A small-tiled geographic mosaic of coevolution between *Eurosta solidaginis* and its natural enemies and host plant

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**Abstract.** We identified a geographic mosaic of *Eurosta solidaginis* fly traits produced by coevolution of the stem gall-forming fly with both its natural enemies and its host plants at small geographic scales in the presence of gene flow. These tritrophic interactions between the fly with its natural enemies and with its host-plant *Solidago altissima* produced what has been termed a small-tiled geographic mosaic of coevolution. Selection on gall diameter and length varies between prairie and forest habitats due to differences in host plants and natural-enemy communities. At the prairie–forest ecotone where prairie and forest habitats are intermixed, we found that geographic selection mosaics on gall diameter and length varied on a scale of a few kilometers. Gall diameter variation among sites correlated with selection on gall diameters, indicating local adaptation. In contrast, gall lengths did not correlate with selection, indicating that gene flow may have prevented local adaptation of this trait. Eastern (forest) and western (prairie) subspecies of *E. solidaginis* have been proposed based on fly wing patterns, and these had intermediate forms in the ecotone indicating gene flow between these subspecies. Variation in wing patterns correlated with gall diameter, indicating that gene flow between prairie and forest fly populations may influence the distribution of gall traits. The ratio of forest to prairie vegetation increases with latitude, but there was no indication of latitudinal clines in gall or wing traits. Our results indicate that selection for differentiation in coevolved traits is strong enough to overcome gene flow in small tiles of habitat. The result is that ecological forces produce a dynamic mosaic of genetically differentiated locally adapted populations. It also indicates that prairie and forest host races of *E. solidaginis* form a mosaic hybrid zone in this region.

**Key words:** *Eurosta solidaginis*; *Solidago altissima*; three trophic level coevolution; local adaptation.

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**INTRODUCTION**

A geographic mosaic of coevolution (GMC; Thompson 1999, 2005) occurs “when local variation in species’ interactions produces a spatially variable pattern of reciprocal adaptation in these species” (Craig 2016). The GMC theory consists of three components: selection mosaics, hot and cold spots in interactions, and interactions among populations in hot and cold spots (Thompson 1999, 2005, 2009, Golmulkiewicz et al. 2000, Nuism et al. 2003). Local variation in the type and strength of selection produces geographic selection mosaics. This local variation produces hot spots where species strongly interact, and cold spots where interactions are weak or absent (Smith 1970, Benkman 1999, Lively 1999, Brodie et al. 2002, Toju 2011). The evolution of local populations depends on gene flow among them, genetic drift, and local extinction of populations within these hot and cold spots. All three of these processes together produce either local adaptation (Toju and Sota 2006a, b, Vergara et al. 2013) or maladaptation due to trait remixing (Zangerl and Berenbaum 2003, Hanifin et al.
Thus, the geographic structure of a coevolutionary mosaic depends on the size and distribution of coevolutionary hot and cold spots and gene flow among them (Golmulkiewicz et al. 2000, 2003, Nuismer et al. 2000, 2003).

To understand the evolutionary dynamics of geographic mosaics of coevolution, it is crucial to determine the spatial structure of selection mosaics (Toju 2008, 2009). Knowledge of this spatial structure will enable evaluation of the relative importance of selection and gene flow on trait evolution, help determine the scale at which ecological and evolutionary dynamics occur, and indicate the underlying abiotic and biotic mechanisms producing geographic mosaics of coevolution (Toju and Sota 2006a, b). A critical unanswered question is the minimum spatial scale at which coevolution can result in local adaptation. Most studies are done at a single geographic scale (Toju and Sota 2006a, b), and only some recent studies have shown that geographic selection mosaics can produce variation in coevolved traits on a small geographic scale (Laine 2005, Rey et al. 2006, Toju and Sota 2006a, b, Toju 2008, 2009, Vergara et al. 2013).

We studied the geographic mosaic of coevolution of a tritrophic interaction at small geographic scales in the gall-inducing fly *Eurosta solidaginis*, (Diptera: Tephritidae) on tall goldenrod *Solidago altissima*, and the gallers’ natural enemies in a region where geographically differentiated populations of all species come in contact with each other. This is a tritrophic interaction (Price et al. 1980) because the interaction between the herbivore and natural enemies is influenced by their interactions with the host plant (Abrahamson and Wise 1997, Craig et al. 2007, Craig and Itami 2011). Abrahamson and Wise (1997) have described the natural history of *E. solidaginis* and its interactions with host plants and natural enemies in detail. The natural enemies of *E. solidaginis* are the parasitoid wasp *Eurytoma gigantea*, the inquiline beetle *Mordelissena convicta*, black-capped chickadees *Poecile atricapillus*, and downy woodpeckers *Dryobates pubescens*.

Craig and Itami (2011) found that *E. solidaginis* in the prairie and forest biomes (Fig. 1) in Minnesota, USA, are partially reproductively isolated host races as a result of local adaptation to both the host plant and to its natural enemies. We refer to these as the “prairie” and “forest” host races. Western and eastern subspecies of *E. solidaginis* (Ming 1989, Foote et al. 1993) have also been defined by differences in wing patterns (Fig. 2). The distribution of these eastern *Eurosta solidaginis solidaginis* and western *Eurosta solidaginis fascipennis* subspecies roughly corresponds with the forest and prairie biomes, respectively (Ming 1989), and so we refer to their wing patterns as “prairie” and “forest.” Determining the relationship between subspecies as defined by wing patterns (Foote et al. 1993), and the host races as defined by host plant adaptation (Craig and Itami 2011) requires further research. *S. altissima* is also geographically differentiated with Semple et al. (2015) defining a forest subspecies, *S. a. altissima*, and a prairie subspecies, *S. a. gilvocanescens*.

