doi:10.1111/evo.13

SSE
SOCIETY for the STUDY of EVOLUTION

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Consumer-resource interactions along urbanization gradients drive natural selection

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Received April 5, 2018
Accepted June 24, 2018

Urbanization is an important component of global change. Urbanization affects species interactions, but the evolutionary implications are rarely studied. We investigate the evolutionary consequences of a common pattern: the loss of high trophic-level species in urban areas. Using a gall-forming fly, *Eurosta solidaginis*, and its natural enemies that select for opposite gall sizes, we test for patterns of enemy loss, selection, and local adaptation along five urbanization gradients. *Eurosta* declined in urban areas, as did predation by birds, which preferentially consume gallmakers that induce large galls. These declines were linked to changes in habitat availability, namely reduced forest cover in urban areas. Conversely, a parasitoid that attacks gallmakers that induce small galls was unaffected by urbanization. Changes in patterns of attack by birds and parasitoids resulted in stronger directional selection, but loss of stabilizing selection in urban areas, a pattern which we suggest may be general. Despite divergent selective regimes, gall size did not very systematically with urbanization, suggesting but not conclusively demonstrating that environmental differences, gene flow, or drift, may have prevented the adaptive divergence of phenotypes. We argue that the evolutionary effects of urbanization will have predictable consequences for patterns of species interactions and natural selection.

KEY WORDS: Cities, eco-evo, *Eurytoma gigantea*, parasitoid, predator-prey, phenotypic selection.

Urban environments are an increasingly dominant component of our landscapes. More than half of all humans now live in urban areas, and urbanization is increasingly being seen as an important driver of global environmental change (Seto et al. 2010). Urban and nonurban areas differ in critical ways. Urban environments tend to be warmer, environmentally homogeneous, and support less diverse ecological communities, with some species being more sensitive to urbanization than others (Seto et al. 2010; Alberti 2015). All of these changes should alter the selective pressures species encounter in urban environments (Johnson et al. 2015; Johnson and Munshi-South 2018). However, while the ecological consequences of urbanization are being well studied, the evolutionary impacts of cities remain largely unexplored (Donihue and Lambert 2014; Johnson and Munshi-South 2018).

Urbanization has been shown to mediate selection in response to various environmental changes. For example, *Crepis sancta* evolved to produce nondispersing seeds in response to small and fragmented habitats in urban areas (Cheptou et al. 2008). Other studies have implicated the materials used to build cities (Winchell et al. 2016), light pollution (Altermatt and Eibert 2016), and temperature (Thompson et al. 2016) as drivers of selection and ultimately local adaptation (for a review see Johnson and Munshi-South 2018). These studies have one commonality: they implicate changes in abiotic conditions as causes of selection and adaptation. However, urban, suburban, and rural areas often differ widely in the species and communities they support (McPhearson et al. 2016). Given that changes in communities can create strong selective pressures, biotic differences along a gradient from rural to downtown core areas are likely to create divergent selective regimes (Johnson et al. 2015; Johnson and Munshi-South 2018). Despite these clear links, surprisingly few studies have probed the evolutionary effects of community change in urban landscapes (Johnson and Munshi-South 2018), and all have focused on plant-pollinator interactions (Irwin et al. 2014).

One of the most pervasive effects of urbanization is changing trophic structure (Faeth et al. 2005) through the loss of high trophic-level species (Christie et al. 2010; Raupp et al. 2010). Indeed, changes in the abundance of high trophic level species across urban-rural gradients have been implicated as determinants of herbivore abundance, and ultimately herbivory (Christie et al. 2010). In short, urbanization can affect species through both direct environmental changes and indirect effects mediated by changes in community composition and species interactions (Johnson et al. 2015; Johnson and Munshi-South 2018). Given that species interactions and particularly consumer-resource interactions are thought to be strong selective forces (Benkman 2013), such changes may result in divergent selective regimes in more and less urbanized areas.

The effects of urbanization on consumer-resource evolution can depend on how high-trophic level species respond to the urban environment. In the simplest case, urbanization may reduce the abundance of a consumer (Christie et al. 2010), lessening their selective effect. If consumer defenses have a cost (Peckarsky and McIntosh 1998; Sih et al. 1998), then selection should favor consumer-resistant phenotypes in rural areas, and poorly defended but more competitive phenotypes in urban areas. However, a species is often attacked by several enemy species, each of which may respond differently to urbanization (Christie et al. 2010; Raupp et al. 2010) and select for different, even opposing phenotypes (W and Abrahamson 1985). In this case, urbanization should favor phenotypes that are well defended against the consumer least sensitive to urbanization (the most common enemy in urban areas), causing directional selection for those phenotypes. Similarly, the loss of one or both consumers selecting for opposite phenotypes can cause a breakdown of stabilizing selection (Weis and Abrahamson 1985). This effect can occur if only one enemy declines in response to urbanization, or if both enemies decline but one does so to a greater extent than the other, leading to an imbalance of opposing selection pressures. Selection across urban-rural gradients should track the density and phenotypic preferences of a species' natural enemies.

Enemies may also cause phenotypic differentiation among populations (Debinski and Holt 2000; Start and Gilbert 2016). Phenotypic shifts can occur when selection is able to overcome gene flow, drift, and correlated environmental differences among populations (Slatkin 1987). While these conditions appear to be commonly met in urban environments (Johnson et al. 2015), they are by no means universal and should be subject to the dispersal ability and population sizes of the evolving species (Slatkin 1987; Debinski and Holt 2000). Ultimately, local adaptation depends on the balance of selection, gene flow, and drift, all of which may be altered by urbanization (Johnson and Munshi-South 2018) but whose effects are likely to depend on the idiosyncrasies of the evolving species.

