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# Little plant, big city: a test of adaptation to urban environments in common ragweed (*Ambrosia artemisiifolia*)

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A full understanding of how cities shape adaptation requires characterizing genetically-based phenotypic and fitness differences between urban and rural populations under field conditions. We used a reciprocal transplant experiment with the native plant common ragweed, (*Ambrosia artemisiifolia*), and found that urban and rural populations have diverged in flowering time, a trait that strongly affects fitness. Although urban populations flowered earlier than rural populations, plants growing in urban field sites flowered later than plants in rural field sites. This counter-gradient variation is consistent adaptive divergence between urban and rural populations. Also consistent with local adaptation, both urban and rural genotypes experienced stronger net selection in the foreign than in the local habitat, but this pattern was not significant for male fitness. Despite the evidence for local adaptation, rural populations had higher lifetime fitness at all sites, suggesting that selection has been stronger or more uniform in rural than urban populations. We also found that inter-population differences in both flowering time and fitness tended to be greater among urban than rural populations, which is consistent with greater drift or spatial variation in selection within urban environments. In summary, our results are consistent with adaptive divergence of urban and rural populations, but also suggest there may be greater environmental heterogeneity in urban environments which also affects evolution in urban landscapes.

## 1. Introduction

Urbanization causes pronounced changes in the biotic and abiotic environment. Urban areas are warmer [1], have increased levels of $CO_2$ and ozone [2], greater salinity stress [3] and altered precipitation patterns [4] compared to non-urban areas. These environmental changes can reduce biodiversity [5], modify plant growth and physiology [6], and change animal behaviours [7]. The evolutionary consequences of urbanization have been studied far less than these ecological consequences [8]. Nevertheless, urban environments are likely to alter both non-adaptive and adaptive evolutionary processes [8]. Urban populations are often smaller and more fragmented than rural populations, thereby increasing genetic drift and genetic differentiation among populations [9,10]. Moreover, environmental differences within and between urban and rural environments may cause spatially-varying natural selection and adaptive divergence among populations.

The results of a handful of studies on animals and plants are suggestive of adaptive divergence between urban and rural populations [11–13]. Three studies have investigated urban–rural divergence of plant populations [14–16]. Those studies found evolutionary changes in dispersal [14], flowering time and fecundity [16], and chemical defence [15]. All three studies indicate that urban plant populations have phenotypically diverged from rural populations. However,

only one of these [15], was conducted under field conditions, limiting our ability to characterize how selection is acting in contemporary populations.

Studies investigating adaptation to urban environments have generally treated urban areas as a single environment, effectively ignoring the diversity of habitats found within cities (e.g. parks, roadsides, mowing, pavements, rail tracks). This environmental heterogeneity, which has been discussed extensively in the urban ecology literature [17], may lead to differing selection pressures and adaptive divergence among subpopulations found within a single urban area. Although several studies have investigated molecular divergence among urban populations [9,10], the extent to which subpopulations from a single urban area have phenotypically diverged from one another remains unexplored.

Our primary objective of this study was to advance our understanding of local adaptation to urban environments in plants using realistic field experiments. Our specific objectives were to: (i) characterize whether urban and rural populations differ in fitness and phenotypic traits that are probably subject to strong selection; (ii) compare the extent of inter-population phenotypic divergence among urban and among rural populations; (iii) determine whether selection acting on these traits differs between urban and rural environments or between urban and rural genotypes; and (iv) test for evidence of local adaptation to urban and rural environments. If urban and rural populations are both locally adapted to their home environments, we expect phenotypic divergence at the selected traits, different patterns of selection between urban and rural environments, weaker selection acting on local genotypes compared to foreign genotypes, and that both urban and rural populations will have higher fitness when grown in their home environment. We compare the extent of inter-population phenotypic divergence among urban and rural populations to gain insight to how environmental heterogeneity within these broadly defined environments may affect evolution.

To achieve these objectives, we used a multi-site (two rural and two urban sites) reciprocal transplant experiment with the plant *Ambrosia artemisiifolia* (common ragweed, Asteraceae) to conduct, to our knowledge, the most comprehensive study of local adaptation to urban environments in plants to date. At each site, we collected data on whole-organism phenotypes and lifetime fitness and used these data to characterize selection acting on local and foreign genotypes. We chose *A. artemisiifolia* because it is native to North America, is widely abundant in urban areas, and is an annual, which makes it feasible to conduct experimental manipulations and collect data on lifetime fitness.

## 2. Methods

We collected seeds from multiple urban and rural populations of common ragweed (*A. artemisiifolia* L.) in the Minneapolis–Saint Paul, Minnesota, USA metropolitan area. We planted these field collected seeds in four common gardens and visited each site regularly to collect data on phenological traits and fitness. *Ambrosia artemisiifolia* is an annual, self-incompatible [18], monoecious, and wind-pollinated [19], early-successional species that is native to and widely distributed in North America, and is invasive in Europe [20], Asia [21], and Australia [22]. The species is abundant in urban areas and in marginal and disturbed habitats [23].