Local adaptation of both species in a coevolved interaction is a key prediction of the geographic mosaic of coevolution (Thompson 2005), and there is evidence of local reciprocal adaptation in both the *E. solidaginis–E. gigantea* and the *E. solidaginis–S. altissima* interactions. Gall diameters are larger (Craig et al. 2007, Craig and Itami 2011) and *E. gigantea* ovipositors longer in the prairie than forest (Craig et al. 2007) as the result of reciprocal selection for local adaptation. Gall diameter is determined by the interaction of fly genotype, plant genotype, and the environment (Weis and Abrahamson 1986). Natural-enemy selection on the gall diameter *E. solidaginis* induces varies geographically (Abrahamson et al. 1989, Weis et al. 1992, Craig et al. 2007, Craig and Itami 2011). Birds cause high mortality on larvae in large diameter galls, selecting for smaller diameter galls, and the parasitoid *E. gigantea* and the inquiline *M. convicta* cause high fly morality in small galls selecting for larger gall diameter. Birds are rare or absent in the prairie, and the parasitoid and the inquiline exert directional selection for the induction of larger gall diameters by *E. solidaginis* (Craig et al. 2007). *E. gigantea* are under selection to have ovipositors that can reach *E. solidaginis* larvae in center of galls, and they are therefore under selection for longer ovipositors in the prairie (Craig et al. 2007). In forests, birds selecting for smaller gall diameters and *E. gigantea* and *M. convicta* selecting for larger galls diameters are all present creating stabilizing selection on *E. solidaginis* gall
diameter induction (Abrahamson et al. 1989, Weis et al. 1992, Craig et al. 2007, Craig and Itami 2011), and as a result, there is also stabilizing selection on *E. gigantea* ovipositor length. These patterns indicate that the prairie is a coevolutionary hotspot for the *E. solidaginis*–*E. gigantea* interaction where there is selection on *E. solidaginis* for induction of increasingly larger gall diameters and selection on *E. gigantea* for increasingly longer ovipositor lengths. In contrast, the forest is a coevolutionary cold spot in the interaction with no directional selection on the gall diameter induced by *E. solidaginis*, and no directional selection on *E. gigantea* ovipositor length (Craig et al. 2007, Craig and Itami 2011; T. P. Craig and J. K. Itami, unpublished manuscript).

Fig. 1. Locations of sample sites in the eastern transect sampled in 2010 eastern transect (E) and 2011 western transect (W). Maps show pre-European settlement habitats. Plots of small-tile sites M and N and sites O and P overlap even in the enlarged portion of the map.
Local differences in the reciprocal adaptation of *E. solidaginis* and *S. altissima* have also been found. As described above, selection by natural enemies results in *E. solidaginis* populations that induce larger gall diameters in the prairie than in the forest. Since *E. solidaginis* galls negatively affect *S. altissima* fitness (Hartnett and Abrahamson 1979), this creates stronger selection for

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**Fig. 2.** Digital images of fly wings were measured for the extent of the proximal hyaline areas in cell r4+5 identified by Ming (1989) as varying between east and west *Eurosta solidaginis* populations. (a) Major wing cell and vein characteristics of *E. solidaginis* based on the ground pattern of dipteran wings developed by McAlpine et al. (1981). (b) Typical forest fly wing with no or only a small hyaline area in wing cell r4+5. (c) Typical prairie fly wing with hyaline areas forming a clear band across wing. (d) Standard hyaline measurement protocol for the hyaline area present in all fly wings in cell r4+5.

1. A straight line was drawn across the wing based on the angle of the inner edge of the hyaline area in cell r2+3, designated Line 1.
2. Line 2 was drawn perpendicular to Line 1 where it intersected with vein R4+5.
3. Line 3 was drawn perpendicular to Line 1 where it intersected with vein M.
4. Total R4+5 cell length was the length of line 1 between Line 2 and Line 3.
5. The hyaline length was the length of Line 1 between Line 2 and the beginning of the pigmented area indicated by the yellow arrow in (b). Partially unpigmented areas were not included in the hyaline length.
6. The hyaline ratio was the length of the R4+5 hyaline spot divided by the total length of the R4+5 cell. The ratio was 0.0 to 1.0.
resistance to gall induction by *S. altissima* in the prairie than in the forest. Craig and Itami (2011) found, in a common garden experiment where the prairie and forest host races of *E. solidaginis* were reared on both *S. a. altissima* and *S. a. gilvocanescens*, that the plant subspecies significantly influenced gall diameter. A subsequent double reciprocal transplant experiment was conducted where the prairie and forest host races of *E. solidaginis* were reared on both *S. a. altissima* and *S. a. gilvocanescens* in sites in the prairie and in the forest (T. P. Craig and J. K. Itami, *unpublished manuscript*). This study demonstrated that *S. a. gilvocanescens*, which are attacked by the prairie host race of *E. solidaginis* that induced large galls, were more resistant to gall growth than *S. a. altissima*, which are attacked by the forest host race of *E. solidaginis* that induces smaller galls. This indicates that the prairie is a coevolutionary hotspot where there is selection for increasing stimulus for gall growth by the fly, and selection for increasing resistance to gall growth by the plant. In contrast, the forest is a coevolutionary coldspot for the fly–plant interaction where neither gall stimulus by the fly nor resistance to gall growth by the plant is under directional selection.

Intermediate wing patterns in the central United States in a transition zone where the western and eastern subspecies of *E. solidaginis* meet (Ming 1989, Foote et al. 1993) indicate that it is a quantitative trait that can be used to measure gene flow. The western subspecies *E. s. solidaginis* has the prairie wing pattern that has an unpigmented band across the wing (Fig. 2). The eastern subspecies *E. s. fascipennis* has a forest wing pattern with a partially pigmented band divided into two distinct spots (Fig. 2). Intermediate wing patterns have been found between the eastern and western subspecies in the midwestern transition zone between the subspecies (Foote et al. 1993, Faust and Brown 1998, Brown and Cooper 2006) consistent with it being a heritable trait. T. P. Craig and J. K. Itami (*unpublished data*) found that in the region covered by this study that all flies from prairie sites distant from the prairie–forest biome border have an *E. s. solidaginis* prairie wing pattern, while all flies from forest sites distant from the prairie–forest border have a *E. s. fascipennis* forest wing pattern. Populations from the prairie–forest ecotone in Minnesota (Fig. 1) have intermediate degrees of pigmentation with a series of spots in the cells that form the pigmented band. When mating occurs within *E. solidaginis* subspecies, their offspring have the wing pattern of their subspecies, and when crosses between the subspecies are made, intermediate spotted bands are produced (T. P. Craig and J. K. Itami, *unpublished manuscript*). Although no studies have been published for Tephritidae, True et al. (1999) found that melanization of wing patterns in *Drosophila* species was a heritable trait. We conclude that there are strong indications that wing patterns are a heritable trait and thus can be used as indicator of gene flow between populations.

