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An urban–rural spotlight: evolution at small spatial scales among urban and rural populations of common ragweed

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Abstract

Urbanization produces similar environmental changes across cities relative to their neighboring rural environments. However, there may be high environmental heterogeneity across an urban–rural gradient. Previous research in Minneapolis, MN, USA, found mixed evidence that urban and rural plant populations of common ragweed have locally adapted and that urban populations exhibit greater among-population divergence in ecologically-important traits. To investigate whether there are parallel patterns of urban–rural trait divergence across different urban areas, we examined trait variation across an urban–rural gradient in a second city, St. Louis, MO, USA. We used growth chamber and greenhouse common environments to investigate variation in six traits within and among 16 populations of common ragweed (eight from each urban and rural area). Urban and rural plants diverged significantly in three of five traits, with rural plants having lower percent germination, greater height and lower leaf dissection index. We also found greater variance in plant height among urban compared to rural populations, potentially driven by heterogeneity in human management practices on urban populations. Patterns of urban-rural trait divergence (e.g. in flowering time) differ substantially from those found previously in the reciprocal transplant experiment in Minneapolis, contradicting the hypothesis of parallel evolution across different metropolitan areas. The results of this study suggest that there is considerable population variation in ecologically-important traits, but that urban populations do not consistently differ from neighboring rural populations.

Key words: urban adaptation, environmental heterogeneity

Introduction

Urban environments differ from neighboring rural environments in a variety of factors (e.g. temperature, Oke 1973; pollutant concentrations, Lovett et al. 2000; CO_2 concentrations, Koerner and Klopatek 2002), which may affect the growth and reproduction of urban plants (e.g. leaf lobing, Vogel 1970; tree growth, Gregg et al. 2003; seed dispersal, Cheptou et al. 2008; plant height, Yakub and Tiffin 2017; flowering time, Yakub and Tiffin 2017; Gorton et al. 2018, 2020). These differences between urban and rural environments are sometimes replicated across different metropolitan areas (Thompson et al. 2016; Yakub and Tiffin 2017; Johnson et al. 2018) and it has been suggested that these predictable differences may lead to parallel evolution (Johnson and Munshi-South 2017). Urban areas also have substantial spatial heterogeneity resulting from the juxtaposition of a wide variety of land-use types, including constructed environments with impervious surfaces and semi-natural areas that are primarily vegetation (Porter et al. 2001; Cadenasso et al.

2007; Niemelä 2011; Pickett et al. 2017). While the ecological consequences of spatial and landscape variability within urban areas have received attention (Melles et al. 2003; Wania et al. 2006; Cadenasso et al. 2007; Hope et al. 2008), little is understood about the evolutionary implications of this environmental heterogeneity despite recent calls for research (Rivkin et al. 2018).

Thus far, studies investigating the evolutionary consequences of within-city environmental heterogeneity have made progress in identifying how particular land cover types may facilitate or impede gene flow among habitat fragments (Munshi-South 2012; Unfried et al. 2013; Beninde et al. 2018). Much less is understood about whether the high heterogeneity of urban environments influences trait evolution and local adaptation. The diverse microenvironments of urban areas (e.g. parks, sidewalks and roadsides, boulevards, maintained yards and greenspaces, and abandoned lots) may cause contrasting selection pressures over fine spatial scales, which in turn may result in phenotypic differentiation among urban populations within a city. For example, Gorton et al. (2018) found greater phenotypic variance among urban populations of common ragweed (*Ambrosia artemisiifolia*), compared to among rural populations, suggesting increased diversification due urban heterogeneity (although genetic drift could also contribute). Therefore, more empirical studies examining divergence among urban populations are necessary to clarify whether high urban habitat heterogeneity predictably results in elevated phenotypic variance.

