RESEARCH ARTICLE

Botany
American Journal of

Pollen limitation of native plant reproduction in an urban landscape

Adrian L. Carper[1] | Paige S. Warren[2] | Lynn S. Adler[3] | Rebecca E. Irwin[1]

[1]Department of Biological Sciences, Dartmouth College, 78 College St, Hanover, New Hampshire 03755, USA

[2]Department of Environmental Conservation, University of Massachusetts, Amherst, Massachusetts 01003, USA

[3]Department of Biology, University of Massachusetts, Amherst, MA 01003, USA

Correspondence
Rebecca E. Irwin, Department of Applied Ecology, North Carolina State University, Raleigh, North Carolina 27695, USA.
Email: reirwin@ncsu.edu

Present address
Adrian L. Carper, Department of Ecology and Evolutionary Biology, University of Colorado, Campus Box 334, Boulder, Colorado 80309-0334, USA.

Rebecca E. Irwin, Department of Applied Ecology, North Carolina State University, Raleigh, North Carolina 27695, USA.

Abstract

Premise: Evidence suggests that bees may benefit from moderate levels of human development. However, the effects of human development on pollination and reproduction of bee-pollinated plants are less-well understood. Studies have measured natural variation in pollination and plant reproduction as a function of urbanization, but few have experimentally measured the magnitude of pollen limitation in urban vs. non-urban sites. Doing so is important to unambiguously link changes in pollination to plant reproduction. Previous work in the Southeastern United States found that urban sites supported twice the abundance of bees compared to non-urban sites. We tested the hypothesis that greater bee abundance in some of the same urban sites translates into reduced pollen limitation compared to non-urban sites.

Methods: We manipulated pollination to three native, wild-growing, bee-pollinated plants: *Gelsemium sempervirens*, *Oenothera fruticosa*, and *Campsis radicans*. Using supplemental pollinations, we tested for pollen limitation of three components of female reproduction in paired urban and non-urban sites. We also measured pollen receipt as a proxy for pollinator visitation.

Results: We found that all three plant species were pollen-limited for some measures of female reproduction. However, opposite to our original hypothesis, two of the three species were more pollen-limited in urban relative to non-urban sites. We found that open-pollinated flowers in urban sites received less conspecific and more heterospecific pollen on average than those in non-urban sites.

Conclusions: These results suggest that even when urban sites have more abundant pollinators, this may not alleviate pollen limitation of native plant reproduction in urban landscapes.

KEYWORDS

Campsis radicans, *Gelsemium sempervirens*, heterospecific pollen, *Oenothera fruticosa*, pollen limitation, pollen receipt, pollination, urban

Humans have drastically altered the landscape to produce food, fuel, and housing. Changes in land use have led to declines in native biodiversity through several mechanisms, including habitat loss and fragmentation, invasion of non-native species, and disruption of population and ecosystem processes (e.g., Gaston et al., 2003). How land-use change will affect the performance of remaining native species, and the mechanisms involved, is less well-studied. For example,

land-use change could have indirect effects on native species persisting in human-dominated environments through changes in species interactions (Shochat et al., 2010). The goal of this study was to test how one form of land-use change, urbanization, affected native plant reproduction through changes in pollination.

Pollination is a ubiquitous species interaction of both ecological and economic importance, given that the

majority of flowering plants, including many agricultural crops, are dependent on animal vectors for pollination (Ollerton et al., 2011). However, pollination is susceptible to ecological perturbation (Knight et al., 2005), and variation in the abundance and diversity of pollinator communities can affect pollen limitation of plant reproduction (Steffan-Dewenter and Westphal, 2008). Land-use change has been implicated in the widespread decline of some native pollinators (e.g., Biesmeijer et al., 2006; Winfree et al., 2009; Burkle et al., 2013), raising concern over the functional link between pollinator loss and the loss of pollination activity (Brosi and Briggs, 2013). However, studies have shown that urban and suburban areas can sustain abundant and diverse pollinator communities, especially bees (reviewed in Hall et al., 2017). For example, in North Carolina, USA, native bees were consistently more abundant and as diverse in urban and suburban compared to non-urban sites across two years, likely due to more abundant and diverse floral resources (Carper et al., 2014). In Britain across four major cities, urban allotments and gardens exhibited similar or higher bee abundance along with more floral resources than nature reserves (Baldock et al., 2019). In Central Europe across nine major cities, urban sites had higher bee species richness and flower visitation rates than non-urban sites, which was attributed to the greater edge density of urban green spaces (Theodorou et al., 2020). These patterns are not universal, however (e.g., Hostetler and McIntyre, 2001; Harrison et al., 2019). The factors that drive variation in pollinator communities are not always known but likely relate to the magnitude of urbanization or length of the urban gradient, the type of non-urban land-use that urban sites are compared to, and fine-scale variation in other biotic and abiotic factors, such as floral resource and nesting site availability, temperature, humidity, water availability, and air quality (reviewed in Irwin et al., 2020).

