Urbanization correlates with genetic and plastic variation of the spotted jewelweed flower morphology

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Abstract

The spectacular diversity of flowers is largely driven by pollinator-mediated selection that favors attractive flowers and effective pollen transfer. Urbanization has the potential to affect floral trait evolution by altering pollinator communities through environmental changes. Additionally, abiotic changes in urban habitats can induce phenotypic plasticity, further shaping evolutionary trajectories. We investigated how urbanization affects the genetic and plastic components of flower morphology of *Impatiens capensis* across four Canadian cities. We found that urbanization influenced the pollinator community composition and the body size of bumblebees, the species' main pollinator, although the magnitude of the size effect varied among cities. Using a combination of field surveys and a common garden experiment, our results suggest that urbanization affects sepal size – a tubular floral organ in which pollinators enter to access the nectar – through both genetic and plastic responses. While plasticity sometimes masked the genetic determination of sepal size in the field, we observed a positive correlation between the genetic component of the sepal size and bumblebee body size. These results suggest that urban habitats may drive evolutionary changes in floral traits by modifying pollinator communities.

Introduction

Flowers show an astounding diversity in shapes, sizes, colors, and scents across angiosperms, mostly resulting from selection imposed by pollinators (Galen, 1999; Harder & Johnson, 2009; Ollerton et al., 2011; Stebbins, 1970; van der Niet & Johnson, 2012; Whittall & Hodges, 2007). Flowers can genetically adapt to changes in pollinator communities or abundance by evolving traits that enhance attraction, such as color, scent, and nectar, that facilitate pollen transfer by improving the mechanical fit with pollinators through shape or size modifications, or that promote self-pollination (Acoca-Pidolle et al., 2024; Gervasi & Schiestl, 2017; Gómez et al., 2009). But flowers can also be modified by their environment through phenotypic plasticity (Caruso, 2006; Gómez et al., 2020), which can be either adaptive or maladaptive depending on whether the environment modifies the phenotype in a way that increases or decreases the mean fitness of the population, respectively (Dorey & Schiestl, 2022). Also, phenotypic plasticity can hide genetic adaptation if it affects the phenotype in a way that opposes natural selection, a concept called counter-gradient variation (Conover et al., 2009; Conover & Schultz, 1995). Despite the important roles of genetics and phenotypic plasticity in determining phenotypes, few studies have tried to tease them apart when investigating evolutionary responses of flower morphology across environmental gradients.

Urban habitats offer a unique opportunity to study evolutionary responses to environmental changes (Johnson & Munshi-South, 2017). The development and spread of cities alter abiotic conditions by remodeling the physical environment and biotic conditions through their effects on species richness, abundance, interactions, and evolution (Alberti et al., 2017; Johnson & Munshi-South, 2017; McKinney, 2008; Santangelo et al., 2022). Furthermore, since urban habitats are generally more similar to each other than to their surrounding natural environments (Santangelo et al., 2022), studying species response to urbanization in different cities is equivalent to a replicated evolutionary experiment (Santangelo et al., 2020), and could uncover parallel or idiosyncratic responses across cities (Diamond et al., 2018; Santangelo et al., 2022).

Urbanization is expected to disrupt mutualistic interactions, such as pollination, because these interactions require compatibility between flowering and pollinator activity and phenotype matching between the plant and pollinator traits (Fisogni et al., 2020; Irwin et al., 2020). Pollinator communities commonly exhibit changes along urbanization gradients (Baldock et al., 2019; Hall et al., 2017; Theodorou et al., 2020; Wenzel et al., 2020), but urban habitats could also affect the

body size of insect pollinators via changes in the pollinator species composition or through evolutionary responses to changes in resource availability and temperature (Goulson, 2010). So far, studies have produced conflicting results regarding how urbanization affects bumblebee sizes (Eggenberger et al., 2019; Theodorou et al., 2021). A greater distance between resource patches, as commonly found in cities, could favor larger insects with greater flight capacities (Greenleaf et al., 2007). On the other hand, high temperatures could favor smaller insects with greater thermoregulation potential (Eggenberger et al., 2019). Such changes in pollinator body size, as well as those in the abiotic environments, can potentially affect flower morphology through both genetic and plastic responses. Yet, few studies have investigated the impact of urbanization on the evolution of flower traits while also considering changes in pollinator communities (Irwin et al., 2018; Rivkin et al., 2020; Ushimaru et al., 2014) and attempted to disentangle genetic from plastic responses (but see Irwin et al., 2014).

We tested whether urbanization impacts, directly or indirectly through its effects on pollinator communities, the size and shape of flowers of the spotted jewelweed, *Impatiens capensis* Meerb. This annual species has a mixed pollination strategy, bearing both closed, green, and tiny self-fertilizing (cleistogamous) flowers, and, open, orange, large protandrous and mainly outcrossing (chasmogamous) flowers (Fig. 1). This reproductive strategy is plastic, as a greater proportion of chasmogamous flowers are produced with more light (Schemske, 1978; Waller, 1980), but it does not seem to be affected by urbanization (Barker & Sargent, 2020). Populations of *I. capensis* growing in sunlight produce numerous chasmogamous flowers pollinated by bumblebees, other bees, wasps, flies, and hummingbirds (Fenster et al., 2004; Rust, 1977, 1979), which all feed on pollen or the nectar secreted in the spur. The spur curvature of *I. capensis* is heritable, and affects pollen removal by hummingbirds (Travers et al., 2003) and the duration of visits by bees (Young, 2008). *Impatiens capensis* evolved smaller sepals under relaxed pollinator-mediated selection (Zhao & Schoen, 2022), and larger flowers under pollinator limitation (Panique & Caruso, 2020). Also, natural populations in Montreal, Canada, had larger sepals and longer nectar spurs compared to their urban counterparts (Faure et al., 2023). Finally, reproductive success was shown to be limited by pollen deposition in urban and natural habitats (Barker & Sargent, 2020), potentially allowing natural selection to drive floral phenotypic changes (Harder & Aizen, 2010). Therefore, the generalist pollination strategy of *I. capensis*, and its reported

capacity to respond to its pollinator communities in both urban and natural habitats make it an excellent system for studying the evolution of flower shape and size in response to urbanization.

In this study, we tested four hypotheses. First, the pollinators of *Impatiens capensis* differ in both their composition and sizes between urban and natural areas habitats. Second, because the pollinators of *I. capensis* must pass through the large tubular posterior sepal to access the nectar produced in the spur (Fig. 1), and because the reproductive organs are situated at the top entrance of the sepal, we expect sepal size to be correlated with the size of pollinators, and thus to differ between habitats, to better fit the local pollinator communities and ensure an effective pollen transfer. Third, we expect the corolla surface to be larger in urban habitats due to its role in pollinator attraction. This is based on previous studies that have shown that increased competition for floral resources (Hennig & Ghazoul, 2011) and reduced pollen dispersal in urban habitats (Rivkin et al., 2020) should result in stronger pollen limitation (Zink et al., 2024). Consequently, selection should favor larger floral displays in pollinator-limited populations to attract more pollinators (Geslin et al., 2013; Thomann et al., 2013). Fourth, sepal shape will differ between urban and natural habitats to maximize pollen transfer, but not corolla shape, as size appears more important than shape for pollinator attraction.

To test these hypotheses, we performed both field investigations and a greenhouse common garden experiment to control for environmental effects. We studied populations of *I. capensis* growing in sunlight to focus on the evolutionary responses of their animal-pollinated flowers. We considered the phenotypes measured in the common garden as the genetic components of the corresponding traits, whereas the phenotypes measured in the field corresponded to joint genetic and plastic components of the traits. We quantified flower shapes and sizes using geometric morphometrics, and we characterized pollinator communities for a total of 16 populations, paired by habitat – urban versus natural – in and around four large Canadian cities ("regions" hereafter): Toronto, Ottawa, Montreal, and Quebec (Fig. 1A).

Materials and Methods

Population selection and characterization

To test whether urbanization impacted the size and shape of *I. capensis* flowers, we compared urban and natural populations of four large Canadian metropolitan areas (+500,000 inhabitants).

We selected two pairs of urban and natural populations with more than 100 individuals growing in open canopies in an area of 75 km around the city center of each region, for a total of 16 populations. Urban populations were selected to have high proportions of impervious surfaces, while natural populations were selected to have fewer impervious surfaces, more vegetation and few crop fields around them. One natural population selected in 2021 in the Toronto region produced too few chasmogamous flowers and was abandoned (only 15 populations were sampled in 2021). It was replaced with the Silver Creek population in 2022 (Table S1). The Oka natural population in the Montreal region in 2021 was replaced by the Mirabel population in 2022 because of access issues (Table S1).

To confirm that urban and natural sites were clearly distinct across regions, we characterized each population with the Normalized Difference Vegetation Index (NDVI) and the percentage of land use/land cover using satellite images from Sentinel-2 (resolution 10m), and the mean Global Man-made Impervious Surface (GMIS) (Brown de Colstoun et al., 2017) using Landsat 7 data (resolution 30m). We measured the mean NDVI, GMIS, and land use percentages for each population with buffer zones of 500 m, 1000 m, and 2000 m radius centered on them. We also characterized each population using bioclimatic variables. We interpolated values of temperature (minimum and maximum, mean, mean minimum and maximum, mean dew), precipitation (total, mean relative humidity, number of wet and dry days), solar radiation, and frost-related metrics (number of days with and without) using BIOSIM 11 (Régnière & Bolstad, 1994), and computed the monthly summary for each population between 2010 and 2022 to obtain average bioclimatic conditions across several years for each population. These values were used to compute worldclim bioclimatic variables using the QBMS package (Al-Shamaa, 2021) for all populations in R version 4.3.3 (R Core Team, 2024). To compare the populations, we ran a scaled principal component analysis (PCA) including all above abiotic variables.

Field sampling

In both 2021 and 2022, we visited each population to photograph flowers, collect pollinators, and record flower visitors with cameras, and a second visit allowed us to collect pollinators and record flower visitors again. Both visits were done during peak flowering (between August 1st and September 10th). In each population, we photographed 50 flowers in profile and front view from randomly selected plants with either a Canon T2i with a EF-S 60mm macro lens (1:2.8 USM,

Canon Inc., Tokyo, Japan) or a Canon 90D with a EF 100mm macro lens (1:2.8 L IS USM, Canon Inc., Tokyo, Japan), placing a ruler beside flowers. We also recorded flower visitors of at least 5 different flowers for one hour using two GoPro Hero 9 cameras (GoPro, San Mateo, California, USA) placed 2 to 50 m apart. The camera was positioned 30 to 50 cm from the flowers, and recordings were done in 4K (3820 x 2160), with 30 frames per second. In 2021, we also sometimes recorded pollinator visits using a Canon T2i with a EF-S 60mm macro lens in 1080p (1920 x 1080) at 30 frames per second.

After the cameras stopped recording, two people collected insect pollinators of *I. capensis* for further identification and measurements during 20-minute sessions, using entomological nets while standing in front of, or walking along a stand of *I. capensis*. Pollinators were caught if they touched the reproductive parts of the flower and were then placed in a 50 mL tube in a cooler and frozen at the end of the day. All pollinator observations and captures were conducted in sunny conditions, with a minimum temperature of 15°C and low to no wind.

Common garden experiment

During the summer of 2021, we collected one seed from at least 30 plants in each population for a common garden experiment. A few days after collection, seeds were stored in Petri dishes on moist filter paper, closed with parafilm, and maintained at 4°C until germination (ca. 4-5 months later). Seedlings were transferred to 2-inch square pots containing BM6 multipurpose soil (Berger, Saint-Modeste, Quebec, Canada) and watered every two days from the bottom of the tray, alternating between clear water and fertilized water (15-5-15 NPK fertilizer from Monday to Thursday and 19-2-19 NPK from Friday to Sunday). When the plants reached 15 cm in height, they were transferred to 8.5 inch round pots with BM6 soil. They were watered and fertilized every two days, and every day from March 1st. Plants were grown with 16 hours of light, with day and night temperatures of 22°C and 18°C, respectively. When plants started flowering, we photographed the front and profile views of one random flower on each plant for phenotyping, using a Canon T2i with an EF-S 60mm macro lens, including a ruler on each picture (Table S1). The common garden experiment aimed to remove the environmental effects on the plants' phenotypes, but because it consisted of the progeny of the natural plants, maternal effects might be expressed.

Pollinator identification and measurement

Frozen pollinators were pinned on foam blocks using needles and dried at room temperature for a week. Apart from one Calliphoridae, one Muscidae, and one wasp specimen (the last being identified at the genus level) pollinators were identified at the species level. Pollinators were deposited at the Ouellet-Robert entomological collection of the Université de Montréal (Burkiewicz, 2025). We used the inter-tegular distance (ITD) as a proxy of pollinator size, as it is a good indicator of insects' dry mass and flight capability (Goulson, 2010; Greenleaf et al., 2007). We measured it by photographing each pollinator under a Zeiss microscope equipped with an AxioCam HRc (Discovery V20) camera and using the software ZENblue v2.5 Pro (Carl Zeiss, Oberkochen, Germany).

Pollinator observations

On the camera recordings, we identified between 5 and 10 open flowers from which we could easily distinguish a pollination event. An animal was considered a pollinator when it touched the reproductive parts of the flowers. Videos were watched at 2 × speed. When an animal was observed, videos were slowed down (0.12 – 0.5 ×) to score pollination events. For each pollinator, we recorded the time of the bout, the species or genus and family, the number of flowers observed in this particular recording, and the number of flowers visited. We identified bumblebees at the genus level, other bees at the species level, and wasps and flies at the family level. When we could not determine the species or genus, we identified taxa at the family level.

We calculated the total observation effort as the sum over all observation units (eg., one camera recording) of the number of hours observed times the number of flowers observed per unit. The pollinator visitation rate was the number of flowers pollinated over the total observation effort. Finally, the relative frequency of each pollinator taxon was the number of pollination events observed for that taxon divided by the total number of pollination events, either at the population level or over all populations.

Flower shape and size characterization

We used geometric morphometrics to characterize the flower shape from flower pictures taken in profile and front views, and measure the posterior sepal size and corolla surface (Collyer et al., 2015; Klingenberg, 2010; Mitteroecker & Gunz, 2009). We used tpsDig v2.32 (Rohlf, 2021) to digitize specimens. We used a similar approach to that of Faure et al. (2023) to extract flower shape (Fig 5A, D), and details on landmarks and semilandmarks positioning are available in

Supplementary Materials. Flower size was estimated from the landmarks and semi-landmarks that delimited the contour of the sepal and the corolla (Fig 3A, E); more details can be found in the Supplementary Materials. To compare flower shape between populations we performed a General Procrustes Analysis (GPA) with the *gpagen* function of the geomorph package (Adams, 2020), minimizing Procrustes distance between individuals (Klingenberg, 2010). As in Faure et al. (2023), semilandmarks were treated as landmarks in the GPA because the high variability of the spur and petals' curvature caused misalignments when treated as semilandmarks. We manually checked for outlier shapes to ensure these were not due to improper landmark positioning.