In this study, we tested the predictions that (1) urban populations of common ragweed exhibit divergence in ecologically important traits compared to neighboring rural populations and (2) there is greater phenotypic variance among urban populations compared to among rural populations, in St. Louis, MO, USA, metropolitan area using a common garden greenhouse experiment. If urban areas experience the urban heat island effect and increased human management practices, we expect urban populations to diverge from rural populations via increased leaf dissection index, earlier flowering time, and shorter plant height. If urban areas have higher variation in microenvironments and corresponding selection pressures than rural areas, we expect urban populations to exhibit greater among-population phenotypic differentiation than rural populations. We also evaluated whether patterns of urban–rural divergence in St. Louis are parallel to those from Minneapolis, MN, USA, where we have conducted a previous reciprocal transplant experiment (Gorton et al. 2018). Studies that have compared urban and rural populations from multiple cities have found some evidence for similar urban–rural phenotypic differences, supporting a possible pattern of parallel evolution in converged urban environments (blackbirds, Mueller et al. 2013; crested anoles, Winchell et al. 2016; white clover, Thompson et al. 2016; Johnson et al. 2018; Virginia pepperweed, Yakub and Tiffin 2017; acorn ants, Diamond et al. 2018). However, the patterns of divergence between urban and rural populations are not always consistent across cities (Thompson et al. 2016; Diamond et al. 2018). Thus, more studies are needed to determine how often urban environments result in phenotypic parallel evolution.

Methods

Study species

Common ragweed (*A. artemisiifolia* L.) is a wind-pollinated, outcrossing, annual plant native to North America. The species is widely distributed and grows in marginal and disturbed habitats that are commonplace in both urban and rural environments (Bassett and Crompton 1975; Marks 1983; Simard and Benoit 2011). Common ragweed is monoecious, producing male and female florets separately on the same plant (Essl et al. 2015). The developmental transition to flowering is photoperiod-sensitive, where it is cued by shortening days (Deen et al.1998).

Site selection and seed collection

In 2014, we collected seeds from 16 populations in and around St. Louis (eight urban and eight rural populations) (see Fig. S1 and Table S1). The St. Louis metropolitan area has a land area of 7863 m^2 (20 366 km^2) and an estimated 2.8 million residents, as of 2018 (U.S. Census Bureau 2018). We defined the urban region we sampled as a 10 × 10 km area centered over the downtown core of St. Louis, and the rural region as a 10 × 10 km area approximately 40–55 km southeast of the downtown where the land is primarily classified as agricultural or pastoral (Homer et al. 2015). We overlaid a grid on each region, including one population per grid cell, in order to spatially stratify our samples. The distance between the closest neighboring populations within a region ranged from 1–8 km. In each region, we used extensive searching to sample ragweed populations from a range of habitats. In the rural area, ragweed was only found along roadsides and agricultural fields. In the urban area, the populations were sourced from roadsides, parks, gardens, bike paths, lawns, railroads and vacant lots. Within populations, seeds were collected haphazardly from individual plants spaced at least 3 m apart.

We assessed land cover within a 100-m radius of each source population using the 2016 National Land Cover Database (Yang et al. 2018; Table S3). We were particularly interested in testing whether land use indicative of urbanization was more frequent in the area that we defined as urban versus that defined as rural. To do this, we summed three land cover categories that included constructed materials and consisted of at least 20% impervious surfaces (developed low intensity, developed medium intensity, developed high intensity). We tested whether developed land cover differed between the two regions using a Wilcoxon rank sum test. We also tested whether variance in developed land cover was greater in urban compared to rural areas using a Levene's test of unequal variances.

Germination experiment

In January 2018, we randomly selected approximately 10 maternal families (mean = 9.7 families, see Table S1) per population and 2–8 seeds per maternal family (mean number of seeds per family = 7.8, total number of seeds = 1216). After 7 weeks of stratification at 4°C in the dark, the seeds were planted in BM2 soil mix (BERGER, Quebec, CA) and transferred to two Conviron E7/2 growth chambers (Controlled Environments Ltd., Manitoba, CA). We assigned maternal families to the two chambers using a stratified random approach so that each population had an equal number of maternal families in each chamber. We then planted the maternal families in a completely randomized design across four 128-slot trays in each chamber. We planted two seeds per maternal family in a given cell and covered each tray with clear plastic domes. The seeds were kept at 22°C days/20°C nights (14-h photoperiod) and were misted daily for 2 weeks.