This article tests how urbanization affects the ecology and evolution of consumer-resource interactions. We use patches of goldenrod (*Solidago altissima*) that support gall-forming parasites (*Eurosta soladiginis*) that in turn are attacked by a community of bird predators and insect parasitoids (Weis and Kapelinski 1994). Previous work has shown that gall size of *Eurosta solidaginis* is under stabilizing selection when a parasitoid and bird predators are present, with the parasitoid and birds selecting for large and small galls, respectively (Weis and Abrahamson 1985; Weis and Abrahamson 1986). We quantified survival, frequency of attack, gall size (trait distributions), and estimates for phenotypic selection on gall size for > 8900 galls in 107 populations across five cities. Our study tested the above ideas by hypothesizing that (i) *Eurosta* will be less abundant in downtown cores, (ii) *Eurosta* attack by parasitoids and birds will decline in urban areas where appropriate habitat has been largely destroyed, but the rate of decline will depend on changes in the habitat availability of each species, (iii) the changes in bird and parasitoid attack will increase directional selection on gall size, but reduce stabilizing selection in urban environments, and (iv) changes in selection on gall-size will cause morphological shifts toward poorly defended phenotypes in urban areas.

Methods

STUDY SYSTEM

Goldenrod is a common old-field plant found throughout much of eastern North America (Root 1996). Goldenrod is attacked by *Eurosta,* fly whose larvae form a spherical stem gall (Uhler 1951). Adults emerge from galls, mate, and oviposit in late May in southern Ontario. The size of the gall is partly determined by *Eurosta* genotype, but is also affected by the genotype and quality of the plant, as well as the timing of gallmaker attack (Weis and Abrahamson 1985; Horner et al. 1999). Nevertheless, gall size is partly heritable, meaning that differences in selection detected in observational surveys may have implications for the course of phenotypic evolution. We may equally expect that selection may drive local adaptation, creating the possibility that observational approaches coupling measurements of selection and phenotype could provide evidence for adaptive evolution (Craig et al. 2007; Start and Gilbert 2016).

Galls are attacked by two species of parasitoid wasp (*Eurytoma sp.*) and *Mordellistena* beetles (Weis and Kapelinski 1994) in early summer, and are attacked by two species of bird (downy woodpeckers and chickadees) starting in late autumn, and through the early spring (Uhler 1951). Of these enemies, birds and *E. gigantea* preferentially attack galls of specific sizes (*Mordellistena* and *E. obtusiventris* do not favor galls of different sizes).

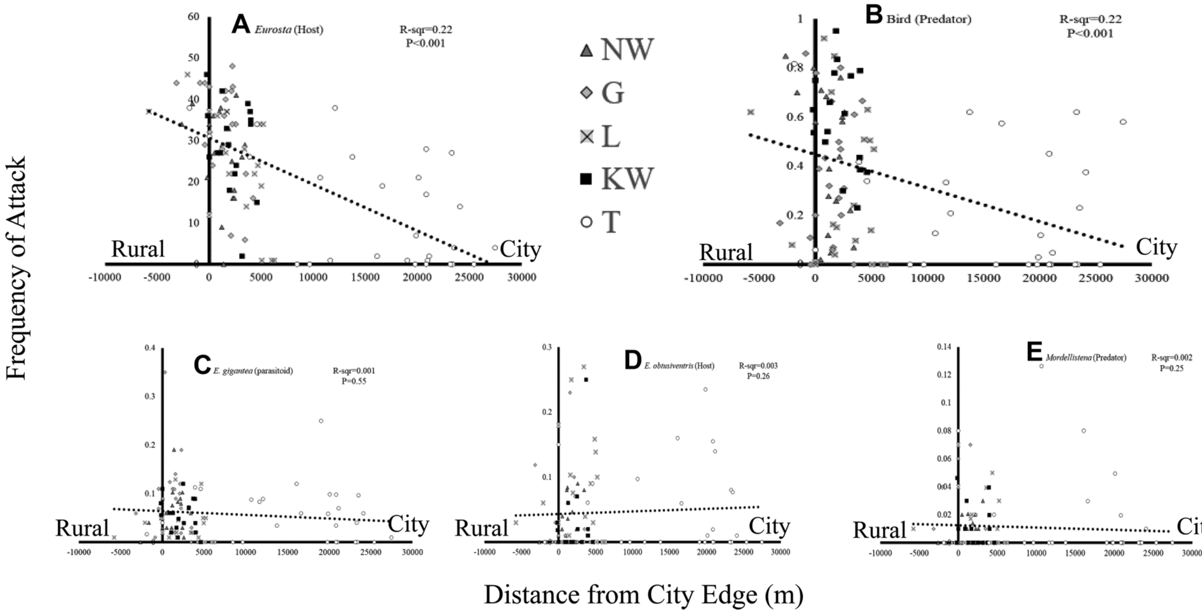

Figure 1. **Frequency of attack of *Eurosta* and its enemies across five urban gradients. *Eurosta* parasitism declined further into cities (A). Bird attack similarly declined towards city centers (B). *E. gigantea* (C), *E. obtusiventris* (D), and *Mordellistena* (E) were all unaffected by urbanization. Best fit lines represent predicted values from GLMMs. Analyses used a binomial measure of attack (except for *Eurosta* parasitism) but we transformed the predicted values to better show our data. As a result each point represents a patch rather than a single gall.**

In particular, birds and *E. gigantea* attack large and small galls, respectively, thus inducing opposing selective forces on galls size (Weis and Abrahamson 1985). Note that while both bird species have similar preferences for larger galls (Weis and Abrahamson 1986), allowing for these species interactions to be grouped for the purposes of statistical analyses, *E. gigantea* is the only parasitoid that prefers galls of a particular size (Uhler 1951; Weis and Abrahamson 1985). When both enemies are present, their combined effect is to cause stabilizing selection, with the optimum gall size depending on the frequency of attack by each enemy (Weis and Kapelinski 1994).

