

Research



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Phenotypic selection on floral traits in an urban landscape

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Native species are increasingly living in urban landscapes associated with abiotic and biotic changes that may influence patterns of phenotypic selection. However, measures of selection in urban and non-urban environments, and exploration of the mechanisms associated with such changes, are uncommon. Plant–animal interactions have played a central role in the evolution of flowering plants and are sensitive to changes in the urban landscape, and thus provide opportunities to explore how urban environments modify selection. We evaluated patterns of phenotypic selection on the floral and resistance traits of *Gelsemium sempervirens* in urban and non-urban sites. The urban landscape had increased florivory and decreased pollen receipt, but showed only modest differences in patterns of selection. Directional selection for one trait, larger floral display size, was stronger in urban compared to non-urban sites. Neither quadratic nor correlational selection significantly differed between urban and non-urban sites. Pollination was associated with selection for larger floral display size in urban compared to non-urban sites, due to the differences in the translation of pollination into seeds rather than pollinator selectivity. Thus, our data suggest that urban landscapes may not result in sweeping differences in phenotypic selection but rather modest differences for some traits, potentially mediated by species interactions.

1. Introduction

Evolutionary ecologists are fundamentally interested in geographical variation in natural selection and the abiotic and biotic selective agents driving those spatial patterns. The majority of studies that have measured landscape variation in natural selection have focused on species in relatively pristine environments, finding mosaics of selection and adaptive evolution across the landscape due to changes in the physical environment and species interactions [1]. Despite the widespread recognition of spatial variation in natural selection [2,3] and its potential to lead to differentiation and adaptive evolution among populations, urban landscapes are one dominant form of land-use change that remain understudied within the context of spatial variation in selection [4]. Urban areas make up 3% of land surfaces on Earth, with over half of the global human population currently living in cities [5]. Urban areas are associated with changes in abiotic factors, including increased temperature and pollution [6], as well as changes in biotic factors, including reduced biodiversity and phylogenetic diversity, increased density of invasive species, community shifts towards human commensal species, and changes in species interactions [7–9]. These ecological changes associated with urbanization have the potential to influence patterns of phenotypic selection for native species remaining in urban habitats.

Many of our clearest examples of selection (and evolutionary response) to the urban environment are in animal systems. For example, beyond classic studies of peppered moths [10], urban populations of fish and mice have evolved tolerance to urban pollutants [11,12]. Urban populations of anole lizards locomoting on artificial surfaces evolved longer limbs and more toe lamellae [13], and urban populations of house finches experience selection on

beak morphology due to altered differences in seeds at bird feeders in urban versus non-urban sites [14]. However, plants as targets of selection in urban landscapes have been less-well studied [15].

Plant–animal interactions have played a key role in the evolution of floral and chemical traits in plants. Pollinator- and herbivore-mediated selection on floral traits and plant secondary chemistry are hallmarks of adaptive evolution. The urban landscape can modify the abundance and biodiversity of pollinators and herbivores, important selective agents, relative to non-urban sites. For example, bee pollinators often decline with an increase in built landscape in urban areas [16], but abundant and/or diverse flowers in urban gardens and open habitats can promote a diverse assemblage of bees [17,18]. Canopy trees can receive more herbivory in urban versus non-urban sites [19], but levels of urban herbivory can be modulated by the physical environment, such as heat island effects [20]. However, we do not know the degree to which ecological changes in plant–animal interactions in urban versus non-urban sites affect patterns of selection on floral and chemical traits [21]. For example, some of the strongest examples of urban evolution in plants focus on the physical environment as a driver (e.g. temperature and habitat fragmentation), but do not identify plant–animal interactions as significant agents of selection [21,22].

The goal of this study was to assess how the urban landscape affected patterns of phenotypic selection on floral and chemical traits, and to evaluate whether plant–animal interactions were associated with changes in patterns of selection. We focused on the native vine *Gelsemium sempervirens* (Loganiaceae) in southeastern North America. We measured plant–animal interactions and phenotypic selection on floral and chemical traits in multiple urban and non-urban sites to address the following questions: (i) do pollination and herbivory vary between urban and non-urban sites? Based on prior data from field observations [23], we predicted that urban sites would have higher florivory, nectar robbing, and heterospecific pollen transfer than non-urban sites, but both urban and non-urban *Gelsemium* would experience pollen limitation of reproduction [24]. (ii) How do patterns of phenotypic selection on floral and chemical (resistance) traits vary between urban and non-urban sites? And (iii) to what degree are pollination and herbivory associated with patterns of phenotypic selection? Prior field observations suggest that floral traits affect the intensity of species interactions with *Gelsemium*, with florivores preferring larger floral displays, nectar robbers preferring flowers with longer corolla tubes and narrower petal lobes, and narrower petal lobes resulting in more heterospecific pollen deposition [23]. Because selection is expected to be strongest when antagonistic interactions have strong negative effects and mutualistic interactions are weaker or variable [25], we predicted to find selection for smaller floral displays and wider, shorter flowers in urban compared to non-urban sites via changes in antagonistic interactions. However, we predicted no selection on the alkaloid gelsemine given that florivores previously did not respond to variation in gelsemine [23]. Consideration of the broader geographical context in which organisms live, including in both urban and non-urban sites, is fundamental to our understanding of the contemporary selection pressures that shape native species.

