Evolutionary Applications

WILEY

Evolutionary Applications

ORIGINAL ARTICLE OPEN ACCESS

Phenotypic and Genomic Signatures of Adaptation in Urban Populations of the Wild Radish *Raphanus raphanistrum*

Y. F. Chan[1] | G. Scopece[1] | L. Guzzetti[2] | L. Pellegrini[1] | D. Cafasso[1] | M. Labra[2] | S. Cozzolino[1]

[1]Department of Biology, University of Naples Federico II, Napoli, Italy | [2]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy

Correspondence: S. Cozzolino (cozzolin@unina.it)

Received: 5 November 2025 | **Revised:** 15 February 2026 | **Accepted:** 19 February 2026

Keywords: evolutionary adaptation | GWAS | *Raphanus raphanistrum* | SNPs outliers | urbanisation

ABSTRACT

By modifying biotic and abiotic environments, urbanisation can influence the evolutionary adaptation of organisms, especially when their distribution is fragmented. However, the effects of urbanisation on evolutionary adaptation remain poorly understood within a metapopulation framework, where high gene flow and recurrent extinctions/colonisations may counteract local adaptation. To bridge this gap, we integrated phenotypic and genomic data and explored how urbanisation shaped the evolutionary processes in the wild radish *Raphanus raphanistrum*, a widely distributed ruderal species. We conducted field surveys and a common garden experiment to compare phenotypic differences between urban and natural populations in a Mediterranean area. Additionally, population genetic analysis and a genome-wide association study (GWAS) were performed to uncover the genetic basis of urban adaptation and population structure. Our findings indicate that urban populations of the wild radish exhibit significantly smaller flowers and earlier flowering times compared to natural populations. Their correlations with fitness suggest that earlier flowering and longer petal length have higher adaptive values. However, a trade-off between these traits indicates that earlier flowering is advantageous in urban environments, whereas larger flowers are favoured in natural habitats. Genomic analyses reveal low genetic differentiation and frequent gene flow among urban and natural populations. The GWAS identified 46 single-nucleotide polymorphisms (SNPs) significantly associated with urbanisation, 32 of which are located within gene regions. Notably, one of these genes, AT3g27390, is associated with the shift to the reproductive phase. Overall, our results demonstrate how natural selection in an urban context promotes rapid phenotypic divergence in *R. raphanistrum* despite ongoing gene flow.

1 | Introduction

The exponential growth of human populations has accelerated the pace of urbanisation, transforming natural habitats into urban environments dominated by anthropogenic activity (Seto et al. 2010; United Nations 2018; Zhou et al. 2015). Urban areas are characterised by environmental changes in the abiotic environment, including elevated temperatures, increased pollution and extensive impervious surfaces (Fenger 1999; Grimm et al. 2008; Oke 1973). Such urban characteristics can also indirectly modify community dynamics, hence altering the biotic environment (Irwin et al. 2020). To persist in these drastically altered habitats, animals and plants must cope with specific urban constraints (Brans et al. 2017; Diamond et al. 2017; Johnson and Munshi-South 2017; Weston et al. 2021). While many species cannot persist against such dramatic changes and go locally extinct, others successfully adapt and thrive in human-dominated landscapes (Cheptou et al. 2008; Fukano

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *Evolutionary Applications* published by John Wiley & Sons Ltd.

et al. 2023; Winchell et al. 2016). Consequently, urbanisation results in a shift of the phenotypic optima of animals and plants (Donihue and Lambert 2015; Palumbi 2001).

An increasing number of studies have documented phenotypic divergence between populations in urban and adjacent natural habitats, highlighting how urbanisation can drive evolutionary adaptation (Alberti et al. 2017; Lambert et al. 2021). For instance, common garden experiments have been employed to show that different urban plant species flowered earlier than their natural counterparts (Gorton et al. 2018; Schmitz et al. 2024). Gorton et al. (2018) further demonstrated that stronger natural selection in urban environments has driven adaptive shifts in flowering phenology. Another classroom example is the evolutionary response of anti-herbivore chemical production in white clover (*Trifolium repens*; Johnson et al. 2018; Thompson et al. 2016). Scientists have observed the parallel adaptation in hydrogen cyanide production across 160 urban areas globally (Santangelo et al. 2022). Collectively, these studies have advanced our understanding of how urban environments shape adaptive phenotypic differentiation.

In addition to adaptive changes, genomic differentiation between urban and non-urban populations can be compounded by non-adaptive evolutionary processes such as gene flow and genetic drift (Donihue and Lambert 2015). Urban infrastructure—including roads and buildings—often fragments natural habitats, reducing population connectivity. This fragmentation can limit gene flow, amplify the effects of genetic drift and local selection, and disrupt the dynamics of metapopulations (defined as networks of spatially discrete populations connected by dispersal; Slatkin 1987). While gene flow tends to homogenise allele frequencies and counteract adaptive divergence, local adaptation can still occur when selection is strong enough, and genetic variation is maintained across populations (Tigano and Friesen 2016). Alternatively, when gene flow is disrupted, selection might act independently in isolated populations, increasing genetic differentiation and promoting local adaptation (Tigano and Friesen 2016; Johnson et al. 2015). Although many studies have examined urban adaptation through a metapopulation framework (Brans and De Meester 2018; Brans et al. 2017; Schmitz et al. 2024; Winchell et al. 2016), few have successfully disentangled the confounding effects of gene flow from adaptive signals.

Several studies have utilised genomic data to assess the non-adaptive processes under urbanisation (Munshi-South et al. 2016; Santangelo et al. 2022; van Dongen et al. 2015). Some of these studies focused on detecting genomic signatures of urban adaptation (Muller et al. 2018; Theodorou et al. 2018), while others integrated phenotypic divergence from common garden experiments with population genetic analyses (Johnson et al. 2018; Yakub and Tiffin 2017). The former highlighted processes such as gene flow and drift, but did not explicitly test for adaptive responses. The latter did not contemporarily link phenotypic traits to fitness and to the amount of gene flow. However, how constant and high gene flow interacts with urban adaptation under a metapopulation framework remains unanswered.

To bridge this knowledge gap, we investigated how urbanisation shaped the evolutionary divergence of *Raphanus raphanistrum* (wild radish). *R. raphanistrum* is a self-incompatible annual to biennial species in the family Brassicaceae. The species has successfully colonised diverse human-dominated environments, including agricultural fields, waste areas and sheltered beaches, making it an ideal model for studying plant adaptation to anthropogenic habitats (Holm et al. 1997; Schmid et al. 1998). *R. raphanistrum* is widely distributed across the Mediterranean basin, ranging from the edge of arable fields and roadsides to flowerbeds along city pavements. The patchy populations experience a rapid turnover due to ongoing human activities (such as ploughing fields and mowing roadside areas), with local extinctions followed by recolonisation from nearby populations, consistent with a metapopulation dynamic (Bullock et al. 2002; Hanski 1999).

Previous studies have suggested that weedy *R. raphanistrum* showed signatures of adaptation to harsh and unpredictable agricultural fields (Somerville and Ashworth 2024; Sun et al. 2020). The species was able to adjust its life cycle within as few as five generations despite maintaining high genetic variability (Ashworth et al. 2016). We therefore hypothesise that *R. raphanistrum* possesses the ability to rapidly adapt to human-dominated environments. In the present study, by investigating nearby urban and natural populations, we aim to test whether the *R. raphanistrum* populations in the metropolitan area are capable of adaptation despite ongoing gene flow. Specifically, we predict that (1) urban and natural populations of *R. raphanistrum* exhibit phenotypic divergence under a metapopulation framework. (2) These divergences are adaptive and allow *R. raphanistrum* to thrive in urban environments. Finally, we aim to understand which genes and pathways are associated with adaptive divergence between urban and natural environments.

2 | Method

2.1 | Phenotyping in the Wild (Field Study) and Sampling Sites

To evaluate the degree of divergence in *R. raphanistrum* traits associated with urbanisation, we recorded morphological and floral traits from six urban and six nearby natural populations in southern Italy, Campania region, between March 2023 and October 2024. The details of the studied populations and their respective locations are displayed in Figure S1 and Table S1. Urbanisation level was quantified using impervious surface data from the CORINE Land Cover database (European Environment Agency 2018), a widely used resource in ecological and evolutionary studies (Feranec et al. 2010). We applied a 1 km^2 buffer around sampling locations to capture the local landscape context relevant to pollinator-mediated gene flow (Jha and Kremen 2013). Populations inhabiting areas with over 75% impervious surface were classified as urban, whereas those with less than 25% impervious surface were classified as natural.

We visited each wild population once in 2023 and once in 2024. Depending on plant availability, we sampled between 5 and 20 individuals per population, resulting in a total of 286 plants. We measured morphological traits and floral traits that may underpin adaptation to urban environments. For the flower sizes, we measured corolla diameter, petal length and petal width. Petal

length was measured as the linear distance from the base to the tip of a petal, and petal width as the widest point perpendicular to the length axis. Corolla diameter was measured as the distance across the flower from the tip of one petal to the tip of the opposite petal, encompassing the central reproductive structures. All measurements were taken on fresh flowers using digital callipers and averaged across three flowers. We also counted the number of floral axes and open flowers per plant. Given the high number of flowers in the wild, we scored the number of open flowers on three floral axes and estimated the total number of open flowers by multiplying by the number of floral axes. Additionally, we collected all available siliques from each plant in 2023. Seeds were stored separately for each maternal plant in labelled bags and kept at 4°C in a climate-controlled storage room.

2.2 | Common Garden Experiment

To detect the evolutionary response to urbanisation, we conducted a common garden experiment at the open experimental area of the Department of Biology, University of Naples Federico II. In April 2024, we selected 59 maternal lines from both natural and urban populations and sowed 890 seeds (20 per maternal line, where available). Seeds were stratified in water at 4°C for 3 days, then germinated in 4 cm wells of plastic trays filled with non-composted soil (TerCompost; pH 6; 90% porosity) under a natural day/night cycle. We watered the plants twice per week to keep the soil moist. Plants were checked daily, and herbivores were removed immediately on detection.

For each maternal line, we randomly selected eight seedlings for transplantation. If the germination rates were low for certain maternal lines, we included all available seedlings. In May 2024, we transferred 388 seedlings into 14-cm-diameter pots (1.1 L) filled with a 2:1 mixture of composted soil (TerCompost; pH 7.5; 85% porosity) and river sand. Then, we arranged the 388 plants with a randomised design on the four benches, with 14 rows and 7 columns. Four plants died within a week of transplantation, and they were replaced with the remaining seedlings on 20 May 2024. We watered the seedlings immediately after transplantation. Afterwards, the plants were regularly watered by flooding the benches with the same quantity of water to ensure uniform soil moisture. The pesticide (Zepi Garden) was applied as needed to control aphid infestation, and caterpillars were removed manually. The plants were exposed to natural pollinators throughout the experiment in the common garden.