Previous research on gall size has concluded that the effect of gall size on fitness manifests through differential mortality but not fecundity (Weis and Abrahamson 1986). Mortality of *Eurosta* in the gall stage is very high (frequently > 70%) and strongly biased toward particular phenotypes, meaning that fitness should be quite reduced and selection should be strong. Although many organisms show size-fecundity relationships, a study that included 33 female *Eurosta* found a negative but nonsignificant relationship between size and fecundity (Weis and Abrahamson 1986). In practice, survival-selection has been shown to drive local adaptation in this system (Craig et al. 2007; Start and Gilbert 2016), strongly suggesting that survival-selection is a dominant source of overall differences in fitness and total selection. Previous work has shown that the parasitoid *E. gigantea* is sensitive to fragmentation (Start and Gilbert 2016) when fragments were separated by forest. Conversely, downy woodpeckers and chickadees have been studied across urban gradients but the effects of urbanization on abundances are not consistent (Beissinger and Osborne 1982; Rottenborn 1999; Zuckerberg et al. 2011).

FIELD METHODS

In Early May 2016, we collected 8890 *Eurosta* galls from 107 populations across five urban-rural gradients. We surveyed galls in Newmarket, Guelph, Kitchener-Waterloo, London, and Toronto (all Ontario, Canada). These cities differ in population size from 80,000 to > 5 million, but are all surrounded by a matrix of farmland and temperate forest. Surveying several cities allows us to be more confident that any observed patterns are not driven by some correlated underlying environmental variable (Thompson et al. 2016).

We selected patches randomly from satellite images, aiming to begin collecting in outlying farmland and moving at relatively regular intervals into the center of each city (Thompson et al. 2016). Our aim was to sample randomly within cities, while still achieving regular coverage from urban to rural areas. As such, we sampled populations regularly, but without using formal transects, by spreading sites haphazardly across cities, except when geographical barriers prevent goldenrod from occurring (e.g., water lies directly south of Toronto; all sites are marked in Fig. S1–S5). Because goldenrod and *Eurosta* gall-makers are patchily distributed we were unable to sample at perfect

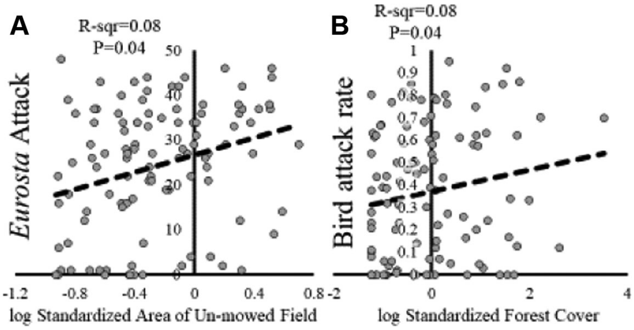

Figure 2. Relationships between ecological variables and the frequency of attack. *Eurosta* parasitism was higher in areas with large amounts of unmowed field (A). Bird attack increased with forest cover (B). Best fit lines represent predicted values from GLMMs. Data points in both figures represent proportion attacked per patch.

intervals (Root 1996), and patches were further apart in larger cities (see nearest neighbor distance between patches for each city in Table S1).

In all, we collected galls from ~20 patches per city (Table S1), with all locations being near paved roads and in human-occupied areas. Within patches, we collected up to 100 galls by haphazardly selecting galls around a central point. In addition to collecting galls, we counted the number of galls on the first one hundred goldenrod stems encountered as a measure of the rate of *Eurosta* attack.

Following collection, we measured gall diameter then dissected each gall to determine its content as per Abrahamson et al. (1989). We scored galls containing *Eurosta* larvae as survivors, those with a large hole as having been killed by birds, and those containing other larvae as having been attacked by the corresponding parasitoid. Empty galls were scored as early larval death (ELD).

MEASURING URBANIZATION

We aimed to measure urbanization in two ways. First, we were simply interested in quantifying distance into a given city, a common measure of urbanization (Thompson et al. 2004, 2016). Distance into a given city clearly has no biological significance, but can be viewed as a simple way to quantify a cline, analogous to known and well-characterized altitudinal and latitudinal patterns. We used Google Maps Pro and our marked GPS points to measure several variables associated with urbanization. Most simply we drew a polygon along the edge of the heavily urbanized area then calculated the minimum distance from that line to each patch. In all cases, cities were predominantly surrounded by farmland, and we used the stark and rapid transition between dense housing and farmland as our measure of urbanization (Fig. S1–S5). We use this as our base measure of urbanization, representing the distance of a patch to the edge of the city (Thompson et al. 2004). Thus, patches in outlying farmland had negative urbanization values.