To assess the technical error in landmark positioning, we placed landmarks on duplicate flowers in some populations (four in front view, five in profile view). The technical error estimated using a Procrustes ANOVA with 999 permutations (*procDlm* function of the geomorph package (Adams, 2020)) was 0.02% for the profile pictures and 2.08% for the front pictures.

Statistical analyses

All analyses were performed in R version 4.3.3 (R Core Team, 2024) using the car (Fox et al., 2001), emmeans (Lenth, 2017), lme4 (Bates et al., 2003), geomorph (Adams, 2020), and vegan (Oksanen et al., 2025) packages. We visually inspected the normality and homoscedasticity of residuals, indicating if these assumptions were unmet and how we addressed these cases.

Pollinator abundance — We evaluated whether pollinator communities differed between urban and natural habitats. Because observation data and collected specimens gave consistent results (Fig. 2A, Table S2), we used only collected specimens for statistical analysis, as most insects were identified at the species level. Redundancy analysis (RDA) was used to analyze community data rather than canonical correspondence analysis (CCA) because in the latter, rare species contribute importantly to the chi-square distance (Legendre and Legendre 2012), and we were more interested in differences in the main pollinators. We used a Hellinger transformation of the data to account for shared absences among sites (Legendre and Legendre 2012). We tested whether regions (cities) or habitats (urban/natural) could explain variation in pollinator abundance. Partial RDA was used to test each factor while removing the effect of the other.

Pollinator visitation rate — We assessed whether urbanization influenced pollinator visitation rates. We used region, habitat and their interaction as fixed factors in an ANOVA to

explain the variation in pollinator visitation rate, assuming a Gaussian distribution. Significance was tested using the *Anova* function from the car package and type II sum of squares.

Bumblebee size and flower size — We tested whether urbanization affected the sizes (ITD) of bumblebees (all species combined), the main pollinator of *I. capensis*, and of flowers (sepal size and corolla surface). We built a linear mixed model that included Year (when applicable), Region, Habitat, and their interactions as fixed effects, including a population random effect and allowing slopes to vary with region, habitat, and their interaction:

$$\text{Size} \sim \text{Year} \times \text{Habitat} \times \text{Region} + (\text{Region} \times \text{Habitat} \mid \text{Population})$$

We first selected the best variance structure by fitting and comparing models with random effects of descending order of complexity and all fixed effects. Models were fitted with Restricted Maximum Likelihood (REML) using the *lmer* function from the lme4 R package and the best variance structure was the one with the lowest AIC (Zuur et al., 2009). Singular models and models that did not converge were excluded from the comparisons. The best variance structure for the three response variables was a simple population random effect, independent of region or habitat:

$$\text{Size} \sim \text{Year} \times \text{Habitat} \times \text{Region} + (1 \mid \text{Population}).$$

Using this variance structure, we then tested fixed effects and their interactions using type II Wald tests (*Anova* function from the car package). We computed estimated marginal means contrasts for urban/natural comparisons within regions within years using the Holm correction for multiple testing.

For flower size, we also tested if the sexual stage of the flowers (male or female) was correlated with the sepal size or the corolla surface, thus eliminating a potential confounding effect. The sexual stage was weakly associated (R^2_{adj} <1%) with sepal size and corolla surface in the field. To further test the robustness of the results to the sexual stage, we compared the results of models that included or excluded female flowers (12.2% of the data) and found consistent results for both analyses (Table S6), indicating that our results were not affected by the sexual stage of the flower.

Plasticity of flower size — We tested whether flower size was affected by phenotypic plasticity and if this effect was influenced by urbanization by comparing the flower size measured in the field to that measured in the common garden. We first analyzed the data from the 2021 field season versus the common garden experiment to compare the phenotype of the parental population and the phenotype of the offspring generation (the parentage of which is unknown), showing both genetic and plastic responses to environmental change. We then analyzed the data from the 2022 field season versus the common garden experiment to compare the phenotypes of kin, representing the plastic response to environmental change.

For each comparison, we built a linear mixed model to analyze variation in flower size with Environment (either field or common garden), Habitat (either urban or natural), and their interaction as fixed effects, including a population random effect, allowing slopes to vary with environment, habitat, and their interaction. We used the same procedure mentioned above to select the best variance structure. The best model included a population random effect that changed the intercept and the slopes were allowed to vary with Environment:

$$\text{Size} \sim \text{Environment} \times \text{Habitat} + (\text{Environment} \mid \text{Population})$$

We then tested fixed effects and interactions using type II Wald tests using the *Anova* function from the car package.

Correlation between bumblebee size and sepal size, and between pollinator visitation rates and corolla surface — We tested whether variation in mean population sepal sizes (or corolla surfaces) was correlated to the variation in mean population bumblebee sizes (ITD) (or mean population visitation rates). We used the sepal sizes and corolla surfaces measured in the common garden because they correspond to the genetic component of the trait, and analyzed their variation with an analysis of covariance with habitat and ITD (or the average pollinator visitation rates) and their interaction as fixed factors:

$$\text{Sepal Size} \sim \text{Habitat} \times \text{ITD}$$

$$\text{Corolla Surface} \sim \text{Habitat} \times \text{Pollinator Visitation Rates}$$

Significance was tested using the *Anova* function of the car package and type II sum of squares as above.

320

321 ***Flower shape***—We used a Procrustes ANOVA (equivalent to a PERMANOVA) with the
322 *procD.lm* function from the geomorph package to test whether flower shape was influenced by
323 size (allometry effect), year (when applicable), sexual stage of the flower (male vs female), region,
324 and habitat, including all possible interactions between these variables:

325

326 $$\text{Shape} \sim \text{Size} \times \text{Year} \times \text{Sex} \times \text{Region} \times \text{Habitat}.$$

327

328 Because Procrustes ANOVA cannot include random factors, we followed (Collyer et al., 2015)
329 and used the interaction of habitat and population as the error term to test for fixed effects on
330 interpopulation variation. We removed all non-significant interactions, except the one between
331 habitat and region, which we were interested in, and ran a Procrustes ANOVA with the remaining
332 terms. We used type 1 Procrustes ANOVA, ordering factors in a way that we tested for the effect
333 of habitat and the interaction between habitat and region after all other factors.

334

335 **Results**

336 **Urban populations were environmentally distinct from natural populations**

337 Urban and natural populations, across the four regions studied, were characterized by different
338 abiotic environments, showing our ability to discriminate those environments in the field and
339 justifying the use of the "habitat" categorical variable in all analyses that quantify the effects of
340 urbanization (Fig. 1 and Fig. S1). The natural populations were associated with higher tree cover
341 and greater vegetation index (NDVI), whereas urban populations were associated with higher
342 proportions of impervious surfaces and built areas, as well as lower isothermality (day-to-night
343 temperature variation compared to annual oscillations) due to a heat island effect (Fig. 1B, C and
344 Fig. S1).

345

346 **Urbanization modifies the pollinator communities of *I. capensis***

347 Bumblebees (*Bombus* spp.) were the most frequent pollinators of *I. capensis*, representing 73% of
348 all pollination events recorded on video (Fig. 2A). *Bombus impatiens* was the most collected
349 species (62.4%, Table S2), followed by *Bombus vagans* (12.7%, Table S2). *Apis mellifera*
350 represented 6.4% of the specimens sampled (Table S2) but primarily collected pollen and may not

be a reliable pollinator, as stigmas are not receptive when anthers are mature. *Anthophora terminalis*, *Vespidae*, and *Augochlora pura* together represented 12.5% of the specimens collected (Table S2), and these less common and less cosmopolitan pollinators did not make contact as consistently with the reproductive structures as bumblebees, according to video recordings.

Pollinator communities of *I. capensis* differed between urban and natural habitats (R^2_{adj} = 0.264, P = 0.013; Fig. 2B, Fig. S2). Urban habitats were more associated with *Bombus impatiens, Vespula maculifrons,* and *Apis mellifera,* while natural habitats were more associated with *Bombus vagans*, *Augochlora pura* and *Rhingia nasica* (Fig. 2B, Fig. S2). Among these pollinators, bumblebees were the most consistently found across all populations, and as such, they best characterized urban and natural pollinator communities of *I. capensis.* We also found that the effect of urbanization on pollinator visitation rates varied among regions (Region × Habitat: $F_{(3, 23)}$ = 3.675, P = 0.026; Table S3A, B).

Finally, we found that the effect of urbanization on bumblebee size varied among regions (Region × Habitat: $\chi^2_{(3)}$ = 8.826, P = 0.031; Fig. 2C, D, Table S5A), with Quebec showing the strongest effect (11.6% increase from natural to urban populations, Fig. 2C, D, Table S5B).

Urbanization affects the genetic component of sepal size

Sepal size was the same for urban and natural populations in the field ($\chi^2_{(1)}$ = 0.239, P = 0.624; Fig. S3, Table S6C). However, in the common garden experiment, sepals from urban populations were on average 7.6% larger than those in natural populations ($\chi^2_{(1)}$ = 8.564, P = 0.003; Table S7A), though this effect varied among regions (Region × Habitat: $\chi^2_{(3)}$ = 8.329, P = 0.039; Table S7A), with Quebec populations showing the largest difference (20.3% increase, $t_{(7.29)}$ = 3.700, P = 0.028; Fig. 3B, C, Table S7B). Together, these results suggest that phenotypic plasticity in the field could hide the genetic differences in sepal size between habitats. To further investigate this phenotypic plasticity, we analyzed the mean response of sepal size per habitat to changes in the environment (field vs. common garden). Compared to the common garden experiment, the sepal size measured in the field was smaller in urban populations and larger in natural populations, thus completely masking the existing genetic differences in sepal size between habitats (Environment × Habitat, 2021: $\chi^2_{(1)}$ = 4.796, P = 0.028; 2022: $\chi^2_{(1)}$ = 3.706, P = 0.054; Fig. 3D, Fig. S4A, Table S8).

380 We found that the genetic component of the mean sepal size per population was positively
381 correlated with the mean population bumblebee size ($F_{(1,11)} = 6.531$, $P = 0.026$, $R^2_{adj} = 0.527$; Fig.
382 4, Table S9).

383 **Urbanization has a limited effect on corolla surface, which is strongly affected by phenotypic**
384 **plasticity**

385 In the field, corolla surface was affected by habitat, but only in certain regions and certain years,
386 suggesting phenotypic plasticity (Table S6C). Under controlled conditions, the effect of
387 urbanization on corolla surface varied among regions (Region × Habitat: $\chi^2_{(3)} = 8.136$, $P = 0.043$;
388 Fig. 3F, G, Table S7A). The corolla surface was 19% larger in the field compared to the common
389 garden, regardless of habitat, confirming strong phenotypic plasticity for this trait (2021: $\chi^2_{(1)} =$
390 8.859, $P = 0.002$; 2022: $\chi^2_{(1)} = 36.761$, $P < 0.001$; Fig. 3H, Fig. S4B, Table S8). The genetic
391 component of the corolla surface was positively correlated with pollinator visitation rates in urban
392 populations, but not in natural populations (Pollinator Visitation Rate × Habitat: $F_{(1,11)} = 14.851$,
393 $P = 0.002$; Fig. S7, Table S10)

394 **Urbanization affects the sepal shape of *I. capensis* through plastic responses**

395 The spur of urban flowers was shorter and less curved compared to the ones in natural habitats in
396 the field ($F_{(1,1518)} = 3.751$, $P = 0.019$; Fig. 5B, C, Table S11A), but not in controlled conditions
397 ($F_{(1,315)} = 0.858$, $P = 0.502$; Fig. 5B, C, Table S11A). In contrast, corolla shape was similar in urban
398 and natural habitats, both in the common garden ($F_{(1,314)} = 0.436$, $P = 0.837$; Fig. 5E, Table S11A)
399 and in the field ($F_{(1,1509)} = 0.886$, $P = 0.472$; Fig. 5F, Table S11A). Corolla shape of flowers from
400 the field populations also differed from those raised in the common garden (Environment: $F_{(1,1839)}$
401 $= 6.198$, $P = 0.002$; Fig. 5E, F, Table S11B), further supporting the effect of phenotypic plasticity
402 on flowers. The shape of the corolla in controlled conditions suggests that they are less open than
403 those in the field (Fig. 5E, F).
404

405 **<u>Discussion</u>**

406 The combination of fieldwork and common garden experiments across four cities in southeastern
407 Canada revealed that urbanization altered the pollinator communities of *I. capensis* and its flower
408 morphology. Different species of pollinators were associated with urban and natural populations
409 of *Impatiens capensis.* Moreover, urbanization affected bumblebee size, the main pollinator,

410 differently among regions, a result explained by both intraspecific and interspecific differences.
411 These differences in pollinator community and size support our initial hypothesis. The genetic
412 component of sepal size was larger overall in urban areas, despite variation among cities. In
413 addition, and supporting our second hypothesis, we found that the genetic component of sepal size
414 was positively correlated with bumblebee size across populations, in both urban and natural
415 habitats. This suggests that, despite substantial phenotypic plasticity in the field that sometimes
416 masked the genetic determination of sepal size, as well as the existence of discrepancies among
417 regions, we may have found an explanation for the sepal size variation of *I. capensis* across
418 urbanization gradients, where sepal size has to fit the size of its bumblebee pollinators to maximize
419 pollen transfer.

420 Urbanization affected the genetic component of the corolla surface differently among
421 regions, with corollas being significantly larger in Quebec's urban areas, partly supporting our
422 third hypothesis. However, corolla surface was positively correlated with pollinator visitation rates
423 in urban populations (Fig. S7), contrary to our expectation that larger corollas should be favored
424 in habitats with reduced pollination services. This finding does not preclude the expected negative
425 relationship between floral display and pollen limitation as our analysis was limited to a single
426 component of floral display, corolla surface, excluding the effect of flower number, and did not
427 account for pollen transfer effectiveness and pollen quality in the estimation of pollen limitation.
428 Trade-offs may also play a role; for example, limited resources could constrain investment in both
429 flower size and number, such that increased flower number compensates for smaller corollas in
430 overall display. Pollinator visitation rates to *I. capensis* may also be driven by the entire plant
431 community through both competitive and facilitated interactions with co-flowering species. For
432 instance, the presence of the large-flowered invasive *Impatiens glandulifera* has been associated
433 with selection for reduced corolla height in *I. capensis* (Beans & Roach, 2015).