We recorded the fraction of seeds that germinated and germination date (as the first date of cotyledon appearance above the soil); we gathered germination data daily for 2 weeks. Previous pilot experiments found that 87.5% of seeds from these

populations germinated within 12 days of planting after 9 weeks of stratification (see Table S2).

Greenhouse experiment

After 2 weeks, we randomly culled germinants to three per maternal family (mean = 2.7 seedlings per family, 9.1 families per population; total number of seedlings = 399). Using the same soil as in the greenhouse experiment, we transplanted the seedlings into individual pots (10.5 cm W × 9 cm H) and transferred to a greenhouse with 14-h photoperiod, 24°C day/22°C night. Pots were spaced out on benches in the same pattern as the slots in the germination trays, thereby maintaining random stratification of source population across the greenhouse benches. We bottom-watered the seedlings whenever topsoil began to dry and we applied liquid fertilizer via bottom-watering during weeks 4, 8 and 13 (20-20-20 fertilizer: 238 ppm, Peters Professional). After 5 weeks, we shortened the photoperiod to 12 h to cue the transition to reproduction.

We collected data on several phenological (initiation of reproduction, male flowering time, female flowering time) and morphological traits (height and leaf dissection). We selected these traits as they are known to be ecologically important, vary within and among populations of *A. artemisiifolia*, and that trait variation has a genetic basis (Hodgins and Rieseberg 2011; Gorton et al. 2018; Gorton et al. 2019). Furthermore, increased leaf dissection index (or leaf lobing) can increase heat dissipation (Vogel 1970), which may be important to avoid heat stress in the warmer conditions of urban areas (i.e. urban heat island, Alcoforado and Andrade 2008). Initiation of reproduction was scored as the date on which a reproductive bud at the apical meristem first appeared. Male flowering time was scored as the number of days from transplanting to first observed open anther. Female flowering time was scored as the number of days from transplanting to the first visible stigma. Plant height was measured during weeks 7, 9, 11 and 13. After 7 weeks, the third fully expanded leaf of each plant was collected to measure leaf dissection index. Leaf area and perimeter were calculated using ImageJ (Schneider et al. 2012), and leaf dissection index was calculated by dividing leaf perimeter by twice the square root of the product of leaf area and π, or the formula: $DI = perimeter/(2\sqrt{area \times \pi})$ (Kincaid and Schneider 1983; Thomas and Bazzaz 1996; Santiago and Kim 2009; Shi et al. 2020). Prior to measurement, leaves were stored at 4°C and dried at 55°C. Data collection and drying occurred within a week of collection for all leaves.

Statistical analyses

Urban–rural phenotypic divergence

To test whether there was divergence between urban and rural genotypes, we used linear mixed-effects models (lme4 package, Bates et al. 2014; linear models are shown in Table S4) with region (urban vs rural) and population nested within region as predictors, and maternal family nested within population as a random effect. The effect of maternal family was not significant for any of the traits measured in the greenhouse experiment and did not affect the significance of other terms in the models, and thus was excluded from the models. For percent germination (total number of germinants divided by number of seeds planted per family) and days to germination (number of days from planting to appearance of radicle), growth chamber was also included as a predictor. In the greenhouse experiment, plant height was highly correlated across all four measurement

dates, as were the three measures of flowering phenology ($r > 0.6$ for all traits, see Tables S5 and S6). Thus, we used height at 13 weeks and female flowering time to represent size and flowering phenology categories, respectively. In total, we conducted analyses on five traits: percent germination, days to germination, height at 13 weeks, female flowering time and leaf dissection index (pairwise correlations are shown in Table S7).