Our second quantification of urbanization aimed to link distances into a city to biologically meaningful change in habitat availability. While we treated distance into a city as analogous to latitude, we next aimed to develop metrics of urbanization that were likely to be important in our study system. We view this as equivalent to discerning the mechanisms underlying latitudinal gradients (e.g., speciation rates may be higher in the tropics). Because species are likely to respond to different components of urbanization we measured a suite of environmental variables that are thought to be relevant to our focal species. We traced an area with a 272 m radius around each patch, the size being chosen to correspond to the diameter of a predatory downy woodpecker's home range (Kellam et al. 2006). Note that galls were typically collected from patches with radii > 20 m, meaning that each patch contributed negligibly to the area within the area sampled for forest cover. Within that area, we calculated the surface cover of trees and unmowed field, the habitats associated with bird predators, and *Eurosta* gall-makers and their parasitoids, respectively (Craig et al. 2007). We also calculated the distance of our patch from the nearest trees, since changes in bird attack rate have been documented over very small spatial scales (e.g., > 5 m; Craig et al. 2007).

STATISTICAL ANALYSES

We used a series of linear models (LMs) to test for the effects of urbanization on frequency of attack of *Eurosta* gall-makers and its natural enemies, selection on gall size caused by differences in attack, and any associated phenotypic shifts. All linear models were run using the "lme4" packages in R (Bates et al. 2015).

PATTERNS OF ENEMY ATTACK

We first calculated the frequency of attack for each species. We used a generalized linear mixed model (GLMM) with a binomial error distribution to test for differences in the attack of *Eurosta* gall-makers on goldenrod, including distance into the city as a main effect and city as a random intercept. We included patch nested in city as a random effect. We repeated this analysis for each enemy species, but while pooling attack by woodpeckers and chickadees into a single category (Weis and Abrahamson 1985; Craig et al. 2007; Start 2017). We then used GLMMs with binomial error distributions to test for the effect of gall size on the frequency of attack by birds and parasitoids, repeating tests for each species independently and including patch nested in city as a random intercept. Due to our reasonably large sample sizes, we tested for significance of all models using log likelihood ratios, with likelihoods determined from the maximum-likelihood solution (Bolker et al. 2009).

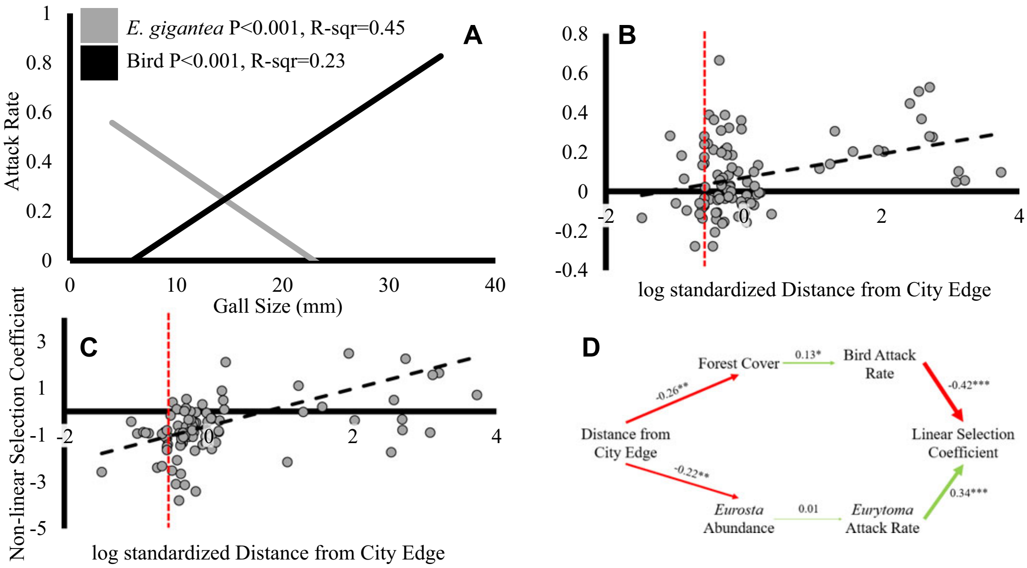

Figure 3. The selective effects of urbanization on gall size. (A) *E. gigantea* (gray) and birds (black) disproportionately attacked small and large galls, respectively (global selection analysis). (B) Directional selection coefficients increased in urban areas, meaning selection favored larger galls (local selection analysis). (C) Stabilizing selection broke down in urban areas, approaching then passing neutrality (local selection analysis), although the apparent disruptive selection in increasingly urban areas resulted from few data points and is not statistically significant. (D) SEM analyses were congruent with results from linear models; urbanization altered patterns of linear selection, but only by altering habitat availability and ultimately bird attack (local selection analysis). In A–C best fit lines are generated from LMs. In B and C each point represents selection in a single patch. In B and C Red dashed lines show where the edges of cities occur, with all points to the right being in cities and all points to left in rural areas. In D, red and green arrows denote negative and positive effects respectively, and the size of the arrow is scaled to show the magnitude of the standardized regression coefficient. Significance codes: *<0.05, **<0.01, ***<0.001.

We then attempted to relate any differences in patterns of attack across urbanization gradients to underlying environmental conditions. We began by log-transforming all independent variables to linearize observed relationship and to meet statistical assumptions (of a later analysis using structural equation modeling), and then standardizing each independent variable by subtracting the mean and dividing by the standard deviation. By using standardized predictors, we aimed to calculate standardized selection coefficients, allowing a comparison of the relative importance of different variables (Lande and Arnold 1983). We first used LMs in univariate analyses to test for relationships between forest surface area, unmowed surface area, and distance to trees to distance into the city. We then used GLMMs with binomial error distributions to test for relationships between the frequency of attack by each enemy and all environmental variables in a fully interactive model, including patch nested in city as a random intercept. We removed non-significant (always $P > 0.15$) terms to arrive at a final model, and estimated the effects of cities by comparing equivalent final models with and without city as a random effect.