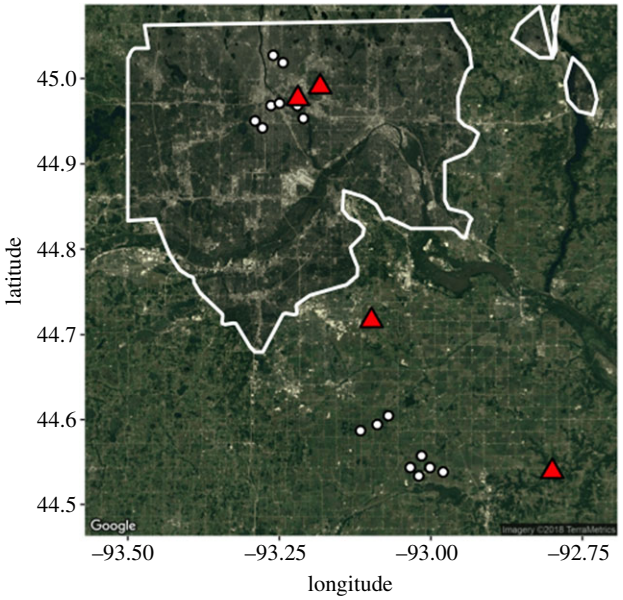


**Figure 1.** Map of sampling locations and field sites in the Minneapolis area. Sampling locations are indicated with white circles, and common garden locations are indicated with red triangles. The white outline indicates urban areas defined by MODIS satellite data at 0.5 km resolution [26]. Map made with *ggmap* [27]. (Online version in colour.)

Staminate capitula (i.e. male flowering heads) are in spike-like racemes, hereafter referred to as 'male flowers', which produce pollen that is one of the primary causes of summer and autumn allergic rhinitis [24]. Pistillate capitula (i.e. female flowering heads) are found in axillary clusters below the male flowers. Individual flowers develop into achenes (small, single seeded fruit) which readily fall off the plant once ripe. The groups of achenes from all flowers on each capitula are hereafter referred to as 'fruits'.

The Minneapolis–Saint Paul metropolitan area has a population of over 3.5 million people with a high-density urban core. Adjacent rural environments are primarily agricultural, with some forest, prairie and wetlands. From 2011 to 2014 the urban core was on average 2.5°C warmer than surrounding rural areas [25].

### (a) Seed collections

In 2014, we collected seeds from eight urban and eight rural populations from within 55 km of the city centre (rural area classified as agricultural or pastoral, figure 1; electronic supplementary material, tables S1 and S2). Seeds were collected from individual maternal plants, each separated by at least 3 m, at each of the 16 collection locales. Seeds from each maternal plant were kept separate from seeds from other maternal plants. Across both regions (*ca* 50 $km^2$), we stratified our sampling to broadly sample environments; the distance between adjacent populations ranged from 1 to 8 km. The urban populations were collected in the city centre core of Minneapolis which has a high concentration of buildings and impervious surfaces (electronic supplementary material, table S2). The urban populations were collected from a range of habitats including bike paths, road medians, abandoned lots and riverbanks. The rural populations were collected from the edges of agricultural fields and in roadside ditches that were outside of the urban heat island [25].

### (b) Reciprocal transplant experiment

In spring 2015, we planted four common gardens: two in urban areas and two in rural areas (figure 1). In each region (i.e. urban,

rural), we established one large (urban: USC; rural: RRO) and one small common garden (urban: USP; rural: RRT). Hereafter, the common gardens are referred to as 'sites'. Except for RRT, which has a sandy loam soil, the field sites have soil that consist of more than 60% clay. All sites were sprayed with herbicide (Roundup, Monsanto, MO) on 19 May 2015 to remove pre-exiting vegetation and we tilled the soil on 22 May 2015 to create an open, highly disturbed environment, which mimics the natural growing conditions of *A. artemisiifolia*.

At each site, we transplanted approximately four seedlings from each of 8–14 maternal families per population (electronic supplementary material, table S1; average number of seeds germinated per line = 7.5 ± 0.5 s.e.). All seeds from each maternal family (i.e. genotype) were weighed to obtain an average weight per seed per maternal family. Seeds were stratified in mesh bags by burying them in moist silica sand and keeping them in the dark at 4°C for 10 weeks. After stratification, seeds were planted in 72-cell trays in a 50 : 50 mix of local field soil and Sunshine Mix no. 1 (Sun Gro, MA), and germinated in the greenhouse under a 14 h day and at a day/night temperature of 22/20°C. After two weeks, we transplanted seedlings directly into the soil at each field site (from 28 May to 3 June, USC: $n = 528$; USP: $n = 228$; RRO: $n = 532$; RRT: $n = 256$). This matches the phenology and approximate size of seedlings previously observed in urban Minneapolis (AJ Gorton 2014, personal observation). For each site, we arranged the seedlings in a completely randomized design with plants spaced 15 cm apart along each of 10 rows, with the distance between columns alternating between 30 and 80 cm. Seedlings were watered immediately after transplanting and once during the first week after transplanting. The plants were not fertilized during the experiment.