A key question is whether changes in pollinator abundance translate into changes in pollination and reproduction of native plants in urban landscapes. Several mechanisms could affect the degree to which pollinator abundance in urban vs. non-urban areas translates into changes in pollination to native flowering plants. Increased pollinator abundance could increase pollination through greater pollinator visitation, leading to higher pollen deposition (Engel and Irwin, 2003) and seed set in pollen-limited species. Pollen-limited species are those that receive inadequate quantity (or quality) of pollen, resulting in reduced plant reproduction (Ashman et al., 2004). Alternatively, differences in competition vs. facilitation for pollination in urban vs. non-urban sites could alter the links between pollinators, pollination, and seed set. For example, the presence of diverse and highly attractive flowering species in urban areas could facilitate pollination to co-occurring native species (Ghazoul, 2006). Conversely, more diverse or abundant floral resources in urban areas could increase competition for pollinators for native flowering species (Hennig and

Ghazoul, 2011), or increase heterospecific pollen transfer, which could reduce fruit and seed set (Morales and Traveset, 2008). Finally, even if pollinator abundance differs between urban and non-urban areas, if plants are not pollen-limited for seed set, differences in pollinator communities may have no effect on fruit or seed set. A growing number of studies have measured natural variation in pollination and reproduction of plants across urbanization gradients (e.g., Rivkin et al., 2020; Theodorou et al., 2020). However, fewer studies have experimentally measured the magnitude of pollen limitation of the same plant species in urban vs. non-urban sites, which is necessary to unambiguously link changes in pollination to plant reproduction. An impressive global meta-analysis of pollen limitation revealed that pollinator-reliant plants were more pollen-limited in urban compared to non-urban habitats, but this analysis did not explicitly consider studies of the same plant species in urban vs. non-urban sites (Bennett et al., 2020). Studies which assessed pollen limitation of the same plant species in urban and non-urban sites reveal conflicting results, finding that plants in urban sites can be more, less, or have similar magnitudes of pollen limitation compared to those in non-urban ones (e.g., Parker, 1997; Verboven et al., 2012; Minnefors, 2016; Barker and Sargent, 2020). These variable results suggest that more pollen-limitation studies are needed, especially using multiple co-occurring flowering species, to assess general patterns in urban relative to non-urban habitats.

The goal of this study was to compare pollen limitation of three native, wild-growing plant species in urban and non-urban sites. We estimated and experimentally manipulated pollination and measured subsequent reproduction (fruit and seed set) in wild-growing plant species across multiple paired urban and non-urban sites. We conducted this study in Raleigh/Durham, North Carolina, USA in 2011; in 2008 and 2009 at many of the same sites, we found that urban sites exhibited nearly twice the abundance of bees relative to nearby non-urban ones (Carper et al., 2014). If increased bee abundance translates into greater pollinator visitation, urban areas could play an important role in conserving pollination mutualisms for native, wild-growing plants. Specifically, we asked: (1) To what degree does pollen-limitation of female plant reproduction vary between urban and non-urban sites? We predicted that plants growing in urban sites would exhibit less pollen limitation of fruit and seed set compared to plants growing in non-urban sites, assuming that higher bee abundance in urban sites translates into greater pollination success; and (2) How does conspecific and heterospecific pollen receipt by flowers compare between urban and non-urban sites? Pollen deposition can provide an index of pollinator visitation (Engel and Irwin, 2003) and insight into pollinator behavior. By manipulating and measuring pollination, this research extends previous studies of bee pollinator abundance to understand pollination and reproduction of native plants in urban landscapes.