PHENOTYPIC SELECTION ANALYSES

We next aimed to relate ecological differences in patterns of attack to resultant selective differences on the gall size of *Eurosta*. Before analyzing selection coefficients, we calculated relative fitness by dividing an individual gall maker's survival by the mean survival measured in each patch (Lande and Arnold 1983). Relativizing within patches assumes that gall-makers are interacting with other individuals in their patch (Start and Gilbert 2016), and thus assumes low levels of dispersal (De Lisle and Svensson 2017).

We used separate models to estimate significance values and regression coefficients because coefficients from GLMMs cannot be directly related to selection, and LMs of transformed binary data cannot be assessed for significance because statistical assumptions are violated (e.g., normality, homoscedasticity, etc.; Start and Gilbert 2016; Start 2017). We tested for significant selection coefficients for gall size by regressing survival against standardized gall size using a GLMM with a binomial error distribution for all data together (global selection), and for each patch separately (local selection). We included patch as a random effect in the global selection analysis (for which fitness was relativized across all gall makers rather than at the patch level). We repeated this analysis using an LM with relative fitness as the response variable to estimate selection coefficients for gall size and their standard errors (Lande and Arnold 1983).

We considered linear selection coefficients to represent estimates of directional selection (Start and Gilbert 2016; Start 2017), but then estimated nonlinear selection coefficients as a measure of nonlinear (stabilizing or disruptive) selection (Lande and Arnold 1983). Both types of selection often occur simultaneously for the same trait in the same system (e.g., Weis and Abrahamson 1985; Start and Gilbert 2016), but are only estimable using different models (Lande and Arnold 1983). Specifically, we estimated nonlinear selection coefficients by including the squared standardized gall size in the above models, then doubled the coefficients from LMs to calculate the actual magnitude of nonlinear selection (Stinchcombe et al. 2008).

We used the above calculated coefficients to test for systematic differences in selection on *Eurosta* gall size. Specifically, we fit a linear mixed model (LMM), regressing distance into the city against our calculated selection coefficients, including city as a random intercept and weighting each coefficient by the inverse of its standard error. Because not all patch-level selection coefficients were significant, this weighting reduces the importance of selection coefficients with only weak statistical support (Start and Gilbert 2016). Thus, weighting should prevent a few nonsignificant selection coefficients from driving patterns of selection across urban gradients. We repeated the analysis for linear and nonlinear selection coefficients separately, with each measure testing for changes in directional and nonlinear (stabilizing or disruptive) selection, respectively (Lande and Arnold 1983; Start and Gilbert 2016).

Structural equation modelling

As an additional step, we aimed to tie together all above analyses using structural equation modeling (SEM; "lavaan"; Rosseel 2012). As above, all variables were log-transformed and standardized to meet assumptions, and to be able to compare standardized selection coefficients. We began with a base model linking distance from city edge to pertinent environmental variables (forest cover and the attack rates (abundance) of *Eurosta* gall-makers) that were in turn linked to the frequency of bird predator and *Eurytoma* parasitoid attack, respectively. These estimates of attack frequency were then used to predict linear selection coefficients for gall size. After creating this base model, we sequentially added or removed potential links (all tested links are summarized in Fig. S6). We always fit links as regressions, ensuring that distance from the city edge was the most ultimate predictor, and that selection coefficients were the most proximate variable. Ultimately, we chose the model with the lowest AIC, but that still retained all links and variables included in the base model.

Phenotypic differences

We investigated phenotypic differences among patches, aiming to link traits to selection on gall size. We tested for changes in gall size across urban gradients, which may have resulted from differences in selection. We used an LMM to regress distance into the city against gall size, including patch nested in city as a random intercept. As a more direct test, we reran the same model, separately using linear and nonlinear selection coefficients for gall size as predictor variables.

Testing for spatial autocorrelation

Finally, since spatial data are not fully independent, the regression approaches used here are only justified when spatial autocorrelation in residuals is insignificant (Diniz Filho et al. 2003). We formally tested for spatial autocorrelation in each factor. Specifically, we saved residuals from each model, then examined spatial autocorrelation using Moran's I correlograms ("ape"; Paradis et al. 2004).

Results

A large proportion of goldenrod stems were attacked by *Eurosta* gall-makers, which were in turn attacked by predatory birds and the parasitoid *E. gigantea*. *Eurosta* formed galls on goldenrod more frequently in rural than urban areas (Fig. 1A; $P < 0.001$), but this pattern did not vary among cities ($P > 0.3$). Attack by bird predators declined in city centers relative to rural areas (1B; $P = 0.005$), but did so consistently in all cities ($P = 0.27$). Conversely, attack by the parasitoids *E. gigantea*, *E. obtusiventris*, and *Mordellistena* was unaffected by a patch's distance into the city (Fig. 1C–E; all $P > 0.25$; see Table S2 for outputs from models). All estimates of attack frequency were within the typical range observed in previous studies (Uhler 1951; Weis and Abrahamson 1985; Abrahamson et al. 1989; Craig et al. 2007; Start and Gilbert 2016; Start 2018).