## (c) Data collection

We visited each site twice weekly to collect data on three phenological traits: date of transition to reproductive phase, date of first male flower and date of first female flower. The transition to reproductive phase was scored as the date on which a reproductive bud at the apical meristem first appeared. The first male flower was scored as the date on which the first anther opened and shed pollen (i.e. the first open male flower). The first female flower was scored as the date at which stigmas first appeared. We also calculated the time between first open male flower and first open female flower (male to female flower). As a proxy for final size, we measured height for each individual between 21 September and 5 October. This is after individuals stopped growing and the weeks during which there is a 50% chance of a frost, based on data from 1981 to 2010 (0°C, US Climate Normals, NOAA). On the same date, we estimated fitness by conducting flower and fruit counts on a subsample of branches on each plant. A.J.G. counted the number of male flowers and number of fruits on every fourth branch of each plant (*ca* one fourth of each plant), starting with the lowest and largest branch of each plant, and moving up towards the apical meristem. Each plant had a minimum of 20 branches. We multiplied this number by four to get a whole-plant estimate of male and female fitness. We recognize that this is an approximation of fitness and subsampling branches may have introduced variance in our estimates that weaken the statistical power. Nevertheless, owing to the size of the plants more precise measures of fitness, e.g. counts of every flower and fruit on every branch of every plant, were not feasible.

## (d) Statistical analyses of phenotypic divergence and adaptation

We tested for phenotypic differences at two spatial scales. At the regional level, we tested for phenotypic differences between urban and rural seed sources, ignoring source population. At the population level, we tested for phenotypic differences among source populations within either the urban or rural region. These tests of regional and population differentiation were conducted using data from each of the four field sites. All model equations for the statistical analyses conducted below are included in the electronic supplementary material, A and all data analyses were conducted in R, v. 3.2.2 [28].

To test for phenology, size, and fitness differences among sites or between urban and rural seed sources (hereafter 'source region'), we fitted linear mixed models (LMMs, *lme4* package [29]). We ran separate models with each of four phenology traits (date of transition to reproductive phase, date of first male flower, date of first female flower, male to female flower) as the dependent variables, average seed weight as a covariate, site, and source region as fixed effects, and the interaction between site and source region, and maternal plant (the plant from which seed was collected) as a random effect (electronic supplementary material, A1). Average seed weight was included as a covariate to account for potential maternal effects, as we used field collected seeds.

For the fitness and size analyses, we scaled male and female fitness and height within each site using *z*-scores (trait value − site mean)/site standard deviation. The *z*-score transformation was used because plants at the USP field site were considerably larger than plants at the other field sites (mean height in cm: USP = 129.1, USC = 61.5, RRO = 79.2, RRT = 55.6, likelihood ratio test (LRT): $p < 0.0001$ for site), and significant interactions can be caused by both change in mean and change in variance [30].

When these initial analyses for fitness and size revealed a significant or marginally significant site × source region interaction, we conducted separate analyses on the urban and rural seed sources within each site. For each site, we used generalized linear mixed models (GLMMs, *lme4* package, [29]) with a Poisson error distribution and a log link function for the fitness variables, and LMMs as above for the size traits. Average seed weight and source region were modelled as fixed effects, and maternal plant as a random effect (electronic supplementary material, A2).

For all analyses, we determined the significance of fixed effects and interactions by comparing sequential nested models with and without the term of interest using LRTs. To simplify analyses, interaction terms with $p > 0.1$ were dropped from the models, but all main effects were retained. Least-square means were extracted from each model using the *lsmeans* package [31]. For both male and female fitness, we also calculated local-foreign contrasts [32] within each site using these least-square means (contrast = local source mean − foreign source mean).

To test for phenology and fitness differences among urban populations and among rural populations, we conducted two series of analyses: one analysis was conducted using data from only urban populations and one using data from only rural populations. These analyses were similar to the urban versus rural analyses, but instead of source region in the model, we used 'source population' (electronic supplementary material, A3) and maternal plant was nested in population as a random effect. In cases where a significant effect of population was found, we used Tukey tests implemented in the *multcomp* package [33] to conduct pairwise comparisons among populations. In addition, we conducted Levene's test (*levene.test*, *car* package [34]) on the least-square means to determine whether the variance among urban populations and among rural populations was equal.

## (e) Aster analysis

We also used 'aster' (*aster* package [35,36]) to compare lifetime fitness of urban and rural populations. Aster explicitly models the dependence of fitness components expressed later in

development (e.g. fecundity) on those expressed earlier (e.g. survival) and allow for different statistical distributions for each fitness component. As such, aster models can be more statistically powerful than traditional GLMMs [37]. For each individual, we used the following graphical model to estimate lifetime female fitness (number of fruits):

$$1 \rightarrow \underset{(0,1;\,\text{Bernouilli})}{\text{survival to 6 weeks}} \rightarrow \underset{(0,1;\,\text{Bernouilli})}{\text{(flowering)}} \rightarrow \underset{(\text{Poisson})}{\text{number of fruit.}}$$

The same model was used for male fitness, except that number of male flowers replaced number of fruits. In addition to the graphical model, our aster analyses included source region, site, and source region × site as fixed effects. We determined the significance of fixed effects and interactions by comparing sequentially nested models with and without the term of interest using LRTs. We predicted male and female lifetime fitness separately for each source region at each site, and calculated local-foreign contrasts (contrast = local source mean − foreign source mean) with these predicted means.