MATERIALS AND METHODS

Study system

Study area

Wake, Durham, and Chatham counties, North Carolina (NC), USA contain the Raleigh-Durham metropolitan area (hereafter RDU). This metropolitan area increased its land area by two-thirds in the 1990s alone, and simulations over the next 50 years project a doubling to tripling of land for urban and suburban growth in this region (Terando et al., 2014). The dominant forest type is mixed-pine woodland, although urban expansion has resulted in an extensive matrix of forests within urban development and publicly protected forested land that provided tractable, replicated study sites.

To determine how urban areas affected pollination, we used paired, naturally forested non-urban and urban sites (Appendix S1, Table S1). Non-urban sites consisted of contiguous forests >10 acres, whenever possible, within protected natural areas such as state and local parks. Corresponding urban sites were located within single-family residential communities with persistent, undeveloped, forested patches. Urban sites were surrounded by residential land (i.e., multi-family and single-family housing development) that was no more than 300 m away from our focal plants. This threshold was selected because many pollinators, such as native bees, have foraging radii within this range (Greenleaf et al., 2007), although we recognize that pollinators can vary greatly in foraging distances. Urban sites had greater than 10 times more impervious surface cover than non-urban sites (Appendix S1, Figures S1, S2). Human population density in urban sites often reached >186 people per km^2 based on the U.S, Bureau of the Census. By some definitions, this level of development is referred to as "urban" (Pickett et al., 2001), which is the terminology we use here (and see Irwin et al., 2018). Residential communities within urban sites contained a range of housing values, styles, and ages, but most were at or above the median housing value for the area (Appendix S1, Table S1). Because of this limited variation in socioeconomic status among urban sites, we did not consider how socioeconomic factors affected pollen limitation. Within each site pair, sites were on average 3.145 km apart (range: 1.518–5.667 km apart) to reduce differences in environmental factors other than urbanization. Among pairs, we ensured that pairs were distinct by choosing them >5 km apart whenever possible (Appendix S1, Table S1, Figure S1). Urban and non-urban site pairs in this study did not differ in abiotic factors, including temperature, relative humidity, light availability, and soil nutrients (Appendix S2; Irwin et al., 2018). Previous work across two years (2008–2009) in some of the same urban and non-urban sites demonstrated that urban forests in this region harbored twice the abundance of bees compared to non-urban forests, with similar rarefied species richness and composition (Carper et al., 2014), and urban warming reduced bee abundance while bee species richness increased with urban flower density (Hamblin et al., 2018).

Focal plant species

We studied three wild-growing, focal plant species that occur in urban and non-urban sites, are self-incompatible and bloom at distinct times during the growing season. *Gelsemium sempervirens* (L.) J.St.-Hil. (Loganiaceae; hereafter *Gelsemium*) is a native perennial, evergreen vine that occurs in forests across the southeastern USA. Plants bloom for approx. 6 weeks in March and April. *Gelsemium* is distylous, with tubular, yellow flowers of two distinct floral morphs: (1) pin plants (long styles and short stamens); and (2) thrum plants (short styles and long stamens; Ornduff, 1970, 1979, 1980); distyly is a trait presumed to promote disassortative pollen transfer. *Gelsemium* is an obligate outcrosser, dependent on the opposite morph for fertilization, and is primarily pollinated by bumble bees (*Bombus bimaculatus* Cresson; Apidae), honey bees (*Apis mellifera* L.; Apidae), blue orchard bees (*Osmia lignaria* Say; Megachilidae) and blueberry bees (*Habropoda laboriosa* Fabricius; Apidae) (Adler and Irwin, 2005). *Gelsemium* growing in non-urban forests in central Georgia, USA, are pollen-limited for female plant reproduction (Adler and Irwin, 2012). In NC, USA *Gelsemium* in non-urban sites range from having higher to lower conspecific and heterospecific pollen receipt relative to *Gelsemium* in urban sites (Irwin et al., 2014, 2018).