Ecologically relevant measures of enemy habitat availability drove patterns of attack across urban-rural gradients. *Eurosta* formed fewer galls when unmowed field was rare (Fig. 2A; $P = 0.04$), as occurred in urban areas ($P < 0.001$). Attack by parasitoids was unaffected by any environmental variable or gall-maker abundance (all $P < 0.3$) but attack by bird predators was positively correlated with forest surface area (Fig. 2B; $P = 0.04$), and forest surface area declined in urban areas ($P = 0.02$). We note that these ecological measures all varied along the urban-rural gradient, such that they are not independent. The rural-urban gradient was a better predictor of *Eurosta* abundance and bird attack, suggesting that the multivariate nature of the urban-rural gradient better captures ecological dynamics than any single ecological variable.

The parasitoid *E. gigantea* and bird predators preferentially attacked small and large galls respectively (Fig. 3A; both $P < 0.001$). Globally, selection favored larger galls ($\beta = 0.14$, $P < 0.001$) and had a stabilizing component ($\gamma = -1.43$,

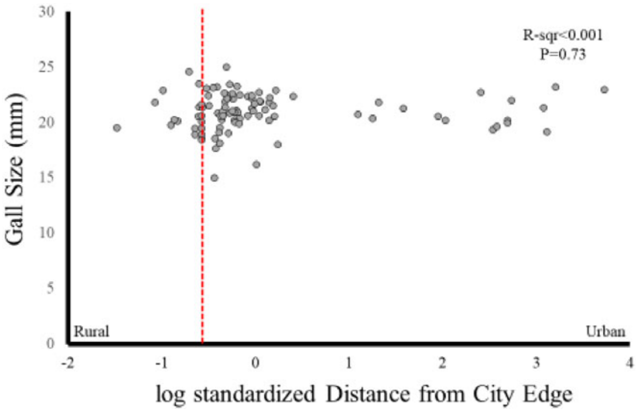

Figure 4. Gall size is unchanging across five urbanization gradients. Analyses used a GLMM to predict gall size (the best fit line) but here we used mean gall to make our data more easily interpretable. Each point therefore represents a single patch rather than a single gall.

$P < 0.001$). At the patch level, directional selection for larger galls increased further into the city (Fig. 3B; $P = 0.002$). Nonlinear selection was stabilizing near city edges, but coefficient values increased to become neutral further into cities (Fig. 3C; $P < 0.001$). SEM analyses (ΔAIC = 21.3) corroborated all above tests; forest cover and gall-maker abundance both declined further into the city (both $P < 0.01$), reducing the frequency with which galls were attacked by birds ($P = 0.02$). Conversely, reduced gall abundance had no effect on the frequency of attack by the parasitoid *E. gigantea* ($P > 0.45$). Ultimately, differences in the frequency of attack by predators and parasitoids mediated patterns of linear selection on gall size (Fig. 3D; both $P < 0.001$).

However, these differences in selection on gall size did not result in changes in mean gall size among patches (both $P > 0.45$), nor did gall size change systematically across urban gradients (Fig. 4; $P = 0.66$). We found no evidence of residual spatial autocorrelation in any test (all $P > 0.2$).

DISCUSSION

Our study demonstrates how urbanization can shape patterns of species interactions and selection. Although it is well known that community composition and species interactions change from rural to downtown areas (Faeth et al. 2005; Raupp et al. 2010; McPhearson et al. 2016), the evolutionary consequences of changing species interactions in urban environments remain largely unexplored (Donihue and Lambert 2015; Johnson et al. 2015; Johnson and Munshi-South 2018). We show that species respond differently to urbanization (Fig. 1), sometimes but not always in response to underlying changes in habitat availability (Fig. 2). Urbanization altered the relative frequency of attack on galls and created selection on gall size for phenotypes resistant to the parasitoid *E. gigantea* (Fig. 3B and D). Reduced attack from bird predators in cities removed selection for smaller galls, causing the breakdown of stabilizing selection (Fig. 3C). Although large selective differences existed between urban and rural sites, selection on gall size did not drive changes in gall size (Fig. 4). Our study suggests that changing species interactions can underlie the evolutionary consequences of urbanization.

Plant parasitism by the gall-maker declined toward the center of cities. *Eurosta* formed fewer galls per goldenrod stem in cities, a pattern that was correlated with a concurrent decline in available habitat (unmowed fields; Figs. 1A and 2A). The decline in attack may have been due to reduction in available habitat, thus causing stochastic extinctions and low mean population sizes (Lande 1993). Alternatively, some other correlated effect of intense urbanization may degrade habitat quality (Sumoski et al. 2009), although it is more plausible that habitat availability had a direct effect on gall-maker abundances. In contrast to our work, other studies have found an increase in gall-maker abundance in cities, seemingly in response to changes in plant quality (Sumoski et al. 2009). These contrasting patterns suggest that the effects of urbanization on gall-makers are likely to depend on the factors limiting their abundance.