## (f) Selection analyses

We tested whether selection differs between urban and rural genotypes at each field site and whether foreign genotypes experienced stronger selection than local genotypes, a pattern consistent with local adaptation. We conducted the selection analyses on four traits (transition to reproductive phase, date of first open male flower, male to female flower, and height), some of which were highly correlated (electronic supplementary material, tables S11 and S12). These traits are all key contributors to plant fitness and are often under strong selection [38]. We estimated selection differentials (net selection) and selection gradients (direct selection) using simple and multiple linear regression, respectively, of male and female relative fitness on standardized traits [39]. Selection differentials measure selection via both direct selection acting on the trait of interest and selection acting through correlated traits; whereas selection gradients provide estimates of selection on a trait after statistically removing indirect selection that results from selection acting on other measured, but not unmeasured, traits. We calculated relative fitness by dividing individual male and female fitness by the site mean. We standardized traits within each site by subtracting the site mean and dividing by the standard deviation. Last, we calculated the genotypic means for relativized fitness metrics and standardized traits. The significance of differentials and gradients were evaluated with Type II sums of squares.

We conducted separate selection analyses on data from each site, and within each site we examined selection separately for urban and rural genotypes. In addition, we asked whether the strength of selection differed between urban and rural genotypes by fitting a linear model with relative male or female fitness as the response variable, source region and the interaction between the two as predictor variables (electronic supplementary material, A4).

We quantified stabilizing and disruptive selection using the same model format but included the square of each trait as an additional variable. When quadratic terms were significant, we fitted nonparametric cubic splines to the data using the *smooth.spline* function in R to determine where there was a fitness minimum or maximum within the phenotypic range [40].

We quantified selection gradients and tested whether they differ between urban and rural genotypes by fitting a linear model for the three phenology traits following the same format as the selection differential models above: relative male or female fitness as the response variable, source region, the three phenology traits, and interactions between source region and each trait (electronic supplementary material, A5). We omitted height in the selection gradients analyses because it was highly correlated with our estimates of fitness (all $r > 0.6$). For all quadratic gradients, we multiplied the regression coefficient by two [41].

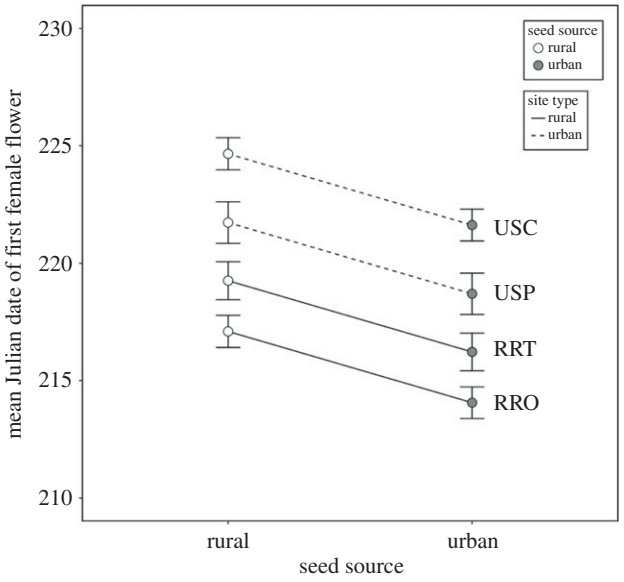


**Figure 2.** Mean Julian date of first open female flower for urban and rural populations. Grey, urban populations; white, rural populations. Dashed lines, urban field sites; solid lines, rural field sites. The bars indicate ± standard error. There was a significant effect of regional seed source ($p = 0.0006$) and site ($p \leq 0.0001$) (electronic supplementary material, table S3). Means were extracted from linear mixed effects models.

To test whether selection was stronger on foreign than local genotypes, we calculated local-foreign contrasts and conducted a one-sided Wilcoxon signed-rank test on these contrasts. We calculated the absolute difference in selection differentials and gradients between local versus foreign genotypes (foreign coefficient − local coefficient) at each field site for each trait. We then used a one-sided Wilcoxon signed-rank test (*wilcox.test*) to determine if the difference in selection was significantly greater than zero, which would indicate stronger selection on the foreign genotype. We also include the results of the two-sided Wilcoxon signed-rank test for comparison. We conducted all selection analyses with the *lme4* package [29], and *car* packages [34], and conducted separate tests for selection differentials and gradients, and for male and female fitness.

# 3. Results

## (a) Urban–rural phenotypic divergence and adaptation

Data from the four sites revealed genetic differences in flowering time between urban and rural populations. Urban populations flowered earlier than rural populations ($p = 0.04$ first open male, $p = 0.0006$ first open female flower; figure 2; electronic supplementary material, table S3), a difference that was consistent across the four field sites ($p > 0.4$ for all site × source region; electronic supplementary material, table S3). By contrast, plants growing in the urban sites (USC, USP) flowered significantly later than plants in rural sites (site: first open male and female flower, $p < 0.0001$; electronic supplementary material, table S3; figure 2).