2. Methods

(a) Study system

We studied the native perennial vine *Gelsemium sempervirens* (Loganiaceae; hereafter *Gelsemium*) in naturally forested patches of urban and non-urban habitat in and around Raleigh-Durham, NC, USA. The plant blooms in open pine forests in March and April, producing up to several hundred yellow, tubular flowers, each with five petal lobes. Individual flowers bloom for 3–5 days [26]. *Gelsemium* is self-incompatible and distylous, with plants having either long styles and short anthers (pin plants) or short styles and long anthers (thrum plants) that are intra-morph incompatible. *Gelsemium* reproduces by seed and is typically not clonal in the field.

Gelsemium is pollinated by a suite of generalist bees [27], and pollination can limit reproduction in wild-growing plants [28]. Flowers are nectar robbed by carpenter bees, *Xylocopa virginica*, that make slits near the corolla base to take nectar. Robbing rates range from 0% to 100% of flowers per plant [26]. Robbing can reduce estimates of plant reproduction via changes in pollinator behaviour (RE Irwin & LS Adler 2004, unpublished data). Flowers are also damaged by larvae of the univoltine generalist *Amphipyra pyramidoides* (Noctuidae). These florivores eat petal tissue, anthers, and stigmas. Florivory has variable effects on pin and thrum morphs [29], reducing the number of pollinator visits to pins but increasing time spent per flower for thrums [30]. Leaf herbivory to *Gelsemium* is rare, likely due to the production of the indole-related alkaloid gelsemine, which has been isolated from leaves, flowers, and nectar [27]. Gelsemine deters pollinators and nectar robbers [27].

(b) Study sites

We studied *Gelsemium* in eight paired forested urban and non-urban sites (electronic supplementary material, table S1) in Wake, Durham and Chatham Counties, NC, USA, which contain the Raleigh-Durham metropolitan area. This metropolitan area has undergone rapid urban growth, increasing its land area by two-thirds in the 1990s alone [31], and simulations over the next 50 years project a doubling to tripling of land for urban and suburban growth in this southeastern USA piedmont region [32]. Counties in this region have experienced sprawling, fragmented ‘leap frog’ development [33], resulting in a mosaic of housing and woodlots. Urban sites consisted of forested areas within single-family residential communities (electronic supplementary material, table S1 and figure S1). We chose urban sites surrounded by residential land within a 300 m radius since some bees have foraging radii within this range [34], although bees vary in foraging distances [35]. Residential communities within urban sites contained a range of housing values, styles, and ages, but most were at or above the median housing value for the area. Because of this limited variation in socioeconomic factors among urban sites, we did not consider how socioeconomic factors might mediate selective agents. Urban sites were paired with comparable, non-urban sites that were unmanaged or managed for their natural resources (hereafter referred to as ‘non-urban’), and had similar *Gelsemium* density. All non-urban sites were >10+ acres whenever possible to minimize effects of adjacent urban growth. Urban and non-urban site pairs did not differ in temperature, relative humidity, or soil nutrients (electronic supplementary material, text S1). Because our sites were limited to single-family housing of moderate to high housing value and we only had eight site pairs, we include site pair as a fixed and not a random factor in statistical analyses [36].

(c) Field methods

At each site in 2009, we haphazardly selected up to 100 *Gelsemium* (electronic supplementary material, table S1), for a

total of 1372 plants. We only used individual plants not connected to others nearby via runners. We measured floral and chemical traits, nectar robbing, florivory, pollination, and plant reproduction. For floral traits, we recorded floral morph (pin or thrum), and two times during the flowering season for each plant we measured floral display size (number of flowers open) and corolla and petal length and width (to the nearest 0.01 mm using digital calipers; electronic supplementary material, figure S2) on up to three flowers per plant. Floral traits were averaged within and then across days to calculate a mean trait value per plant. As a resistance trait, we measured the concentration of the alkaloid gelsemine using gas chromatography (electronic supplementary material, text S2).