Gene flow between fly host races depends on a combination of intrinsic reproductive isolation between the prairie and forest host races and extrinsic geographic isolation. Prairie and forest fly host races are partially pre- and post-reproductively isolated due to host-plant adaptation (Craig and Itami 2011). Mating occurs on the host plant, and there is significant, but relatively weak, assortative mating due to host-plant preference. Fly host races had 64% assortative mating in the presence of the host plant, but there was no significant assortative mating in the absence of host plants. In addition, there is post-zygotic isolation between the prairie and forest populations, with hybrid survival being less than 25% of that of pure host races in an experiment (Craig and Itami 2011). Finally, immigrant inviability (Nosil et al. 2005) between fly populations also limits gene flow between populations because migrants have low survival on the non-natal plant subspecies (Craig and Itami 2011).

Geographic isolation also limits gene flow between the host races. *E. solidaginis* has a relatively low mean lifetime dispersal range, but a high potential for longer-range dispersal. Cronin et al. (2001) found in a mark and recapture study that 50% of females would have a lifetime dispersal within a patch of ≤50 m, and that 95% within a patch ≤130 m. However, much longer-range dispersal does occur. McCrea and Abrahamson (1987) removed all *E. solidaginis* by mowing a goldenrod field isolated by approximately 1 km from any other *E. solidaginis* habitat, and colonization restored the population to 63% of the pre-treatment population within a year.
Eurosta solidaginis gall diameters are adapted to the large-scale geographic selection mosaic imposed by large patches of prairie and forest habitats distant from the biome border (Craig et al. 2007). In this study, we tested the hypothesis that fly gall diameter and length are locally adapted to selection mosaics on a smaller spatial scale. No one has previously evaluated selection on gall length. We will refer to patches in the selection mosaic as tiles (Thompson 2005, Toju 2008), to be consistent with the mosaic analogy. We will refer to the distribution of gall traits as tiles in a “trait mosaic.” If the tiles in the trait mosaic are correlated with the tiles in the selection mosaic, then the trait distribution is adaptive, but if they are not correlated, then the distribution is maladaptive. At the biome border, there is a mosaic of small patches of prairie and forest habitats (Fig. 1) producing the potential for a small-tiled trait mosaic of coevolutionary hot spots for selection on gall morphology due to interactions with different natural enemies and host subspecies, and for trait remixing due to increased opportunities for gene flow. We examined variation on two scales: among local sites on the scale of a few kilometers, and across the entire transition from prairie to forest. Specifically, we asked: (1) What is the scale of the geographic selection mosaics of coevolution by natural enemies and plants on fly gall diameter, and length? (2) What are the geographical trait mosaics of gall diameters, gall lengths, and gall shapes? (3) Is there a correlation between the geographic mosaic of selection and the distribution of gall traits indicating local adaptation? (4) What is the distribution of a putatively neutral trait wing patterns: Does it indicate gene flow among sites?

**Methods**

**Study sites**

To measure selection mosaics by natural enemies and host plants on E. solidaginis, and the corresponding mosaics of fly traits where gene flow was facilitated by lack of geographical isolation we sampled populations at regular intervals across the prairie-forest ecotone in Minnesota and Iowa, USA. In order to sample areas where forest and prairie vegetation was intermixed on a small geographic scale, we chose locations for transects based on maps of pre-European settlement vegetation that were produced using data from Government Land Office surveys done at the time of European settlement in Minnesota (Marschner 1974) and Iowa (Anderson 1996). These maps provide remarkably detailed information on habitat distributions. We produced a new map (Fig. 1) based on this data and pooled the vegetation classifications into four categories: forest, prairie, wetland, and open water. Recent surveys by the Minnesota Department of Natural Resources (2005) confirm that these areas retain the mixture of forest, prairie, and savanna vegetation recorded on these maps. The Minnesota DNR surveyed a limited number of sites in detail, and extrapolated from these points to produce maps with broadly overlapping vegetation designations that do not allow us to determine their specific classification of vegetation at each of our sites. Consistent with the land office surveys, these maps indicate a mix of vegetation types throughout the entire region surveyed. The Minnesota DNR classified predominate types in this area as forested (southern mesic maple-basswood forest type, southern wet mesic hardwood forest), savanna (southern dry savanna and southern mesic savanna), and prairie (southern mesic prairie). During our collections, we observed a patchy mix of habitats with prairie and forest vegetation that roughly matched those on the land office maps, which were intermixed with disturbed land (T. P. Craig, personal observation). We ran transects through the center of prairie–forest transition where habitats were highly intermixed as indicated by the more detailed land office record maps (Fig. 1), and we have plotted the sites on those maps. These sites have a fine-grained mix of prairie and forest sites as indicated by both current and past surveys, but the dynamic nature of the prairie–forest ecotone boundary (Loehle et al. 1996, Ratajczak et al. 2014) and the decline of the sharp boundaries between forest and prairie vegetation (Broderick and Heilman 2018) mean that the current habitat at the sites where we collected galls may not precisely match the habitat descriptions on our map. However, all sources agree that these areas have a mix of prairie and forest plant species at a small geographic scale, which was our goal in selecting sites for this study.
We collected galls along East (E) and West (W) transects running from southern areas that were predominately prairie through an area where prairie and forest habitats formed a small-tiled mosaic to northern areas that were predominately forest (Fig. 1). We chose sites in areas where there were no strong geographical barriers to fly dispersal as with few gaps in their distribution as possible. The eastern transect followed the right of way between a road and a railway with nearly uninterrupted populations of *S. altissima*. The western transect were roadside populations. Using the Google Maps street view, we examined photographs taken in 2015, at every 0.1-km interval. Goldenrods were easily identifiable, and we found no gaps in visible goldenrod stands greater than 0.5 km.

We collected galls from 21 eastern transect sites in 2009, and five eastern micro-transects and eight western transect sites in 2010. Each sample site covered an area of less than 20 × 100 m but varied in size due to gall density. We walked a haphazard path parallel to the road collecting galls until we had collected at least 350 galls. We attempted to space the samples at 20 km intervals, but there was some variation due to the need to find sites containing sufficient numbers of galls in areas where we could obtain permission to collect. As a result, the eastern and western transects were spaced at (mean ± standard error [SE]) 19.89 ± 4.22 km and 20.11 ± 3.68 km, respectively. We conducted a second survey within the eastern transect (termed the micro-transect) to examine variation at an even smaller scale and these micro-transect sites were spaced (mean ± SE) 4.168 ± 0.688 km apart.