In contrast to the phenological traits, for which there were no significant site × source region interactions (electronic supplementary material, table S3), there was evidence that fitness and height of urban and rural populations differed among sites (site × source region interaction). Although these interaction terms were not significant in the GLMMs

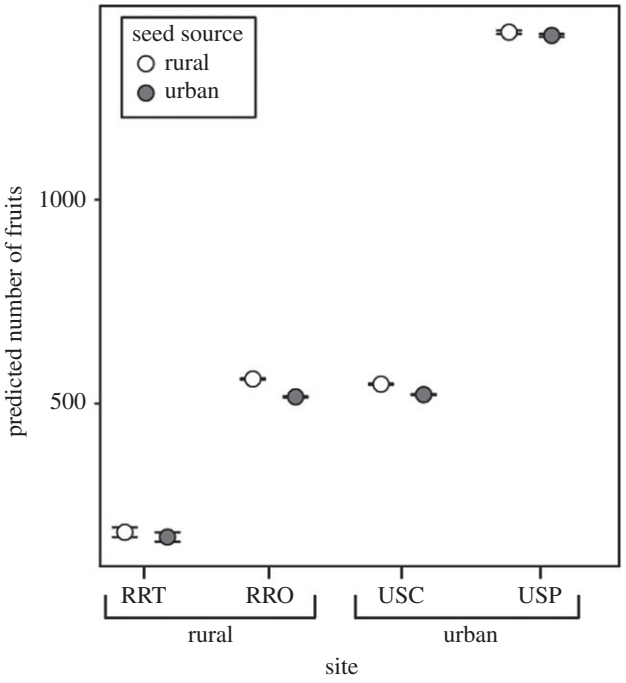


**Figure 3.** Mean number of predicted fruits produced by urban and rural populations at each field site. White, rural populations; grey, urban populations. The bars indicate ±standard error. Predicted means were extracted from separate aster models for each site. There was a significant effect of source region (see the electronic supplementary material, table S9). Rural field sites: RRT, RRO; urban field sites: USC, USP.

(LRT; flowers: $\chi^2 = 5.38$, $p = 0.15$, fruits: $\chi^2 = 0.89$, $p = 0.83$, height: $\chi^2 = 6.49$, $p = 0.09$), the interaction was highly significant in the aster analyses (male and female lifetime fitness: site × source region: $p < 0.0001$; electronic supplementary material, table S9). This reflects the greater statistical power of aster models, obtained by including multiple fitness components and modelling each with a different statistical distribution [34,35].

Within each site, the GLMMs indicated there were no significant differences between urban and rural populations in either male fitness, female fitness or height (source region: $p > 0.1$ at all sites; electronic supplementary material, table S4; see the electronic supplementary material, table S5 for local-foreign contrasts), with the exception of the rural site RRO, where rural populations were 7 cm taller, on average, than urban populations (RRO: $p = 0.006$; electronic supplementary material, table S4 and figure S3). By contrast, the aster models revealed that male and female lifetime fitness of rural populations was significantly higher than urban populations at all four sites (source region: all $p < 0.003$; figure 3; see the electronic supplementary material, table S10 for local-foreign contrasts), except for USP, where urban populations had slightly higher male lifetime fitness than rural populations.

## (b) Inter-population divergence within urban and rural region

The phenological traits, transition to reproductive phase and all flowering time traits, differed significantly among urban populations (source population: all $p < 0.005$; figure 4; electronic supplementary material, table S6) and tended to differ among rural populations (source population: all $p < 0.09$; electronic supplementary material, table S6), with no strong evidence for the site affecting

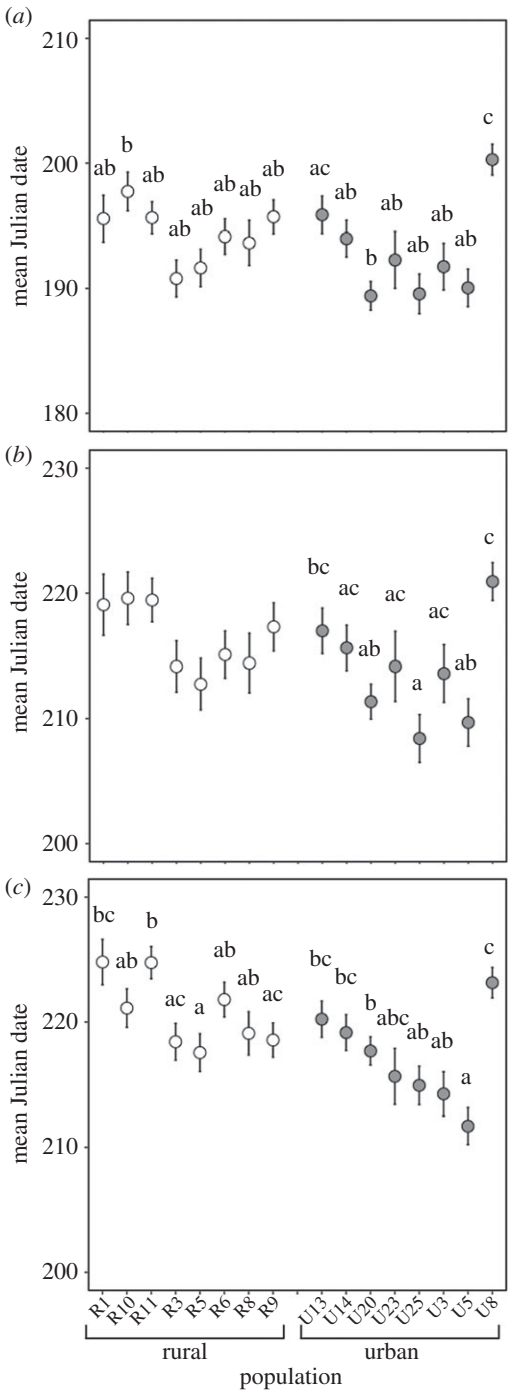


**Figure 4.** Mean Julian date of (*a*) transition to reproduction, (*b*) first open male flower, and (*c*) first open female flower of urban populations and rural populations averaged across field sites. White, rural populations; grey, urban populations. The bars indicate ±standard error. Letters indicate Tukey test results conducted among rural or among urban populations, where unique letters indicate significantly different groups. Urban and rural population names are listed along the *x*-axis in an arbitrary order based on the numerical identity of the population. There was a significant effect of population seed source on all flowering time variables (electronic supplementary material, table S6). Means were extracted from linear mixed effects models for each trait.