We measured robbing, florivory, and pollination twice during the flowering season. On all census days, we counted the number of flowers open per plant. For robbing, we counted the number of flowers per plant with robbing holes and the number of holes per flower. For florivory, we counted the number of flowers with florivore damage per plant, and on damaged flowers the proportion of petal area removed and whether anthers and stigmas were consumed. Because *Gelsemium* is a vine that can grow up into trees, for some plants we could not reach all of the open flowers to census them; for these plants, we estimated the proportion of flowers we could census out of the total open. For each plant, we calculated the mean proportion robbing and mean proportion florivory (number of flowers with robber holes or florivore damage divided by the number of flowers censused). To assess the magnitude of florivory, we calculated two additional metrics: (i) the mean proportion of flowers with reproductive structure damage, and (ii) the proportion of petal area removed on plants with florivore damage (including damaged and undamaged flowers on plants with damage).

For pollination, we measured stigma-pollen loads on emasculated flowers. Increased pollinator visitation to *Gelsemium* is associated with increased pollen receipt [23]. Stigmas were collected once corollas senesced from up to three flowers per plant per census and stained in basic fuchsin. We counted the number of conspecific and heterospecific pollen grains with a compound microscope and calculated mean conspecific and heterospecific pollen receipt per flower per plant.

To estimate female components of plant fitness, we counted the number of flowers, fruits, and seeds produced per plant. Measuring male plant fitness was beyond the scope of this study. Because *Gelsemium* produce flowers throughout the flowering season and flowers that abort leave no scar, we marked all flowers using small pieces of tape on the stems. At the end of the season, we counted the number of marks to estimate total flowers per plant; this approach ensured that we did not double-count flowers or miss aborted flowers. Because we could not reach all of the flowers on some plants, we recorded the proportion of flowers we marked relative to the total. For each plant, we calculated proportion fruit set (number of seed-bearing fruits/total flowers marked), mean seeds produced per seed-bearing fruit, and total seeds per plant. Flower, fruit, and seed production were corrected by the proportion of the plant marked. The corrected values for fruit and seed set assume that the likelihood of fruit and seed set were the same for flowers we could reach and those we could not; this is a parsimonious assumption given there was no evidence of differential fruit or seed set based on flower location within a plant.

(d) Statistical analyses

(i) Species interactions

To assess how the urban landscape, plant traits, and their interactions influenced species interactions, we used ANCOVAs with site type (urban or non-urban), block (pair), floral morph,

floral and resistance traits and interactions as factors, and measures of florivory and pollination as responses. Non-significant interactions between site type and traits were removed from final models ($p > 0.05$). Because the floral morphological traits (corolla and petal length and width) were all positively correlated within and among sites (electronic supplementary material, table S2), we used principal component analysis within each site to reduce the number of dimensions in the morphological traits. The first two principal components (PCs) accounted for at least 76% of the variation per site and were used in these analyses and to calculate selection gradients (below). PC1 had positive loadings for all traits, reflecting flower size; PC2 had positive and negative loadings, reflecting flower shape (electronic supplementary material, table S3). We transformed some species interaction variables to improve normality of residuals: arcsine square-root for proportion of flowers with florivory and reproductive structure damage, log for florivore tissue removal, square-root for conspecific and $\log(x + 1)$ for heterospecific pollen receipt. A significant effect of site type indicates that the frequency or intensity of interactions varies in urban versus non-urban sites. A significant trait \times site-type interaction suggests that the effect of traits on interactions varies with site type. Robbing levels were low in the year of study (see Results); thus, for robbing, we only examined the probability of plants experiencing robbing (yes/no) between urban versus non-urban sites using a Chi-squared test. Statistical analyses (here and below) were performed in SAS version 9.4 and JMP version 13.0.0.

(ii) Plant reproduction and floral traits

We explored how the urban landscape affected flower production, proportion fruit set, mean seeds per fruit, and total seeds per plant. We used a model with type (urban versus non-urban), block (pair), and floral morph as factors for each response variable. To improve normality of residuals, we used a $\log(x + 1)$ transformation for total flowers and total seeds, and arcsine square-root transformation for proportion fruit set. We used similar models to assess how floral traits (corolla length and width, and petal length and width) and alkaloid production varied with site type.

(iii) Patterns of phenotypic selection

We estimated selection gradients within each site using relative total seeds per plant as our fitness estimate regressed on standardized floral and resistance traits [37]. Relative fitness (individual fitness divided by mean site fitness) and standardized trait values (with a mean of 0 and a variance of 1) were calculated separately for each site because we were most interested in comparing the strength and direction of selection among sites and within the context of other studies [38]. To improve normality of the residuals, we transformed relative total seeds via $\log(x + 1)$. The selection gradients are from the untransformed models, but the p -values are from the transformed models [39]. We quantified directional selection gradients β_i from multiple regression models including linear terms only for each site. We calculated quadratic (γ_{ii}) and correlational (γ_{ij}) selection gradients from the quadratic and cross-product terms from the full regression models. The quadratic selection gradients were calculated by doubling the regression coefficients from the full regression model [40]. All variance inflation factors were less than 2, suggesting that multi-collinearity did not strongly affect the results.