434 For both sepal size and corolla surface, the impact of urbanization varied among cities, but
435 it aligned with the impact of urbanization on pollinator communities. For example, pollinators
436 were the largest in urban populations of Quebec, where the genetic component of flower size was
437 the most impacted by urbanization. Previous studies on flower evolution in urban areas have
438 focused on phenotypic changes associated with pollinator availability and pollen movement (Irwin
439 et al., 2018; Ushimaru et al., 2014), but they left aside a more thorough characterization of

440 pollinator communities. Here, we show that characterizing the pollinator community helps to
441 understand the floral evolution in an urbanization context.

442 Urbanization had a limited effect on flower shape, partly supporting our fourth hypothesis.
443 In the field, urban sepals had shorter and less curved nectar spurs, which partially supports the
444 results of Faure et al. (2023), who found that urban nectar spurs were less curved in the region of
445 Montreal. However, the lack of difference in the common garden experiment suggests that
446 urbanization affected sepal shape only through phenotypic plasticity, although the statistical power
447 to detect a genetic response was small for the common garden experiment (Table S1). In contrast,
448 corolla shape was not affected by urbanization in both field and common garden conditions.
449 Overall, flower shape—excluding size—shows no evidence of genetic adaptation and seems to be
450 strongly influenced by environmental factors. These findings may seem surprising, as spur
451 curvature, a component we characterize through sepal shape, has been linked to improved pollen
452 removal by hummingbirds (Travers et al., 2003) and longer visit durations by bees (Young, 2008).
453 However, in our populations, bumblebees were the main pollinators, and hummingbirds were rare,
454 potentially limiting their role as selective agents. Finally, there is no reason to believe that visit
455 duration by bumblebees differs between urban and natural habitats, so the lack of difference in our
456 common garden experiment appears logical.

457 Phenotypic plasticity can be adaptive or maladaptive depending on whether it moves
458 phenotypes towards or away from the fitness optimum of populations. It can also decrease the
459 efficiency of natural selection as the phenotypes with the highest fitness represent both the genetic
460 and plastic components of the corresponding traits. We found that for the sepal size of *I. capensis*,
461 phenotypic plasticity masked the genetic differences detected in the common garden experiment
462 (Fig. 3D; Fig S4), suggesting a counter-gradient variation in which plasticity typically modifies
463 the phenotype in a direction opposite to natural selection (Conover & Schultz, 1995). Assuming a
464 similar plastic response for all genotypes, such as larger flowers in the common garden are also
465 larger in the field conditions, we infer that the sepal size genetically respond to its pollinator
466 community. Therefore, bumblebee selection for sepal size along an urbanization gradient appears
467 to be a case of compensatory adaptive change in which selection counteracts the homogenizing
468 effect of phenotypic plasticity (Conover et al., 2009; Conover & Schultz, 1995; Hendry, 2016).
469 Indeed, natural selection seems to favor larger flowers in some cities because of the presence of
470 larger bumblebees, but plasticity masked the genetic differences in the field (Fig. 3D, Fig. S4). We

also highlighted that the corolla size and shape were significantly affected by the environment through phenotypic plasticity, which is particularly evident from the observed differences between the common garden and field conditions. This plasticity could be caused by developmental constraints, weather conditions, and pollinator visits (by pushing the petals apart), and may at least partly explain the variation among years (Table S6C), and between field and controlled conditions. Our results demonstrate that it is critical to disentangle plastic from genetic responses to fully understand the causes of phenotypic changes and adequately interpret the evolutionary responses of organisms to environmental changes (de Villemereuil et al., 2016; Diamond & Martin, 2021; Lambert et al., 2021; Thompson et al., 2025).

Strong environmental changes associated with urbanization impact living organisms, their interactions, and their evolution. Although our knowledge of eco-evolutionary dynamics in these environments has increased in the last decade, integrative approaches that assess plastic and genetic components of traits while considering species interactions are still scarce, but they are necessary to understand rapid evolution to changing environments. Only with this will we be able to understand adaptation to urbanization and pave the way for conservation policies in these habitats.

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Conceptualization: SJ, JB

Methodology: JB, SJ, CD

Investigation: JB, JC, SC, JL, EN, SJ

Visualization: JB, SJ, CD, JL

Funding acquisition: SJ

Project administration: JB, SJ

Writing – original draft: JB

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Supplementary Materials

Supplementary Methods

Supplementary Figures (Figs. S1 to S7)

Supplementary Tables (Tables S1 to S22)

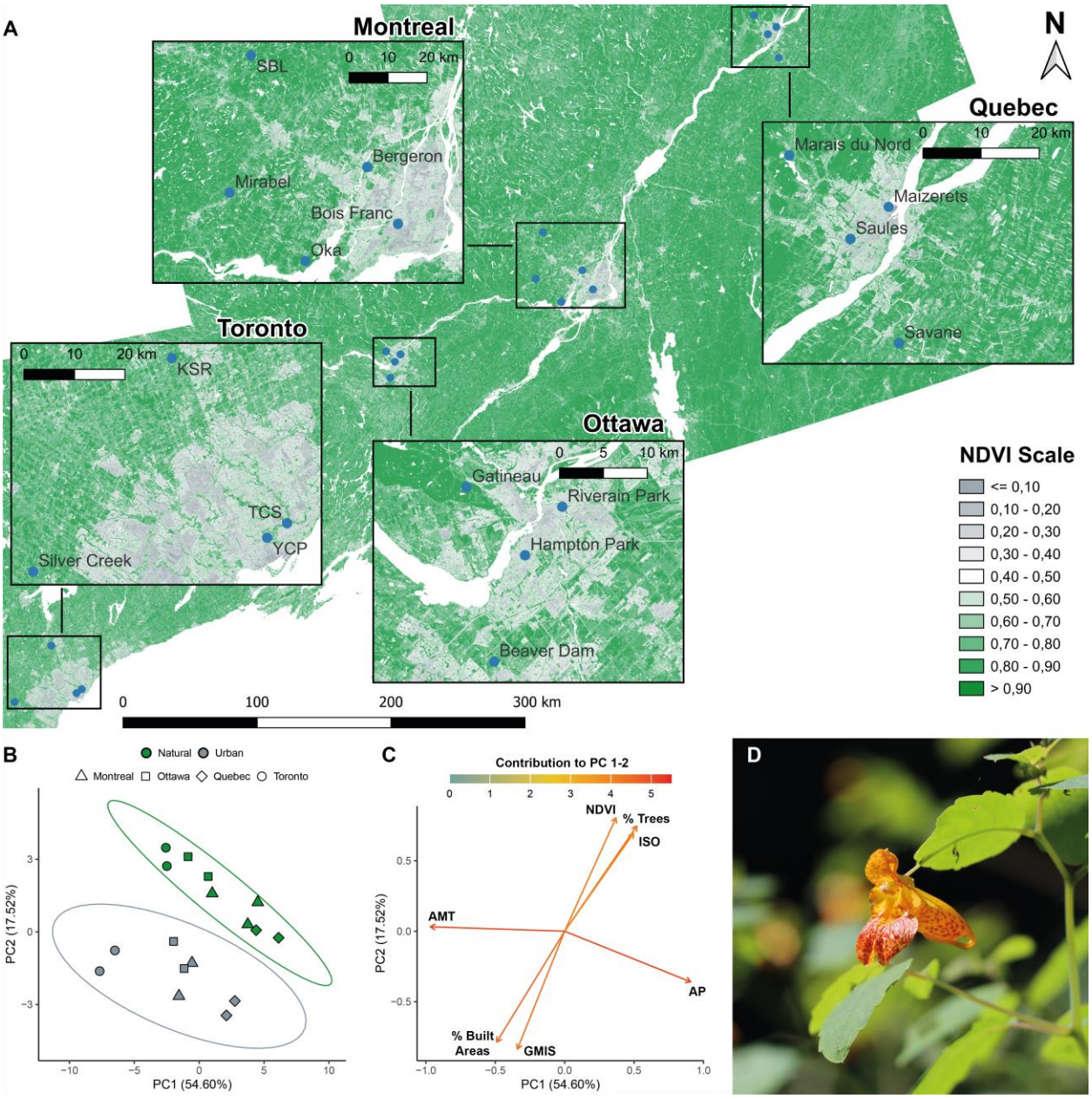


Fig. 1. Study sites and system. Flowers from populations of *Impatiens capensis* were sampled in four regions in 2021 and 2022. (A) Normalized-Difference Vegetation Index (NDVI) map showing the sampled populations; high NDVI values (green) indicate more important vegetation cover. (B) Populations and (C) variables plots of a Principal Component Analysis (PCA) conducted on the mean values of environmental variables from a 500m radius circle around each population. Only the most contributing variables are represented: annual mean temperature (AMT), annual precipitation (AP), isothermality (ISO), NDVI, global man-made impervious surfaces (GMIS), percentage of trees (% Trees) and percentage of built areas (% Built Areas). Ellipses represent 95% confidence interval (CI). PCAs for 1000m and 2000m radius buffers are available in the Supplementary Material. (D) Flower of *Impatiens capensis* (credits: J. Burkiewicz).

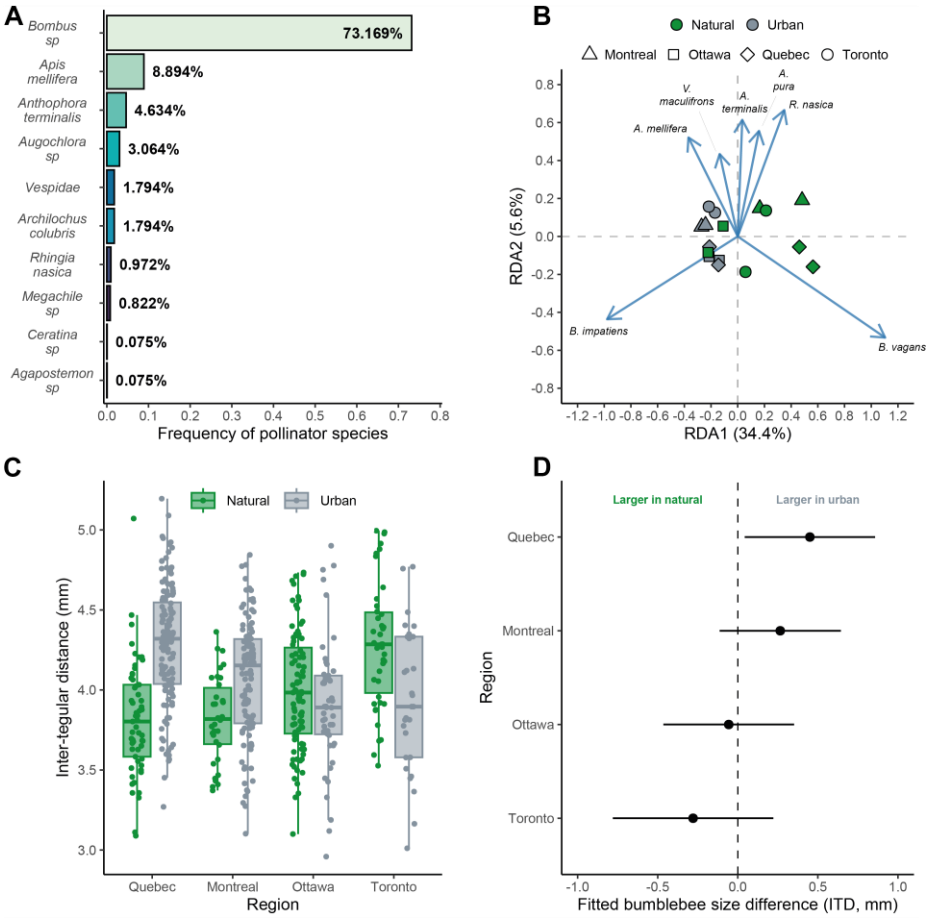


Fig. 2. Characterization of the *I. capensis* pollinator community and size of bumblebees. (A) Relative frequencies of pollinator species computed from video observation data, although frequencies are similar to those obtained with collected specimens (Table S2). (B) Redundancy analysis of pollinator abundance (collected specimens) by habitat and region for the 2022 field season. Only the most abundant species were represented on the graph for clarity purposes. The redundancy analysis on combined field seasons (2021 and 2022) is available in Fig. S2. (C) Boxplots of bumblebee inter-tegular distances by habitat and region, averaged over the two field seasons. Dots represent individual bumblebee measurements; sample sizes are available in Table S4. (D) Estimated marginal means contrasts of bumblebee size between urban and natural habitats for each region from the linear mixed model and associated 95% confidence intervals. Measures of bumblebee size include all *Bombus sp.* collected.