population differences (site × source population: all $p > 0.2$), except for male to female flower for rural populations ($p = 0.06$; electronic supplementary material, table S6). There was no evidence for differences in the variance among urban versus among rural populations (Levene's test: $p > 0.4$ for all phenological traits).

**Table 1.** Selection differentials from linear regression models. (Differentials were estimated separately for urban and rural genotypes. S, linear regression coefficient. Selection differentials in bold are significant at $p < 0.05$.)

| | | transition to reproduction | | first open male flower | | male to female flower | | height | |
|---|---|---|---|---|---|---|---|---|---|
| | | urban | rural | urban | rural | urban | rural | urban | rural |
| urban | | | | | | | | | |
| USC | $S_{male}$ | −0.14 | **−0.20** | **−0.13**[a] | **−0.32**[a] | **0.18** | **0.28** | **0.33** | **0.48** |
| | $S_{female}$ | −0.09[a] | **−0.30**[a] | −0.05 | **−0.23** | −0.05[a] | **0.18**[a] | **0.29** | **0.41** |
| USP | $S_{male}$ | −0.15 | 0.04 | −0.06 | −0.18 | 0.17 | 0.02 | **0.38** | 0.19 |
| | $S_{female}$ | −0.14 | 0.05 | −0.05 | −0.20 | 0.10 | −0.06 | **0.31** | 0.14 |
| rural | | | | | | | | | |
| RRO | $S_{male}$ | −0.12 | **−0.18** | **−0.23** | **−0.15** | **0.36** | **0.23** | **0.40** | **0.24** |
| | $S_{female}$ | **−0.28** | −0.10 | **−0.22** | −0.07 | **0.25** | 0.09 | **0.30** | **0.15** |
| RRT | $S_{male}$ | −0.12 | −0.18 | −0.22[a] | 0.10[a] | 0.13 | −0.06 | **0.40** | 0.28 |
| | $S_{female}$ | −0.24 | −0.27 | **−0.38**[a] | −0.07[a] | −0.07 | 0.02 | **0.28** | **0.34** |

[a]Selection differentials which are significantly different between urban and rural seed sources at $p < 0.05$.

Male fitness also differed significantly among urban populations (source population: all $p < 0.05$; electronic supplementary material, figure S1 and table S7) with no evidence for site affecting population differences (site × urban population: LRT, $\chi^2_{13} = 14.69$, $p = 0.33$). By contrast, there was evidence for a site × urban population for female fitness and height (LRT: female fitness, $\chi^2_{13} = 22.41$, $p = 0.05$, height, $\chi^2_{13} = 26.65$, $p = 0.01$), although the ranking among the urban population was similar across sites (electronic supplementary material, figures S2 and S7). Within each site, there was a significant effect of urban population for female fitness and height (source population: all $p < 0.05$; electronic supplementary material, table S7).

There were no differences in either male or female fitness among rural populations (source population: $p > 0.2$ for both traits; electronic supplementary material, table S8 and figures S1 and S2), nor did site affect this pattern (site × rural population: $p > 0.3$ for both traits). Mean height of rural populations varied significantly among sites (site × rural population: $\chi^2_{13} = 33.93$, $p = 0.001$), and among rural populations at the USP field site (electronic supplementary material, table S8).

There was some evidence for unequal variance in fitness among urban and rural populations, however this was only true at USP (Levene's test: male fitness, d.f. = 6, $p = 0.001$; female fitness, d.f. = 6, $p = 0.003$), and RRT (Levene's test: female fitness, d.f. = 6, $p = 0.004$). This is not surprising given each site had 4–6 populations per region, and therefore there was little statistical power to detect unequal variance.

## (c) Selection analyses

Selection differentials (net selection), calculated for both urban and rural genotypes for data from each of the four experimental sites, revealed that selection tended to favour larger plants, earlier transition to reproduction, earlier flowering, and delayed time between first open male and female flower (table 1). Although not all estimated differentials were statistically significant ($p < 0.05$), the estimates of selection were generally in the same direction as the patterns described above. At all field sites selection strongly favoured large plants (table 1). Consistent with past adaptation to local environments, assuming that local genotypes are near a fitness optimum, net selection tended to be stronger on the foreign seed source at all sites, although the pattern was not as strong for male fitness (Wilcoxon one-sided sign test: male fitness, $p = 0.07$, female fitness, $p = 0.01$; Wilcoxon two-sided sign test: male fitness, $p = 0.14$ female fitness, $p = 0.02$).