We explored whether patterns of directional selection differed between urban and non-urban sites using ANCOVA. We included site type (urban or non-urban), block (pair), floral morph, standardized traits, and interaction terms as factors, and relative total seeds as the response ($\log(x + 1)$ transformed).

We calculated standardized traits and relative fitness within sites. We were most interested in interactions between trait and site type, indicating that the trait–fitness relationship changed between urban and non-urban sites. Across sites, there were overlapping variances in traits and fitness, as well as overlapping data ranges and broadly similar trait distributions. We used a similar ANCOVA model with quadratic terms \times site type and trait \times trait \times site-type interactions to assess whether quadratic and correlational selection varied between urban and non-urban sites.

(iv) Species interactions and patterns of selection

To assess whether species interactions were associated with differences in phenotypic selection in urban versus non-urban sites, we used ANCOVAs with type (urban or non-urban), block (pair), floral morph, standardized traits, species interactions, and their interactions as factors, and relative seed set ($\log(x + 1)$ transformed) as the response. We were most interested in three-way interactions between site type \times trait \times species interactions, which suggests that how species interactions affect trait–fitness relationships varies in urban versus non-urban sites. We only considered traits with significant differences in selection between urban and non-urban sites (see *Results*). We focused on directional selection because considering quadratic and correlational selection between traits made the model too complex.

3. Results

(a) Species interactions

(i) Overview

Nectar robbing was low, with only 10% of plants across all sites experiencing any nectar robbing (111 of 1 088 plants censused for robbing). Plants growing in urban versus non-urban sites did not vary in their likelihood of experiencing robbing ($\chi^2_1 = 0.38$, $p = 0.54$). Given the low levels of nectar robbing, it was not considered further in analyses. Florivory was more common, with 52% of plants receiving floral herbivory (566 of 1 088 plants). On damaged plants, florivory levels ranged from 2% to 100% of flowers damaged, with $10\% \pm 1\%$ of flowers having anthers or stigmas damaged by florivores, and $20\% \pm 1\%$ of total petal area (across damaged and undamaged flowers) removed. Mean *Gelsemium* pollen deposition per stigma ranged from 13 to over 4 000 pollen grains and mean heterospecific pollen from 1 to over 1 500 pollen grains. On average, $11\% \pm 1\%$ of the total pollen received was heterospecific pollen. There was no evidence that florivory affected *Gelsemium* or heterospecific pollen deposition, nor that this lack of effect varied with site type ($F < 3.26$, $p > 0.07$ in all cases).

(ii) Comparison of species interactions between urban and non-urban sites

The urban landscape had significant effects on both florivory and pollination. For florivory, we found no difference in the proportion of flowers damaged by florivores ($F_{1,903} = 0.02$, $p = 0.90$) or the proportion of flowers with reproductive structures damaged ($F_{1,901} = 0.59$, $p = 0.44$) between urban and non-urban sites. However, if plants had florivore damage, the magnitude of damage was greater in urban sites, with florivores removing 20% more floral tissue in urban than non-urban sites ($F_{1,462} = 9.26$, $p = 0.003$). Some

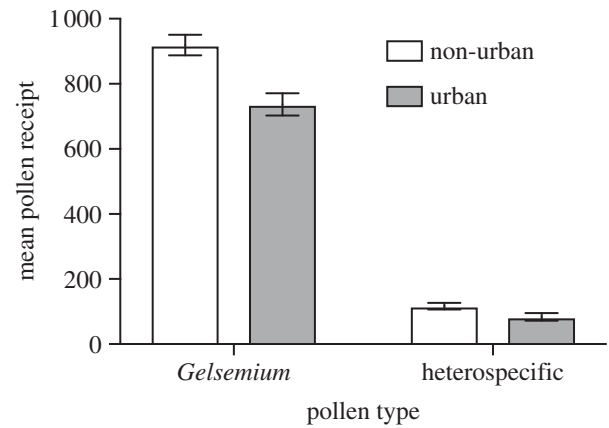


Figure 1. Conspecific and heterospecific pollen receipt to plants of *Gelsemium sempervirens* was significantly lower in urban compared to non-urban sites.

floral traits influenced plant susceptibility to florivory (electronic supplementary material, text S3). Floral display size, and PC1 (flower size) were negatively related to the proportion of tissue removed (respectively, $F_{1,462} = 50.45$, $p < 0.0001$; $F_{1,462} = 9.10$, $p = 0.003$), likely because consumption of flower tissue could not keep pace with increased floral displays and tissue area. We also found a significant floral display size \times site-type interaction for floral tissue removal ($F_{1,462} = 3.80$, $p = 0.052$); in both urban and non-urban sites, increasing floral display resulted in less tissue removal, but the magnitude of effect was stronger in non-urban compared to urban sites. Florivory did not respond to variation in alkaloids or PC2 ($p > 0.05$ in all cases).