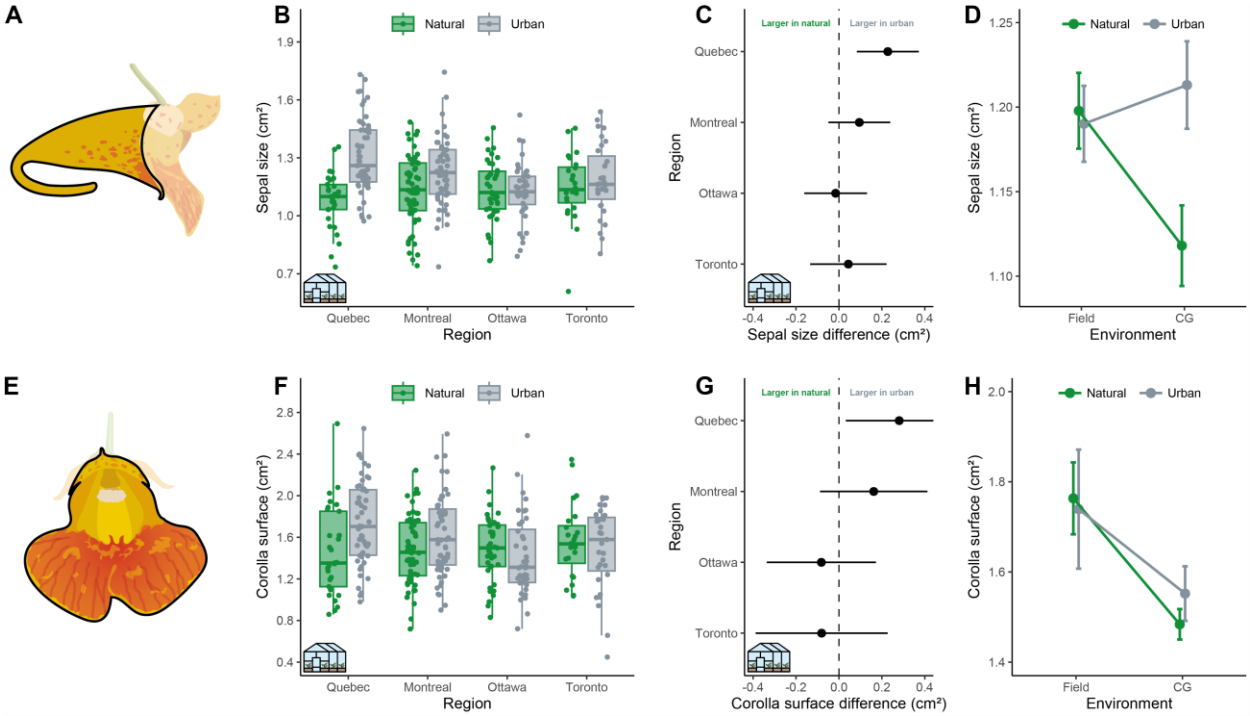


Fig. 3. Sepal size (A-D) and corolla surface (E-H) comparisons between habitats. (A) Drawing showing the measure used to estimate the sepal size of *Impatiens capensis* in black outline. (B) Boxplots of the sepal size measured in the common garden (as indicated by the greenhouse icon) with dots representing individual flowers. (C) Estimated marginal means contrasts of the sepal size between urban and natural habitats per region and associated 95% confidence intervals in the common garden experiment. (D) Mean response of sepal size, grouped by habitats, to environmental change for the 2021 field season. Error bars represent standard errors of the mean. Results with two field seasons combined are available in Fig. S5A. (E) Drawing showing the measure used to estimate the corolla surface of *Impatiens capensis* in black outline. (F) Boxplots of the corolla surface measured in the common garden with dots representing individuals. (G) Estimated marginal means contrasts of the corolla surface between urban and natural habitats per region and associated 95% confidence intervals in the common garden experiment. (H) Mean response of corolla surface, grouped by habitats, to environmental change for the 2021 field season. Error bars represent standard errors of the mean. Results with two field seasons combined are available in Fig. S5B. Credits for the drawings: J. Lafond.

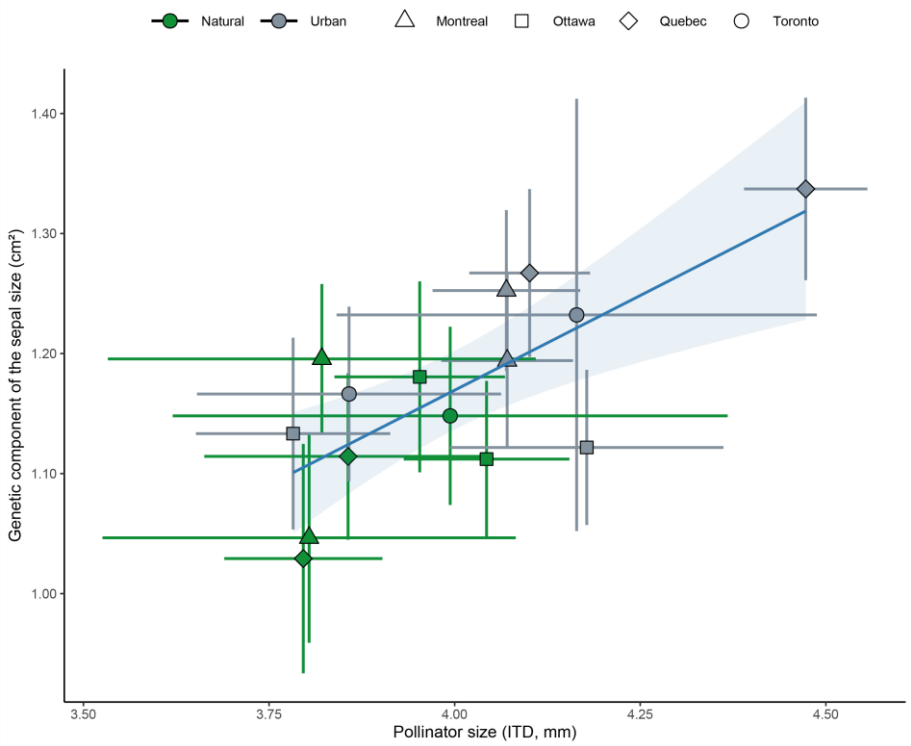


Fig. 4. Correlation between the genetic component of the sepal size and bumblebee inter-tegular distances. The points represent the population mean of the sepal size in the common garden experiment and the population mean bumblebee size averaged across the 2021 and 2022 field seasons. Error bars for the points indicate the 95% confidence intervals of the means. The line shows the significant positive linear relationship with its standard error. Measures of bumblebee size include all *Bombus sp.* collected.

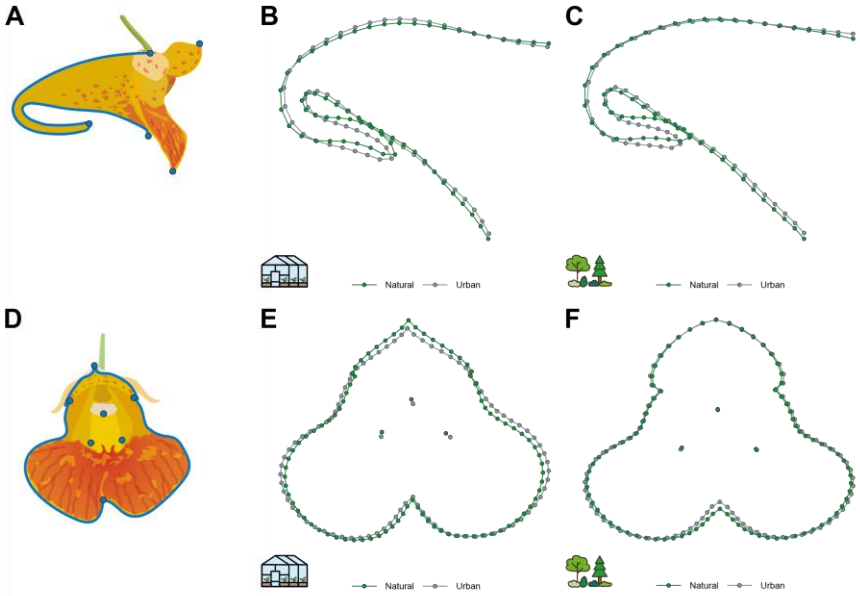


Fig. 5. Predicted posterior sepal and corolla shape comparison between habitats. (A) Drawing showing the landmarks (blue dots) and semilandmarks (blue curves) position on the posterior sepal of *Impatiens capensis*. (B) Predicted shape of the posterior sepal in urban and natural habitats for the common garden experiment. (C) Predicted shape of the posterior sepal in urban and natural habitats for the 2021 field season. (D) Drawing showing the landmarks (blue dots) and semilandmarks (blue curves) position on the corolla of *Impatiens capensis*. (E) Predicted corolla shape in urban and natural habitats for the common garden experiment. (F) Predicted corolla shape in urban and natural habitats for the 2021 field season.

References

Acoca-Pidolle, S., Gauthier, P., Devresse, L., Deverge Merdrignac, A., Pons, V., & Cheptou, P.-O. (2024). Ongoing convergent evolution of a selfing syndrome threatens plant–pollinator interactions. *New Phytologist*, *242*(2), 717–726. https://doi.org/10.1111/nph.19422

Adams, D. C., M. L. Collyer, and A. Kaliontzopoulou. (2020). *Geomorph: Software for geometric morphometric analyses* [Computer software]. https://cran.r-project.org/package=geomorph

Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., Hunt, V. M., Apgar, T. M., & Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences*, *114*(34), Article 34. https://doi.org/10.1073/pnas.1606034114

Al-Shamaa, K. (2021). *QBMS: Query the Breeding Management System(s)* (p. 1.5.0) [Data set]. https://doi.org/10.32614/CRAN.package.QBMS

Baldock, K. C. R., Goddard, M. A., Hicks, D. M., Kunin, W. E., Mitschunas, N., Morse, H., Osgathorpe, L. M., Potts, S. G., Robertson, K. M., Scott, A. V., Staniczenko, P. P. A., Stone, G. N., Vaughan, I. P., & Memmott, J. (2019). A systems approach reveals urban pollinator hotspots and conservation opportunities. *Nature Ecology & Evolution*, *3*(3), Article 3. https://doi.org/10.1038/s41559-018-0769-y

Barker, C. A., & Sargent, R. D. (2020). Pollination Services to Impatiens Capensis (Balsaminaceae) Are Maintained across an Urbanization Gradient. *International Journal of Plant Sciences*, 000–000. https://doi.org/10.1086/710488

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2003). *lme4: Linear Mixed-Effects Models using 'Eigen' and S4* (p. 1.1-35.5) [Data set]. https://doi.org/10.32614/CRAN.package.lme4

Beans, C. M., & Roach, D. A. (2015). An invasive plant alters pollinator-mediated phenotypic selection on a native congener. *American Journal of Botany*, *102*(1), Article 1. https://doi.org/10.3732/ajb.1400385

Brown de Colstoun, E. C., Huang, C., Wang, P., Tilton, J. C., Tan, B., Phillips, J., Niemczura, S., Ling, P.-Y., & Wolfe, R. E. (2017). *Global Man-made Impervious Surface (GMIS) Dataset From Landsat* [Data set]. NASA Socioeconomic Data and Applications Center (SEDAC). https://doi.org/10.7927/H4P55KKF

Burkiewicz. (2025). *Pollinators of Impatiens capensis in southeastern Canada* (Version 2.1) [Samplingevent dataset]. https://doi.org/10.5886/wj63qz

Caruso, C. M. (2006). Plasticity of inflorescence traits in Lobelia siphilitica (Lobeliaceae) in response to soil water availability. *American Journal of Botany*, *93*(4), Article 4. https://doi.org/10.3732/ajb.93.4.531

Collyer, M. L., Sekora, D. J., & Adams, D. C. (2015). A method for analysis of phenotypic change for phenotypes described by high-dimensional data. *Heredity*, *115*(4), 357–365. https://doi.org/10.1038/hdy.2014.75

Conover, D. O., Duffy, T. A., & Hice, L. A. (2009). The covariance between genetic and environmental influences across ecological gradients. *Annals of the New York Academy of Sciences*, *1168*(1), 100–129. https://doi.org/10.1111/j.1749-6632.2009.04575.x

Conover, D. O., & Schultz, E. T. (1995). Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology & Evolution*, *10*(6), 248–252. https://doi.org/10.1016/S0169-5347(00)89081-3

de Villemereuil, P., Gaggiotti, O. E., Mouterde, M., & Till-Bottraud, I. (2016). Common garden experiments in the genomic era: New perspectives and opportunities. *Heredity*, *116*(3), Article 3. https://doi.org/10.1038/hdy.2015.93

Diamond, S. E., Chick, L. D., Perez, A., Strickler, S. A., & Martin, R. A. (2018). Evolution of thermal tolerance and its fitness consequences: Parallel and non-parallel responses to urban heat islands across three cities. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1882), Article 1882. https://doi.org/10.1098/rspb.2018.0036

Diamond, S. E., & Martin, R. A. (2021). Evolution in Cities. In *Annual Review of Ecology, Evolution, and Systematics* (Vol. 52, Issue Volume 52, 2021, pp. 519–540). Annual Reviews. https://doi.org/10.1146/annurev-ecolsys-012021-021402

Dorey, T., & Schiestl, F. P. (2022). Plant phenotypic plasticity changes pollinator-mediated selection. *Evolution*, *76*(12), 2930–2944. https://doi.org/10.1111/evo.14634

Eggenberger, H., Frey, D., Pellissier, L., Ghazoul, J., Fontana, S., & Moretti, M. (2019). Urban bumblebees are smaller and more phenotypically diverse than their rural counterparts. *Journal of Animal Ecology*, *88*(10), Article 10. https://doi.org/10.1111/1365-2656.13051

Faure, J., Volz, V., & Joly, S. (2023). Variation in flower size and shape of Impatiens capensis is correlated with urbanization in Montreal, Canada. *Ecology and Evolution*, *13*(12), e10826. https://doi.org/10.1002/ece3.10826

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., & Thomson, J. D. (2004). Pollination Syndromes and Floral Specialization. *Annual Review of Ecology, Evolution, and Systematics*, *35*(1), Article 1. https://doi.org/10.1146/annurev.ecolsys.34.011802.132347

Fisogni, A., Hautekèete, N., Piquot, Y., Brun, M., Vanappelghem, C., Michez, D., & Massol, F. (2020). Urbanization drives an early spring for plants but not for pollinators. *Oikos*, *129*(11), Article 11. https://doi.org/10.1111/oik.07274

Fox, J., Weisberg, S., & Price, B. (2001). *car: Companion to Applied Regression* (p. 3.1-3) [Data set]. https://doi.org/10.32614/CRAN.package.car

Galen, C. (1999). Why do flowers vary?: The functional ecology of variation in flower size and form within natural plant populations. *BioScience*, *49*(8), Article 8. https://doi.org/10.2307/1313439

Gervasi, D. D. L., & Schiestl, F. P. (2017). Real-time divergent evolution in plants driven by pollinators. *Nature Communications*, *8*(1), Article 1. https://doi.org/10.1038/ncomms14691

Geslin, B., Gauzens, B., Thébault, E., & Dajoz, I. (2013). Plant Pollinator Networks along a Gradient of Urbanisation. *PLOS ONE*, *8*(5), Article 5. https://doi.org/10.1371/journal.pone.0063421

Gómez, J. M., Abdelaziz, M., Camacho, J. P. M., Muñoz-Pajares, A. J., & Perfectti, F. (2009). Local adaptation and maladaptation to pollinators in a generalist geographic mosaic. *Ecology Letters*, *12*(7), Article 7. https://doi.org/10.1111/j.1461-0248.2009.01324.x

Gómez, J. M., Perfectti, F., Armas, C., Narbona, E., González-Megías, A., Navarro, L., DeSoto, L., & Torices, R. (2020). Within-individual phenotypic plasticity in flowers fosters pollination niche shift. *Nature Communications*, *11*(1), 4019. https://doi.org/10.1038/s41467-020-17875-1

Goulson, D. (2010). *Bumblebees: Behaviour, ecology, and conservation*. Oxford University Press.