**Natural-enemy selection, gall morphology, and wing patterns**

To measure the selection mosaic by natural enemies and the distribution of coevolved traits, we measured gall length, gall diameter, and fly survival and calculated phenotypic selection on those traits. Galls were stored together in outdoor cages over the winter when flies were in diapause and then reared indoors the following spring. We measured gall length and diameter with dial calipers and divided diameter by length to obtain gall shape. Diameter was measured as the widest dimension of the gall, and length was determined as the point where the gall began to alter the curvature of the stem. After fly emergence, we dissected each gall and recorded fly emergence or cause of fly mortality. In spring 2011, we reared galls collected from the 2010 cohort in individual condiment cups in order to obtain the relationship between gall morphology and wing patterns.

To assess gene flow, we measured variation in fly wing pigmentation as described by Ming (1989) to distinguish between the prairie *E. s. solidaginis* wing pattern and the forest *E. s. fascipennis* wing pattern. We measured the ratio of non-pigmented area to pigmented area in the r4+5 cell (Fig. 2) and termed this the “hyaline ratio.” Prairie wings have an unpigmented r4+5 cell creating a hyaline band across the width of the wing, and forest wings have a largely pigmented r4+5 cell dividing the hyaline band into two discrete spots (Ming 1989); at the biome border, intermediate hyaline ratios are found (T. P. Craig and J. K. Itami, *unpublished manuscript*). The method for measuring the hyaline ratio is described in Fig. 2. Hyaline ratios were arcsine-square-root-transformed before analysis to normalize variance. Wing patterns have been hypothesized to play a role in reproductive isolation due to assortative mating in Tephritidae (Bush 1969), but there has been no support for this hypothesis (Sivinski and Pereira 2005). Wing hyaline ratio had no significant impact on mate preference in prairie and forest host race flies (Craig and Itami 2011), and we are assuming it to be a neutral trait.

**Selection analysis**

The covariance between a trait and the expected relative fitness of phenotypic traits such gall diameter can be used as a measure of expected natural selection on a trait (Brodie et al. 1995). Lande and Arnold (1983) proposed using multiple regression to do this, with the standardized regression coefficient, or beta function used as a measure of directional selection, and the gamma coefficient used to indicate nonlinear stabilizing or disruptive selection (Brodie et al. 1995). We used logistic regression as it is more appropriate for analysis of categorical responses such as fly survival or mortality (Brodie et al. 1995, Janzen and Stern 1998). The selection by natural-enemy analysis was completed on galls in which flies survived or were killed by natural enemies and the distribution of coevolved traits, we measured gall length, gall diameter, and fly survival and calculated phenotypic selection on those traits. Galls were stored together in outdoor cages over the winter when flies were in diapause and then reared indoors the following spring. We measured gall length and diameter with dial calipers and divided diameter by length to obtain gall shape. Diameter was measured as the widest dimension of the gall, and length was determined as the point where the gall began to alter the curvature of the stem. After fly emergence, we dissected each gall and recorded fly emergence or cause of fly mortality. In spring 2011, we reared galls collected from the 2010 cohort in individual condiment cups in order to obtain the relationship between gall morphology and wing patterns.

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enemies. We used gall diameter and length in our analysis because they directly influence fitness by determining vulnerability to natural enemies. We visualized the pattern of selection by using the best-fit regression model in Minitab 18 (Systat Software, San Jose, California, USA).

Geographic mosaics of selection by host plants
To determine distributions of S. altissima and S. a. gilvocanescens, we grew plants from 12 sites from the central section of our eastern transect in a common garden and then measured morphological traits to determine their subspecies. We collected seeds from the 2009 growing season, germinated the seeds in a greenhouse, and planted seedlings at the University of Minnesota Jean Duluth Research site in Duluth in 11.4-L pots filled with Promix BX commercial potting mixture. Plants were watered as needed and given one application of 20N-20P-20K soluble fertilizer and one application of 20N-20P-20K slow-release fertilizer pellets.

We constructed a discriminate functional analysis using morphological data reported by Craig and Itami (2011) to classify goldenrod plants as either forest S. a. altissima or prairie S. a. gilvocanescens, using the subspecies designations of Semple et al. (2015). We used data from S. altissima populations distant from the forest–prairie border to construct the discriminate function analysis. We measured the total height, stem diameter at 5 cm from soil level, leaf width and length of the 10th leaf below the apical meristem, and total number of leaves of the tallest ramet in each pot, which are a subset the traits used in the Semple et al. (2015) analysis. The discriminate function correctly identified 72.2% of the plants in Craig and Itami (2011) as being from prairie or forest. We then used this discriminate function, to calculate a subspecies index score for plants in this study. Higher index scores indicate plants that had more prairie, or S. a. gilvocanescens characteristics, and lower scores indicated plants with more forest or S. a. altissima, characteristics.

Statistical analysis
All analyses were completed using Minitab 18 Statistical Software. The specific analysis utilized is listed in the methods description and results for each analysis.

RESULTS
Plant selection mosaic
Plant morphology.—There is a fine-tiled mosaic of the S. altissima subspecies (Fig. 3) with no significant correlation between latitude and the subspecies index score (Table 1). The plant subspecies index score showed significant differences among sites, and adjacent sites were frequently significantly different from each other (Fig. 3, ANOVA $F_{10,1032} = 8.42$, $P < 0.0001$). Plants within sites varied widely in their traits as indicated by the wide range of subspecies scores within sites (Fig. 3), and plants showing predominately prairie and forest characters often co-occurred in the same site. For example, sites M-E and R-E had significantly different mean subspecies scores, with each site having plants classified as having 100% probability of coming from both prairie and forest populations.

Natural-enemy selection mosaic
Selection mosaic on gall diameter and gall length by natural-enemy mortality.—Selection by natural enemies on gall diameter (Table 2) and gall length (Table 3) varied strongly along transects with sharp shifts in the strength of directional and stabilizing selection over short distances along transects. These differences in selection can be visualized in the site survival curves in relation to gall diameter (Fig. 4) and gall length (Fig. 5).