For pollination, flowers on plants in urban sites received 20% less *Gelsemium* pollen ($F_{1,491} = 11.86$, $p = 0.0006$) and 28% less heterospecific pollen than flowers on plants in non-urban sites ($F_{491} = 8.53$, $p = 0.004$; figure 1). Thrum flowers received 18% more *Gelsemium* pollen ($F_{1,491} = 19.27$, $p < 0.0001$) and 31% more heterospecific pollen than pin flowers ($F_{1,491} = 14.65$, $p < 0.0001$). Both floral display size ($F_{1,491} = 18.64$, $p < 0.0001$) and PC1 (flower size) increased *Gelsemium* pollen receipt ($F_{1,491} = 6.95$, $p < 0.009$). There were no other significant relationships between floral traits and *Gelsemium* or heterospecific pollen receipt ($p > 0.05$ in all cases), and there were no significant interactions between traits and site type ($p > 0.05$ in all cases), indicating that effects of traits on pollination did not vary with urbanization. *Gelsemium* and heterospecific pollen receipt were positively correlated ($r > 0.24$, $p < 0.0001$). Because of this correlation and because heterospecific pollen only made up 10% of total pollen receipt, we only consider *Gelsemium* pollen receipt in subsequent analyses.

(b) Plant reproduction

Plants in non-urban sites produced 8% more total flowers ($F_{1,979} = 4.64$, $p = 0.03$; electronic supplementary material, figure S3a) than in urban sites. However, the urban landscape had no effect on proportion fruit set, mean seeds per fruit, or total seeds per plant ($F < 2.10$, $p > 0.15$ in all cases; electronic supplementary material, figure S3b–d). Floral morph (pin versus thrum) did not affect any measures of plant reproduction ($F < 1.40$, $p > 0.24$ in all cases). There were significant effects of block (site pair) for all measures of plant reproduction ($F > 2.65$, $p < 0.01$ in all cases), suggesting that aspects of the landscape drive block-level differences in performance.

(c) Patterns of phenotypic selection

(i) Directional selection

The most consistent directional selection we observed was for larger floral display size, with all sites showing selection for larger floral displays (electronic supplementary material, tables S4 and S5). The selection for larger floral display sizes was significant in all sites except for three non-urban sites. However, the strength of selection for larger floral display differed significantly between urban and non-urban sites (ANCOVA: trait \times site-type interaction: $F_{1,884} = 9.12$, $p = 0.003$; figure 2; electronic supplementary material, table S5), with 28% stronger selection for larger floral display size in urban versus non-urban sites. Mean floral display size differed significantly between urban and non-urban sites, with significantly larger floral display size in non-urban compared to urban sites (electronic supplementary material, figure S4a).

We found weaker directional selection for flower size (PC1) and shape (PC2) (figure 2; electronic supplementary material, table S4). For flower size (PC1), none of the selection gradients were significant in urban and non-urban sites (electronic supplementary material, table S4). For flower shape (PC2), only one non-urban site showed significant selection on flower shape (electronic supplementary material, table S4). We found no interactions between flower size or shape and site type, suggesting no differences in selection between urban and non-urban sites ($p \geq 0.82$ in both cases; figure 2; electronic supplementary material, table S5). Flower morphology did not differ significantly between urban and non-urban sites (electronic supplementary material, figure S4b).

Across all sites and types, there was significant selection on reduced alkaloid production ($F_{1,884} = 3.77$, $p = 0.052$; electronic supplementary material, table S4). However, the strength and pattern of selection on alkaloids did not vary between urban and non-urban sites (ANCOVA: trait \times site-type interaction: $F_{1,884} = 0.20$, $p = 0.66$; figure 2; electronic supplementary material, table S5), and alkaloid production did not differ significantly between urban and non-urban sites (electronic supplementary material, figure S4c).