Greenleaf, S. S., Williams, N. M., Winfree, R., & Kremen, C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia*, *153*(3), 589–596. https://doi.org/10.1007/s00442-007-0752-9

Hall, D. M., Camilo, G. R., Tonietto, R. K., Ollerton, J., Ahrné, K., Arduser, M., Ascher, J. S., Baldock, K. C. R., Fowler, R., Frankie, G., Goulson, D., Gunnarsson, B., Hanley, M. E., Jackson, J. I., Langellotto, G., Lowenstein, D., Minor, E. S., Philpott, S. M., Potts, S. G., … Threlfall, C. G. (2017). The city as a refuge for insect pollinators. *Conservation Biology*, *31*(1), Article 1. https://doi.org/10.1111/cobi.12840

Harder, L. D., & Aizen, M. A. (2010). Floral adaptation and diversification under pollen limitation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1539), 529–543. https://doi.org/10.1098/rstb.2009.0226

Harder, L. D., & Johnson, S. D. (2009). Darwin's beautiful contrivances: Evolutionary and functional evidence for floral adaptation. *New Phytologist*, *183*(3), Article 3. https://doi.org/10.1111/j.1469-8137.2009.02914.x

Hendry, A. P. (2016). Key questions on the role of phenotypic plasticity in eco-evolutionary dynamics. *Journal of Heredity*, *107*(1), 25–41. https://doi.org/10.1093/jhered/esv060

Hennig, E. I., & Ghazoul, J. (2011). Plant–pollinator interactions within the urban environment. *Perspectives in Plant Ecology, Evolution and Systematics*, *13*(2), 137–150. https://doi.org/10.1016/j.ppees.2011.03.003

Irwin, R. E., Warren, P. S., & Adler, L. S. (2018). Phenotypic selection on floral traits in an urban landscape. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1884), Article 1884. https://doi.org/10.1098/rspb.2018.1239

Irwin, R. E., Warren, P. S., Carper, A. L., & Adler, L. S. (2014). Plant–animal interactions in suburban environments: Implications for floral evolution. *Oecologia*, *174*(3), 803–815. https://doi.org/10.1007/s00442-013-2797-2

Irwin, R. E., Youngsteadt, E., Warren, P. S., & Bronstein, J. L. (2020). The Evolutionary Ecology of Mutualisms in Urban Landscapes. In *Urban Evolutionary Biology* (pp. 111–129). Oxford University Press. https://doi.org/10.1093/oso/9780198836841.003.0007

Johnson, M. T. J., & Munshi-South, J. (2017). Evolution of life in urban environments. *Science*, *358*(6363), Article 6363. https://doi.org/10.1126/science.aam8327

Klingenberg, C. P. (2010). Evolution and development of shape: Integrating quantitative approaches. *Nature Reviews Genetics*, *11*(9), Article 9. https://doi.org/10.1038/nrg2829

Lambert, M. R., Brans, K. I., Des Roches, S., Donihue, C. M., & Diamond, S. E. (2021). Adaptive Evolution in Cities: Progress and Misconceptions. *Trends in Ecology & Evolution*, *36*(3), 239–257. https://doi.org/10.1016/j.tree.2020.11.002

Lenth, R. V. (2017). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (p. 1.10.5) [Data set]. https://doi.org/10.32614/CRAN.package.emmeans

McKinney, M. L. (2008). Effects of urbanization on species richness: A review of plants and animals. *Urban Ecosystems*, *11*(2), Article 2. https://doi.org/10.1007/s11252-007-0045-4

Mitteroecker, P., & Gunz, P. (2009). Advances in Geometric Morphometrics. *Evolutionary Biology*, *36*(2), Article 2. https://doi.org/10.1007/s11692-009-9055-x

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Borman, T., Carvalho, G., Chirico, M., Caceres, M. D., … Weedon, J. (2025). *vegan: Community Ecology Package* [Data set]. https://vegandevs.github.io/vegan/

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), Article 3. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Panique, H., & Caruso, C. M. (2020). Simulated pollinator declines intensify selection on floral traits that facilitate selfing and outcrossing in Impatiens capensis. *American Journal of Botany*, *107*(1), Article 1. https://doi.org/10.1002/ajb2.1403

R Core Team. (2024). *_R: A Language and Environment for Statistical Computing_* (Version 4.3.3) [Computer software]. R Foundation for Statistical Computing. <https://www.R-project.org/>

Régnière, J., & Bolstad, P. (1994). Statistical Simulation of Daily Air Temperature Patterns Eastern North America to Forecast Seasonal Events in Insect Pest Management. *Environmental Entomology*, *23*(6), 1368–1380. https://doi.org/10.1093/ee/23.6.1368

Rivkin, L. R., Nhan, V. J., Weis, A. E., & Johnson, M. T. J. (2020). Variation in pollinator-mediated plant reproduction across an urbanization gradient. *Oecologia*, *192*(4), Article 4. https://doi.org/10.1007/s00442-020-04621-z

Rohlf, F. (2021). *tpsDig2* (Version 2.32) [Computer software].

Rust, R. W. (1977). Pollination in Impatiens capensis and Impatiens pallida (Balsaminaceae). *Bulletin of the Torrey Botanical Club*, *104*(4), Article 4. JSTOR. https://doi.org/10.2307/2484781

Rust, R. W. (1979). Pollination of Impatiens capensis: Pollinators and nectar robbers. *Journal of the Kansas Entomological Society*, *52*(2), Article 2. JSTOR.

Santangelo, J. S., Miles, L. S., Breitbart, S. T., Murray-Stoker, D., Rivkin, L. R., Johnson, M. T. J., & Ness, R. W. (2020). Urban Environments as a Framework to Study Parallel Evolution. In *Urban Evolutionary Biology* (pp. 36–53). Oxford University Press. https://doi.org/10.1093/oso/9780198836841.003.0003

Santangelo, J. S., Ness, R. W., Cohan, B., Fitzpatrick, C. R., Innes, S. G., Koch, S., Miles, L. S., Munim, S., Peres-Neto, P. R., Prashad, C., Tong, A. T., Aguirre, W. E., Akinwole, P. O., Alberti, M., Álvarez, J., Anderson,

J. T., Anderson, J. J., Ando, Y., Andrew, N. R., … Johnson, M. T. J. (2022). Global urban environmental change drives adaptation in white clover. *Science*, *375*(6586), Article 6586. https://doi.org/10.1126/science.abk0989

Schemske, D. W. (1978). Evolution of reproductive characteristics in Impatiens (Balsaminaceae): The significance of cleistogamy and chasmogamy. *Ecology*, *59*(3), Article 3. https://doi.org/10.2307/1936588

Stebbins, G. L. (1970). Adaptive Radiation of Reproductive Characteristics in Angiosperms, I: Pollination Mechanisms. *Annual Review of Ecology and Systematics*, *1*(1), Article 1. https://doi.org/10.1146/annurev.es.01.110170.001515

Theodorou, P., Baltz, L. M., Paxton, R. J., & Soro, A. (2021). Urbanization is associated with shifts in bumblebee body size, with cascading effects on pollination. *Evolutionary Applications*, *14*(1), Article 1. https://doi.org/10.1111/eva.13087

Theodorou, P., Radzevičiūtė, R., Lentendu, G., Kahnt, B., Husemann, M., Bleidorn, C., Settele, J., Schweiger, O., Grosse, I., Wubet, T., Murray, T. E., & Paxton, R. J. (2020). Urban areas as hotspots for bees and pollination but not a panacea for all insects. *Nature Communications*, *11*(1), Article 1. https://doi.org/10.1038/s41467-020-14496-6

Thomann, M., Imbert, E., Devaux, C., & Cheptou, P.-O. (2013). Flowering plants under global pollinator decline. *Trends in Plant Science*, *18*(7), 353–359. https://doi.org/10.1016/j.tplants.2013.04.002

Thompson, M. J., Réale, D., Chenet, B., Delaitre, S., Fargevieille, A., Romans, M., Caro, S. P., & Charmantier, A. (2025). The city and forest bird flock together in a common garden: Genetic and environmental effects drive urban phenotypic divergence. *Evolution*, *79*(5), 800–822. https://doi.org/10.1093/evolut/qpaf028

Travers, S. E., Temeles, E. J., & Pan, I. (2003). The relationship between nectar spur curvature in jewelweed (Impatiens capensis) and pollen removal by hummingbird pollinators. *Canadian Journal of Botany*, *81*(2), Article 2. https://doi.org/10.1139/b03-014

Ushimaru, A., Kobayashi, A., & Dohzono, I. (2014). Does urbanization promote floral diversification? Implications from changes in herkogamy with pollinator availability in an urban-rural area. *The American Naturalist*, *184*(2), Article 2. https://doi.org/10.1086/676855

van der Niet, T., & Johnson, S. D. (2012). Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology & Evolution*, *27*(6), Article 6. https://doi.org/10.1016/j.tree.2012.02.002

Waller, D. M. (1980). Environmental determinants of outcrossing in Impatiens capensis (Balsaminaceae). *Evolution*, *34*(4), Article 4. JSTOR. https://doi.org/10.2307/2408029

Wenzel, A., Grass, I., Belavadi, V. V., & Tscharntke, T. (2020). How urbanization is driving pollinator diversity and pollination – A systematic review. *Biological Conservation*, *241*, 108321. https://doi.org/10.1016/j.biocon.2019.108321

Whittall, J. B., & Hodges, S. A. (2007). Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature*, *447*(7145), Article 7145. https://doi.org/10.1038/nature05857

Young, H. J. (2008). Selection on spur shape in Impatiens capensis. *Oecologia*, *156*(3), Article 3. https://doi.org/10.1007/s00442-008-1014-1

Zhao, Y., & Schoen, D. J. (2022). Relaxed selection and the evolution of the chasmogamous flower of Impatiens capensis (Balsaminaceae). *Evolutionary Ecology*, *36*(2), 233–250. https://doi.org/10.1007/s10682-022-10155-y

Zink, A., CaraDonna, P. J., Larsen, K., & Iler, A. M. (2024). Pollination deficits increase with urbanization in Chicago. *Ecosphere*, *15*(12), e70100. https://doi.org/10.1002/ecs2.70100

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R* (Vol. 574). Springer.

768 **Supplementary Materials**

769

770 **Supplementary Methods**

771

772 Geometric morphometric

773

774 We identified five landmarks (homologous points) for the posterior sepal: 1) the tip of the upper
775 petal, 2) the insertion of the pedicel on the perianth, 3) the extremity of the spur, 4) the lower
776 extremity of the opening of the posterior sepal, and 5) the lower point of the lower petal closer to
777 the camera (Fig. 5A). We also defined two curves with 30 semilandmarks each to capture the shape
778 of the posterior sepal. The first curve followed the outline of the posterior sepal between landmarks
779 2 and 3 (dorsal curve, Fig. 5A), and the second one followed the outline of the posterior sepal
780 between landmarks 3 and 4 (ventral curve, Fig. 5A).

781 We placed seven landmarks for the front views of the flowers: 1) the tip of the upper petal,
782 2) the intersection between the upper petal and the right lateral petal (left for landmark 6, which is
783 symmetrical), 3) the right widest point of the corolla opening (left for landmark 5, which is
784 symmetrical), 4) the intersection of the two lower petals or, if the two petals do not intersect, the
785 center of a segment drawn between the uppermost part of each lower petal, and, 7) the lower point
786 of the stamens or the stigma (Fig. 5D). Note that points 4 and 7 are better defined as semi-
787 homologous landmarks. We also defined four semilandmarks curves to capture the shape of the
788 corolla. The first and fourth followed the outline of the upper petal between landmarks 1 and 2, or
789 6 and 1, and each contained 10 points. The second and third contained 35 points and followed the
790 lateral and lower petal outline between landmarks 2 and 4, or 4 and 6 (Fig. 5D). Curves were
791 numbered clockwise starting from the top of the flower.

792 We removed flower pictures that were too blurry or for which critical features of the flowers
793 were masked, preventing us from precisely locating landmarks or semilandmarks. For all curves,
794 we removed the first and last semilandmarks because they were redundant with the landmarks. For
795 profile pictures, we also removed landmarks 1 and 5 because they were redundant with the
796 landmarks and semilandmarks of the front pictures and were sometimes challenging to locate
797 precisely. Different persons placed the landmarks, but one person (JB) validated all points and
798 curves of all flowers.

For the profile view, the sepal size corresponded to the area of a polygon created by concatenating all curves and landmarks 2, 3, and 4 (Fig. 3A, Fig. 5A). For the front view, the corolla surface corresponded to the area of a polygon created by concatenating all curves and landmarks 1, 2, 4 and 6 (Fig. 3E, Fig. 5D).