Gall morphology and wing patterns
Gall diameter, gall length, gall shape, and hyaline ratio differed significantly among sites along the eastern transect (one-way ANOVA, gall diameter Fig. 6a, $F_{19,3801} = 54.15$, $P < 0.0001$, gall length Fig. 6b, $F_{19,3801} = 28.47$, $P < 0.0001$, gall shape Fig. 6c, $F_{19,3801} = 57.92$, $P < 0.0001$, hyaline ratio Fig. 6d, $F_{17,1813} = 57.08$, $P < 0.0001$). At a smaller scale among the eastern micro-transect sites, these traits also differed significantly (ANOVA, gall diameter Fig. 7a, $F_{4,369} = 52.58$, $P < 0.0001$; gall length Fig. 7b, $F_{4,369} = 19.81$, $P < 0.0001$; gall shape Fig. 7c, $F_{4,369} = 16.31$, $P < 0.0001$; wing pattern Fig. 7d, $F_{4,437} = 271.86$, $P < 0.0001$). These traits also differed significantly along the western transect (gall diameter Fig. 8a, $F_{7,810} = 23.61$, $P < 0.0001$; gall length Fig. 8b, $F_{7,810} = 8.19$, $P < 0.0001$; gall
shape, Fig. 8c, $F_{7, 810} = 43.96, P < 0.0001$; hyaline ratio Fig. 8d, $F_{7, 135} = 35.21, P < 0.0001$). There was no correlation of any of the gall traits with decreasing latitude, which is associated with an increasing proportion of prairie habitat (Fig. 1). In either transect, such a correlation would have indicated a cline (Table 1). Instead, there was a geographic mosaic of patches with abrupt and statistically significant shifts between adjacent sites on a small geographic scale, as indicated by Tukey’s multiple range tests (Figs. 6–8, Table 4). For example, fly populations at site pairs H-E and I-E are 6.82 km and M-E and R-E are 15.92 km apart, respectively (Fig. 1), but they are significantly different from their neighbors in gall diameter, gall length, gall shape, and wing patterns (Fig. 6).

Hyaline ratios were non-normally distributed in both the eastern and western transects with larger-than-expected number of individuals at the extremes with a lower number of intermediates than would be expected from a normal distribution (Fig. 9a, b). In the eastern transect, hyaline ratios significantly departed from a normal distribution (Anderson-Darling normality test $= 92.489, P < 0.005, n = 2207$) with an excess of individuals with 0% or 100% pigment in the hyaline area. The western transect showed a similar pattern with hyaline ratios significantly departing from normality (Anderson-Darling normality test $= 2.677, P < 0.005, n = 209$), with an even greater excess of complete hyaline bands.

**Relationship between gall trait mosaic and selection mosaic**

To determine whether there was a correspondence between the selection mosaics for gall diameter and length and the site mosaics of gall diameter and length, we regressed observed gall morphology at a site on the gall diameter or length at a site where fitness was highest. If these were correlated, it would indicate that there was local adaptation by *E. solidaginis* due to selection by natural enemies on gall traits. We found a highly significant relationship between observed gall

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**Table 1. Correlation and P-values of site means for gall traits and latitude for East, East microsite, and West transects. Subspecies index was measured at 12 sites on the eastern transect.**

| Trait          | Latitude | Gall shape | Gall diameter | Gall length | Hyaline ratio |
|----------------|----------|------------|---------------|-------------|---------------|
| Gall shape     | −0.215   |            |               |             |               |
| Gall diameter  | −0.225   | 0.732***   |               |             |               |
| Gall length    | −0.070   | −0.166     | 0.549*        |             |               |
| Wing ratio     | −0.120   | 0.744***   | 0.505*        | −0.196      |               |
| Subspecies index | −0.131 | 0.604*     | 0.682*        | 0.443       | 0.743**       |

*P < 0.05, **P < 0.01, ***P < 0.001."
Table 2. Equations for the best-fit curves for the relationship between gall diameter and larval survival to emergence, and the chi-square values from logistic regression analysis on the significance of directional and non-directional selection on gall diameter at each site from all transects.

| Site | Equation | $\chi^2$ directional selection | $\chi^2$ non-directional selection |
|------|----------|-------------------------------|-----------------------------------|
| A-E  | $y = -1.448 + 0.1780x - 0.03947x^2$ | 7.11** | 4.31* |
| B-E  | n.s. | n.s. | n.s. |
| C-E  | $y = -3.526 + 0.4736x - 0.01411x^2$ | 9.59** | 10.68*** |
| D-E  | n.s. | n.s. | n.s. |
| E-E  | $y = -0.417 + 0.0128x - 0.000167x^2$ | 28.55*** | n.s. |
| F-E  | $y = 3.291 + 0.38x - 0.00841x^2$ | 28.3*** | n.s. |
| G-E  | $y = -6.118 + 0.6994x - 0.01787x^2$ | 12.76*** | 11.57*** |
| H-E  | $y = -5.116 + 0.8171x + 0.03245x^2$ | 50.52*** | n.s. |
| I-E  | $y = -0.7495 + 0.07176x$ | 30.08*** | n.s. |
| J-E  | $y = -6.777 + 0.7460x - 0.017296x^2$ | 62.69*** | n.s. |
| K-E  | $y = -5.032 + 0.5945x - 0.01485x^2$ | 40.52*** | n.s. |
| L-E  | $y = -7.5976 + 0.8336x - 0.020227x^2$ | 8.47** | 5.38* |
| M-E  | $y = -1.424 + 0.09732x$ | 14.92*** | 10.82 |
| N-E  | $y = -6.912 + 0.8374x - 0.02364x^2$ | 3.40 | 6.60** |
| O-E  | $y = -0.428 + 0.1490x - 0.004686x^2$ | 14.54*** | 14.138*** |
| P-E  | $y = -14.45 + 1.62231x - 0.04343x^2$ | 4.54* | 4.09* |
| Q-E  | $y = -11.07 + 1.223x - 0.03087x^2$ | 35.84*** | n.s. |
| R-E  | $y = 0.1484 + 0.0897x - 0.002658x^2$ | 5.01* | 4.58* |
| S-E  | $y = -7.895 + 1.029x - 0.03004x^2$ | 5.27 | 4.25* |
| T-E  | $y = -6.646 + 0.8752x - 0.025512x^2$ | 19.34*** | 19.56*** |
| U-E  | n.s. | n.s. | n.s. |
| V-E  | n.s. | n.s. | n.s. |
| W-E  | $y = -2.560 - 0.2181x + 0.005254x^2$ | 28.35*** | n.s. |
| A-W  | $y = 2.223 - 0.2846x + 0.009101x^2$ | 40.32*** | n.s. |
| B-W  | $y = -0.3934 + 0.06817x$ | 9.71** | n.s. |
| C-W  | $y = -0.4817 + 0.08274x$ | 9.71** | n.s. |
| D-W  | $y = 1.502 + 0.1306x$ | 8.79 | 7.75** |
| E-W  | $y = 5.279 - 0.6168x + 0.01788x^2$ | 11.10** | 8.72** |
| F-W  | $y = 2.715 - 0.3335x - 0.009028x^2$ | 3.40 | 3.85* |
| G-W  | $y = -0.2440 - 0.03202x$ | 6.03 | n.s. |
| H-W  | $y = -3.725 + 0.4608x - 0.01240x^2$ | 8.24** | 6.95* |