(ii) Quadratic selection

Across all sites and traits, we only found six instances of significant quadratic selection (electronic supplementary material, table S4); five were for significant quadratic selection for floral display size (one non-urban site and four urban sites) and one was significant quadratic selection for flower shape in an urban site (PC2). However, there were no traits for which the strength or pattern of quadratic selection varied between urban and non-urban sites ($p > 0.56$ in all cases; electronic supplementary material, figure S5).

(iii) Correlational selection

We found 13 significant correlational selection gradients across all sites and trait correlations (electronic supplementary material, table S6), but we found no significant trait \times trait \times site-type interactions ($p > 0.21$ in all cases; electronic supplementary material, figure S6), suggesting that the urban landscape did not differentially affect patterns of correlational selection. However, across both urban and non-urban sites, there was significant correlational selection for floral display size by flower shape (PC2) (ANCOVA: display \times PC2

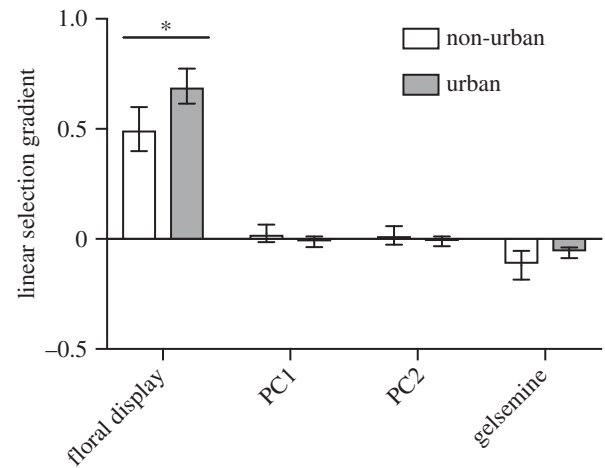


Figure 2. Standardized directional selection gradients (mean \pm s.e.) for floral and resistance traits in urban and non-urban sites of *Gelsemium sempervirens*. The line and asterisk above bars indicate that ANCOVA documented significant differences among urban and non-urban sites in floral display size (trait \times site-type interaction; $p < 0.05$).

interaction: $F_{1,872} = 9.88$, $p = 0.002$) and floral display size by alkaloid production (ANCOVA: display \times gelsemine interaction: $F_{1,872} = 7.95$, $p = 0.005$). For display by floral shape, plants with larger floral display and reduced floral shape were favoured, and for display by alkaloids, plants with larger floral display with less alkaloids were favoured.

(d) Species interactions and patterns of selection

We found evidence to suggest that pollination may be associated with differences in the strength of selection on larger floral displays in urban versus non-urban sites. We found a three-way interaction between pollen receipt, floral display size, and site type for relative total seeds (ANCOVA: pollen \times display \times site-type interaction: $F_{1,478} = 8.71$, $p = 0.003$), suggesting that pollination differentially modifies the trait–fitness relationship in urban versus non-urban sites. We also found a significant interaction between pollen receipt and site type (ANCOVA: pollen \times site-type interaction: $F_{1,478} = 9.24$, $p = 0.003$). Seed production increased with pollen receipt in urban sites ($p = 0.006$) but not in non-urban sites ($p = 0.13$; figure 3a,b). These results indicate variation in how pollen receipt translates into seed production; pollination only has the potential to exert phenotypic selection on floral display in urban sites. Thus, plants in both urban and non-urban sites experience selection for larger floral displays, but the stronger selection on floral display documented in urban sites may be associated with the translation of pollination into seed production. There was no evidence that florivory was involved in differential patterns of selection (ANCOVA, three-way interactions: $p > 0.05$ in all cases).

4. Discussion

Here, we demonstrate that urban landscapes can alter patterns of directional selection on floral display size in a native plant species. It is important to note that we did not find differences in selection on all traits. Instead, the patterns of selection we document are modest, and suggest that urban landscapes may not result in sweeping changes in phenotypic