Oenothera fruticosa L. (Onagraceae, hereafter *Oenothera*) is a perennial herb native to eastern North America and commonly found near forest edges, in meadows, and along roadsides. In NC, it flowers in June and is primarily pollinated by honey bees and soldier beetles (Cantharidae) (Primack and Silander, 1975). Flowers are self-incompatible and open for a single day before senescing. Seed set is often low in natural areas of NC in spite of adequate pollen receipt, presumably due to the transfer of incompatible pollen (Silander and Primack, 1978).

Campsis radicans Seem. (Bignoniaceae, hereafter *Campsis*) is a woody vine endemic to the southeastern US which flowers along forest edges and roadsides from June to August. *Campsis* flowers are primarily self-incompatible, but self-pollen can fertilize ovules on occasion when outcrossed pollen is also present (Bertin and Sullivan, 1988). Hummingbirds are the most effective pollinators of *Campsis*; however, honeybees and bumble bees also visit and pollinate *Campsis*, resulting in fruit and seed set (Bertin, 1982a, b), and visitation by solitary bee species also occurs in NC (A. Carper, *personal observation*). *Campsis* plants in Illinois are pollen-limited for reproduction (Bertin, 1982a).

Field methods

(1) To what degree does pollen-limitation of female plant reproduction vary between urban and non-urban sites?
We conducted hand-pollination experiments in the spring and summer of 2011. We manipulated pollination in eight urban/non-urban site pairs for *Gelsemium*, four site pairs for *Oenothera*, and three site pairs for *Campsis* (Appendix S1,

Table S1). Although plant reproduction can be a function of an interaction between resource availability and pollen limitation (e.g., Campbell and Halama, 1993), testing for such an interaction across multiple replicated urban and non-urban sites and in multiple plant species was beyond the scope of this study but could be assessed in future research. For each species, we haphazardly chose up to 10 plants per site and manipulated pollination across their respective blooming seasons (*Gelsemium* in April, *Oenothera* in June, and *Campsis* in July).

To compare pollen limitation of plant reproduction, we supplemented pollen receipt by individual flowers on plants in each site. On each plant, we haphazardly selected up to 10 pairs of flowers and assigned one member of each pair to receive either supplemental hand-pollination (treatment) or open pollination (control). We paired flowers within plants because *Gelsemium* and *Campsis* can produce up to several hundred flowers per plant, making plant-level manipulations infeasible, and we kept the paired approach for *Oenothera* to standardize methodology across species. We paired flowers as close to each other as possible to control for phenological stage and to account for variation among branches. Plants that did not have 10 pairs of flowers when we first visited were revisited 2 to 3 days later when new flowers had opened. Studies manipulating pollination on fractions or parts of plants can inflate the magnitude of pollen limitation compared to studies manipulating whole plants, although general patterns of pollen limitation between the two methods are similar (Knight et al., 2005). Because we standardized manipulations across all sites, any potential inflation of pollen limitation should be consistent across sites and should not affect differences detected between site types. We hand-pollinated flowers using camel hair brushes to deposit supplemental pollen directly onto stigmas. Supplemental pollen was collected from haphazardly chosen non-experimental plants in close proximity to study plants and combined from at least three donors (of the opposite morph for *Gelsemium*) to ensure adequate out-crossing. Open-pollinated flowers received no supplemental pollen but were left open to natural pollinator visitation and were handled in a similar manner as hand-pollinated flowers. All flowers were labeled with small pieces of white tape placed on the petiole or branch below the flower for identification during fruit collection. In total across sites, we hand-pollinated 1522 pairs of flowers of *Gelsemium* (10 plants per site), 170 pairs of flowers of *Oenothera* (10–12 plants per site), and 200 pairs of flowers of *Campsis* (9–11 plants per site), for a total of 3784 experimental flowers. All flowers were also exposed to natural pollination.

We collected fruit from all hand-pollinated and open-pollinated flowers after fruits had fully expanded but before fruits dehisced. Fruits were dissected, and we counted the number of expanded seeds and aborted ovules using a dissecting microscope. The term 'fruit' refers only to seed-bearing fruits hereafter. We measured three estimates of female reproduction: (1) proportion fruit set (total fruits/total treated flowers); (2) mean seed set per fruit; and (3) mean proportion seed set (total seeds/total ovules per fruit).