Supplementary Figures

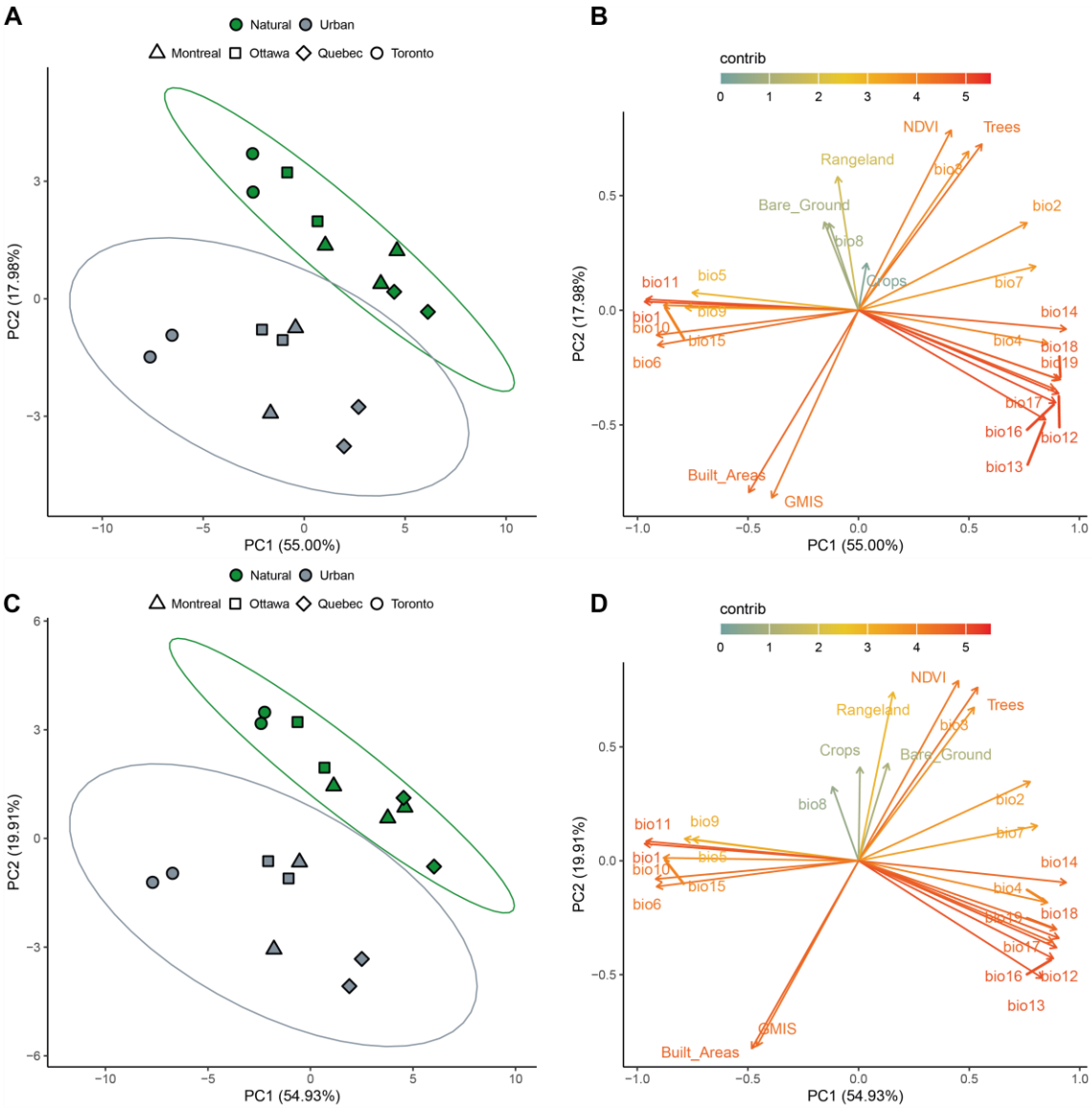


Fig. S1. Population characteristics at different spatial scales. (A-B) Individual and variables plot of a Principal Component Analysis (PCA) on urbanization and bioclimatic variables computed using a circle of 1000m radius around each population. (C-D) Individual and variables plot of a Principal Component Analysis (PCA) on urbanization and bioclimatic variables computed using a circle of 2000m radius around each population. Urban and natural populations mainly differ in the percentage of impervious surfaces, trees and isothermality (BIO3) in both analyses, whereas cities vary according to temperatures and precipitations.

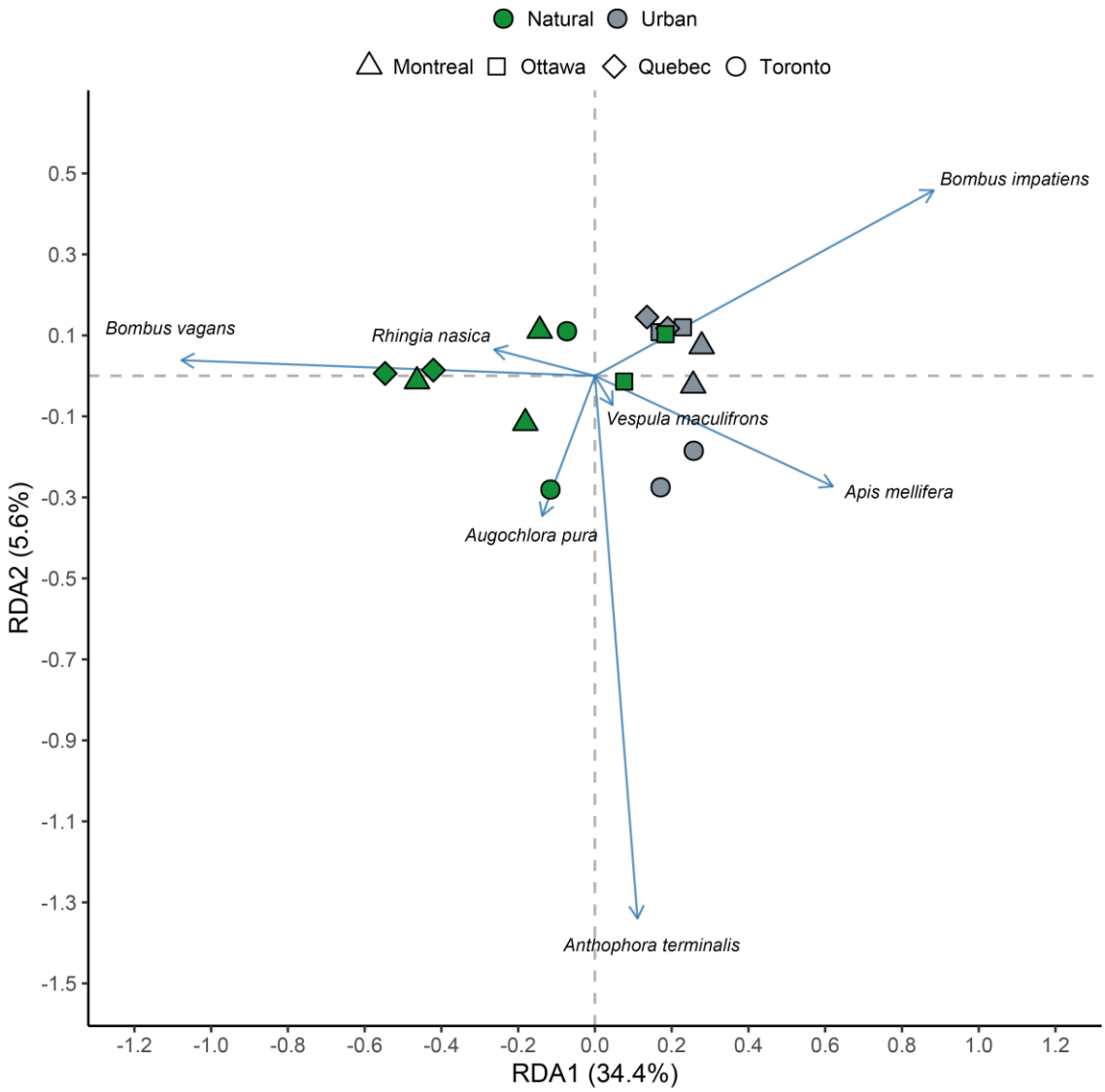


Fig. S2. Redundancy analysis (RDA) evaluating the impact of urbanization on the *Impatiens capensis* pollinator community in 2021 and 2022. When both years are considered together, the main difference between urban and natural habitats is the bumblebee species pollinating *I. capensis*. *Anthophora terminalis* separates the Toronto region because it was overrepresented in this region in 2021.

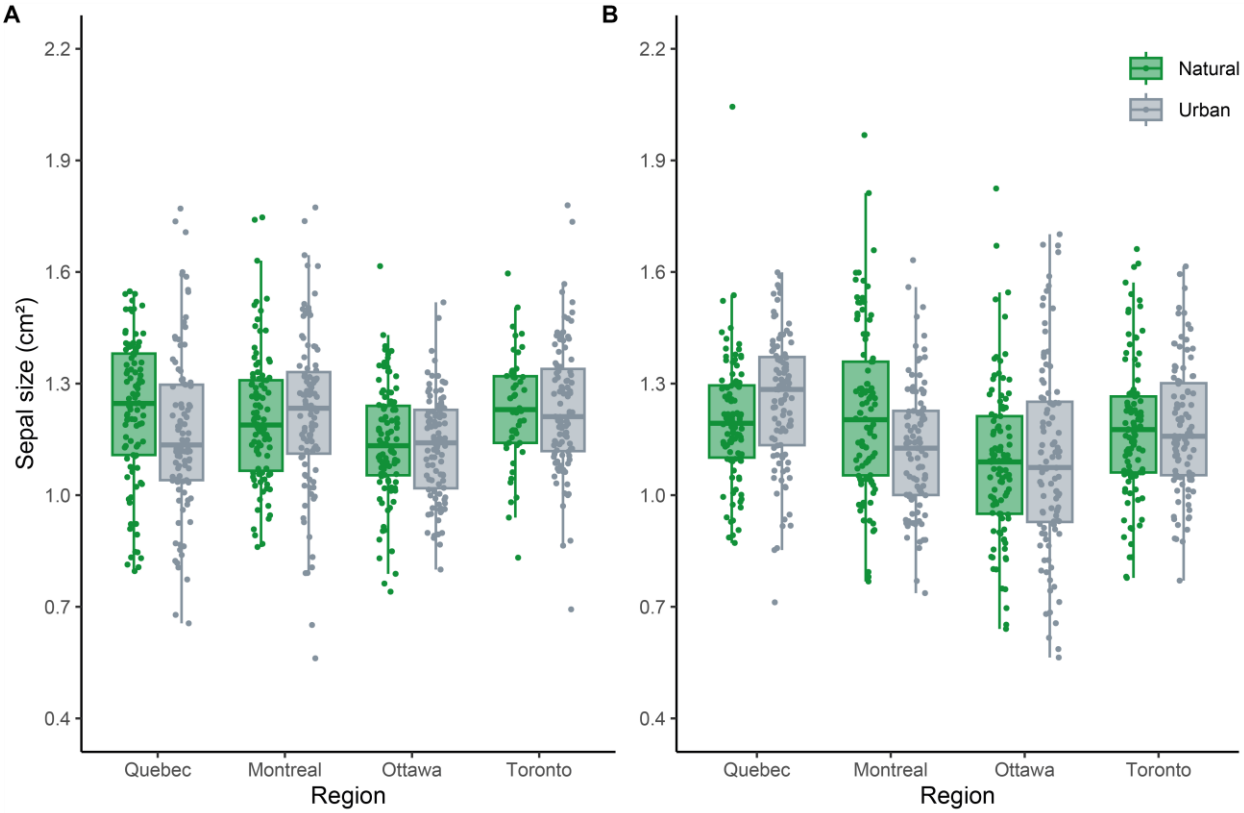


Fig. S3. Sepal size in the field according to region and habitat type for 2021 (A) and 2022 (B). See Table S1 for more information about sampling.

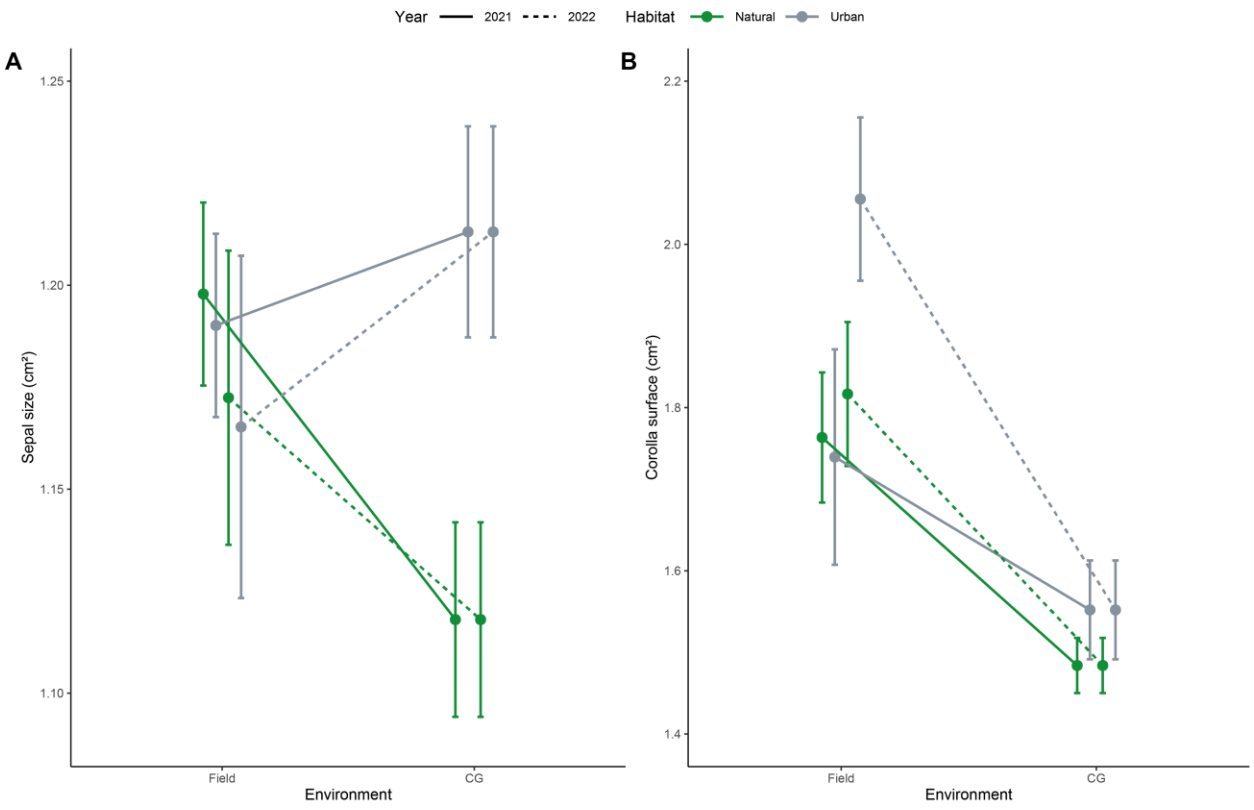
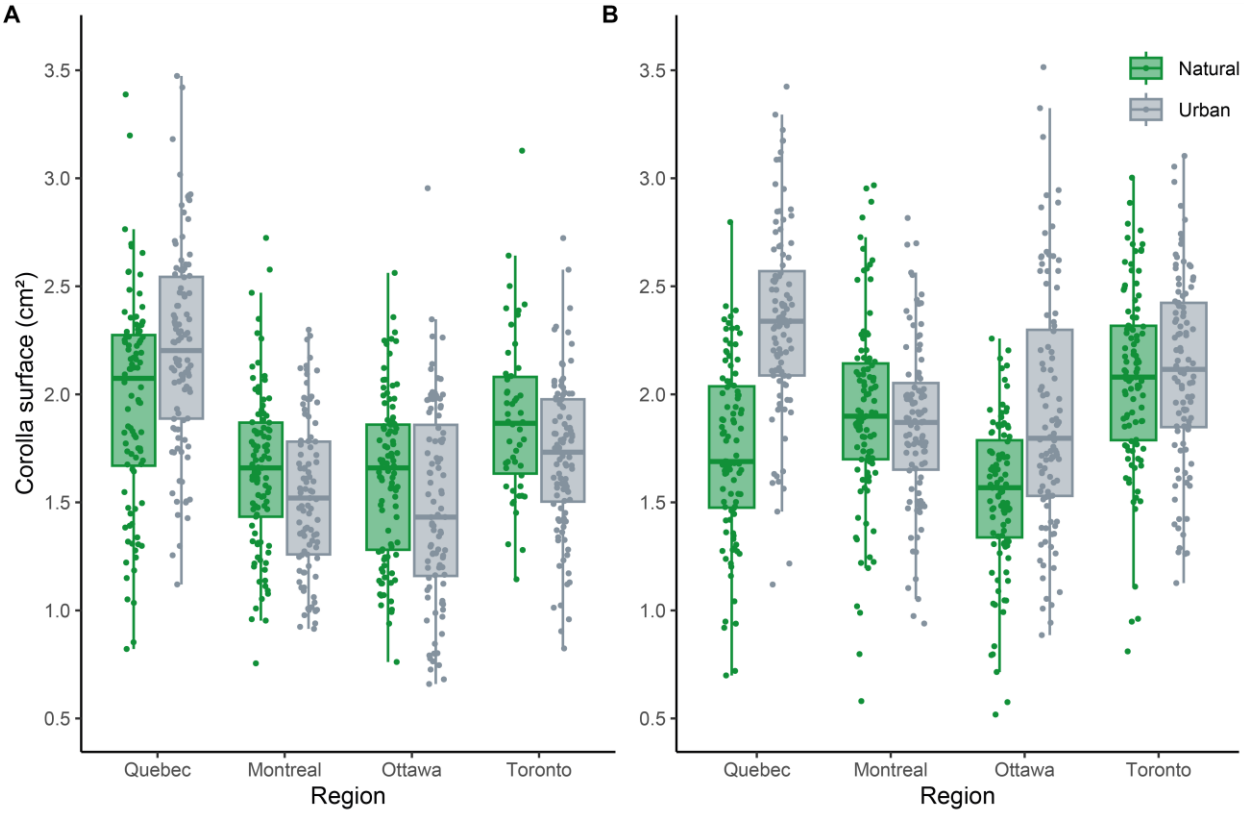