We collected fitness traits that were relevant for urban adaptation and flower traits that were linked to biotic (pollinator) interactions. The flowering time (i.e., days to first flower) was determined as the time span between the transfer of the seedling to the pots and the first flower with open petals. We determined the flower sizes by measuring the corolla length, petal length and petal width following the same procedures in the field study. We also collected nectar using 2 μL glass capillaries (Hirschmann, Germany) fitted with a rubber bulb to extract nectar directly from the flowers. The capillaries containing nectar were analysed for the concentrations of glucose, fructose and sucrose (Supporting Information). We continued to collect the siliques until the end of September 2024. Then, the siliques were kept at room temperature until they dried, and then we weighed them using a laboratory balance (Mettler Toledo).

2.3 | Statistical Analysis of Phenotypic Divergence

To test the effects of urbanisation on phenotypic traits in *R. raphanistrum*, we conducted linear mixed-effect models or generalised linear mixed-effect models with the lme4 and glmmTMB packages in R, version 4.3.3 (Bates et al. 2015; Brooks et al. 2017; R Core Team 2023). The lmerTest package was used to compute the *p* value for fixed effects (Kuznetsova et al. 2017). We ran separate models for each trait recorded as the response variables, origin environments (urban or natural) as fixed effects. For the field study, the date of recording was included as a random effect. For the common garden experiment, we included the maternal line nested within population to control maternal effects and the position of plants as random effects. For the continuous data, we conducted linear mixed-effect models. The flowering time (days to first flower) was log-transformed to ensure normal distribution. We checked model assumptions by plotting the residuals vs. fitted values. Additionally, we tested the deviation of residuals from normality using the Shapiro–Wilk test. For count data and binomial data, we performed general linear mixed-effect models, using Poisson or binomial error distributions, respectively. When applying Poisson as the family for count data, we used the performance package (Lüdecke et al. 2021) in R to check if there was any overdispersion, and qqmath in lattice (Sarkar 2008) to check the normal distribution of our random factors. If overdispersion was detected, we used the negative binomial distribution as a family to handle overdispersion. When it comes to seed number and fruit weight, there were numerous zero values. To handle zero-inflation in our dataset, we conducted generalised linear mixed-effects models via glmmTMB.

2.4 | Selection Analysis

To understand whether these divergences were adaptive, we conducted selection analyses to investigate the association between divergent traits (flowering time and petal length) and maternal fitness (Lande and Arnold 1983). Since petal length and corolla diameter were highly correlated and indicated flower size, we only used the petal length in the following analyses. We estimated selection differentials and selection gradients using univariate and multivariate regressions, respectively. Selection differentials measure both direct and indirect selection on a single trait, whereas selection gradients estimate partial regression coefficients that quantify direct selection while accounting for trait correlations. We selected seed number as a proxy for fitness, as it reflects the reproductive output of plants. We calculated the maternal mean of the traits (Rausher 1992). Then, we standardised them by subtracting their global mean and dividing by the standard deviation. To estimate selection differentials, each trait was included as a fixed effect and relative fitness was included as the response variable in a linear mixed-effects model. To estimate selection gradients, relative fitness was included as the response variable and flowering time and petal length were included as fixed effects. The position and population were included as random effects for the models of selection differentials and selection gradients.

2.5 | DNA Extraction and Sequencing

We collected leaf tissue from 60 *R. raphanistrum* individuals grown in the common garden from nine studied populations. These plants were selected based on extreme phenotypic values (top 10%) observed for traits of interest and represented the most divergent individuals in our studied populations. Afterwards, we extracted their genomic DNA using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. DNA quality and purity were assessed using a NanoDrop ND 1000 Spectrophotometer (Thermo Fisher Scientific, Delaware, USA), and concentrations were quantified with a Qubit fluorometer and the Quant-iT ds-DNA HS Assay kit (Invitrogen, USA).

We then constructed paired-end DNA libraries using the Illumina DNA prep kit (Illumina, San Diego, CA, USA), following the manufacturer's protocol, and sequenced them from both ends on the Illumina NovaSeq platform. The genome size of *R. raphanistrum* is around 421 Mb and the expected coverage given our sequence design was 15. We conducted quality checks with the raw reads using FASTQC (Andrews 2010). The Illumina adaptors and a minimum length of 36 bp bases were trimmed using Trimmomatic 0.39 (Bolger et al. 2014). After filtering, we removed 10 low-coverage samples, resulting in 50 genomes for downstream analysis from nine populations.

2.6 | Genomic Alignment and Single Nucleotide Polymorphism Genotyping

We aligned the trimmed reads from the 50 samples to the *Raphanus raphanistrum* reference genome (accession No. GWHANWM00000000; Zhang et al. 2021) using the BWA-MEM (Li and Durbin 2009) with the default settings. We performed variant calling using GATK HaplotypeCaller in GVCF mode, followed by joint genotyping across all individuals (DePristo et al. 2011; McKenna et al. 2010; Van der Auwera and O'Conner 2020). SNPs were filtered based on the following criteria: QD < 2.0 || FS > 60.0 || MQ < 40.0. We conducted additional filtering using VCFtools v0.1.16 (Danecek et al. 2011) to retain SNPs with a minor allele frequency greater than 5%, a calling rate of 90%, and a minimum read depth of 5 per sample.

2.7 | Population Genetics Analysis and Genome-Wide Association Study

To assess the population genetics, we used Pixy v2.0.0 (Korunes and Samuk 2021) to calculate genome-wide nucleotide diversity, absolute divergence (dXY) and pairwise population fixation statistics (F_{ST}) between populations. Pixy analyses were conducted on the full all-sites VCF using non-overlapping 10 kb windows across the genome. To avoid bias, we excluded two populations (FAIT and CARR) from the population genetic analysis since they have fewer than two individuals each. Following that, we conducted principal component analysis (PCA) using PLINK v. 1.90 (Purcell et al. 2007) and ADMIXTURE v. 1.3.0 to evaluate population structure. For ADMIXTURE (Alexander et al. 2009), we set the number of genetic groups from $K = 1$ to $K = 7$ and used cross-validation error to determine the optimal genetic groups.

We also investigated historical population splits and spatial patterns of gene flow using a combination of Treemix v. 1.13 (Pickrell and Pritchard 2012) and Fast Estimation of Effective Migration Surfaces (FEEMS; Marcus et al. 2021; Petkova et al. 2016). Likewise, CARR and FAIT were excluded from this analysis due to small sample sizes per population. TreeMix was used to infer population relationships and historical migration events by fitting models with increasing numbers of migration edges (m 1 to m 9) and different SNP blocks (k), with model fit assessed based on log-likelihood. In parallel, FEEMS was used to characterise spatial variation in effective migration by modelling covariance in allele frequencies across a discretised landscape, allowing the identification of regions where genetic connectivity is higher or lower than expected given geographic distance alone. Effective migration rates were estimated on a 500 km spatial grid with a smoothness parameter of $\lambda = 2.0$ to balance local genetic variation with global spatial trends. The results were expressed as relative (mean-centred) log-transformed effective migration, such that negative values indicated reduced connectivity and positive values indicated elevated connectivity relative to the landscape average.

To investigate genetic variants associated with urbanisation, we performed GWAS using GEMMA (Zhou and Stephens 2012, 2014). The filtered SNPs and environmental origins (urban vs. natural) of each sample were used as input. GEMMA allows us to fit a linear mixed-effect model while accounting for population stratification and relatedness through a kinship matrix. A kinship matrix was calculated with a built-in GEMMA function and was included as a random effect in the linear mixed-effects model. The GWAS result was plotted with the qqman package in R (Turner 2014). To assess the performance of the linear mixed-effects model, we investigated QQ plots to control the potential inflation of p values due to population structure. We defined the outlier SNPs as those with −log(P) larger than 5.

To investigate the biological functions of candidate SNPs, we identified their associated genes and inferred orthologs using SONICPARANOID version 1.3.0 (Cosentino et al. 2024), with the peptide sequences from *Raphanus raphanistrum* (Zhang et al. 2021) and *Brassica napus* (Handa 2003). The orthologs of candidate genes associated with SNPs were annotated and analysed for functional enrichment using the Database for Annotation, Visualisation and Integrated Discovery (DAVID) Functional Annotation tool (Huang et al. 2009; Sherman et al. 2022).

3 | Results

3.1 | Phenotypic Divergence Between Urban and Natural Populations

Urban populations exhibited consistent phenotypic divergence from natural populations, characterised by reduced flower size and shifts in flowering phenology. In both field surveys and the common garden experiment, urban populations produced smaller flowers and flowered earlier than natural populations. Across the 12 field sites, the natural populations exhibited significantly larger corolla diameters ($p = 0.021$) and petal lengths ($p < 0.01$), but not petal widths

TABLE 1 | Phenotypic results of the generalised linear mixed-effect models for urbanisation in the field.

Model	Response variable	Parameter	Estimated	Standard error	*Z* value	*p*
glmer.nb	Flower number	Intercept	3.1384	0.5485	5.722	<0.001***
		Urban	−0.1346	0.6670	−0.202	0.84
glmer.nb	Floral axis number	Intercept	1.0556	0.3073	3.434	<0.001***
		Urban	0.1890	0.3623	0.522	0.602
lmer	Corolla diameter	Intercept	17.0323	0.4181	40.740	<0.001***
		Urban	−1.2513	0.5082	−2.462	0.021*
lmer	Petal length	Intercept	7.6793	0.1765	43.51	<0.001***
		Urban	−0.6191	0.2164	−2.86	0.009**
lmer	Petal width	Intercept	5.4497	0.1389	39.239	<0.001***
		Urban	−0.2592	0.1703	−1.522	0.142

Note: $p < 0.5$*; $p < 0.01$**; $p < 0.001$***.