Selection gradients revealed some similar patterns for the phenological traits, although few selection gradients were statistically significant (table 2) and there was no evidence for stronger direct selection on foreign genotypes than rural genotypes (Wilcoxon one-sided sign test of local-foreign contrasts: male fitness, $p = 0.90$, female fitness, $p = 0.38$; Wilcoxon two-sided sign test: male fitness, $p = 0.23$, female fitness, $p = 0.73$). The weaker statistical support for the gradients is not surprising given that there is some correlation among all traits (electronic supplementary material, tables S11 and S12) and the statistical significance of each trait is evaluated after accounting for variance in fitness that can be explained by other traits. In other words, our analysis does not provide evidence for selection acting on any specific trait, but rather the cumulative effects of selection acting on a trait and those traits which are correlated with it. Although we detected significant quadratic selection differentials and gradients (electronic supplementary material, tables S13 and S14), the non-parametric cubic splines revealed that there were no fitness minima or maxima within the range of the data. Thus, the significant quadratic parameters may reflect curvilinearity to the fitness function rather than stabilizing or disruptive selection.

# 4. Discussion

To advance our understanding of adaptation to urban environments, we conducted a series of reciprocal transplant experiments, using the native annual plant *A. artemisiifolia*.

**Table 2.** Selection gradients from multiple linear regression models. (Gradients were estimated separately for urban and rural genotypes. $\beta$, linear multiple regression coefficient. Selection gradients in bold are significant at $p < 0.05$.)

| | | transition to reproduction | | first open male flower | | male to female flower | |
|---|---|---|---|---|---|---|---|
| | | urban | rural | urban | rural | urban | rural |
| urban | | | | | | | |
| USC | $\beta_{male}$ | −0.06 | 0.07 | −0.02 | **−0.25** | 0.13 | 0.14 |
| | $\beta_{female}$ | −0.09 | −0.06 | −0.08 | −0.18 | −0.14 | 0.01 |
| USP | $\beta_{male}$ | −0.31 | 0.14 | 0.34 | −0.25 | 0.29[b] | −0.03[b] |
| | $\beta_{female}$ | −0.28 | 0.11 | 0.27 | −0.33 | 0.19 | −0.17 |
| rural | | | | | | | |
| RRO | $\beta_{male}$ | −0.06 | −0.10 | −0.01 | 0.11 | **0.32** | **0.26** |
| | $\beta_{female}$ | −0.12 | −0.09 | −0.06 | 0.05 | 0.16 | 0.09 |
| RRT | $\beta_{male}$ | −0.14[b] | **−0.42[b]** | −0.29[a] | 0.21[a] | 0.03 | −0.09 |
| | $\beta_{female}$ | 0.01 | **−0.43** | **−0.54[a]** | 0.03[a] | **−0.40** | −0.11 |

[a]Selection gradients which are significantly different between urban and rural seed sources at $p < 0.05$.
[b]Selection gradients which are different between urban and rural seed sources at $p < 0.10$.

Previous investigations of plant adaptation to urban environments have revealed evidence for adaptive divergence between urban and rural populations [14–16]. However, ours is, to our knowledge, the first study to examine selection and adaptation of urban populations using whole organism phenotypes, lifetime fitness, and data collected from plants planted directly into the ground under semi-natural field conditions. Our results suggest local adaptation of both urban and rural populations: urban and rural populations have genetically diverged in multiple phenological traits—including flowering time, a trait that is often under strong selection [38]. Moreover, across both urban and rural sites net selection acting on phenological traits was stronger on foreign genotype, particularly with female fitness. This is consistent with local genotypes being closer to a selective optimum, as expected if populations have adapted to local conditions. However, this pattern of selection was not as strong for male fitness, nor was this result detected in the selection gradients and thus we cannot differentiate the effects of selection acting directly on our measured traits from the effects of direct selection and indirect selection acting through other measured traits.

Although the pattern of phenological divergence and the evidence of stronger selection acting on foreign genotypes are both consistent with local adaptation of urban and rural populations, the aster analyses revealed that rural populations had higher lifetime fitness than urban populations at all sites. There are several possible reasons for this apparent mismatch: selection on phenological traits may have contributed to the divergence of urban and rural populations but selection on other unmeasured traits, may be more important to lifetime fitness [39]. Alternatively, selection and the adaptive response to selection may be stronger in the rural than in the urban environment, perhaps because the rural environment is more uniform (see discussion of spatial heterogeneity below) or because of larger effective population sizes in the rural than in the urban populations.

## (a) Counter-gradient variation

Phenotypic differences between urban and rural environments reflect both environmentally-induced, plastic effects, and genetic divergence between urban and rural populations. We found that the plants grown in the urban sites flowered later than plants in the rural sites, reflecting a plastic response that affected both urban and rural populations. However, urban populations tended to flower earlier than rural populations: a pattern of counter-gradient variation, whereby genetic differences are in the opposite direction of plastic responses [37,42]. The earlier flowering of urban populations might be owing to the drier conditions and reduced water availability of urban environments compared to non-urban areas [4,43]. Earlier reproduction to avoid drought conditions has been commonly observed, and is probably a common adaptation in annual, ruderal species such as ragweed [44,45].