Fig. S4. Mean response of sepal size (A) and corolla surface (B) to environment change, grouped by habitat. Solid lines represent 2021 data in the field compared to the unique common garden experiment, and dashed lines represent 2022 data in the field compared to the unique common garden experiment.



833

834 **Fig. S5. Corolla surface in the field according to region and habitat type for 2021 (A) and**
835 **2022 (B).** See Table S1 for more information about sampling.

836

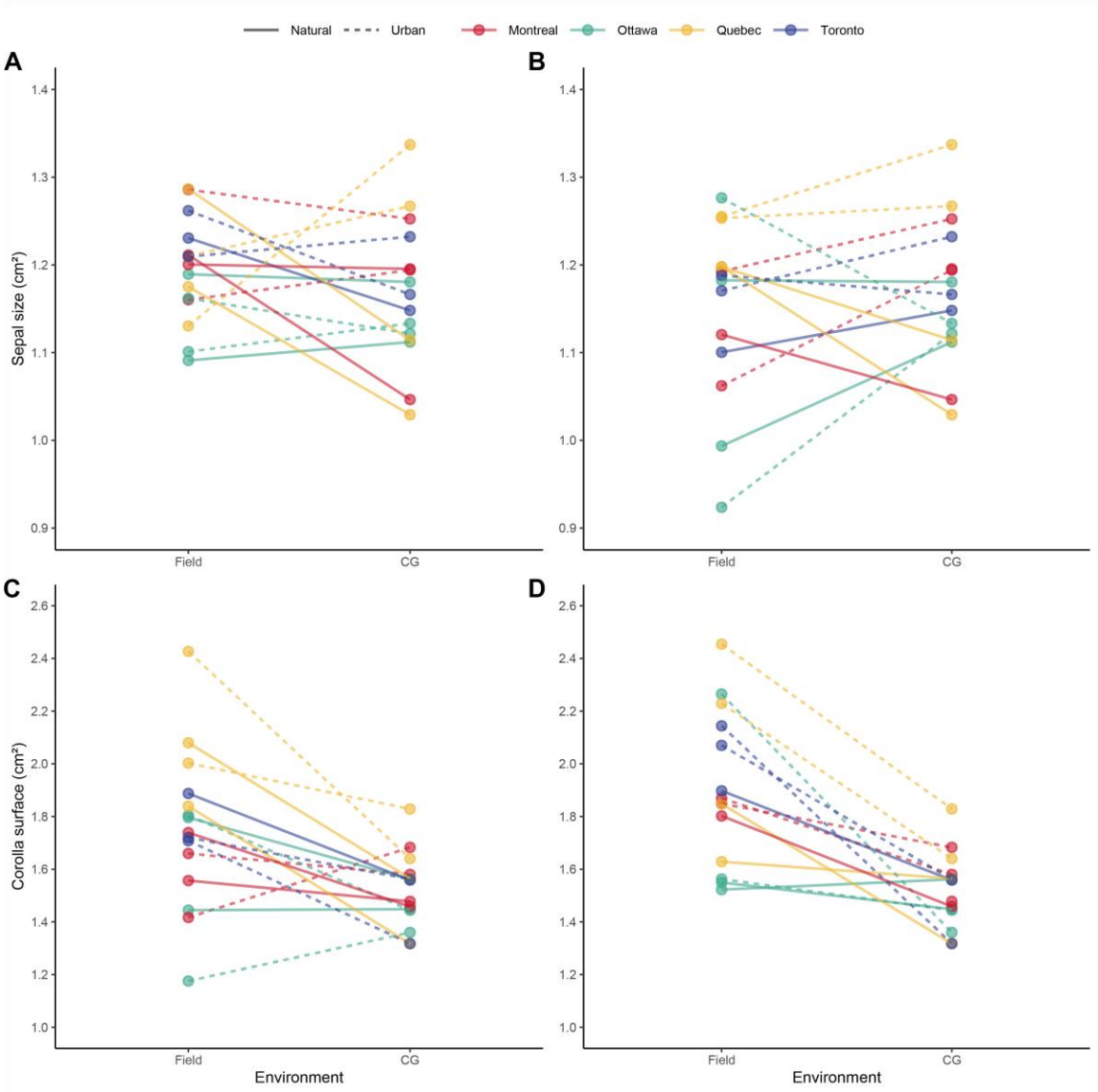


Fig. S6. Plastic response of sepal size and corolla surface, grouped by region. Lines represent populations colored according to their region. Solid lines represent natural populations, and dashed lines represent urban populations. (A) Sepal size response for 2021 field data vs common garden data. (B) Sepal size response for 2022 field data vs common garden data. (C) Corolla surface response for 2021 field data vs common garden data. (D) Corolla surface response for 2022 field data vs common garden data.

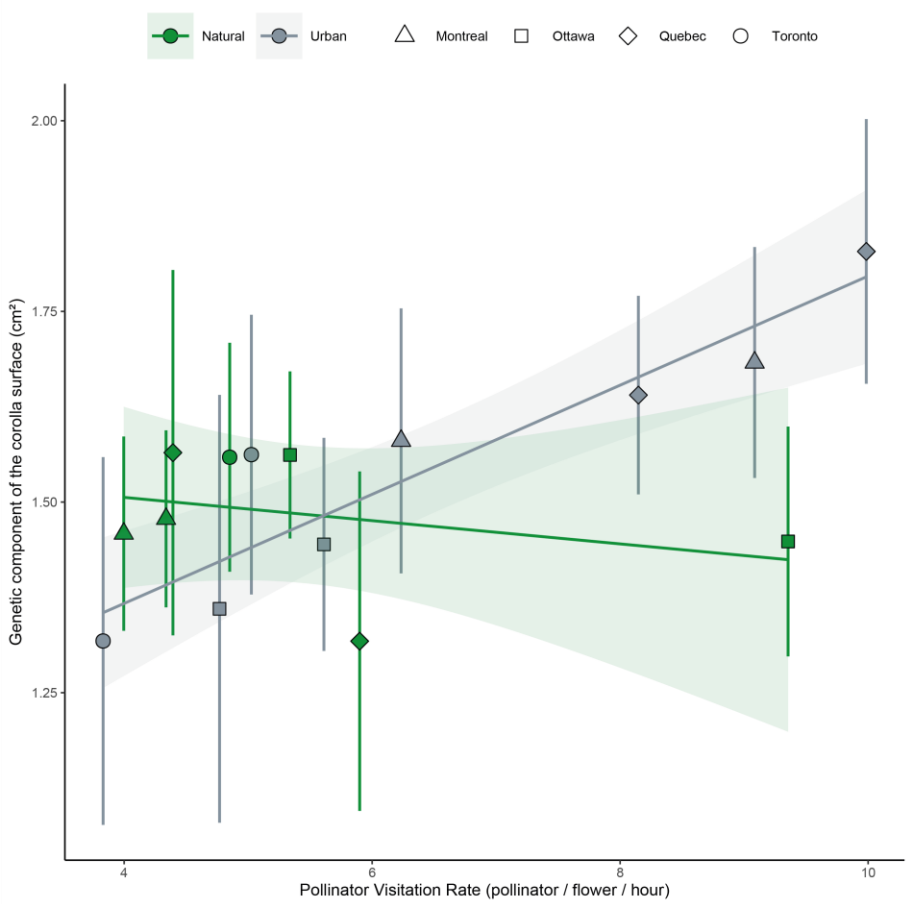


Fig S7. Correlation between the genetic component of the corolla surface and the pollinator visitation rate. There was a significant effect of the pollinator visitation rate on corolla surface, and a significant interaction between pollinator visitation rate and habitat. Regression lines for urban and natural populations are shown, with their standard errors. The points represent the population mean of the corolla surface in the common garden experiment (with 95% confidence intervals of the mean) and the population mean of the pollinator visitation rate averaged across the 2021 and 2022 field seasons.

Supplementary Tables

Table S1. Populations studied. The first number in the sample size column is for the 2021 field season, the second one for the 2022 field season, and the third for the common garden experiment. The first line in the sample size column for each population is for profile pictures (sepal), and the second one is for front pictures (corolla).

Population	Region	Habitat	Latitude	Longitude	Sample size
Bergeron (BER)	Montreal	Urban	45.651	-73.753	50/50/26 44/50/27
Bois Franc (BFC)	Montreal	Urban	45.505	-73.708	49/49/28 49/49/28
Mirabel (MIR)	Montreal	Natural	45.683	-74.213	NA/50/NA NA/50/NA
Oka (OKA)	Montreal	Natural	45.482	-74.035	49/NA/39 48/NA/39
SBL (SBL)	Montreal	Natural	45.974	-74.020	50/50/21 49/49/21
Beaver Dam (DAM)	Ottawa	Natural	45.293	-75.823	50/49/25 50/50/25
Gatineau (GAT)	Ottawa	Natural	45.474	-75.798	50/50/14 50/50/14
Hampton Park (HAM)	Ottawa	Urban	45.391	-75.740	50/50/27 47/50/27
Riverain Park (RIV)	Ottawa	Urban	45.429	-75.670	50/50/13 49/50/13
Maizerets (MAI)	Quebec	Urban	46.838	-71.211	50/50/30 50/50/28
Marais du Nord (MDN)	Quebec	Natural	46.963	-71.388	47/50/17 48/50/17
Saules (SAU)	Quebec	Urban	46.81	-71.316	47/50/25 50/50/25
Savane (SAV)	Quebec	Natural	46.633	-71.287	50/49/12 48/49/12
KSR (KSR)	Toronto	Natural	44.029	-79.528	50/50/24 50/50/24
Silver Creek (SCK)	Toronto	Natural	43.703	-79.964	NA/50/NA NA/50/NA
TCS (TCS)	Toronto	Urban	43.701	-79.328	49/50/9 50/50/9
YCP (YCP)	Toronto	Urban	43.682	-79.383	50/50/22 50/50/22

Table S2. Pollinator relative frequency calculated on collected specimens. The 2022 collection effort was more important (see Material and Methods), and this explains why more specimens were observed. NA are for species not found in either 2021 or 2022. Values are rounded.

Species	Frequency		
	2021 (n = 171 insects)	**2022** (n = 521 insects)	**Cumulated** (n=692 insects)
Agapostemon virescens	NA	0.002	0.001
Ancistrocerus adiabatus	NA	0.002	0.001
Anthophora terminalis	0.070	0.036	0.045
Apis mellifera	0.088	0.056	0.064
Augochlora pura	0.018	0.015	0.016
Bombus borealis	0.012	NA	0.003
Bombus fervidus	0.006	0.002	0.003
Bombus impatiens	0.596	0.633	0.624
Bombus ternarius	0.018	0.002	0.006
Bombus vagans	0.099	0.136	0.127
Calliphoridae	NA	0.004	0.003
Ceratina calcarata	NA	0.002	0.001
Dolichovespula arenaria	0.023	0.012	0.014
Ectemnius continuus	NA	0.004	0.003
Epistrophe sp.	NA	0.002	0.001
Megachile mendica	0.006	0.002	0.003
Megachile relativa	NA	0.002	0.001
Muscidae	NA	0.002	0.001
Platycheirus nearcticus	NA	0.013	0.010
Rhingia nasica	NA	0.019	0.014
Toxomerus geminatus	NA	0.004	0.003
Unknown Syrphidae	NA	0.002	0.001
Unknown Vespula	0.006	NA	0.001
Vespula acadica	0.006	NA	0.001
Vespula alascensis	NA	0.002	0.001
Vespula consobrina	0.041	0.004	0.013
Vespula flavipilosa	NA	0.002	0.001
Vespula germanica	NA	0.004	0.003
Vespula maculifrons	0.012	0.036	0.030

870 **Table S3. Analysis of the pollinator visitation rate variation**

871

A) Analysis of variance table for Pollinator Visitation Rate

	Pollinator Visitation Rate			
Effect	Sum of Squares	df	F	P
Region	18.75	3	1.433	0.258
Habitat	3.77	1	0.865	0.362
Region × Habitat	48.09	3	3.675	**0.026**

B) Estimated marginal means contrasts (Urban - Natural)

Urban - Natural contrast	**estimate**	**t**	**df**	**P**
Quebec	3.92	2.654	23	0.056
Montreal	2.07	1.399	23	0.430
Ottawa	-2.24	-1.515	23	0.430
Toronto	-1.23	-0.769	23	0.449

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Table S4. Sample size and summary of bumblebee sizes. This table summarizes the 2021 and 2022 sampling years combined. n is the sample size for each population, and N is the sample size for each habitat within a region.

