and Orr 2004). Our results indicate that the prairie and forest fly populations are adapted to their local *S. altissima* populations and so hybrid flies may have low hybrid larval survival because they have intermediate genotypes not well adapted to either host plant population, although intrinsic isolation could also play a role.

The interaction with natural enemies in natural settings could strongly influence hybrid fitness in ways that we did not measure in our experiments. For example, hybrid flies induced large galls on both host plants in 2003 where larvae would suffer higher rates of bird predation in the forest and lower rates of mortality from the parasitoid and inquiline influencing fly survival rate in the field. The interaction of the host plant with the local abiotic environment could also have a strong impact on hybrid fitness, and we minimized the impact of this interaction by watering the plants and growing them in potting soil. Only reciprocal transplant experiments in the two parental environments can adequately test these hypotheses. Our study also did not examine other aspects of fitness such as adult viability, mating, and oviposition success that could influence the relative fitness of the hybrids and parental population.

Assortative mating due to host plant preference produced some prezygotic isolation. Prairie and forest flies preferred to mate on their own host plants, and this could reduce gene flow in areas where the populations meet. Assortative mating was relatively weak, but our experimental design maximized the potential for nonassortative mating by placing the forest and prairie populations in the cages with host plants physically touching. Geographic isolation in the parapatrically distributed populations, and any differences in emergence times would increase prezygotic isolation.

### ECOLOGICAL DIVERSIFICATION

The prairie and forest *E. solidaginis* populations have become partially reproductively isolated populations in response to diversifying selection for adaptation to different habitats without a host shift. The populations currently have a parapatric distribution consisting of intermixed patches in the habitat mosaic along the forest-prairie biome border (T. P. Craig and J. K. Itami, unpubl. data). There is partial reproductive isolation between the populations despite the potential for gene flow between the populations. We do not know, and it may be impossible to determine, whether parts of the population were geographically isolated at some point in the past, and whether some of the divergence in traits was initiated in allopatry. What is clear is that there is currently strong selection for adaptation to different habitats that is maintaining divergence of the two populations. It is also not clear whether the populations are sufficiently reproductively isolated to be species. The partial reproductive isolation seems to be consistent with the designation of these populations as geographical host races. The current differentiation could be part of an ongoing process of ecological speciation or the populations may be in an intermediate stage of differentiation that is in a dynamic equilibrium along a continuum from undifferentiated populations to "good" species. The degree of reproductive isolation between these two populations may shift as ecological conditions fluctuate along a continuum through time and space.

A repeated pattern of genetic diversification of herbivorous insects and their natural enemies on *S. altissima* and *S. gigantea* has been demonstrated. *Eurosta solidaginis* has diversified in response to within and between host species variation into partially reproductively isolated host races on *S. gigantea*, forest

*S. altissima*, and prairie *S. altissima*. The differentiation of prairie and forest populations on *E. solidaginis* on *S. gigantea* has not yet been investigated. The populations of *E. gigantea* on prairie and forest populations of *E. solidaginis* also show evidence of diversification (Dixon et al. 2009). If the host-associated diversification of insects on *Solidago* are representative of a general pattern, then it indicates that diversification of host-associated populations may be a much stronger force for the generation of biological diversity than has been previously recognized. Many plant–herbivore interactions cross habitat boundaries and these studies indicate that there may be diversifying selection without herbivores undergoing a host shift and without geographical barriers between populations.

## ACKNOWLEDGMENTS

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