Proportion fruit set can provide an index of pollinator visitation in self-incompatible species, since unvisited flowers do not produce fruit. Seed set per fruit and proportion seed set per fruit provide an assessment of the ability of the plant to utilize the pollen deposited on stigmas (Waser and Price, 1989) and allow inference about the quality of pollen deposited. Finally, while proportional estimates of plant reproduction (proportion fruit set and proportion seed set) provide comparison of reproductive measures as a function of pollen supplementation, we used seed set per fruit as an assessment of the plant reproductive consequences of pollen supplementation and site type. Assessing the demographic consequences of differences in seed set were beyond the scope of this study.

Statistical Analyses. To test for pollen limitation of plant reproduction, we fit separate generalized linear mixed models (GLMM) for each plant species using site type (urban/non-urban) and treatment (hand-/open-pollinated) as fixed factors. We included site pair as a random effect in all models to account for the paired selection of sites, and plant as a random effect to account for non-independence between flowers on the same plant. We did not include plant as a random effect in proportion fruit set models because proportion fruit set was measured at the plant and not the flower level. Analyses were conducted in SAS 9.2 software (PROC GLIMMIX; SAS Institute Inc., Cary, NC. USA). Proportion fruit and seed set were analyzed with a binomial distribution and logit link function. Seed set per fruit was analyzed with a Poisson distribution and log link function. We ran full models for each analysis including site type, treatment, and their interaction, followed by a series of three reduced models and an intercept only model. To compare models, we used Akaike's Information Criterion (AICc) and quasi-Akaike's Information Criterion (QAICc). Models of proportion fruit set were compared using AICc, while those for seed set per fruit and proportion seed set per fruit were compared using QAICc to account for overdispersion. We ranked models based on the lowest AICc or QAICc value (see Appendix S3) and evaluated the best-fit models for pretending variables, factors that were present in models with low AICc scores but that contributed little to the overall deviance when compared to reduced models (Bolker et al., 2009). We found little evidence for pretending variables and report the results from the best-fit models.

To understand differences in plant reproductive potential among the site types, we also compared metrics of plant reproduction in hand-pollinated flowers only between urban and non-urban sites. To do so, we used similar GLMMs for each species and metric of reproduction with site type as a fixed factor and site pair and plant as random effects (excluding plant as a random factor for proportion fruit set, which was measured at the plant level).

(2) How do conspecific and heterospecific pollen receipt by flowers differ between urban and non-urban sites?

To gain insight into pollinator visitation, we used pollen receipt as a proxy. Pollen receipt is often correlated with

pollinator visitation rates (Engel and Irwin, 2003) and can be used to estimate both the quantity and quality of pollen deposited by visiting pollinators. In *Gelsemium*, higher pollinator visitation to flowers increased pollen deposition to emasculated pin and thrum flowers (Irwin et al., 2014), and the number of pollen grains deposited on *Oenothera* stigmas increased with the number of pollinator visits (Silander and Primack, 1978). Pollen receipt includes both pollinator visitation rates and pollinator efficiency at depositing pollen onto the stigmas of flowers, and allowed us to quantify both conspecific and heterospecific pollen deposition. While increased conspecific pollen deposition can increase fruit and seed set in pollen-limited species (Adler and Irwin, 2012), increased heterospecific pollen deposition can reduce seed set if deposition is high enough to impede conspecific pollen tube growth (Galen and Gregory, 1989). We measured pollen receipt by flowers of *Gelsemium* and *Oenothera* given previous research documenting relationships between visitation and pollen receipt, and because these two plant species showed contrasting effects of pollen limitation in urban relative to non-urban sites (see Results). We were unable to quantify pollen receipt for *Campsis*, as the stigmas close in response to adequate pollen donation, making stigma visualization ineffective.