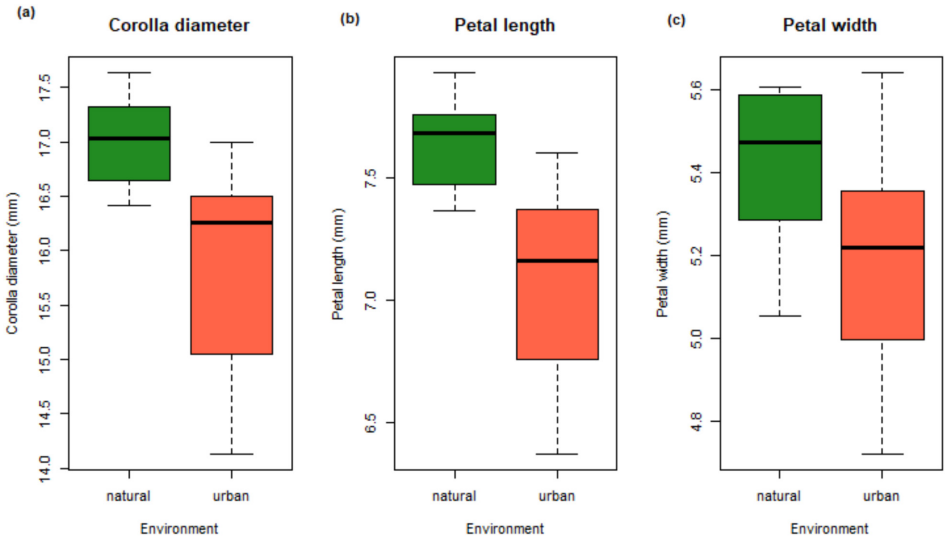


FIGURE 1 | Boxplots of floral traits of *R. raphanistrum* in natural and urban environments across 12 field sites. Green indicates natural populations and orange indicates urban populations.

($p = 0.142$, Table 1, Figure 1) than urban populations. In contrast, no significant differences were found in the number of open flowers ($p = 0.84$) and the number of floral axes ($p = 0.60$) between urban and natural populations (Table 1). Together, these field results indicate that urbanisation is associated with a targeted reduction in flower size rather than a broad shift in reproductive allocation.

Phenotypic divergence between urban and natural populations persisted under common garden conditions, indicating a genetic basis to the observed field patterns. Urban populations differed from natural populations in flowering probability, flowering time, flower size and fruit weight, whereas seed number and nectar traits did not differ between environments (Figure 2). Specifically, urban populations flowered significantly earlier, with flowering starting on average at 53.86 days compared to 78.76 days in natural populations ($p = 0.019$; Figure 2a; Table 2). Urban populations had a significantly higher flowering probability (90.7%) than the natural populations (60.6%, $p < 0.001$; Figure 2b; Table 2). No significant difference in seed number between urban and natural populations ($p = 0.213$), when only flowering plants were included in the analysis (Figure 2e; Table 2). The total seed count predicted by the model was similar between natural (21.02) and urban populations (18.91). When considering only flowering plants, natural populations had significantly higher fruit weight (0.536 g) in comparison to urban populations (0.386 g, $p = 0.0034$, Figure 2f; Table 2).

Consistent with field observations, urban populations produced significantly smaller flowers in the common garden. Corolla diameter ($p = 0.035$; Figure 2c; Table 2) and petal length ($p = 0.0207$; Figure 2d; Table 2) were both decreased in urban populations, whereas petal width did not differ between environments ($p = 0.806$; Table 2). The model predicted average corolla diameters of 15.94 mm and 15.29 mm for natural and urban populations, respectively, and average petal lengths of 7.16 mm and 6.77 mm (Figure 2). Since corolla diameter and petal length

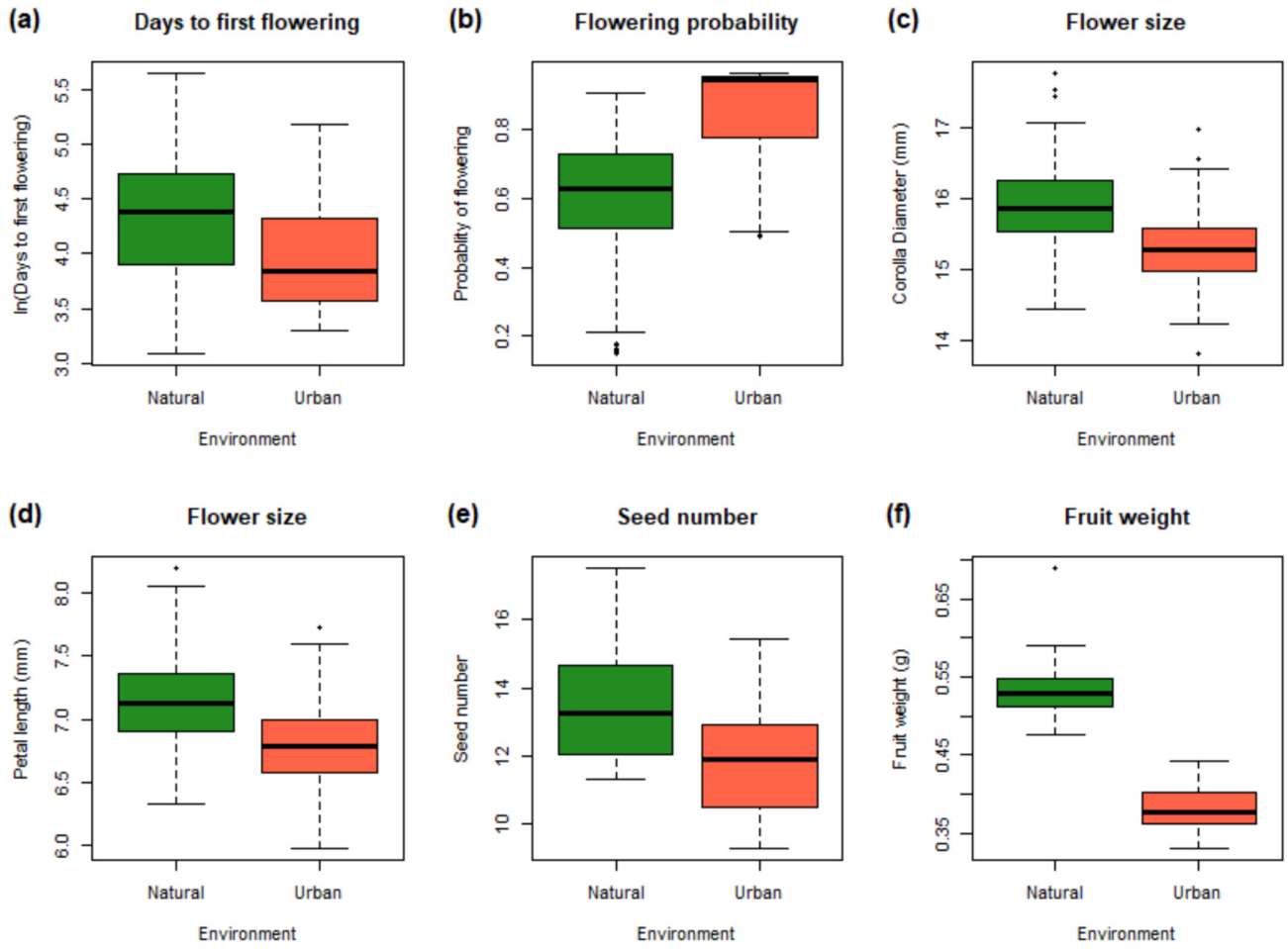


FIGURE 2 | Boxplots of phenotypic means of *R. raphanistrum* between natural and urban populations in the common garden experiment. Green denotes natural populations, while orange denotes urban populations.

were highly correlated (Table S2) and both indicate overall flower size, we focused on petal length in subsequent analysis and discussion. We did not find any significant differences in the nectar volume between urban and natural environments. Additionally, there was also no significant difference in the concentration of glucose, fructose and sucrose in the flower nectar (Figure S3).

Overall, the common garden experiment demonstrates that urban populations combined earlier flowering and reduced flower size with similar reproductive output compared to natural populations. These results indicate that urban-associated phenotypic divergence reflects genetically based shifts in life-history and floral traits rather than environmentally induced plasticity.

3.2 | Selection Analysis

To evaluate whether phenotypic divergence between urban and natural populations was adaptive, we quantified selection on flowering time and flower size using seed number as a proxy for fitness. Both traits showed consistent associations with relative fitness, indicating that the traits diverging under urbanisation were linked to fitness differences. Selection differentials revealed that selection favoured both earlier flowering and larger flowers (Table 3). Specifically, plants with longer petals produced significantly more seeds ($p = 0.024$; Figure 3a), indicating positive directional selection on flower size. Similarly, earlier flowering individuals achieved higher reproductive success, as shown by the significant negative relationship between flowering time and seed number ($p = 0.0486$; Figure 3b). Notably, days to first flower and flower size were negatively correlated (Table S2), with earlier flowering plants tending to produce smaller flowers. This relationship suggests a trade-off between phenological advancement and investment in floral morphology.

When both traits were included simultaneously in selection gradient analyses, neither days to first flower nor petal length showed statistically significant independent effects on fitness (Table 3; $p > 0.05$). However, the estimated gradients followed the same directions as the selection differentials, with a positive effect of petal length and a negative effect of days to first flower on relative fitness. The reduced statistical power of the selection gradients likely reflected the correlation between days to first flower and petal length (Table S2), as each trait's effect on fitness was evaluated after accounting for variance explained by

TABLE 2 | Phenotypic results of the (generalised) linear mixed-effect models for urbanisation in the common garden.

Model	Response variable	Parameter	Estimated	Standard error	*Z* value	*p*
lmer	Flowering time	Intercept	4.4422	0.1331	33.367	<0.001***
		Urban	−0.4878	0.2014	−2.422	0.019*
glmer	Flowering Probability	Intercept	0.4518	0.3219	1.404	0.1605
		Urban	1.8814	0.589	3.194	0.0014*
lmer	Corolla diameter	Intercept	15.885	0.2014	78.887	<0.001***
		Urban	−0.6056	0.2785	−2.175	0.035*
lmer	Petal length	Intercept	7.1332	0.1094	65.182	<0.001***
		Urban	−0.3631	0.1515	−2.396	0.0207*
lmer	Petal width	Intercept	5.02112	0.10803	46.477	<0.001***
		Urban	−0.0378	0.15317	−0.247	0.806
glmmTMB	Seed number	Intercept	2.675	0.0803	33.31	<0.001***
		Urban	−0.1445	0.116	−1.25	0.213
glmmTMB	Fruit weight	Intercept	−0.6255	0.077	−8.122	<0.001***
		Urban	−0.3528	0.1153	−3.06	<0.0034**

Note: $p < 0.05$*; $p < 0.01$**; $p < 0.001$***.