Note: n.s., non-significant.  
*P < 0.05, **P < 0.01, ***P < 0.001.

diameter and the gall diameter where survival was highest for each site ($y = 9.428 + 0.4874x$, $F_{1,26} = 16.11$, $P < 0.0001$, $r^2 = 0.38$ Fig. 10a). There was a slightly negative, but significant, relationship between the gall length at a site with the highest survival rate and gall length at the site (Fig. 10b, $y = 33.30 - 0.2427x$, $F_{1,26} = 4.31$, $P < 0.048$, $r^2 = 0.14$).

Correlations among traits  
For individual flies, hyaline ratio, gall diameter, gall length, and gall shape were all significantly correlated except for the correlation of gall length and shape (Table 5, Fig. 11a). Gall diameter was a significant predictor of hyaline ratio with the polynomial model ($y = 0.855 + 0.077x + 0.002813x^2$, $r^2 = 28.9\%$, $P < 0.0001$, $n = 158$) being a better fit than the linear model (Akaike’s information criterion [AIC] linear = 33.78, AIC quadratic = 26.3).

Gall diameter was significantly correlated to mean gall length, mean shape, and mean hyaline ratio, in both the eastern and western transects (Table 1). Gall length was not significantly correlated with any other trait except gall diameter.

Gall diameter was a highly significant predictor of hyaline ratio among sites with a polynomial model (Fig. 11b, $y = 4.76 - 0.571x + 0.01789x^2$, $P < 0.0001$, $r^2 = 0.48$, $n = 29$) being a better fit than the linear model (AIC...
linear = −2.97, quadratic −7.43). The curvilinear relationship indicates that sites can be roughly divided into three groups: a small diameter group with small hyaline ratios, an intermediate gall diameter group with a wide variation in hyaline ratios, and group with large gall diameters and large hyaline ratios. We interpret this as representing in order, pure forest host race flies, hybrids between the forest and prairie races, and pure prairie host race flies.

**Discussion**

*Eurosta solidaginis* showed evidence of small-scale local adaptation in gall diameter, but not gall length, due to geographic mosaics of coevolution with its natural enemies and its host plant. There is evidence of gene flow among locally adapted populations that may have influenced local adaptation. This local adaptation to the selection mosaic imposed by natural enemies supports a key assumption of geographic mosaic of coevolution theory (Thompson 2005). The overall pattern of variation is consistent with the hypothesis that there is a mosaic hybrid zone between forest and prairie *E. solidaginis* host races.

**Adaptive gall diameter geographic mosaic**

Local adaptation was indicated by correlation between the mean gall diameter at a site and the diameter with the highest fly survival at the site.
Fig. 4. Relationship between gall diameter and the proportion of *Eurosta* survival on the eastern and western transect surveys. Sites vary showing directional, stabilizing or little selection. Axes scales are 0.0–1.0 for proportion survived and 12–24 mm for gall diameter.
Fig. 5. Relationship between gall length and the proportion *Eurosta* survival on the eastern and western transect surveys. Sites vary, showing directional, stabilizing or little selection. Axes scales are 0.0–1.0 for proportion survived and 14–38 mm for gall length.
Selection by natural enemies on gall diameter differed in patches that were intermixed on a scale of a few kilometers producing a small-tiled selection mosaic, and this covaried with the distribution of gall diameters that also formed a small-tiled trait mosaic. The high frequency of intermediate hyaline ratios indicates gene flow among fly populations in different habitat tiles. The strong differentiation of gall diameters among adjacent sites indicates that selection is stronger than gene flow. Gall diameter differed as strongly among geographically adjacent sites in this small-tiled geographic selection mosaic scale as it did in the much larger habitat tiles of forest and prairie habitat away from the biome border where geographic isolation of the fly host races was much larger (Craig et al. 2007).

Host-plant variation among sites also influenced gall diameter as indicated by the significant positive correlation between gall diameter and *S. a. gilvocanescens* plant characteristics. Our data are consistent with the hypothesis that prairie host race flies that induce larger galls

Fig. 6. Box and whisker plots of sites on the eastern transect for (a) gall diameters, (b) gall length, (c) gall shapes, and (d) fly wing hyaline ratio with additional outlier points plotted as closed circles. Site means indicated by plus symbols. Tukey’s multiple range test values for site means are given in Table 4.
oviposited more frequently on *S. a. gilvocanescens*, and forest host race flies that induce smaller galls oviposited more frequently on *S. a. altissima* (Craig and Itami 2011). The positive correlation of plants with *S. a. gilvocanescens* characteristics and flies with prairie hyaline ratios supports this hypothesis. Prairie fly oviposition on *S. a. gilvocanescens*, and forest fly oviposition on *S. a. altissima* is adaptive because survival of each host race is higher on their “own” subspecies than on the “alternate” subspecies (Craig and Itami 2011). Craig and Itami (2011) showed that this occurs even though *S. a. gilvocanescens* is more resistant to gall diameter growth than *S. a. altissima* indicating the strong influence of the fly on gall diameter.

In contrast to gall diameter, gall lengths showed a lack of local adaptation to natural-enemy selection. As in gall diameter there was a small-tiled natural-enemy selection mosaic on gall length, and a small-tiled gall length-trait mosaic. However, the mean gall lengths were slightly negatively correlated to the gall lengths with the highest fitness. This lack of local adaptation could be the result of trait remixing or interactions of the fly with the host plant. Gene
flow among sites could produce trait remixing of gall lengths adapted to different natural-enemy communities resulting in maladaptation. The combination of local adaptation in gall diameter and maladaptation in gall length would require that the balance of gene flow and selection differ in these traits. Why this would occur is not obvious since there are small-tiled selection mosaics of similar strengths for both traits, and we would expect gene flow to be similar for both traits.