To estimate pollen receipt, we used the same site pairs and plants as in Question 1. We collected up to three stigmas per plant from each of the sites after corollas abscised from non-experimental, un-emasculated flowers. Stigmas were collected at peak flowering for each species, and represent total pollen receipt across the lifetime of individual flowers. Stigmas were mounted on microscope slides and fixed in fuchsin dye (Kearns and Inouye, 1993). We counted the number of conspecific and heterospecific pollen grains per stigma under a compound microscope. We identified conspecific and heterospecific pollen by comparison to a pollen reference library, made using anthers from focal species and co-flowering species at the sites. We calculated the proportion of conspecific pollen per stigma by dividing the number of conspecific pollen grains by the total number of conspecific and heterospecific pollen grains on each stigma.

Statistical analyses. We compared total conspecific and heterospecific pollen deposition, and the proportion of conspecific pollen deposited on stigmas, between urban and non-urban sites using GLMMs. We included plant and site as random factors to account for multiple samples taken from each plant and the paired nature of study sites, and included site type (urban or non-urban) as a fixed effect. We modeled the numbers of conspecific and heterospecific pollen grains using a lognormal distribution and logit-transformed the proportion of conspecific pollen grains per stigma to facilitate convergence of models using a Gaussian distribution. We conducted separate analyses for *Gelsemium* and *Oenothera*.

RESULTS

(1) To what degree does pollen-limitation of female plant reproduction vary between urban and non-urban sites?

Across both urban and non-urban sites, pollen limitation was common across all three plant species and all three metrics of female plant reproduction measured (Table 1, Figure 1; Appendix S4). However, the magnitude of pollen limitation varied by species. For example, hand-pollination increased overall seed set per fruit by 7.5% in *Gelsemium*, 15% in *Campsis*, and 24% in *Oenothera*.

We found significant interactions between site type and pollen supplementation in seed set per fruit and proportion seed set per fruit for two of the plant species, *Gelsemium* and *Campsis* (Table 1; Appendix S4). However, the direction of the interaction was opposite to our prediction. Pollen supplementation benefited plants more in urban relative to non-urban sites in both plant species for seed set per fruit and proportion seed set per fruit (Figure 1B, C, H, I). In *Gelsemium*, the response to hand-pollination was three times higher in urban compared to non-urban sites for seed set per fruit (Figure 1B), and for *Campsis*, the response was nearly two times greater (Figure 1H). For *Oenothera*, the best-fit models for all responses only included positive effects of hand-pollination and did not include site type or interactions between pollination treatment and site type (Table 1).

To understand the reproductive potential of plants in the absence of pollen limitation, we compared reproduction of hand-pollinated flowers only (excluding open-pollinated) in urban vs. non-urban sites. Plants growing in urban sites had higher plant reproduction in *Gelsemium* and *Campsis*; for *Oenothera*, there was no difference in estimates of plant reproduction from hand-pollinated flowers in urban vs. non-urban sites (Figure 1). For *Gelsemium*, urban hand-pollinated flowers had 32.3% higher proportion fruit set ($F_{1,143} = 34.04$, $P < 0.001$) than hand-pollinated flowers in non-urban sites, although they did not differ in either seed set per fruit ($F_{1,543} = 1.27$, $P = 0.260$) or proportion seed set ($F_{1,529} = 0.30$, $P = 0.582$). For *Campsis*, urban hand-pollinated flowers had 40.4% higher proportion fruit set ($F_{1,51} = 8.94$, $P = 0.004$) and 25% higher seed set per fruit ($F_{1,31} = 6.99$, $P = 0.013$), with no difference in proportion seed set ($F_{1,28} = 3.21$, $P = 0.084$).

(2) How do conspecific and heterospecific pollen receipt by flowers differ between urban and non-urban sites?

Gelsemium flowers in urban sites received 18% fewer conspecific pollen grains per stigma ($F_{1,236} = 13.17$, $P < 0.001$) and 20% more heterospecific pollen, on average, than *Gelsemium* flowers in non-urban sites, though the latter was not statistically significant ($F_{1,236} = 0.32$, $P = 0.572$).

TABLE 1 Effect structure and parameter estimates from the best-fit generalized linear mixed models (GLMMs) for three estimates of female reproduction in three native flowering species, *Gelsemium sempervirens*, *Oenothera fruticosa*, and *Campsis radicans*, in urban and non-urban sites. Fixed effects include site type (type; urban or non-urban), hand-pollination treatment (treat; hand-pollinated or control), and site type by pollination treatment interactions. Site pair and plant were included as random effects. Dashes indicate factors not included in the best-fit model.