Population	Habitat	Region	n	ITD	sd	se	ci	N
MDN	Natural	Quebec	19	3.857	0.402	0.092	0.194	57
SAV	Natural	Quebec	38	3.796	0.324	0.053	0.106	
MAI	Urban	Quebec	63	4.473	0.33	0.042	0.083	128
SAU	Urban	Quebec	65	4.101	0.328	0.041	0.081	
MIR	Natural	Montreal	19	3.828	0.239	0.055	0.115	34
OKA	Natural	Montreal	7	3.821	0.311	0.118	0.288	
SBL	Natural	Montreal	8	3.804	0.333	0.118	0.278	
BER	Urban	Montreal	30	4.071	0.238	0.043	0.089	102
BFC	Urban	Montreal	72	4.07	0.423	0.05	0.099	
DAM	Natural	Ottawa	45	4.043	0.371	0.055	0.112	87
GAT	Natural	Ottawa	42	3.953	0.368	0.057	0.115	
HAM	Urban	Ottawa	16	4.178	0.345	0.086	0.184	49
RIV	Urban	Ottawa	33	3.782	0.369	0.064	0.131	
KSR	Natural	Toronto	6	3.994	0.356	0.145	0.374	42
SCK	Natural	Toronto	36	4.331	0.382	0.064	0.129	
TCS	Urban	Toronto	8	4.164	0.387	0.137	0.323	29
YCP	Urban	Toronto	21	3.858	0.45	0.098	0.205	

880 **Table S5. Analysis of the bumblebee size variation.**

881

A) Wald χ^2 test

	Inter-Tegular Distance (ITD, mm)		
Effect	**χ^2**	**df**	**P**
Year	0.109	1	0.74
Region	2.075	3	0.556
Habitat	2.414	1	0.12
Year × Region	1.989	3	0.574
Year × Habitat	0.302	1	0.582
Region × Habitat	8.826	3	**0.031**
Year × Region × Habitat	1.494	3	0.683

B) Estimated marginal means contrasts (Urban - Natural)

	Inter-Tegular Distance (ITD, mm)			
Urban - Natural contrast	**estimate**	**t**	**df**	**P**
Quebec	0.45	2.599	7.21	0.138
Montreal	0.264	1.584	9	0.443
Ottawa	-0.056	-0.324	7.45	0.754
Toronto	-0.279	-1.141	28.29	0.526

882
883 We tested whether bumblebee inter-tegular distance (ITD) was explained by Year (2021 or 2022), Region (Quebec, Montreal, Ottawa, or Toronto),
884 Habitat (Urban or Natural), and all interactions between these factors, using linear mixed models. **A)** Result of the Wald chi-square test **B)** We
885 estimated the effect of habitat on inter-tegular distance for each region using post-hoc tests comparing estimated marginal means contrasts. We
886 corrected for multiple comparisons using the holm correction.
887

Table S6. Analyses of the sepal size and corolla surface variation in the field.

A) Wald χ2 test, male only

	Sepal Size			Corolla Surface		
Effect	χ^2	**df**	**P**	χ^2	**df**	**P**
Year	8.385	1	**0.003**	33.597	1	**<0.001**
Region	3.834	3	0.279	37.237	3	**<0.001**
Habitat	0.344	1	0.557	2.191	1	0.138
Year × Region	28.021	3	**<0.001**	25.117	3	**<0.001**
Year × Habitat	8.146	1	**0.004**	54.977	1	**<0.001**
Region × Habitat	0.302	3	0.959	16.120	3	**0.001**
Year × Region × Habitat	6.980	3	0.072	10.690	3	**0.013**

B) Estimated marginal means contrasts (Urban - Natural), male only

	Sepal Size				Corolla surface			
Urban - Natural contrast	**estimate**	**t**	**df**	**P**	**estimate**	**t**	**df**	**P**
Quebec 2021	-0.060	-0.646	9.36	1.000	0.293	2.363	10.3	0.234
Quebec 2022	0.067	0.719	9.43	1.000	0.623	4.992	10.6	**0.004**
Montreal 2021	-0.059	-0.678	10.08	1.000	-0.167	-1.386	12.2	0.572
Montreal 2022	-0.068	-0.781	10.15	1.000	-0.007	-0.056	12.4	1.000
Ottawa 2021	-0.027	-0.291	9.45	1.000	-0.223	-1.776	10.8	0.416
Ottawa 2022	-0.008	-0.080	9.48	1.000	0.364	2.905	10.7	0.103
Toronto 2021	-0.081	-0.826	11.39	1.000	-0.271	-1.905	16.2	0.374
Toronto 2022	0.012	0.130	9.66	1.000	0.018	0.140	11.4	1.000

C) Wald χ2 test, male and female

	Sepal Size			Corolla Surface		
Effect	χ^2	**df**	**P**	χ^2	**df**	**P**
Year	17.529	1	**<0.001**	45.646	1	**<0.001**
Region	3.720	3	0.293	33.065	3	**<0.001**
Habitat	0.239	1	0.624	2.279	1	0.131
Year × Region	24.444	3	**<0.001**	25.008	3	**<0.001**
Year × Habitat	6.151	1	**0.013**	63.618	1	**<0.001**
Region × Habitat	0.302	3	0.959	14.239	3	**0.002**
Year × Region × Habitat	6.806	3	0.078	6.638	3	0.084

D) Estimated marginal means contrasts (Urban - Natural), male and female

	Sepal Size				Corolla surface			
Urban - Natural contrast	**estimate**	**t**	**df**	**P**	**estimate**	**t**	**df**	**P**
Quebec 2021	-0.060	-0.672	9.48	1.000	0.255	2.005	10.4	0.358
Quebec 2022	0.057	0.630	9.44	1.000	0.604	4.756	10.4	**0.006**
Montreal 2021	-0.044	-0.527	10.14	1.000	-0.183	-1.501	12.1	0.636
Montreal 2022	-0.066	-0.784	10.14	1.000	0.003	0.021	11.9	1.000
Ottawa 2021	-0.009	-0.097	9.43	1.000	-0.137	-1.078	10.4	0.916
Ottawa 2022	0.012	0.131	9.44	1.000	0.378	2.975	10.3	0.094
Toronto 2021	-0.068	-0.728	10.92	1.000	-0.311	-2.234	14	0.254
Toronto 2022	-0.001	-0.009	9.43	1.000	0.034	0.266	10.3	1.000

We tested whether sepal size or corolla surface was explained by Year (2021 or 2022), Region (Quebec, Montreal, Ottawa, or Toronto), Habitat (Urban or Natural), and all interactions between these factors using linear mixed models. Because the Sex factor was associated with sepal size and corolla surface in the field, we decided to run the same models with males only **(A-B)** and male and female **(C-D)** to assess the effect of females on our results**.** For each test (either male only or male and female combined), we estimated the effect of habitat on the sepal size and corolla surface for each region using post-hoc tests comparing estimated marginal means contrasts. We corrected for multiple comparisons using holm correction.

897 **Table S7. Analyses of the sepal size and corolla surface variation in the common garden**
898 **experiment.**

A) Wald χ^2 test

	Sepal size			Corolla surface		
Effect	χ^2	**df**	**P**	χ^2	**df**	**P**
Region	2.270	3	0.084	5.113	3	0.163
Habitat	8.564	1	**0.003**	2.683	1	0.101
Region × Habitat	8.329	3	**0.039**	8.136	3	**0.043**

B) Estimated marginal means contrasts (Urban - Natural)

	Sepal Size				Corolla surface			
Urban - Natural contrast	**estimate**	**t**	**df**	**P**	**estimate**	**t**	**df**	**P**
Quebec	0.227	3.700	7.29	**0.028**	0.281	2.617	7.78	0.126
Montreal	0.095	1.660	5.42	0.459	0.162	1.709	4.61	0.459
Ottawa	-0.015	-0.249	7.13	1	-0.082	-0.762	7.02	0.942
Toronto	0.043	0.585	6.91	1	-0.081	-0.623	6.84	0.942

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900
901 We tested whether the sepal size or corolla surface was explained by Region (Quebec, Montreal, Ottawa, or Toronto), Habitat (Urban or Natural),
902 and the interaction between these factors, using linear mixed models. **A)** Result of the Wald chi-square test **B)** We estimated the effect of habitat
903 on the sepal size and corolla surface for each region using post-hoc tests comparing estimated marginal means contrasts. We corrected for multiple
904 comparisons using holm correction.
905
906

907 **Table S8. Analysis of the mean responses of sepal size and corolla surface, grouped by**
908 **habitats, to environment change.**

909

	Sepal Size						Corolla size					
	2021			2022			2021			2022		
Effect	χ^2	**df**	**P**	χ^2	**df**	**P**	χ^2	**df**	**P**	χ^2	**df**	**P**
Environment	0.916	1	0.338	0.047	1	0.837	8.859	1	**0.002**	36.761	1	**<0.001**
Habitat	2.003	1	0.156	4.052	1	**0.044**	1.445	1	0.229	2.492	1	0.114
Environment × Habitat	4.796	1	**0.028**	3.706	1	0.054	0.519	1	0.471	1.486	1	0.222

910
911 We tested whether the sepal size or corolla surface was explained by Environment (Field or Common Garden), Habitat (Urban or Natural), and the
912 interaction between these factors, using linear mixed models. The comparison between the common garden and the 2021 field season represents
913 the effect of genetic and plastic changes on the two components of flower size (in the common garden, we measure the offspring of seeds collected
914 in the field in 2021). In 2022, we compared the same generation to one another (assessing the plasticity more than the genetic changes). Therefore,
915 we ran separate models for 2021 and 2022.

Table S9. Analysis of the relationship between the genetic component of sepal size variation and bumblebee inter-tegular distances

	Sepal size			
Effect	Sum of Squares	df	F	P
Habitat	0.006	1	1.742	0.213
ITD	0.022	1	6.531	**0.026**
Habitat × ITD	0.000005	1	0.001	0.971

921 **Table S10. Analysis of the relationship between the genetic component of corolla surface**
922 **variation and the visitation rate of pollinators**
923

	Corolla surface			
Effect	Sum of Squares	df	F	P
Habitat	0.001	1	0.288	0.601
Pollinator Visitation Rate	0.085	1	13.2	**0.003**
Habitat × Pollinator Visitation Rate	0.096	1	14.851	**0.002**

924
925

Table S11. Analysis of the flower shape variation in the common garden experiment and in the field.

A) Procrustes ANOVA on flower shape, excluding the "environment" factor.

		Factors	Df	MS	Rsq	F	P
Sepal	**Common garden**	Size	1	0.48813	0.03662	5.7409	**0.002**
		Sex	1	0.09369	0.00703	1.1019	0.350
		Region	3	0.10132	0.02280	1.1916	0.350
		Habitat	1	0.07298	0.00547	0.8583	0.502
		Region×Habitat	3	0.08595	0.01934	1.0108	0.454
		Habitat×Population	7	0.08503	0.04465	1.0000	1.000
	Field	Size	1	3.01023	0.04240	6.6537	**0.007**
		Year	1	0.17214	0.00242	0.3805	0.854
		Sex	1	0.27642	0.00389	0.6110	0.695
		Region	3	1.74981	0.07394	3.8677	**0.005**
		Habitat	1	1.69706	0.02390	3.7511	**0.019**
		Region×Habitat	3	0.56702	0.02396	1.2533	0.337
		Habitat×Population	9	0.45242	0.05735	1.0000	1.000
Corolla	**Common garden**	Size	1	1.37494	0.10318	13.5472	**0.001**
		Sex	1	0.49330	0.03702	4.8604	**0.007**
		Region	3	0.03944	0.00888	0.3886	0.980
		Habitat	1	0.04430	0.00332	0.4365	0.837
		Region×Habitat	3	0.04251	0.00957	0.4189	0.972
		Habitat×Population	7	0.10149	0.05331	1.0000	1.000
	Field	Size	1	2.07279	0.04042	8.8874	**0.002**
		Year	1	0.42192	0.00823	1.8090	0.154
		Sex	1	0.52744	0.01029	2.2615	0.104
		Region	3	0.55914	0.03271	2.3974	**0.036**
		Habitat	1	0.20683	0.00403	0.8868	0.472
		Region×Habitat	3	0.38019	0.02224	1.6301	0.156
		Habitat×Population	10	0.23323	0.04548	1.0000	1.000

B) Procrusts ANOVA on corolla surface only, including the "environment" factor

Factors	Df	MS	Rsq	F	P
Size	1	4.0716	0.0605	17.4631	**0.001**
Year	1	0.8022	0.01192	3.4406	0.043
Sex	1	0.6576	0.00977	2.8204	0.059
Region	3	0.5287	0.02357	2.2674	0.061
Habitat	1	0.3917	0.00582	1.6800	0.162
Environment	1	1.4451	0.02147	6.1981	**0.002**
Region×Habitat	3	0.2377	0.0106	1.0194	0.424
Habitat×Population	9	0.2332	0.03118	1	1

A) We used Procrustes ANOVA (PERMANOVA) to assess the effect of habitat on flower shape. We constructed a model with Size, Year, Sex, Region, Habitat, and the interactions between these factors as explanatory variables of sepal or corolla shape. We omitted the Year term for the common garden as we ran the experiment once. We compute results using type I Procrustes ANOVA, meaning that the effect of Habitat is assessed after all other factors. We also added population nested in habitat in the model; this way, we were able to define the error of the ANOVA as the mean square of population nested in habitats instead of residuals, therefore considering populations as the statistical individual. This conservative approach offers us more confidence in detecting differences between habitats. **B)** We assessed the effect of the environment factor (Field vs Common Garden) on corolla shape using the same rationale as in **A)**

Tables S12-S22 are provided in supplementary file Table_S12_S22.xlsx

Table S12. The effect of habitat, region, year and all interactions on bumblebee inter-tegular distance. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S13. The effect of habitat, region and all interactions on sepal size in the common garden experiment. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S14. The effect of habitat, region, year and their interaction on sepal size in the field, for male only flowers. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S15. The effect of habitat, region, year and interactions on sepal size in the field, for male and female flowers. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S16. The effect of environment, urbanization and their interaction on sepal size in 2021. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S17. The effect of environment, urbanization and their interaction on sepal size in 2022. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S18. The effect of habitat, region and all interactions on corolla surface in the common garden experiment. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S19. The effect of habitat, region, year and their interaction on corolla surface in the field, for male only flowers. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S20. The effect of habitat, region, year and interactions on corolla surface in the field, for male and female flowers. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S21. The effect of environment, urbanization and their interaction on corolla surface in 2021. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.

Table S22. The effect of environment, urbanization and their interaction on corolla surface in 2022. We show the estimate, standard error and t value for each factor level and interaction, compared to the intercept.