Trophic dynamics were altered by urbanization, but enemies responded differently and only sometimes predictably. Bird attack on gall-makers declined in cities, a pattern possibly driven by reduced forest habitat in urban areas (Figs. 1B and 2B). Conversely, parasitoid abundance was unaffected by urbanization and was uncorrelated to any measured environmental variable (Fig. 1C–E), despite the inclusion of biologically plausible quantifications of parasitoid habitat (unmowed field) and host (*Eurosta*) abundance. Curiously, this pattern is opposite to another study (Faeth et al. 2005) that suggests that parasitoids but not birds decline in urban areas. That study occurred in desert conditions, meaning that the irrigated cityscape with abundant food subsidies may have represented a higher quality habitat for many bird species (Faeth et al. 2005). Contrasting these studies raises an important point: shifts in ecology and evolution will depend on which resources become more limiting over the rural urban gradient's direction. As a consequence, it will be difficult to make generalizations about the effects of urbanization (Raupp et al. 2010). Furthermore, habitat availability in urban environments is not necessarily a strong indicator of species abundances (e.g., for parasitoids; Fig. 1C–E). The breakdown of the habitat availability-species abundance relationship may occur either if habitat is always abundant enough to support large and self-sustaining populations (Lande 1993), or if some other variable relating to habitat quality is primarily responsible for driving species abundances. In sum, species respond differently to urbanization, and these responses are only sometimes predictable using coarse-scale environmental correlates such as habitat or host availability.

The differential sensitivity of bird and parasitoid enemies to city-associated habitat differences created changes in their relative frequencies of attack, ultimately altering patterns of selection on gall size in urban environments. In many cases, defensive traits impose a cost, creating selection against resistance when consumers are absent (Peckarsky and McIntosh 1998; Sih et al. 1998; Start and Gilbert 2016). For example, Mikolajewski et al. (2006) showed that defensive spines on dragonfly larvae improved survival in the presence of fish, but were detrimental when faced with predation by invertebrates. In our study, bird predators and the parasitoid *E. gigantea* selected for opposite defensive phenotypes (Fig. 3A), meaning that traits that confer an advantage against *E. gigantea* (large gall size) will be costly when defending against birds. As a result, when bird attack declined but attack by the parasitoid remained constant in urban areas, directional selection favored larger galls, which are impervious to attack by *E. gigantea* but sensitive to predatory bird attack (Fig. 3B and D). Interestingly, the SEM analysis demonstrated that the impact of urbanization on selection on gall size was entirely driven by changes in habitat availability, and thus differences in frequency of attack by bird predators (Fig. 3D). We may then surmise that the afforestation of cities would eliminate the detrimental effect of urbanization for birds, and therefore cancel selective differences between populations in urban versus rural habitats.

The second selective effect of predatory bird loss is the weakening of stabilizing selection on gall size (Fig. 3C). When both enemies that impose selection are present in approximately equal abundances, their preferences for opposite defensive traits favor intermediate phenotypes (Weis and Abrahamson 1985). Correspondingly, when one enemy, in this case bird predators, is absent or reduced in abundance, stabilizing selection on gall size breaks down because only one of two selective pressures remain (Fig. 3C; Start and Gilbert 2016; Start 2017). While changes in the direction of directional selection are likely to be idiosyncratic, depending on the particular order of species loss, a breakdown of stabilizing selection may be a general phenomenon resulting from the loss of one or both enemies. While the maintenance of both enemies in urban systems is possible, three of four possibilities (the loss of one or the other or both species) will result in weakened stabilizing selection. In short, stabilizing selection should break down in urban environments whenever the sensitivities of enemies to urbanization and associated habitat differences diverge (Faeth et al. 2005; Christie et al. 2010; Raupp et al. 2010), or when both enemies are particularly sensitive to urbanization and therefore absent from urban areas. However, many biological systems have higher levels of complexity, with many biotic and abiotic factors shaping selection. In more complex systems, the loss of one selective pressure may be compensated for by gains in another, maintaining stabilizing selection in urban environments. Ultimately, many communities are far more complex than the focus of our study, potentially making a priori predictions about changes to species interactions and selection difficult.

An important question arising from our results is why there were no detectable differences in gall size in response to the differences in selection. Put simply, why has urbanization not caused gall sizes to diverge? First, observational approaches are often insufficient to detect local adaptation (Kawecki and Ebert 2004; Blanquart et al. 2013), meaning that local adaptation may well have occurred, but is undetectable using phenotypic surveys. For example, genetic differentiation could have occurred among populations with respect to gall size, but the environmental contribution to phenotype may be large enough to swamp any signal of local adaptation (Kawecki and Ebert 2004). If we had grown *Eurosta* gall-makers in a common environment, we may well have been able to detect among-patch variation in gall size consistent with patterns of selection on the same trait (Craig et al. 2007; Start and Gilbert 2016). However, even if we had conducted a common-garden experiment to test for local adaptation, it may be unlikely for adaptation to occur in this system. The potential difficulties associated with adaptive evolution arise because gall size is determined by the genotype of *Eurosta*, but also the genotype and quality of its goldenrod host (i.e., an insect genotype × plant host genotype × environment interaction; Craig et al. 2007). This complex interaction underlying phenotype raises intriguing possibilities about the likelihood of adaptation and the response of populations to urbanization. First, we may expect that such complex interactions will minimize the likelihood of local adaptation by reducing the heritability of gall size (Craig et al. 2007). More interestingly, plant genotype or environmental quality may itself change along an urbanization gradient, requiring changes in *Eurosta* genotype simply to maintain the same gall size (i.e., counter gradient or canalizing selection; Kawecki and Ebert 2004). We may then hypothesize that selection should be driven by urbanization not just because abiotic or ecological conditions change, but also because the genotypes or phenotypes of other, closely interacting species, are altered by urbanization (i.e., urban coevolution; Johnson et al. 2015; Johnson and Munshi-South 2018). In sum, local adaptation could have occurred in this system, but is masked by environmental and host-genotype differences. This creates the interesting possibility that abiotic, ecological, and evolutionary factors ultimately and interactively determine selection in urban environments.