Inter-population phenotypic divergence within urban and rural regions

To test whether there was greater phenotypic variance among urban than among rural populations, we first subsetted our data into urban and rural populations. We then ran linear models for each trait with population as a predictor (lme4 package, Bates et al. 2014). For the germination experiment, we analyzed percent germination on a per family basis, and days to germination on an individual basis. Chamber was included as a predictor for both of these traits. For the greenhouse experiment, we analyzed height at 13 weeks, female flowering time and leaf dissection index.

We used Tukey tests (multcomp package, Hothorn et al. 2008) to test for pairwise differences among population means within a region in both the germination and greenhouse experiment. We also tested whether the variance among urban populations and among rural populations was unequal using Levene's tests. We conducted Levene's tests on population means and genotypic means to acknowledge that phenotypic variance could manifest due to differences among populations or due to the maintenance of phenotypic variance across multiple populations (car package, Fox and Weisberg 2019).

For all analyses, we evaluated the significance of the predictors using F-tests and Type II sums of squares. All data analyses were conducted in R, v. 3.6.1 (R Core Team 2019).

Results

Land cover

Land cover indicative of developed land (including constructed materials and >20% impervious surfaces) was more frequent in urban (mean = 0.75; SE = 0.11, range = 0.19–1.00) compared to rural sites (mean = 0.11, SE = 0.03, range = 0.00–0.22; Wilcoxon rank sum test, $Z = 3.1$, $P = 0.002$). We also found greater variance in developed land cover around urban sites relative to rural sites (Levene's test for land cover, $F = 16.1$, $P = 0.001$).

Urban–rural phenotypic divergence

Three of the five traits (percent germination, plant height and leaf dissection index) differed significantly between urban and rural populations (Table 1, Figs. 1 and 2). Both urban and rural populations germinated within 4–5 days (mean days to germination: urban = 4.7 days, rural = 4.5 days; $P = 0.681$) but had marginally different rates of germination (mean percent germination: urban = 61%, rural = 68%; $P = 0.049$, Table 1, Fig. 1). Growth chamber did not have a significant effect on percent germination ($P = 0.698$) nor days to germination ($P = 0.158$). Rural plants were on average 3.8 cm taller than urban plants at 13 weeks ($P = 0.004$, Table 1, Fig. 2). Urban plants had larger leaf dissection indices than rural plants, indicating that they had greater leaf lobing per unit area (mean leaf dissection indices: urban = 4.66, rural = 4.46, $P = 0.039$, Table 1, Fig. 2). Urban plants flowered 1.6 days later than rural plants, on average,

Table 1: Results of linear regression models testing the effect of source region (urban vs rural) and population on phenotypic traits. Population was nested in source region

Trait	Source of variation	SS	df	F	P-value
Percent germination	Region	0.37	1	3.91	**0.049**
	Region: population	9.23	14	6.96	**<0.0001**
	Chamber	0.01	1	0.15	0.698
Days to germination	Region	0.69	1	0.17	0.681
	Region: population	523.24	14	9.19	**<0.0001**
	Chamber	8.11	1	2.00	0.158
Height at 13 weeks	Region	1291	1	8.30	**0.004**
	Region: population	20531	14	9.43	**<0.0001**
Leaf dissection index	Region	6	1	4.30	**0.039**
	Region: population	20	14	1.08	0.378
Female flowering time	Region	368	1	2.92	0.089
	Region: population	3899	14	2.21	**0.007**

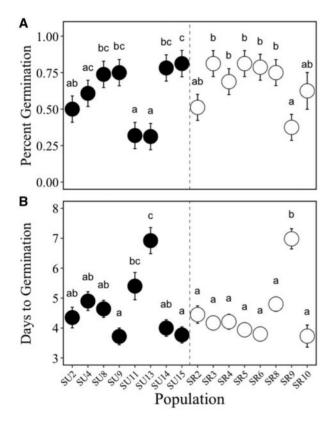Terms in bold are significant at $P < 0.05$.