TABLE 3 | Selection differentials (S) and selection gradients (β) from linear mixed-effects models. A positive β indicates a positive relationship between fitness and the trait whereas a negative β indicates a negative relationship between fitness and the trait.

Trait	*S*	*p*	*β*	*p*
Petal length	0.123	0.024	0.09638	0.131
Days to first flower	−0.107	0.0486	−0.05736	0.352

the other trait. Such collinearity is expected for traits that are developmentally and functionally linked.

Together, these analyses indicate that earlier flowering and larger flower size are both associated with increased fitness. Given that urban populations flower earlier but produce smaller flowers, these results indicate an adaptive shift towards phenological escape in urban environments, potentially at the cost of reduced investment in floral morphology.

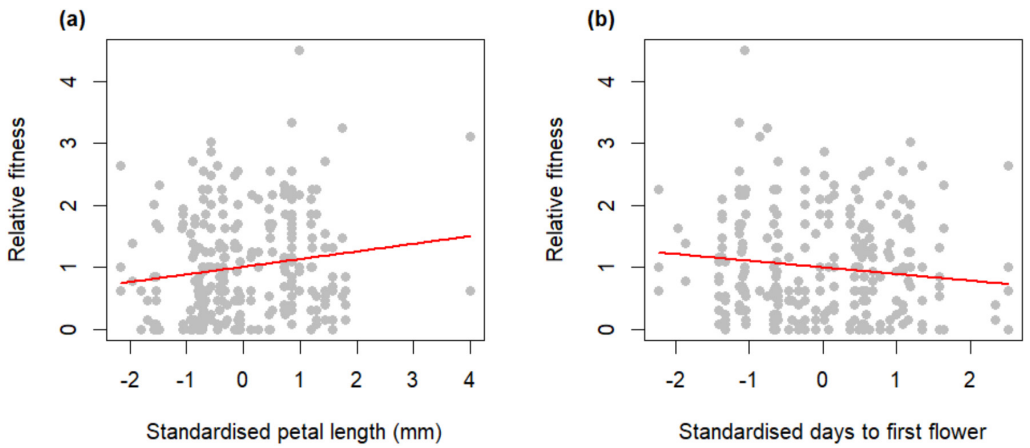


FIGURE 3 | Selection differentials of petal length and days to first flower. (a) The positive relationship between relative fitness and petal length. (b) The negative relationship between relative fitness and flowering time (days to first flower). The *x*-axis and *y*-axis represent the standardised trait values (0 corresponds to the population mean, and ±1 corresponds to one standard deviation from the mean).

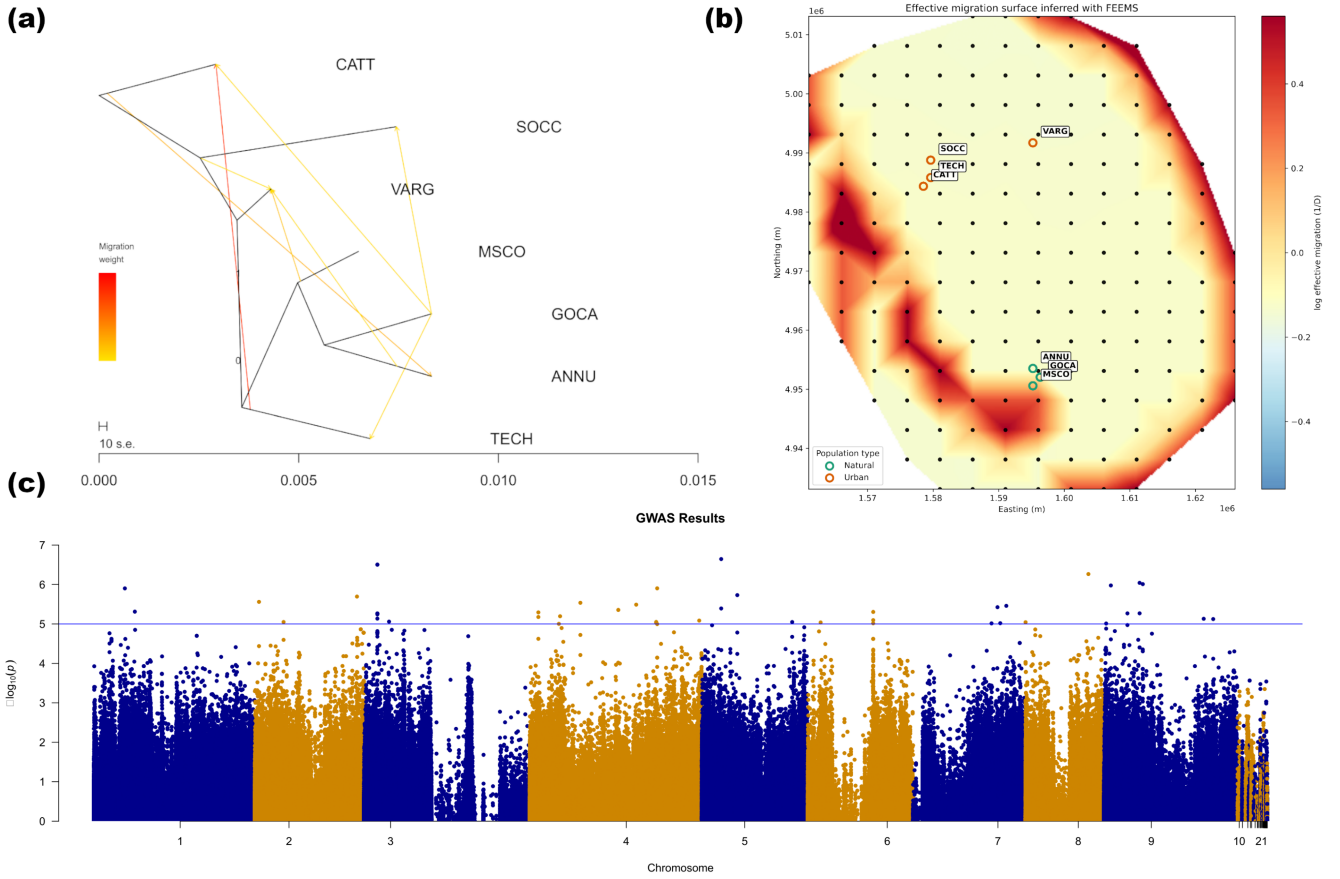


FIGURE 4 | (a) TreeMix analysis of urban and natural *Raphanus raphanistrum* populations. The maximum likelihood population tree shows the inferred relationships among seven populations, with branch lengths proportional to the amount of genetic drift. Migration events are shown as arrows, with colour indicating relative weight. (b) Effective migration surface inferred using FEEMS. Colours indicate relative, mean-centred log effective migration (log(1/D)), with warmer colours representing elevated connectivity and cooler colours reduced connectivity. Black points show deme centres, and coloured circles indicate natural (green) and urban (orange) populations. (c) The Manhattan plot of the GWAS in relation to urban and natural environments. The *x*-axis indicates the chromosome numbers, and the *y*-axis indicates −log(P) values from the GWAS. The blue line indicates the significance threshold generated via the R package qqman.

3.3 | Population Genetics Analysis

Despite pronounced phenotypic divergence between urban and natural populations, genome-wide genetic differentiation was low, indicating extensive connectivity among populations. Across the seven populations retained for population-genetic analyses, patterns of relative and absolute genetic differentiation consistently suggested high gene flow and weak population structure. Pairwise genetic differentiation was generally low but heterogeneous among population pairs. Pairwise F_{ST} ranged from 0.0248 to 0.0965 (Table S3), with the highest differentiation observed between the natural population GOCA and several urban populations (e.g., GOCA–SOCC 0.0965, GOCA–CATT 0.0942, GOCA–TECH 0.0862). In contrast to relative differentiation, absolute divergence (dXY) varied slightly among population pairs (approximately 0.01142–0.01233; Table S4), indicating shallow genome-wide divergence. Similarly, nucleotide diversity (Table S5) was broadly comparable across populations (≈0.00965–0.01185), with the highest diversity in MSCO (0.01185) and the lowest in GOCA (0.00965) and TECH (0.00986). Together, these metrics indicate that population differentiation primarily reflects subtle shifts in allele frequencies rather than long-term isolation or strong demographic separation.

PCA revealed a weak but continuous pattern of genetic differentiation across populations (Figure S2). The first two principal components explained 14.26% (PC1) and 12.31% (PC2) of the total genetic variance, separating the urban and natural populations along a diagonal gradient. Several populations (ANNU, CARR, TECH and VARG) clustered near the centre of the ordination, whereas two urban populations (CATT and SOCC) and two natural populations (MSCO and GOCA) were more genetically distinct, occupying opposite ends of the gradient. ADMIXTURE analysis identified $K = 1$ as the most likely number of genetic clusters, suggesting little to no genetic differentiation between individuals among the studied populations.

To further investigate historical relationships and gene flow, we reconstructed population graphs using TreeMix. The maximum-likelihood tree inferred multiple migration events, with a strong migration edge detected between two urban populations (Figure 4a), indicating substantial recent gene flow. Additional weaker migration events were inferred among other populations, consistent with widespread connectivity. Spatial modelling of effective migration using FEEMS revealed heterogeneous patterns of connectivity across the landscape, with regions of

both elevated and reduced effective migration (Figure 4b). In particular, natural populations were located close to, or within, regions of elevated effective migration, whereas urban populations were generally situated in areas of near-average effective migration. Areas of reduced migration were spatially limited and did not align with a clear urban–natural boundary. Together with low genome-wide differentiation and shallow divergence among populations, these results indicate that effective migration varies spatially across the landscape while remaining broadly continuous.

3.4 | Genome-Wide Association Study and Candidate Genes for Adaptation

To identify genomic variants potentially underlying phenotypic divergence between urban and natural populations, we conducted a genome-wide association study contrasting individuals from the two environments. The GWAS detected a total of 46 SNPs significantly related to environments (urban versus natural, Figure 4c). The QQ plot (Figure S4) showed that the linear mixed-effects model controlled for the false positive rate. Among these SNPs, 32 are physically located within 21 genes (Table 4). The largest number of outlier SNPs was on chromosome 3 and chromosome 4, with seven SNPs on both chromosomes (Figure 4c). The functional annotation implemented in DAVID allowed us to annotate 17 out of 21 loci for adaptation (Table 4). One of the loci is orthologous to *Arabidopsis thaliana*'s AT3g27390, which is functionally related to the transition from vegetative to reproductive phase. Some of the loci were related to stress response, such as disease resistance-like protein DSC1 and heat shock 70 kDa protein BIP2-like. Enrichment analysis conducted via DAVID showed that the 21 genes were overrepresented in nucleotide binding (three genes) and ATP-binding (three genes) in terms of UniProt Knowledgebase Keywords (Table S6). The two loci related to stress response were both in these two categories.