Beyond the complexity of selection and potentially overwhelming environmental differences, there are reasons to suspect that local adaptation is fundamentally less likely in urban environments, preventing selection from driving distributions of gall sizes in our study (Johnson et al. 2015; Johnson and Munshi-South 2018). First, rapid evolution is not a ubiquitous process, and the relative novelty and shifting mosaics of urban systems may mean that populations simply did not have time to adaptively respond to selection. Similarly, while the direction of selection appears to be remarkably consistent in this system (Weis and Abrahamson 1985; Abrahamson 1989; Craig et al. 2007), temporally variable selection may nevertheless prevent strong directional responses in phenotype (Hairston et al. 2005). Finally, selection acting through other components of fitness may counter-balance selection caused by differential survival. For instance, while unlikely in this system (Weis and Abrahamson 1986), there is often a trade-off between survival and fecundity, causing the measurement of selection on just one component of fitness to provide a biased view of total selection. In sum, the complexities of selection acting on many components of fitness and varying through time may lead to a lack of phenotypic differentiation among populations that experience strongly divergent selection regimes.

Beyond the speed of evolution and the consistency of selection, other evolutionary processes may act to speed or retard the rate of evolutionary change in urban environments (Johnson and Munshi-South 2018). For instance, gene flow may have swamped any selection on gall size occurring in individual patches (Slatkin 1987). Relatively small amounts of gene flow, as little as one individual per generation, can prevent local adaptation (Lenormand 2002). However, local adaptation has been observed in a number of other studies of urban evolution in dispersive species (Johnson et al. 2015), and dispersal and thus gene flow is likely reduced in cities and in some cases may even be selected against, further reducing gene flow over time (Cheptou et al. 2008). Second, genetic drift may prevent selection from removing maladapted genotypes from a population, thus hampering local adaptation (Kawecki and Ebert 2004). This possibility is intriguing because genetic drift may be particularly strong in urban environments, owing to the small and fragmented nature of populations in cities (Donihue and Lambert 2014; Johnson and Munshi-South 2018). For example, in our system, unmowed areas are less prevalent and goldenrod is attacked by *Eurosta* gall-makers less frequently in cities, suggesting that *Eurosta* populations are smaller and hence more susceptible to drift in urban areas. It is also possible that the above three hypotheses are jointly contributing to the lack of local adaptation (Johnson et al. 2015; Johnson and Munshi-South 2018). We suggest that while reduced dispersal in urban environments may facilitate local adaptation, this pattern can be negated by drift in small populations.

By incorporating trophic dynamics with phenotypic selection, our study has begun to investigate the intimate links between ecology and evolution in cities. We have shown that species respond differently to urbanization and that those differences are sometimes but not always predicted by changes in habitat availability (Figs. 1 and 2). These differences strengthened upward directional selection on gall size by relaxing the opposing downward selective force (Fig. 3), which thereby weakened stabilizing selection. The loss of stabilizing selection may be common in cities if opposing selective agents often respond differently to urbanization (Johnson et al. 2015). However, these selective differences did not obviously cause phenotypic differences among populations (Fig. 4), perhaps because of overwhelming environmental differences, the slow speed of adaptive evolution, inconsistent selection, gene flow, or drift, the latter of which is likely to be particularly important in urban environments. Overall, the repeatability of species interactions and selection across cities suggests that these links may be widespread, and that selection may act similarly in geographically distant but environmentally similar urban areas (Thompson et al. 2016). Nevertheless, the lack of a response to selection suggests that eco-evolutionary dynamics in urban areas may be more complex then previously appreciated (Johnson and Munshi-South 2018). We suggest that ecology can underlie evolutionary dynamics in urban systems, and that eco-evolutionary dynamics are common in cities.

AUTHOR CONTRIBUTIONS

All authors devised the study. D.S. and C.B. conducted field work. D.S. analyzed the data and wrote the manuscript. All authors edited and approved of the final version of the manuscript.

ACKNOWLEDGMENTS

We thank Kaitlyn Brown for accommodation. We also thank Isabela Borges for help in dissecting galls. We partly sampled at the Koffler Scientific Reserve. Research funding was provided by NSERC (B.G.) and personal funding was awarded by OGS (D.S.).

DATA ARCHIVING

Data are available through Dryad (https://doi.org/10.5061/dryad.4d12c92).

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Associate Editor: N. Emery
Handling Editor: P. Tiffin

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1: A map of Guelph. Red points mark sampling sites. The orange line denotes our demarcation of the shift from urban to rural habitat. Distance into the city was measure from the nearest edge
Figure S2: A map of Kitchener-Waterloo. Red points mark sampling sites. The orange line denotes our demarcation of the shift from urban to rural habitat. Distance into the city was measure from the nearest edge
Figure S3: A map of London. Red points mark sampling sites. The orange line denotes our demarcation of the shift from urban to rural habitat. Distance into the city was measure from the nearest edge
Figure S4: A map of Newmarket. Red points mark sampling sites. The orange line denotes our demarcation of the shift from urban to rural habitat. Distance into the city was measure from the nearest edg
Figure S5: A map of Toronto. Red points mark sampling sites. The orange line denotes our demarcation of the shift from urban to rural habitat. Distance into the city was measure from the nearest edge
Figure S6: A figure showing all possible combinations of paths in our path model. Paths retained in the final model are colored in green (positive) or red (negative) and their size is scaled to the magnitude of the effect. Black dashed lines represent paths that were tested but not retained in the final model
Table S1: Summary of sampling effort for five cities
Table S2: Outputs from GLMMs of attack rates