Figure 1: Growth chamber experiment: Least square mean values of (A) percent germination and (B) days to germination. Bars indicate ±standard error. Black, urban populations; white, rural populations. Letters indicate Tukey test results conducted among urban or rural populations, where unique letters indicate significantly different groups. Populations are listed along the x-axis based on the numerical identity of each population. Least square means were extracted from linear regression models.

although this difference was not statistically significant ($P = 0.089$, Table 1, Fig. 2).

Inter-population divergence within urban and rural regions

Three of the five traits (percent germination, days to germination and plant height) varied significantly among urban and among rural populations ($P < 0.015$ for all three traits; Table 2, Figs. 1 and 2). Germination time varied significantly among both urban and rural populations ($P < 0.0001$ for both, Table 2) and growth chamber had an effect on the germination time of rural populations ($P = 0.052$, Table 2). Female flowering time varied significantly among urban populations ($P = 0.013$, Table 2) and tended to differ among rural populations but was not significant ($P = 0.101$, Table 2).

We found greater variance in height among urban genotypes (Levene's test for height at 13 weeks, $P = 0.034$ genotype means, $P = 0.086$ population means, Table 3) relative to their rural counterparts. For all other traits, there was no significant difference in variance between urban and rural genotypes or populations (all $P > 0.121$, Table 3).

Discussion

Urban heterogeneity has long been recognized in the context of urban ecology (Cadenasso et al. 2007), and recent reviews of the field of urban evolutionary ecology have identified environmental heterogeneity within and among cities as a key characteristic to be considered in studies going forward (Rivkin et al. 2018; Alberti et al. 2020). Previous research on common ragweed found that urban and rural plant populations has phenotypically diverged, and that phenotypic variance was greater among urban than among rural populations (Minneapolis: Gorton et al. 2018). Here, we tested whether there have been parallel patterns of differentiation in a second city, St. Louis, and therefore potentially indicative of parallel evolution to urban environments. While we found some evidence for phenotypic divergence between urban and rural plant populations, there was no evidence for parallel changes in phenotypes between the two cities. In addition, we found evidence for phenotypic divergence among populations within both urban and rural regions of St. Louis. Furthermore, we found some evidence that one trait (plant height) exhibited greater variance among urban than rural populations of St. Louis. Taken together, these results suggest that (1) habitat variation across urban-rural environmental gradients may differ between these two cities and (2) habitat variation may vary at fine spatial scales within regions to a greater extent than between urban-rural environments.

Urban environments are distinct from rural environments and affect plant traits related to growth and reproduction (Gregg et al. 2003; Cheptou et al. 2008; Thompson et al. 2016;
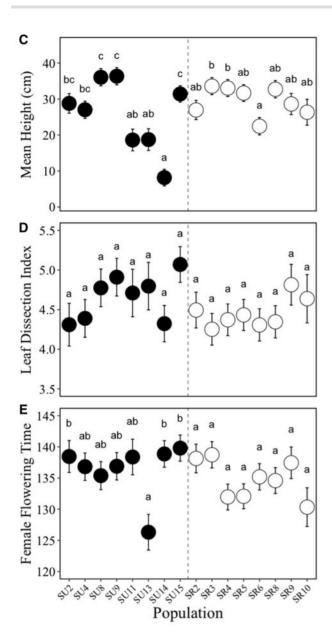
Figure 2: Greenhouse experiment: Least square mean values of (A) height at 13 weeks, (B) leaf dissection index, and (C) Julian date of first open female flower of urban and rural populations. Bars indicate ±standard error. Black, urban populations; white, rural populations. Letters indicate Tukey test results conducted among urban or rural populations, where unique letters indicate significantly different groups. Populations are listed along the x-axis based on the numerical identity of each population. Least square means were extracted from linear regression models.