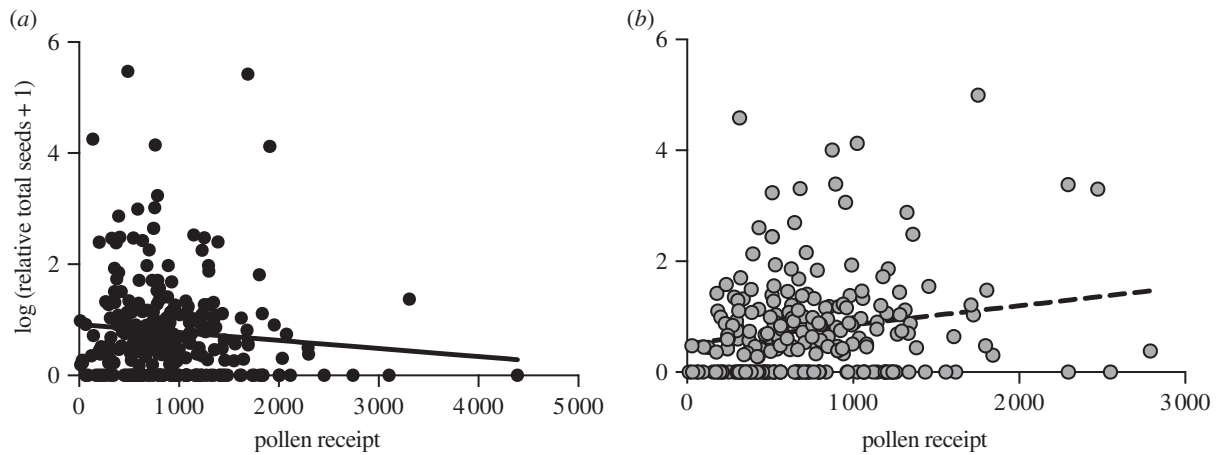


Figure 3. Relationship between pollen receipt and relative total seed production ($\log(x + 1)$) in (a) non-urban and (b) urban sites. There was no significant relationship between pollen receipt in relative total seeds in non-urban sites ($p = 0.13$), but a significant positive relationship in urban sites ($p = 0.006$).

selection. Thus, while some organisms may rapidly adapt to urban landscapes [4] and exhibit marked differences in phenotypes [41], for others, differences in selection between urban and non-urban areas may not be as pronounced. Unfortunately, too few studies report explicit measures of phenotypic selection in replicate urban and non-urban sites to draw general conclusions. Our data suggest that pollination may be associated with selection for larger floral display size in urban compared to non-urban sites, potentially due to differences in the translation of pollination into seeds rather than pollinator selectivity. That urban sites are associated with small changes in selection on floral traits may seem inconsequential; however, pollination is a critical ecosystem service. Thus, understanding how floral traits may be selected upon and the degree to which they can adapt and evolve has important implications for the sustainability of species in urban landscapes.

(a) Species interactions

The patterns of species interactions documented in this study were different from those in our prior research [23], even though we used many of the same sites. In particular, we found very little nectar robbing in this study and so could not consider robbers as important agents of selection. Temporal variation in robbing levels is common in other systems [42], potentially due to variation in the abundance of robbers and alternative host plants. Within the context of this study, this means that robbers may not be consistent agents of selection across years. We found that *Gelsemium* and heterospecific pollen receipt were higher in non-urban sites in the current study, but in prior research we found higher levels of pollen receipt, especially heterospecific pollen, in urban sites [23] and higher bee abundance in urban sites [17]. Higher pollination in non-urban sites in the current study matches research showing that urban landscapes can erode pollinator abundance and pollination services [16,43]. These differences in patterns of pollen receipt among our two studies may reflect differences in the pollinator and heterospecific plant communities among years, as well as differences in other factors that can affect pollinator abundance, such as the availability of nest sites, parasites,

and pesticides [44]. These data suggest that if any of these species interactions are important agents of selection, then high temporal variation in selection may erode the evolution of differential phenotypes among sites. Given that we found little relationship between phenotypes and estimates of selection (electronic supplementary material, figure S7), this could reflect temporal variation in the direction and magnitude of selection, although we cannot rule out other possibilities. Thus, while studies have documented differences in species abundance and interactions between urban and non-urban areas and along urban gradients, temporal variation in these interactions also needs to be considered.

(b) Patterns of plant reproduction

One surprising finding is that estimates of plant fitness did not differ strongly between urban and non-urban sites. Plants in non-urban sites produced significantly more flowers, but these plants did not have a higher proportion of fruit set, seed set per fruit, or total seed set. Our results contrast with studies that show negative effects of fragmentation or urbanization on seed production in plants [45], although some plants show no effect or even benefit from fragmentation or urbanization [46]. We found that plant reproduction varied widely among sites, suggesting a role for landscape context and other unmeasured factors affecting plant reproduction. However, the lack of strong difference between urban and non-urban sites in reproduction suggests no extreme maladaptation to the urban landscape in the year of study.

(c) Phenotypic selection

In prior research, we used field observations and a common-garden experiment in one urban/non-urban site pair to develop predictions for how the urban landscape may alter patterns of selection in *Gelsemium* via changes in species interactions [23]. We predicted selection for smaller floral displays and wider, shorter flowers in urban compared to non-urban sites via changes in antagonistic interactions, and no selection on the alkaloid gelsemine given that florivores previously did not respond to variation in gelsemine [23]. However, we instead found stronger selection for

larger floral displays in urban compared to non-urban sites although the difference was modest, and no differences in selection between site types for any other trait. Typically, the strength of pollinator-mediated selection is positively (although sometimes nonlinearly) related to the strength of pollen limitation [47]. Urban sites had lower pollen receipt, and there was a significant, positive association between pollen receipt and seed production in urban sites (figure 3*b*), but not in non-urban sites (figure 3*a*). These results suggest that unmeasured factors are likely associated with selection for larger floral displays in both urban and non-urban sites, but the stronger selection on floral display size in urban compared to non-urban sites may be associated with differences in the strength of the link between pollination and seed production.