An alternate hypothesis to explain the distribution of gall lengths is that there is a lack of heritable variation between fly host races for gall length and that plant variation primarily determines gall length. Gall diameter is a heritable trait of *E. solidaginis* (Weis and Abrahamson 1986), but the heritability of gall length has not been measured. An experiment where three geographic fly populations of *E. solidaginis* were reciprocally placed on *S. altissima* plants from these populations showed gall length was...
influenced only by plant origin and not by fly origin (T. P. Craig and J. K. Itami, unpublished data). This indicates a lack of genetic differences among fly populations in the gall length they induce. Therefore, the differences in gall length found in this study could be predominately due to differences among sites in host plants. Sites were highly variable in their plant subspecies index, but this was not correlated with gall length. However, our subspecies index may not measure the plant variation that influences gall length, and further research is needed to understand how plant subspecies variation influences gall length.

We found strong geographic variation in gall shape and hypothesize that these differences result from an interaction of gall diameter, which is strongly influenced by *E. solidaginis* genetic variation resulting from local selection by natural enemies, and gall length that is strongly influenced by variation between the *S. altissima* subspecies. Since gall diameter is predominately determined primarily by the fly and gall length predominately by the plant, then differences among sites result from the interaction of natural-enemy selection on gall diameter and variation in the host plant for gall length producing variation in gall shape. In the larger prairie and forest tiles, selection for adaptation to natural enemies and selection for adaptation to the host plant subspecies are correlated (Craig and Itami 2011). Spherical galls are found in the prairie because the prairie fly host race induces galls with large diameters and long lengths, and they oviposit on *S. a. gilvocanescens* that produces shorter gall lengths than *S. a. altissima*. Ellipsoid galls are found in the forest where the forest fly host race induces intermediate gall diameters and long lengths, and *S. a. altissima* produce longer gall lengths than *S. a. gilvocanescens*. However, in the small-tiled geographic mosaic, different combinations of host plant subspecies and fly host races may occur producing different gall shapes. In addition, gene flow among the

| Site | N   | Gall diameter† | Gall length‡ | Gall shape§ | Wing ratio¶ | Probability of being prairie plant# |
|------|-----|----------------|--------------|-------------|-------------|-------------------------------------|
| A    | 65  | 18.48 cdef     | 25.01 efg    | 0.75 cde    | 0.16 def    |
| B    | 50  | 18.07 efghi    | 22.52 j      | 0.82 a      | 0.75 a      |
| C    | 36  | 19.53 bc       | 28.93 a      | 0.68 gh     | 0.07 f      |
| D    | 13  | 18.91 bcddef   | 27.93 ab     | 0.68 gh     | 0.19 cdef   |
| E    | 84  | 19.97 b        | 24.83 fghi   | 0.81 ab     | 0.81 a      |
| F    | 148 | 19.53 bc       | 27.87 ab     | 0.71 fg     | 0.27 cd     |
| G    | 75  | 19.35 bcd      | 26.03 cdefg  | 0.75 cde    | 0.21 cdef   | 72 | 0.45 abc |
| H    | 51  | 13.61 k        | 23.36 hij    | 0.59 i      | 0.23 cdef   | 149 | 0.28 de |
| I    | 138 | 19.81 b        | 25.55 defg   | 0.78 abc    | 0.58 b      | 150 | 0.52 ab |
| J    | 238 | 18.12 efghi    | 23.10 ij     | 0.82 a      | 0.25 cde    |
| K    | 182 | 17.83 fghi     | 24.71 ghi    | 0.73 def    | 0.24 cde    | 60 | 0.26 de |
| L    | 106 | 19.11 bcd      | 24.84 fghi   | 0.77 bc     | 0.28 cd     | 25  | 0.26 cde |
| M    | 209 | 21.64 a        | 26.84 bcde   | 0.82 a      | 146 | 0.58 a |
| O    | 100 | 19.21 bcd      | 26.93 bcd    | 0.72 efg    | 0.30 c      | 65  | 0.36 cde |
| R    | 205 | 15.65 j        | 22.79 j      | 0.69 gh     | 164 | 0.25 e |
| S    | 145 | 17.02 i        | 22.66 j      | 0.76 cd     | 0.20 cdef   | 69  | 0.31 cde |
| T    | 139 | 17.74 ghi      | 25.91 cdefg  | 0.70 fgh    | 0.17 ef     | 67  | 0.34 cde |
| U    | 133 | 17.28 hi       | 25.55 defg   | 0.68 gh     | 0.17 ef     | 80  | 0.40 bcd |
| V    | 41  | 17.81 fghi     | 27.32 abc    | 0.66 h      | 0.17 cdef   |
| W    | 87  | 18.35 delgh    | 26.37 bcd    | 0.70 fg     | 0.19 cdef   |

Notes: Means that do not share a lowercase letter are significantly different. In general, prairie traits have the low Tukey’s letters and forest have the high Tukey’s letters.
† Mean for all sites = 18.35.
‡ Mean for all sites = 25.55.
§ Gall shape is gall diameter divided by gall length; spherical prairie galls have a ratio closest to 1.0.
¶ Prairie flies wing hyaline ratio is 1.0, Forest flies hyaline ratio is <0.5, and intermediates exist between.
# Probability of being a prairie plant for all sites, mean = 0.5.
subspecies host plants and the fly host races may contribute to shape variation. It is also possible that shape itself is under selection. For example, birds could have a preference for particular gall shapes exerting selection on gall shape, but this hypothesis has not been tested.

The continuous variation in wing hyaline ratios in *E. solidaginis* populations at the prairie–forest ecotone supports the hypothesis that this is a polygenic character (Brown and Cooper 2006) and that there is gene flow between the prairie and forest host races that may correspond to the

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Fig. 9. Pooled frequency of hyaline ratios from (a) eastern transect sites including microsites and (b) western transect sites from 2011. Bar colors give a sense of gene flow, pure prairie is dark orange, pure forest is dark blue, prairie leaning intermediates are light orange, forest leaning intermediates are light blue, and intermediates are yellow.