TABLE 4 | Functional annotations of 21 genes, where outlier SNPs are located. The annotation was conducted via DAVID, using the orthologous genes of *Brassica napus*.

Gene ID	Species	Gene name
106400314	*Brassica napus*	Uncharacterised membrane protein At3g27390
106450554	*Brassica napus*	Protease Do-like 9
106393567	*Brassica napus*	Uncharacterised LOC106393567
106426849	*Brassica napus*	Uncharacterised LOC106426849
106395349	*Brassica napus*	Cysteine-rich repeat secretory protein 9
125581425	*Brassica napus*	Uncharacterised LOC125581425
106407008	*Brassica napus*	Outer mitochondrial transmembrane helix translocase
106367025	*Brassica napus*	Disease resistance-like protein DSC1
106364055	*Brassica napus*	Uncharacterised LOC106364055
106367042	*Brassica napus*	Embryogenesis-associated protein EMB8
106381363	*Brassica napus*	60S ribosomal protein L6-3
106353419	*Brassica napus*	Photosystem II 10 kDa polypeptide, chloroplastic
106354117	*Brassica napus*	Trafficking protein particle complex subunit 11
106437568	*Brassica napus*	Heat shock 70 kDa protein BIP2-like
111199790	*Brassica napus*	Microtubule-associated protein 70-2-like
106412416	*Brassica napus*	Myosin-binding protein 7
125592403	*Brassica napus*	Uncharacterised LOC125592403
106400093	*Brassica napus*	Delta-9 acyl-lipid desaturase 1-like
106417108	*Brassica napus*	Putative F-box protein At3g47150
106439759	*Brassica napus*	NEDD8 ultimate buster 1-like
106410774	*Brassica napus*	Glutamate-1-semialdehyde 2,1-aminomutase, chloroplastic-like

4 | Discussion

The rapid expansion of urban areas has raised concerns about the capacity of plant populations to adapt to anthropogenic environments. While numerous studies have addressed evolutionary responses to urbanisation, none of them addressed urban adaptation under a metapopulation framework and how gene flow confounds the effect of evolutionary adaptation. Our study demonstrates that urban populations of *R. raphanistrum* have evolved earlier flowering times and shorter petals compared to nearby natural populations despite strong gene flow and low population genetic structure.

The low pairwise F_{ST} observed among populations indicates marginal genetic differentiation and high gene flow among populations. Likewise, absolute divergence (dXY) between populations was uniformly low, consistent with shallow divergence rather than long-term isolation. High levels of genetic variation, as reflected in nucleotide diversity further support large effective population sizes and frequent gene exchange among our study populations (Leffler et al. 2012). Previous studies have suggested that low genetic variation and genetic structure among populations of *R. raphanistrum* may be due to the high dispersal of the seeds (Kercher and Conner 1996; Wasserstrom et al. 2022).

Consistent with this interpretation, the absence of clear genetic structure in the ADMIXTURE analysis (best $K=1$), combined with the multiple inferred migration events in the TreeMix analysis, supports the idea that our populations formed part of a large metapopulation. As a generalist species, *R. raphanistrum* is widely distributed across diverse habitats. In highly disturbed urban environments—characterised by stressors such as summer drought and frequent mowing—local extinctions are likely common. These vacant patches are likely recolonised by individuals from nearby populations, including natural ones, helping to maintain metapopulation connectivity. This dynamic is consistent with the spatial patterns revealed by FEEMS, which showed broadly continuous effective migration across the landscape, with localised regions of elevated connectivity associated primarily with natural populations. Such spatial heterogeneity suggests that gene flow is not uniformly distributed but instead varies across the landscape, likely reflecting geographic or environmental features that modulate dispersal without constituting strong, genome-wide barriers to connectivity. The multiple weak migration events indicated by yellow lines (Figure 4a) may therefore reflect gene flow mediated through unstudied intermediate populations that facilitate silique dispersal. Additionally, pollinator movement across fragmented green patches might further maintain connectivity. Importantly, the absence of extensive regions of markedly reduced effective migration in FEEMS is consistent with the relatively uniform nucleotide diversity (π) observed among populations, indicating that gene flow remains sufficient to maintain genetic variation across the study area despite spatial variation in effective migration.

Although high gene flow can homogenise populations and counteract local adaptation, we still observed phenotypic divergence between urban and natural populations. The selection differentials of longer petals and earlier flowering with higher seed number (Figure 3) indicate these phenotypic divergences are adaptive. This suggests that environmental selection pressures in urban environments are strong enough to drive adaptive differentiation.

The selection pressures in urban environments can stem from altered abiotic and biotic interactions. In the Naples metropolitan area, summers are typically characterised by higher temperatures, reduced water availability and frequent disturbances (e.g., mowing and cutting). Populations of *R. raphanistrum* in the urban areas likely evolved earlier flowering as an adaptive response to avoid these harsh conditions (Grime 1977; Wolfe and Tonsor 2014). Several studies on *R. raphanistrum* have suggested that rapid flowering is an adaptive strategy against highly disturbed environments (Ashworth et al. 2016; Charbonneau et al. 2018; Wasserstrom et al. 2022). In agricultural fields, the evolution of earlier flowering in *R. raphanistrum* has successfully enabled it to escape weed management and become a major agricultural weed (Sahli et al. 2008). In particular, *R. raphanistrum* was able to advance its flowering time to avoid harvest weed seed control, an agricultural weed management practice that intercepts weed seeds at harvest before they enter the soil seed bank (Somerville and Ashworth 2024; Sun et al. 2020). Similar to agricultural habitats, urban environments impose selection against late-flowering plants due to harsh summer conditions and frequent mowing. The evolution of earlier flowering in the urban populations may represent an optimal survival strategy that mirrors the patterns observed in studies in agricultural environments. Interestingly, *R. raphanistrum* in agricultural stands, and our studied populations, harbours high standing genetic variation and could advance its flowering time in response to only five generations of artificial selection (Ashworth et al. 2016). This further reinforces our hypothesis that *R. raphanistrum* is capable of adapting to urban environments in only a few generations of urban life and even in the face of gene flow from surrounding natural populations.

Studies on other flowering species demonstrated that anthropogenic activities could influence flowering phenology (Lambrecht et al. 2016; Schmitz et al. 2024; Yakub and Tiffin 2017). For example, Gorton et al. (2018) demonstrated that urban populations of the common ragweed (*Ambrosia artemisiifolia*) flowered earlier than natural populations in a reciprocal transplant experiment. Similarly, Schmitz et al. (2024) found that *Arabidopsis thaliana* populations from disturbed habitats flowered earlier than those from less-disturbed sites. These findings align with the idea that urban environments could potentially favour earlier flowering.

Additionally, the environmental pressure in urban areas could alter the pollinator community, modifying the direction of pollinator-mediated selection (McKinney 2006; Pickett et al. 2001). Evidence suggests that pollinators generally prefer larger flowers, which are linked to higher reproductive success (Irwin et al. 2018; Stanton and Preston 1988; Young and Stanton 1990). In the natural populations, pollinator-mediated selection can lead to increased flower size in *R. raphanistrum*, and larger flowers are typically linked to greater reproductive output (Conner et al. 1996). In our study, we found that natural populations of *R. raphanistrum* had significantly longer petals than urban populations (Figure 3). This finding aligns with studies conducted on other plant species, showing that plants in anthropogenic environments tend to exhibit smaller floral traits. For instance, Faure et al. (2023) found that urbanisation was negatively correlated with flower sizes in *Impatiens capensis*. Similarly, Dubois and Cheptou (2017) reported smaller capitulum diameters and shorter flower stalks in fragmented urban populations of *Crepis sancta*. Limited resources, such as water and nutrients, and a decline in pollinators may relax this pollinator-mediated selection on flower size in urban environments (Kuppler and Kotowska 2021; Lambrecht and Dawson 2007; Panique and Caruso 2020), leading to the observed smaller floral traits in *R. raphanistrum*. A previous study found that the realised heritability of flower size in *R. raphanistrum* was high ($h^2=0.49$), indicating substantial potential for rapid evolutionary change in floral size (Lehtilä and Bränn 2007). Additionally, selection for earlier flowering in urban environments may indirectly select for shorter petal lengths and corolla diameters, as a previous study of *R. raphanistrum* found a negative correlation between flower size and the onset of flowering (Lehtilä and Bränn 2007).

Overall, our study demonstrates that there are phenotypic divergences in flower size and flowering time between urban and natural populations. These divergences are likely to be adaptive, and there is a negative correlation between these two traits. Despite these differences, there was no significant difference in seed number between these populations. We may speculate that: (1) Urban and natural populations achieved comparable

reproductive success through an ecological trade-off: natural populations leverage larger flowers to attract pollinators, whereas urban populations focus on earlier flowering to cope with the challenges of urban environments and (2) *R. raphanistrum* may rely on a generalist pollination strategy, minimising dependence on specific pollinators (Sahli et al. 2008). The similar nectar content and composition (glucose, fructose, sucrose) across populations supports this latter hypothesis.

Under frequent gene flow, such phenotypic divergence may not arise from large differences in allele frequencies at single loci, but rather through small, coordinated allele frequency shifts across many loci—a hallmark of polygenic adaptation (Josephs et al. 2017; Le Corre and Kremer 2012). In 2016, Ashworth et al. conducted an artificial selection for earlier flowering in *R. raphanistrum* and they observed progressive shifts in flowering time over successive generations. They proposed that this selection was driven by the polygenic accumulation of minor alleles with incomplete dominance underlying the genetic architecture of flowering time (Conner 1993). This interpretation aligns with our findings that the traits under adaptation, such as flower size (Weiss et al. 2005) and flowering time, are likely polygenic (Dong et al. 2025; Zan and Carlborg 2019). Notably, PCA revealed genetic divergence between urban and natural populations, reinforcing the idea that selection—rather than demographic history—may be the primary driver of the observed divergences. Overall, our findings highlight that urbanisation can promote adaptive phenotypic divergence even in the presence of substantial gene flow, particularly when traits under selection are polygenic.