Table 2: Results of linear regression models testing the effect of source population on phenotypic differences within urban and rural regions separately

Trait	SS	df	F	P value
Urban				
Percent germination	5.78	7	8.78	**<0.0001**
Effect of chamber	0.0003	1	0.003	0.958
Days to germination	260	7	7.83	**<0.0001**
Effect of chamber	0.14	1	0.03	0.863
Height at 13 weeks (cm)	17 704	7	16.37	**<0.0001**
Leaf dissection index	15	7	1.43	0.195
Female flowering time	2439	7	2.62	**0.013**
Rural				
Percent germination	3.45	7	5.15	**<0.0001**
Effect of chamber	0.04	1	0.37	0.543
Days to germination	263	7	10.97	**<0.0001**
Effect of chamber	13	1	3.79	**0.052**
Height at 13 weeks (cm)	2827	7	2.58	**0.015**
Leaf dissection index	5	7	0.60	0.756
Female flowering time	1460	7	1.75	0.101

Terms in bold are significant at $P < 0.05$.

Table 3: Results of Levene's tests for homogeneity of variance among source region (urban vs rural)

Trait	Source of variation	df	F	P-value
Percent germination	Population	14	1.08	0.317
	Genotype	61	1.24	0.270
Days to germination	Population	14	0.13	0.721
	Genotype	56	0.86	0.359
Height at 13 weeks	Population	14	3.40	0.086
	Genotype	56	4.75	**0.034**
Leaf dissection index	Population	14	1.35	0.266
	Genotype	56	0.07	0.799
Female flowering time	Population	14	<0.01	0.981
	Genotype	56	0.37	0.548

Terms in bold are significant at $P < 0.05$.

Yakub and Tiffin 2017; Johnson et al. 2018; Gorton et al. 2018; Gorton et al. 2020). Our results suggest that urban and rural plants in St. Louis have genetically diverged in three ecologically important traits: percent germination, leaf dissection index and plant height. Urban plants had lower percent germination, as well as greater leaf lobing (leaf dissection index) than rural plants; leaves with greater dissection dissipate heat more readily (Vogel 1970). After all, environmental factors such as elevated temperature and CO_2 concentrations may act as selective forces driving phenotypic divergence between urban and rural plant populations (Johnson et al. 2018). Although more study is needed, these results suggest the possibility that greater leaf lobing in urban genotypes may have been selected for in response to the urban heat island effect. The urban heat island in St. Louis is well established: the city is on average 4°C hotter than rural areas during the summer (Vukovich and King 1980; Climate Central 2020). In our experiments and at one common garden in the experiment by Gorton et al. (2018) in Minneapolis, urban plants were shorter than rural plants. One potential explanation for these shared cases of smaller plant height in urban populations in both cities is similar human management pressures, such as mowing, which can lead to selection for shorter plants (Milakovic et al. 2014).

Differences in urban–rural flowering phenology have been a focus in several field (Ziska et al. 2003; Neil and Wu 2006; Gorton et al. 2018) and greenhouse experiments (Lambrecht et al. 2016; Yakub and Tiffin 2017). Observational field studies, which do not account for environmental effects on phenotypes, have observed earlier flowering time in spring-blooming plants in urban compared to rural field areas (Neil and Wu 2006), including common ragweed (Ziska et al. 2003). However, it is unknown whether those trends are driven by environmental or genetic differences. In our greenhouse experiment, urban populations and rural populations did not differ significantly in their

flowering time. These results conflict with the overall trend of earlier flowering among urban compared to rural populations of common ragweed from the reciprocal transplant experiment in Minneapolis (Gorton et al. 2018). Our results, when compared with those of Gorton et al. (2018), suggest that selective pressures across urban–rural environmental gradients may differ among cities even in the same region of a continent with similar climates.