Fig. 10. Regression analysis of observed gall morphology for each site on the gall morphology with highest fitness for each site. (a) Mean gall diameter and (b) mean gall length.
western *E. s. solidaginis* and eastern *E. s. fascipennis* subspecies defined by wing patterns. Local populations are a mix of pure prairie race individuals with an unbroken hyaline band across their wings, pure forest host race individuals with a large intervening pigmented area between hyaline wing spots, and hybrids between the host races with intermediate wing patterns. Instead of the normal distribution of wing patterns expected for high gene flow homogeneous populations, we found significantly non-normal distributions of wing hyaline ratios with higher than expected numbers of forest (0.0 ratio) and prairie (1.0 ratio) wing patterns. Populations varied in the degree of gene flow between the host races as indicated by this marker, and there is wide variation in hyaline ratios in a small geographic area. The low, but significant, correlation between the adaptive trait of gall diameter and the putatively neutral trait of hyaline ratio indicates that gene flow has reduced, but not eliminated, the association between these polygenic traits seen in the larger tile of prairie and forest biomes.

The correlation between wing patterns and gall morphology indicates that the prairie and forest geographic host races (Craig and Itami 2011) and the subspecies of *E. solidaginis* defined by wing patterns (Ming 1989, Foote et al. 1993) may be the same entities. The geographic host races proposed by Craig and Itami (2011) resulted from adaptation to both the host plant and natural enemies while the wing patterns are assumed to be a neutral trait. Therefore, further research on host preference is needed to establish the correlations among all three traits. If all three traits are all correlated, then wing pattern differentiation could have evolved as the result of genetic drift in prairie and forest *Eurosta* populations that are partially reproductively isolated due to adaptation to geographic variation in *S. altissima*, and natural enemies.

**Evidence for a mosaic hybrid zone**

Our results indicate that there is a mosaic hybrid zone between the prairie and forest host races of *E. solidaginis* on *S. altissima*. Two kinds of hybrid zones exist: clinal and mosaic (Harrison 1990, Ross and Harrison 2002). Clinal

![Fig. 11. (a) Regression of wing hyaline ratio, the proportion of pigment in wing cell r4+5 of individually reared flies on the diameter of their gall. (b) Regression of mean wing hyaline ratio per site on the mean gall diameter at each site.](image)

Table 5. Pearson correlation and *P* values of traits from individual flies reared from 10 sites sampled in 2011.

| Trait       | Wing ratio | Gall diameter | Gall length |
|-------------|------------|---------------|-------------|
| Gall diameter | 0.494***   |               |             |
| Gall length  | 0.315***   | 0.764***      |             |
| Gall shape   | 0.316***   | 0.586***      | -0.054      |

*P < 0.05, **P < 0.01, ***P < 0.001.
hybrid zones occur when populations come in contact and hybridize to form a smooth transition between parental types. In contrast, mosaic hybrid zones consist of a patchwork of alternating pure populations with rapid transitions in characters of individuals and populations through space. *E. solidaginis* forest and prairie host race populations clearly exhibit the second pattern with abrupt changes in traits among patches and lack of a cline over the forest–prairie transition. Hybrid zones can be mosaic when examined on a fine scale and clinal when examined on larger scale (Ross and Harrison 2002). However, we found no indication of a larger clinal scale as there was no correlation with latitude which is correlated with change in the ratio of prairie to forest habitat.

Mosaic hybrid zones are maintained by extrinsic selection in a heterogeneous environment such as we found in this study. Our data indicate that geographic selection mosaics on gall traits are determined by the mosaic of prairie and forest habitats that differ in host plant subspecies and natural enemies (Craig et al. 2007, Craig and Itami 2011, this study). The small-tiled distribution of these habitats in the prairie–forest ecotone may produce a variety of combinations in the selection mosaics by the natural-enemy community and the host plants. These selection mosaics are correlated in the larger tiles of prairie and forest (Craig et al. 2007), but they may not be in the smaller habitat tiles in the ecotone where prairie and forests habitats are intermixed. In the prairie, *E. solidaginis* on *S. a. gilvocanescens* experience directional selection for larger galls by the parasitoid, and the inquiline, while in the forest, flies on *S. a. altissima* experience stabilizing selection due to the combination of birds, parasitoids, and inquilines. When prairie and forest habitats are closely intermixed either naturally, or due to human habitat alteration, then trees may be growing adjacent to habitats with *S. a. gilvocanescens* increasing bird predation on flies on these plants. Conversely, human removal of trees from previously forested areas could reduce bird predation of flies on *S. a. altissima*. This produces combinations of top-down and bottom-up selection mosaics not seen in the large-tiled mosaics of prairie and forest habitats. This variation in extrinsic selection combined with gene flow may produce flies with a range of intermediate traits between the prairie and forest host races in the mosaic of habitats in the prairie–forest ecotone. The evolution of gall morphology in mosaic hybrid zone may be the result of evolution within each host race, gene flow between the host races, or combination of the two.

In addition to the mixing of selection mosaics, gene flow between *S. a. gilvocanescens* and *S. a. altissima* in the prairie–forest ecotone may produce plants with intermediate characters that influence gall diameter and length. Our results indicate variation among sites among plants in their subspecies index that may indicate gene flow between the plant subspecies, but the degree of reproductive isolation among these subspecies is poorly understood as is its impact on *Eurosta* gall morphology.

**Reciprocal evolution in other species**

For coevolution to occur there must be reciprocal variation in the interacting species. We predict that the local variation in *E. solidaginis* should impact the local coevolved traits of the other species in its interactions with *S. altissima* and the parasitoid *E. gigantea* as had been found on broader geographic scale (Craig et al. 2007, Craig and Itami 2011; T. P. Craig and J. K. Itami, unpublished data), but we did not measure their local variation in this study. The coevolution of traits of the host plants and parasitoid species with *E. solidaginis* will again depend on the interplay of gene flow and the strength of selection in these species, which may differ from those that found in *E. solidaginis*.

**Conclusion**

We found that a geographic mosaic of *E. solidaginis* traits was produced by local coevolution with both its natural enemies and its host plants. By examining coevolution among pairs of species in a tritrophic interaction, we were able to identify the forces leading to locally adapted and maladapted of *E. solidaginis* traits at small geographic scales in the presence of gene flow. Our results agree with those of other recent studies (Rey et al. 2006, Toju 2011, Vergara et al. 2013) that indicate that coevolution may be a powerful force producing genetic variation in interacting species at small spatial scales. This new recognition of the importance of small-tile
coevolutionary diversification has important implications for studies of the interactions of ecological and evolutionary processes. It indicates that misleading conclusions can be drawn about species and their interactions from either pooling results from large areas or by considering results only from a single location. It also has conservation implications as even small-scale disturbances can obliterate unique combinations of coevolved interactions.

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