The GWAS offered further insights into potential genes under evolutionary adaptation in *R. raphanistrum*. One notable candidate gene, At3g27390, is involved in transforming a vegetative meristem into a reproductive meristem (Swarbreck et al. 2008; The Arabidopsis Information Resource (TAIR) 2023). In a recent genotype by environment analysis conducted on related *Brassica incana* in the same geographic area, Frachon et al. (2023) found that the same gene At3g27390 was associated with local adaptation to hoverflies, likely to adjust phenological synchronisation between plants and pollinators. Although our experimental design did not allow us to test for it, this finding suggested that biotic interactions might have played a role in shaping earlier flowering. Enrichment analysis shows that the 21 genes are overrepresented in the functional annotation clusters: nucleotide binding and ATP-binding in terms of UniProt Knowledgebase Keywords. Both of these clusters have three genes: disease resistance-like protein DSC1, heat shock 70 kDa protein BIP2-like (LOC106437568), and outer mitochondrial transmembrane helix translocase. These genes are integral to plant response to stress and could imply a footprint of evolutionary response to deal with stress in urban environments.

4.1 | Limitations and Potential Caveats

While our study integrates phenotypic, fitness and genomic data to investigate urban adaptation in *Raphanus raphanistrum*, several limitations should be considered when interpreting the results. First, the GWAS was conducted with a limited sample size (50 individuals), which constrains statistical power to detect loci of small effects, particularly for complex and polygenic traits. Consequently, the result should be regarded as hypothesis-generating rather than definitive evidence of causal variants. This limitation is expected given that adaptation to contrasting environments such as urban and natural habitats is likely to involve many traits and pathways, producing diffuse genomic signals. Larger sample sizes and replication across independent systems will be necessary to robustly characterise the genomic basis of urban adaptation. Second, selection analyses were performed in a common garden, and therefore quantify associations between trait variation and reproductive output under controlled conditions rather than measuring ongoing selection in natural populations. These analyses capture evolved differences in trait–fitness relationships among populations, but do not reflect the full spatial and temporal complexity of selection acting in the wild. Finally, although we document divergence in flowering time and floral traits, these traits should not be interpreted as proxies for genome-wide genetic divergence. Consistent with this, population-genetic analyses reveal weak structure and extensive gene flow. Our conclusions therefore concern trait-specific adaptive divergence maintained despite genome-wide connectivity, rather than broad genetic differentiation.

Overall, our findings highlight how urbanisation has influenced the adaptive patterns of *R. raphanistrum* populations. Despite high gene flow between populations, divergent selection seems to maintain adaptive differences in flowering time and floral traits. Our findings lay the groundwork for testing parallel evolution in urban habitats and examining whether similar patterns appear in other natural-urban contexts. However, our understanding of whether local adaptation occurred remains limited. Future research should include reciprocal transplant experiments to explicitly test local adaptation and expand the geographic scope to evaluate the generality of these findings. Integrating gene flow into these frameworks will be essential for uncovering the evolutionary trajectories in human-dominated landscapes.

Acknowledgements

This research was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of the Italian Ministry of University and Research funded by the European Union—NextGenerationEU; Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP H43C22000530001, Project title 'National Biodiversity Future Center—NBFC'. G.S. was also funded in the frame of the PRIN2022PNRR-URBANPOLL funded by the European Union—NextGenerationEU, Mission 4 Component 1 CUP E53D23014450001. We thank Teresa Rosa Galise for helping manage the computer resources for bioinformatics analysis. We also thank Emilio Petrone for assisting with the field work. Open access publishing facilitated by Universita degli Studi di Napoli Federico II, as part of the Wiley - CRUI-CARE agreement.

Funding

This work was supported by European Union—NextGenerationEU (CUP H43C22000530001).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data and computer code used for this study are available at Dryad Digital Repository: https://doi.org/10.5061/dryad.sxksn03g5.

References

Alberti, M., J. Marzluff, and V. M. Hunt. 2017. "Urban Driven Phenotypic Changes: Empirical Observations and Theoretical Implications for Eco-Evolutionary Feedback." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 372, no. 1712: 20160029. https://doi.org/10.1098/rstb.2016.0029.

Alexander, D. H., J. Novembre, and K. Lange. 2009. "Fast Model-Based Estimation of Ancestry in Unrelated Individuals." *Genome Research* 19, no. 9: 1655–1664. https://doi.org/10.1101/gr.094052.109.

Andrews, S. 2010. *FastQC: A Quality Control Tool for High Throughput Sequence Data*. Babraham Bioinformatics. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Ashworth, M. B., M. J. Walsh, K. C. Flower, M. M. Vila-Aiub, and S. B. Powles. 2016. "Directional Selection for Flowering Time Leads to Adaptive Evolution in *Raphanus raphanistrum* (Wild Radish)." *Evolutionary Applications* 9, no. 4: 619–629. https://doi.org/10.1111/eva.12350.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67, no. 1: 1–48. https://doi.org/10.18637/jss.v067.i01.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. "Trimmomatic: A Flexible Trimmer for Illumina Sequence Data." *Bioinformatics* 30, no. 15: 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Brans, K. I., and L. De Meester. 2018. "City Life on Fast Lanes: Urbanization Induces an Evolutionary Shift Towards a Faster Lifestyle in the Water Flea *Daphnia*." *Functional Ecology* 32, no. 9: 2225–2240. https://doi.org/10.1111/1365-2435.13184.

Brans, K. I., M. Jansen, J. Vanoverbeke, N. Tüzün, R. Stoks, and L. De Meester. 2017. "The Heat Is on: Genetic Adaptation to Urbanization Mediated by Thermal Tolerance and Body Size." *Global Change Biology* 23, no. 12: 5218–5227. https://doi.org/10.1111/gcb.13784.

Brooks, M. E., K. Kristensen, K. J. van Benthem, et al. 2017. "glmmTMB Balances Speed and Flexibility Among Packages for Zero-Inflated Generalized Linear Mixed Modeling." *R Journal* 9, no. 2: 378–400. https://doi.org/10.32614/rj-2017-066.

Bullock, J. M., I. L. Moy, and R. F. Pywell. 2002. "Plant Dispersal and Colonization Processes at Local and Landscape Scales." In *Dispersal Ecology*, edited by J. M. Bullock, R. E. Kenward, and R. S. Hails, 279–302. Blackwell.

Charbonneau, A., D. Tack, A. Lale, et al. 2018. "Weed Evolution: Genetic Differentiation Among Wild, Weedy, and Crop Radish." *Evolutionary Applications* 11, no. 10: 1964–1974. https://doi.org/10.1111/eva.12699.

Cheptou, P. O., O. Carrue, S. Rouifed, and A. Cantarel. 2008. "Rapid Evolution of Seed Dispersal in an Urban Environment in the Weed *Crepis sancta*." *Proceedings of the National Academy of Sciences of the United States of America* 105, no. 10: 3796–3799. https://doi.org/10.1073/pnas.0708446105.

Conner, J. 1993. "Tests for Major Genes Affecting Quantitative Traits in Wild Radish, Raphanus-Raphanistrum." *Genetica* 90, no. 1: 41–45. https://doi.org/10.1007/bf01435177.

Conner, J. K., S. Rush, and P. Jennetten. 1996. "Measurements of Natural Selection on Floral Traits in Wild Radish (*Raphanus raphanistrum*). 1. Selection Through Lifetime Female Fitness." *Evolution* 50, no. 3: 1127–1136. https://doi.org/10.2307/2410653.

Cosentino, S., S. Sriswasdi, and W. Iwasaki. 2024. "SonicParanoid2: Fast, Accurate, and Comprehensive Orthology Inference With Machine Learning and Language Models." *Genome Biology* 25, no. 1: 195. https://doi.org/10.1186/s13059-024-03298-4.

Danecek, P., A. Auton, G. Abecasis, et al. 2011. "The Variant Call Format and VCFtools." *Bioinformatics* 27, no. 15: 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.

DePristo, M. A., E. Banks, R. Poplin, et al. 2011. "A Framework for Variation Discovery and Genotyping Using Next-Generation DNA Sequencing Data." *Nature Genetics* 43, no. 5: 491. https://doi.org/10.1038/ng.806.

Diamond, S. E., L. Chick, A. Perez, S. A. Strickler, and R. A. Martin. 2017. "Rapid Evolution of Ant Thermal Tolerance Across an Urban-Rural Temperature Cline." *Biological Journal of the Linnean Society* 121, no. 2: 248–257. https://doi.org/10.1093/biolinnean/blw047.

Dong, H. L., S. C. Qi, Q. Shen, et al. 2025. "Elucidation of the Genetic Basis of Variation in Flowering Time in *Brassica napus* via Genome-Wide Association Studies and Gene Coexpression Analysis." *BMC Plant Biology* 25, no. 1: 350. https://doi.org/10.1186/s12870-025-06253-y.

Donihue, C. M., and M. R. Lambert. 2015. "Adaptive Evolution in Urban Ecosystems." *Ambio* 44, no. 3: 194–203. https://doi.org/10.1007/s13280-014-0547-2.

Dubois, J., and P. O. Cheptou. 2017. "Effects of Fragmentation on Plant Adaptation to Urban Environments." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 372, no. 1712: 20160038. https://doi.org/10.1098/rstb.2016.0038.

European Environment Agency. 2018. "CORINE Land Cover." https://land.copernicus.eu/pan-european/corine-land-cover.

Faure, J., V. Volz, and S. Joly. 2023. "Variation in Flower Size and Shape of *Impatiens capensis* Is Correlated With Urbanization in Montreal, Canada." *Ecology and Evolution* 13, no. 12: e10826. https://doi.org/10.1002/ece3.10826.

Fenger, J. 1999. "Urban Air Quality." *Atmospheric Environment* 33, no. 29: 4877–4900. https://doi.org/10.1016/s1352-2310(99)00290-3.

Feranec, J., G. Jaffrain, T. Soukup, and G. Hazeu. 2010. "Determining Changes and Flows in European Landscapes 1990-2000 Using CORINE Land Cover Data." *Applied Geography* 30, no. 1: 19–35. https://doi.org/10.1016/j.apgeog.2009.07.003.

Frachon, L., L. Arrigo, Q. Rusman, et al. 2023. "Putative Signals of Generalist Plant Species Adaptation to Local Pollinator Communities and Abiotic Factors." *Molecular Biology and Evolution* 40, no. 3: msad036. https://doi.org/10.1093/molbev/msad036.

Fukano, Y., K. Uchida, and Y. Tachiki. 2023. "Urban-Rural Gradients: How Landscape Changes Drive Adaptive Evolution of Plant Competitive Traits." *Evolutionary Ecology* 37, no. 2: 215–232. https://doi.org/10.1007/s10682-022-10215-3.