Urban environments have substantial spatial heterogeneity that may increase divergence among plant populations (Porter et al. 2001; Cadenasso et al. 2007; Niemelä 2011; Pickett et al. 2017; Gorton et al. 2018). Our results suggest that, in both urban and rural settings, phenotypic divergence is present among populations even at small spatial scales. This result may appear surprising given that ragweed is wind-pollinated and pollen has been reported to travel up to hundreds of kilometers (Cecchi et al. 2006, 2007), which could potentially facilitate gene flow among populations and limit the degree of divergence. Indeed, a recent population genomics study found minimal population structure across the entire Midwest of the United States (Hämälä et al. 2020). However, the majority of ragweed pollen is dispersed within 10 m of the source plant (Nakahara et al. 2018), and in urban settings, ragweed populations within 10 m to 1 km of pollen traps determine ragweed pollen concentrations (Katz and Carey 2014). Our results suggest that divergence in ecologically important traits can occur over fine spatial scales even with possible long-distance gene flow caused by some ragweed pollen.

Previous work has highlighted the high spatial variation of urban environments at small scales (Niemelä 2011; Pickett et al. 2017). Therefore, it is interesting that variation among urban populations was found to be greater than that among rural populations for only one of our five traits, and not more. Divergence in phenotypes among urban populations could also be due to neutral evolutionary processes, such as genetic drift, because of higher levels of fragmentation in cities. The issue of fragmentation within urban environments, and its potential effects on gene flow among urban plant populations, cannot be disentangled based upon our current studies and must be considered when interpreting the results. This is especially important given that, under strong selection, phenotypic divergence between populations may still be possible even with low to moderate gene flow (De Wet and Harlan 1975).

Human management in urban environments may be a stronger selecting force than we had anticipated. Although studies have found greater habitat heterogeneity among urban compared to rural areas (Porter et al. 2001; Cadenasso et al. 2007), we did not find strong evidence to suggest that urban heterogeneity has resulted in elevated phenotypic variance among urban populations relative to rural. Our results show greater phenotypic variance among urban populations compared to rural for height, but not for any other measured growth or reproductive trait. Urban areas vary from heavily managed to entirely neglected (Goldstein et al. 2001), which may select for plants of different heights. In our experiments, and in the reciprocal transplant experiment by Gorton et al. (2018), urban plants were shorter than rural plants. One potential explanation for these shared cases of shorter plant height among urban populations sourced from Minneapolis and St. Louis is similar human management pressures, such as mowing, which can lead to selection for shorter plants (Milakovic et al. 2014). Our shortest urban population, SU14, was sourced from an intensely managed city park, and averaged under 4 cm in height; these genotypes added biomass by branching very low to the soil surface. It may be

that a history of mowing favored genotypes that could grow and reproduce without growing tall. It is therefore likely that heterogenous human management practices in urban areas, such as mowing or lack of mowing, are a major driver of increased height variation among urban compared to rural plant populations. It is also worth noting that, although there has not been a targeted effort of ragweed removal, these cities have experienced general weed management that could have selected for chemical resistance, thereby introducing different fitness-related traits.

In sum, in line with previous studies, we found evidence for phenotypic divergence between urban and rural plant populations. However, there was no evidence to support the proposition that urban populations of *A. artemisiifolia* have undergone parallel evolution in response to the common selection pressures across urban areas, suggesting that selective pressures across urban–rural environmental gradients differ among cities. In addition, variation in the selective pressures introduced by heterogenous management practices may cause greater variation in plant traits in urban environments, relative to rural. Future studies on the impacts of urbanization on the evolution of urban populations should focus on manipulation of specific environmental factors to assess their contribution to population divergence. Further, future studies should systematically (rather than haphazardly) sample individuals at fine spatial scales to account for potentially unrecognized sources of environmental heterogeneity to urban and rural environments. Given that urban heterogeneity can be identified in the form of varied urban developed land cover, it would also be worthwhile to investigate whether this is tied to variation in selection strength among populations within an urban environment.

Supplementary data

Supplementary data are available at *JUECOL* online.

Acknowledgements

Conflict of interest statement

None declared.

Data availability

The raw data are archived in the Data Repository for the University of Minnesota (DRUM) (https://hdl.handle.net/11299/217706) (Kostanecki et al. 2021).

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