The one prediction that was supported by our results was no variation in selection on the alkaloid gelsemine. Higher concentrations of gelsemine in floral nectar reduce both pollination and nectar robbing [27], and gelsemine in petal tissue is associated with reduced pollination [26]. We found a significant alkaloid \times site-type interaction for proportion of flowers with reproductive damage, with increased alkaloids reducing the proportion of flowers with reproductive damage, but only in the urban sites. However, we found selection for reduced gelsemine via total seeds in both urban and non-urban sites, which may reflect costs of production [48].

The magnitude and variability in selection gradients that we observed between urban and non-urban sites is comparable to that in other systems documenting spatial variation in selection. For example, Caruso *et al.* [2] and Chapurlat *et al.* [3] documented significant spatial variation in selection on floral traits, with strong selection for increased flower number and weaker more variable selection for other traits relating to flower size and shape. We observed similar patterns, with respect to the types of traits that exhibited spatial variation in selection and the magnitudes of differences in selection gradients between sites. Strong selection for larger floral display size makes sense within the context of frequent, positive correlations between floral display size, number of flowers, and fruit and seed set [49], although inflorescence size can also be selected through male function. We found spatial variation in selection not only on single floral traits, but also on combinations via correlational selection. Studies that have found correlational selection on floral traits often find selection on one trait that influences the rate of pollinator visitation and a second trait that influences pollinator efficiency, given that pollination is a function of both processes [3]. We found a similar pattern, documenting significant correlational selection on display \times flower shape (PC2).

Four caveats are important to the interpretation of our phenotypic selection results. First, we did not manipulate species interactions. Thus, our correlative approach only allows us to associate patterns of selection with putative biotic agents. Studies are needed that manipulate pollen receipt to determine the proportion of selection that can be attributed to pollination (akin to [50]) and how that varies in urban versus non-urban sites. Second, we used wild-growing plants to estimate selection and so any trait differences could be due to a plastic response to environmental or genetic differences [51]. While urban and non-urban sites did not differ in temperature, relative humidity, or soil nutrients

(electronic supplementary material, text S1), we cannot rule out the possibility of other unmeasured environmental differences between the site types or differences in plant quality, including age and genetic structure. A powerful approach for future research would be to use a genotypic selection analysis with genotypes from urban and non-urban sites in replicate common gardens to isolate the effects of urbanization on patterns of selection [23]. Third, we used a paired-site approach to compare selection in urban versus non-urban sites. While this approach was advantageous in allowing us to control for some environmental and socioeconomic heterogeneity within site pairs, it did not provide an opportunity to make generalizations across a broader range of urban sites nor to assess selection across multiple metropolitan areas [21]. Moreover, with only eight site pairs, we had limited sample size and so did not examine associations between selection gradients and site-level factors. Additional insights into patterns and agents of selection may be drawn by work in more sites across gradients of urbanization and in multiple urban and urbanizing metropolitan areas [4]. Fourth, our selection analyses only encompass one episode of selection in a long-lived plant.

5. Conclusions

Surprisingly few studies have assessed how urban landscapes affect patterns of phenotypic selection in plants and the degree to which plant–animal interactions may be involved in patterns of selection. Here, we document that floral display size varied in the magnitude of selection between urban and non-urban sites, with stronger selection for larger floral display size in urban sites. Pollination was the one species interaction associated with differences in patterns of phenotypic selection. However, patterns of species interactions were widely different between this study and prior research in many of the same study sites [23], and we found little difference in phenotypes between urban and non-urban sites. Given the strong temporal variation in species interactions that we observed, measuring selection in more years may offer insights into the degree to which patterns and agents of selection vary spatially and temporally, and whether temporal variation in selection erodes phenotypic differentiation in the urban landscape.

Ethics. Field research was conducted in accordance with local legislation.

Data accessibility. Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.81k9m23> [52].

Authors' contributions. R.E.I., P.S.W., and L.S.A. designed the study and collected the data. R.E.I. and L.S.A. processed the data. R.E.I. analysed the data and wrote the first manuscript draft. All authors contributed significantly to revisions and approved the submission.

Competing interests. We declare we have no competing interests.

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