Gorton, A. J., D. A. Moeller, and P. Tiffin. 2018. "Little Plant, Big City: A Test of Adaptation to Urban Environments in Common Ragweed (*Ambrosia artemisiifolia*)." *Proceedings of the Royal Society B: Biological Sciences* 285, no. 1881: 20180968. https://doi.org/10.1098/rspb.2018.0968.

Grime, J. P. 1977. "Evidence for Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory." *American Naturalist* 111, no. 982: 1169–1194. https://doi.org/10.1086/283244.

Grimm, N. B., S. H. Faeth, N. E. Golubiewski, et al. 2008. "Global Change and the Ecology of Cities." *Science* 319, no. 5864: 756–760. https://doi.org/10.1126/science.1150195.

Handa, H. 2003. "The Complete Nucleotide Sequence and RNA Editing Content of the Mitochondrial Genome of Rapeseed (*Brassica napus* L.): Comparative Analysis of the Mitochondrial Genomes of Rapeseed and *Arabidopsis thaliana*." *Nucleic Acids Research* 31, no. 20: 5907–5916. https://doi.org/10.1093/nar/gkg795.

Hanski, I. 1999. "Habitat Connectivity, Habitat Continuity, and Metapopulations in Dynamic Landscapes." *Oikos* 87, no. 2: 209–219. https://doi.org/10.2307/3546736.

Holm, L., J. Doll, E. Holm, J. Pancho, and J. Herberger. 1997. *World Weeds. Natural Histories and Distribution*. Wiley.

Huang, D. W., B. T. Sherman, and R. A. Lempicki. 2009. "Systematic and Integrative Analysis of Large Gene Lists Using DAVID Bioinformatics Resources." *Nature Protocols* 4, no. 1: 44–57. https://doi.org/10.1038/nprot.2008.211.

Irwin, R. E., P. S. Warren, and L. S. Adler. 2018. "Phenotypic Selection on Floral Traits in an Urban Landscape." *Proceedings of the Royal Society B: Biological Sciences* 285, no. 1884: 20181239. https://doi.org/10.1098/rspb.2018.1239.

Irwin, R. E., E. Youngsteadt, P. S. Warren, and J. L. Bronstein. 2020. "The Evolutionary Ecology of Mutualisms in Urban Landscapes." In *Urban Evolutionary Biology*, edited by M. Szulkin, J. Munshi-South, and A. Charmantier, 111–129. Oxford Academic. https://doi.org/10.1093/oso/9780198836841.003.0007.

Jha, S., and C. Kremen. 2013. "Urban Land Use Limits Regional Bumble Bee Gene Flow." *Molecular Ecology* 22, no. 9: 2483–2495. https://doi.org/10.1111/mec.12275.

Johnson, M. T. J., and J. Munshi-South. 2017. "Evolution of Life in Urban Environments." *Science* 358, no. 6363: eaam8327. https://doi.org/10.1126/science.aam8327.

Johnson, M. T. J., C. M. Prashad, M. Lavoignat, and H. S. Saini. 2018. "Contrasting the Effects of Natural Selection, Genetic Drift and Gene Flow on Urban Evolution in White Clover (*Trifolium repens*)." *Proceedings of the Royal Society B-Biological Sciences* 285, no. 1883: 20181019. https://doi.org/10.1098/rspb.2018.1019.

Johnson, M. T. J., K. A. Thompson, and H. S. Saini. 2015. "Plant Evolution in the Urban Jungle." *American Journal of Botany* 102, no. 12: 1951–1953. https://doi.org/10.3732/ajb.1500386.

Josephs, E. B., J. R. Stinchcombe, and S. I. Wright. 2017. "What Can Genome-Wide Association Studies Tell Us About the Evolutionary Forces Maintaining Genetic Variation for Quantitative Traits?" *New Phytologist* 214, no. 1: 21–33. https://doi.org/10.1111/nph.14410.

Kercher, S., and J. K. Conner. 1996. "Patterns of Genetic Variability Within and Among Populations of Wild Radish, *Raphanus raphanistrum* (Brassicaceae)." *American Journal of Botany* 83, no. 11: 1416–1421. https://doi.org/10.2307/2446096.

Korunes, K. L., and K. Samuk. 2021. "Pixy: Unbiased Estimation of Nucleotide Diversity and Divergence in the Presence of Missing Data." *Molecular Ecology Resources* 21, no. 4: 1359–1368. https://doi.org/10.1111/1755-0998.13326.

Kuppler, J., and M. M. Kotowska. 2021. "A Meta-Analysis of Responses in Floral Traits and Flower-Visitor Interactions to Water Deficit." *Global Change Biology* 27, no. 13: 3095–3108. https://doi.org/10.1111/gcb.15621.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software* 82, no. 13: 1–26. https://doi.org/10.18637/jss.v082.i13.

Lambert, M. R., K. I. Brans, S. Des Roches, C. M. Donihue, and S. E. Diamond. 2021. "Adaptive Evolution in Cities: Progress and Misconceptions." *Trends in Ecology & Evolution* 36, no. 3: 239–257. https://doi.org/10.1016/j.tree.2020.11.002.

Lambrecht, S. C., and T. E. Dawson. 2007. "Correlated Variation of Floral and Leaf Traits Along a Moisture Availability Gradient." *Oecologia* 151, no. 4: 574–583. https://doi.org/10.1007/s00442-006-0617-7.

Lambrecht, S. C., S. Mahieu, and P. O. Cheptou. 2016. "Natural Selection on Plant Physiological Traits in an Urban Environment." *Acta Oecologica* 77: 67–74. https://doi.org/10.1016/j.actao.2016.09.002.

Lande, R., and S. J. Arnold. 1983. "The Measurement of Selection on Correlated Characters." *Evolution* 37, no. 6: 1210–1226. https://doi.org/10.1111/j.1558-5646.1983.tb00236.x.

Le Corre, V., and A. Kremer. 2012. "The Genetic Differentiation at Quantitative Trait Loci Under Local Adaptation." *Molecular Ecology* 21, no. 7: 1548–1566. https://doi.org/10.1111/j.1365-294X.2012.05479.x.

Leffler, E. M., K. Bullaughey, D. R. Matute, et al. 2012. "Revisiting an Old Riddle: What Determines Genetic Diversity Levels Within Species?" *PLoS Biology* 10, no. 9: e1001388. https://doi.org/10.1371/journal.pbio.1001388.

Lehtilä, K., and K. H. Bränn. 2007. "Correlated Effects of Selection for Flower Size in *Raphanus raphanistrum*." *Canadian Journal of Botany* 85, no. 2: 160–166. https://doi.org/10.1139/b07-007.

Li, H., and R. Durbin. 2009. "Fast and Accurate Short Read Alignment With Burrows-Wheeler Transform." *Bioinformatics* 25, no. 14: 1754–1760. https://doi.org/10.1093/bioinformatics/btp324.

Lüdecke, D., M. Ben Shachar, I. Patil, P. Waggoner, and D. Makowski. 2021. "Performance: An R Package for Assessment, Comparison and Testing of Statistical Models." *Journal of Open Source Software* 6: 3139. https://doi.org/10.21105/joss.03139.

Marcus, J., W. Ha, R. F. Barber, and J. Novembre. 2021. "Fast and Flexible Estimation of Effective Migration Surfaces." *eLife* 10: e61927. https://doi.org/10.7554/eLife.61927.

McKenna, A., M. Hanna, E. Banks, et al. 2010. "The Genome Analysis Toolkit: A MapReduce Framework for Analyzing Next-Generation DNA Sequencing Data." *Genome Research* 20, no. 9: 1297–1303. https://doi.org/10.1101/gr.107524.110.

McKinney, M. L. 2006. "Urbanization as a Major Cause of Biotic Homogenization." *Biological Conservation* 127, no. 3: 247–260. https://doi.org/10.1016/j.biocon.2005.09.005.

Muller, J. C., H. Kuhl, S. Boerno, J. L. Tella, M. Carrete, and B. Kempenaers. 2018. "Evolution of Genomic Variation in the Burrowing Owl in Response to Recent Colonization of Urban Areas." *Proceedings of the Royal Society B: Biological Sciences* 285, no. 13: 3629–3637. https://doi.org/10.1111/mec.12288.

Munshi-South, J., C. P. Zolnik, and S. E. Harris. 2016. "Population Genomics of the Anthropocene: Urbanization Is Negatively Associated With Genome-Wide Variation in White-Footed Mouse Populations." *Evolutionary Applications* 9, no. 4: 546–564. https://doi.org/10.1111/eva.12357.

Oke, T. R. 1973. "City Size and the Urban Heat Island." *Atmospheric Environment* 7, no. 8: 769–779. https://doi.org/10.1016/0004-6981(73)90140-6.

Palumbi, S. R. 2001. "Evolution - Humans as the World's Greatest Evolutionary Force." *Science* 293, no. 5536: 1786–1790. https://doi.org/10.1126/science.293.5536.1786.

Panique, H., and C. M. Caruso. 2020. "Simulated Pollinator Declines Intensify Selection on Floral Traits That Facilitate Selfing and Outcrossing in *Impatiens capensis*." *American Journal of Botany* 107, no. 1: 148–154. https://doi.org/10.1002/ajb2.1403.

Petkova, D., J. Novembre, and M. Stephens. 2016. "Visualizing Spatial Population Structure With Estimated Effective Migration Surfaces." *Nature Genetics* 48, no. 1: 94–100. https://doi.org/10.1038/ng.3464.

Pickett, S. T. A., M. L. Cadenasso, J. M. Grove, et al. 2001. "Urban Ecological Systems: Linking Terrestrial Ecological, Physical, and Socioeconomic Components of Metropolitan Areas." *Annual Review of Ecology, Evolution, and Systematics* 32, no. 1: 127–157. https://doi.org/10.1146/annurev.ecolsys.32.081501.114012.

Pickrell, J. K., and J. K. Pritchard. 2012. "Inference of Population Splits and Mixtures From Genome-Wide Allele Frequency Data." *PLoS Genetics* 8, no. 11: e1002967. https://doi.org/10.1371/journal.pgen.1002967.

Purcell, S., B. Neale, K. Todd-Brown, et al. 2007. "PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses." *American Journal of Human Genetics* 81, no. 3: 559–575. https://doi.org/10.1086/519795.

R Core Team. 2023. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. http://www.R-project.org/.