Counter-gradient variation has been found in other transplant experiments of local adaptation [46,47]. Indeed, Thompson *et al*. reported a similar pattern in *Trifolium repens* across an urban–rural gradient: they observed the frequency of cyanogensis declined towards the urban core, but a potted transplant experiment revealed selection for increased cyanogensis in urban areas [15]. The evidence for counter-gradient variation in both our study and that of Thompson *et al*. [15]—the two studies that have conducted field experiments to investigate urban adaptation in plants—suggest that selection and adaptation to urban environments might often be opposite to plastic responses to environmental variation. In other words, plastic responses may not always reflect underlying genetic differences. This result underscores the importance of manipulative experiments for properly characterizing the role of selection and adaptation in driving divergence between urban and rural populations.

Our finding of earlier flowering in rural compared to urban field sites is opposite to ecological and herbaria data suggesting

that plants growing in urban areas, including *A. artemisiifolia*, tend to flower earlier than plants growing in rural areas [48,49]. The reasons for this are unclear. It could be related to different growth conditions, either in weather during the year we conducted our experiment, or at each of our field sites. Alternatively, such a pattern may be driven by differences in the germination timing of urban and rural populations. We exposed all seeds to the same stratification length and temperature, and then germinated them under common greenhouse conditions. However, in natural populations the germination environment and cues of urban and rural populations may vary. For example, the urban heat island [1] may cause plants to germinate earlier than those in rural locations. As germination timing determines the environment that subsequent life stages experience (e.g. flowering), it can influence the evolution and adaptation of plant populations [50]. Our results suggest exploring the potential urban–rural differences in germination and post-germination traits could be a promising direction of future research in urban plant adaptation.

### (b) Spatial heterogeneity and phenotypic differences among urban populations

Much of the experimental work in urban adaptation has treated populations collected from within the same urban area as being similar to one another [12,14,16] or predicted that an 'urban phenotype' will become more common in more urbanized environments [15]. Urban areas are, however, spatially heterogeneous [17] and this heterogeneity can be important ecologically and evolutionarily [9,10]. Consistent with this heterogeneity being evolutionarily important, we found phenological divergence and fitness differences among urban populations at a scale of 2–3 km. Across all field sites, both urban and rural populations had differences in phenology among populations, but the pattern was stronger among urban populations. Furthermore, there were differences in both male and female fitness among urban populations, but not for rural populations. Thus, the urban populations appear to display greater variance in both phenological and fitness traits, although this should be viewed with caution given that unequal variances are not statistically well supported (Levene's test). However, with only a maximum of eight populations per region, there is little statistical power to detect unequal variances. In addition, the range of phenological differences among urban populations was often greater than those at the regional level, i.e. among all urban and all rural populations. These results suggest that selection and adaptation may vary across spatial scales in urban environments, a pattern which has been found in other species in natural or non-urban environments [51,52]. By ignoring these fine-scale differences within cities, we may be limiting our ability to explain phenotypic variation and determine which environmental variables are ultimately driving urban plant adaptation.

Our reciprocal transplant was reciprocal at the scale of urban and rural environments: we did not have field sites in the specific habitats from which we sampled each source population. Therefore, we cannot determine if there is microgeographical adaptation within either the urban or rural environment, nor which environmental variables may drive such patterns of adaptation. Furthermore, patterns of phenotypic differentiation among urban *A. artemisiifolia* populations could be driven by reduced gene flow and greater genetic drift; we cannot disentangle whether these patterns were caused by neutral processes or selection. Nonetheless, there were some notable environmental variables that varied among our collection locales and field sites. Urban collection locales varied from parks and low-density housing to highly developed areas (see the electronic supplementary material, table S2). Furthermore, populations collected from sandy habitats flowered earlier and there was strong selection for earlier flowering at the field site with the highest per cent sand (RRT), suggesting selection in response to sandy habitats may favour earlier flowering genotypes. Our results also support the hypothesis that mowing may lead to delayed flowering in *A. artemisiifolia* [53]: the U8 population was sampled from an area that is mowed frequently and individuals from this population flowered later, and had lower fitness, than other urban populations. While these habitat differences alone do not indicate microgeographical adaptation, they do indicate heterogeneity within urban areas, which in turn may shape patterns of adaptation among urban populations.

### (c) Conclusions

In conclusion, we found evidence for genetic divergence in ecologically-important traits between urban and rural environments, but support for local adaptation was mixed. The selection analyses provided some support for selection against foreign genotypes, which is consistent with local adaptation, but rural populations overall had higher fitness at all field sites, which is not consistent with local adaptation. In addition, we found greater phenotypic divergence among urban populations than rural populations, which we hypothesize may be driven by the higher environmental heterogeneity present in urban areas. Our results suggest that urban environments are fundamentally shaping the phenotypic evolution of plant populations. In addition, future work should consider including both multi-year experiments and fine-scale sampling to explicitly incorporate micro-environmental variation among sites and genotypes in cities.

Data accessibility. The raw data and scripts used to analyse data are archived on Dryad; (http://dx.doi.org/10.5061/dryad.3kv50) [54].

Authors' contributions. A.J.G., D.A.M. and P.T. conceived and designed the experiment and wrote the manuscript. A.J.G. conducted all seed collections, and conducted the field experiment. A.J.G. conducted all data analyses, with advice and assistance from D.A.M. and P.T.

Competing interests. We declare we have no competing interests.

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