Rausher, M. D. 1992. "The Measurement of Selection on Quantitative Traits - Biases due to Environmental Covariances Between Traits and Fitness." *Evolution* 46, no. 3: 616–626. https://doi.org/10.2307/2409632.

Sahli, H. F., J. K. Conner, F. H. Shaw, S. Howe, and A. Lale. 2008. "Adaptive Differentiation of Quantitative Traits in the Globally Distributed Weed, Wild Radish (*Raphanus raphanistrum*)." *Genetics* 180, no. 2: 945–955. https://doi.org/10.1534/genetics.107.085084.

Santangelo, J. S., R. W. Ness, B. Cohan, et al. 2022. "Global Urban Environmental Change Drives Adaptation in White Clover." *Science* 375, no. 6586: 1275–1281. https://doi.org/10.1126/science.abk0989.

Sarkar, D. 2008. *Lattice: Multivariate Data Visualization With R*. Springer-Verlag.

Schmid, R., L. Holm, J. Doll, E. Holm, J. Pancho, and J. Herberger. 1998. *World Weeds: Natural Histories and Distribution*. International Bureau for Plant Taxonomy and Nomenclature.

Schmitz, G., A. Linstädter, A. S. K. Frank, et al. 2024. "Environmental Filtering of Life-History Trait Diversity in Urban Populations of *Arabidopsis thaliana*." *Journal of Ecology* 112, no. 1: 14–27. https://doi.org/10.1111/1365-2745.14211.

Seto, K. C., R. Sánchez-Rodríguez, and M. Fragkias. 2010. "The New Geography of Contemporary Urbanization and the Environment." *Annual Review of Environment and Resources* 35: 167–194. https://doi.org/10.1146/annurev-environ-100809-125336.

Sherman, B. T., M. Hao, J. Qiu, et al. 2022. "DAVID: A Web Server for Functional Enrichment Analysis and Functional Annotation of Gene Lists (2021 Update)." *Nucleic Acids Research* 50, no. W1: W216–W221. https://doi.org/10.1093/nar/gkac194.

Slatkin, M. 1987. "Gene Flow and the Geographic Structure of Natural Populations." *Science* 236, no. 4803: 787–792. https://doi.org/10.1126/science.3576198.

Somerville, G. J., and M. B. Ashworth. 2024. "Adaptations in Wild Radish (*Raphanus raphanistrum*) Flowering Time, Part 2: Harvest Weed Seed Control Shortens Flowering by Twelve Days." *Weed Science* 72, no. 2: 143–150. https://doi.org/10.1017/wsc.2024.4.

Stanton, M. L., and R. E. Preston. 1988. "Ecological Consequences and Phenotypic Correlates of Petal Size Variation in Wild Radish, *Raphanus sativus* (Brassicaceae)." *American Journal of Botany* 75, no. 4: 528–539. https://doi.org/10.2307/2444218.

Sun, C., M. B. Ashworth, K. Flower, M. M. Vila-Aiub, R. L. Rocha, and H. J. Beckie. 2020. "The Adaptive Value of Flowering Time in Wild Radish (*Raphanus raphanistrum*)." *Weed Science* 69, no. 2: 203–209. https://doi.org/10.1017/wsc.2021.5.

Swarbreck, D., C. Wilks, P. Lamesch, et al. 2008. "The Arabidopsis Information Resource (TAIR): Gene Structure and Function Annotation." *Nucleic Acids Research* 36: D1009–D1014. https://doi.org/10.1093/nar/gkm965.

The Arabidopsis Information Resource (TAIR). 2023. https://www.arabidopsis.org/locus?key=37778.

Theodorou, P., R. Radzeviciute, B. Kahnt, A. Soro, I. Grosse, and R. J. Paxton. 2018. "Genome-Wide Single Nucleotide Polymorphism Scan Suggests Adaptation to Urbanization in an Important Pollinator, the Red-Tailed Bumblebee (*Bombus lapidarius* L.)." *Proceedings of the Royal Society B-Biological Sciences* 285, no. 1877: 20172806. https://doi.org/10.1098/rspb.2017.2806.

Thompson, K. A., M. Renaudin, and M. T. J. Johnson. 2016. "Urbanization Drives the Evolution of Parallel Clines in Plant Populations." *Proceedings of the Royal Society B-Biological Sciences* 283, no. 1845: 20162180. https://doi.org/10.1098/rspb.2016.2180.

Tigano, A., and V. L. Friesen. 2016. "Genomics of Local Adaptation With Gene Flow." *Molecular Ecology* 25, no. 10: 2144–2164. https://doi.org/10.1111/mec.13606.

Turner, S. D. 2014. "Qqman: An R Package for Visualizing GWAS Results Using Q-Q and Manhattan Plots." *bioRxiv*, 005165. https://doi.org/10.1101/005165.

United Nations. 2018. *World Urbanisation Prospects*. Department of Economic and Social Affairs, Population Divisions.

Van der Auwera, G. A., and B. D. O'Conner. 2020. *Genomics in the Cloud: Using Docker, GATK, and WDL in Terra*. 1st ed. O'Reilly Media.

van Dongen, W. F. D., R. W. Robinson, M. A. Weston, R. A. Mulder, and P. J. Guay. 2015. "Variation at the DRD4 Locus Is Associated With Wariness and Local Site Selection in Urban Black Swans." *BMC Evolutionary Biology* 15: 253. https://doi.org/10.1186/s12862-015-0533-8.

Wasserstrom, H., J. Z. Berger, M. Barzilai, K. Mummenhoff, and O. Barazani. 2022. "Phenotypic Variation of Wild Radishes *Raphanus pugioniformis* and *R. raphanistrum* Associated With Local Conditions in the Southeast Mediterranean." *Flora* 287: 151997. https://doi.org/10.1016/j.flora.2021.151997.

Weiss, J., L. Delgado-Benarroch, and M. Egea-Cortines. 2005. "Genetic Control of Floral Size and Proportions." *International Journal of Developmental Biology* 49, no. 5–6: 513–525. https://doi.org/10.1387/ijdb.051998jw.

Weston, L. M., K. Z. Mattingly, C. T. C. Day, and S. M. Hovick. 2021. "Potential Local Adaptation in Populations of Invasive Reed Canary Grass (*Phalaris arundinacea*) Across an Urbanization Gradient." *Ecology and Evolution* 11, no. 16: 11457–11476. https://doi.org/10.1002/ece3.7938.

Winchell, K. M., R. G. Reynolds, S. R. Prado-Irwin, A. R. Puente-Rolón, and L. J. Revell. 2016. "Phenotypic Shifts in Urban Areas in the Tropical Lizard *Anolis cristatellus*." *Evolution* 70, no. 5: 1009–1022. https://doi.org/10.1111/evo.12925.

Wolfe, M. D., and S. J. Tonsor. 2014. "Adaptation to Spring Heat and Drought in Northeastern Spanish *Arabidopsis thaliana*." *New Phytologist* 201, no. 1: 323–334. https://doi.org/10.1111/nph.12485.

Yakub, M., and P. Tiffin. 2017. "Living in the City: Urban Environments Shape the Evolution of a Native Annual Plant." *Global Change Biology* 23, no. 5: 2082–2089. https://doi.org/10.1111/gcb.13528.

Young, H. J., and M. L. Stanton. 1990. "Influences of Floral Variation on Pollen Removal and Seed Production in Wild Radish." *Ecology* 71, no. 2: 536–547. https://doi.org/10.2307/1940307.

Zan, Y. J., and Ö. Carlborg. 2019. "A Polygenic Genetic Architecture of Flowering Time in the Worldwide *Arabidopsis thaliana* Population." *Molecular Biology and Evolution* 36, no. 1: 141–154. https://doi.org/10.1093/molbev/msy203.

Zhang, X. H., T. J. Liu, J. L. Wang, et al. 2021. "Pan-Genome of *Raphanus* Highlights Genetic Variation and Introgression Among Domesticated, Wild, and Weedy Radishes." *Molecular Plant* 14, no. 12: 2032–2055. https://doi.org/10.1016/j.molp.2021.08.005.

Zhou, X., and M. Stephens. 2012. "Genome-Wide Efficient Mixed-Model Analysis for Association Studies." *Nature Genetics* 44, no. 7: 821–824. https://doi.org/10.1038/ng.2310.

Zhou, X., and M. Stephens. 2014. "Efficient Multivariate Linear Mixed Model Algorithms for Genome-Wide Association Studies." *Nature Methods* 11, no. 4: 407. https://doi.org/10.1038/nmeth.2848.

Zhou, Y. Y., S. J. Smith, K. G. Zhao, et al. 2015. "A Global Map of Urban Extent From Nightlights." *Environmental Research Letters* 10, no. 5: 054011. https://doi.org/10.1088/1748-9326/10/5/054011.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The geographical locations of the studied populations. Blue dots indicate the natural populations where the impervious surfaces are less than 25% whereas red dots indicate the urban populations where the impervious surfaces are more than 75%. Population PALE is located outside the mapped area. The coordinates of the population are (41.8150287; 14.5188085). **Figure S2:** The PCA plot from the SNPs of four urban and four natural populations. Each colour represents different populations (▲ ANNU ▲ MSCO ▲ GOCA ▲ FAIT ● CATT ● CARR ● SOCC ● TECH ● VARG). PC1 accounts for 14.26% and PC2 accounts for 12.31% of the total variance. **Figure S3:** Concentrations of nectar sugars in *Raphanus raphanistrum* from the common garden experiment: (a) glucose, (b) fructose and (c) sucrose. Green indicates populations originating from natural environments; orange indicates populations from urban environments. **Figure S4:** The Q–Q plot of p values from the GWAS. The x-axis indicates the expected −log10(P) values and the y-axis indicates the empirical −log10(P) from the GWAS. **Table S1:** Field site information of 12 source populations from which *Raphanus raphanistrum* were collected and their sample sizes (N) used in the common garden experiment. **Table S2:**. Pairwise correlations among the six studied traits measured in the common garden experiment. Values represent Spearman's rank correlation coefficients. Correlations were calculated using pairwise complete observations. **Table S3:** Pairwise F_{st} of the seven studied populations. **Table S4:** Pairwise absolute divergence (dXY) of the seven studied populations. **Table S5:** Nucleotide diversity of the seven studied populations. **Table S6:** Significant annotation terms identified by DAVID functional enrichment analysis. Each row lists an annotation cluster, the associated enriched term, the corresponding p value and the gene names annotated with that term. Terms shown are from the UniProt Keyword (UP_KW) category. Only terms with $p < 0.05$ are shown.