Fixed Effect	Gelsemium sempervirens			Oenothera fruticosa			Campsis radicans		
	Num, Den *DF*	*F*	P	Num, Den *DF*	*F*	P	Num, Den *DF*	*F*	P
Proportion fruit set									
type	1, 149	8.11	0.005	–	–		1, 53	1.322	0.256
treat	1, 149	9.53	0.002	1, 65	14.04	<0.001	1, 53	36.34	<0.001
type × treat	1, 149	2.66	0.105	–	–		1, 53	2.66	0.109
Seed set per fruit									
type	1, 1143	0.11	0.745	–	–		1, 44	6.58	0.014
treat	1, 1143	20.53	<0.001	1, 202	207.69	<0.001	1, 44	26.91	<0.001
type × treat	1, 1143	4.33	0.038	–	–		1, 44	83.68	<0.001
Proportion seed set per fruit									
type	1, 1117	0.00	0.961	–	–		1, 36	0.54	0.469
treat	1, 1117	59.21	<0.001	1, 202	530.35	<0.001	1, 36	38.01	<0.001
type × treat	1, 1117	13.99	<0.001	–	–		1, 36	333.46	<0.001

These differences also resulted in lower proportions of conspecific pollen deposition to stigmas of plants growing in urban compared to non-urban sites ($F_{1,236} = 7.39$, $P = 0.007$, Figure 2A).

Although site type did not affect the magnitude of pollen limitation in *Oenothera*, we found similar patterns of pollen receipt as in *Gelsemium*. *Oenothera* growing in urban sites had 25% less conspecific pollen deposition than those growing in non-urban sites, although the effect was not significant ($F_{1,77} = 1.95$, $P = 0.166$). However, heterospecific pollen deposition was twice as high in urban sites ($F_{1,74} = 4.85$, $P = 0.031$), resulting in lower proportions of conspecific pollen deposition to stigmas of plants growing in urban compared to non-urban sites ($F_{1,74} = 7.45$, $P = 0.008$, Figure 2B). Taken together, these results indicate that flowers are receiving lower proportions of conspecific pollen deposition in urban compared to non-urban sites.

DISCUSSION

As landscapes become increasingly affected by urban development, it is important to assess how urban habitats will affect pollination and plant reproduction. We found that *Gelsemium*, *Oenothera*, and *Campsis* were generally pollen-limited for plant reproduction, indicating that pollination services are important for reproduction in these native species. Because prior research documented higher bee abundance in urban relative to non-urban sites in 2008 and 2009, two years before the current study (Carper et al., 2014), we predicted that plants growing in urban sites would be less pollen-limited for reproduction than those growing in non-urban sites. However, our results were opposite to this initial prediction. For *Gelsemium* and *Campsis*, we found that the magnitude of pollen limitation was higher in urban compared to non-urban sites, and for *Oenothera*, site type had no effect on the magnitude of pollen limitation. When we measured pollen receipt as a proxy for pollinator visitation and pollination, we found that both *Gelsemium* and *Oenothera* received lower proportions of conspecific pollen deposition, suggesting lower fidelity of pollinators to focal species in urban landscapes. Finally, when we examined reproductive potential (estimated as reproduction of hand-pollinated flowers), we found evidence of either no difference in reproductive potential (*Oenothera*) or higher reproductive potential of plants growing in urban relative to non-urban sites (*Gelsemium* and *Campsis*), suggesting that urban sites hold conservation value not only for native bees (Carper et al., 2014) but also native plants. However, increased pollinator abundance in these urban landscapes, estimated two years prior to this hand-pollination study, did not alleviate pollen limitation of native plant reproduction.

The number of studies examining how urban landscapes affect pollen limitation of plant reproduction is growing, but yielding conflicting findings. For example, the intensity of urbanization had no effect on pollinator visitation to experimental populations of *Digitalis purpurea* L. (Plantaginaceae) across three urban-rural gradients in Belgium, where pollen limitation was ubiquitous (Verboven et al., 2012). By contrast, non-urban populations of *Cytisus scoparius* (L.) Link (Fabaceae) received fewer pollinator
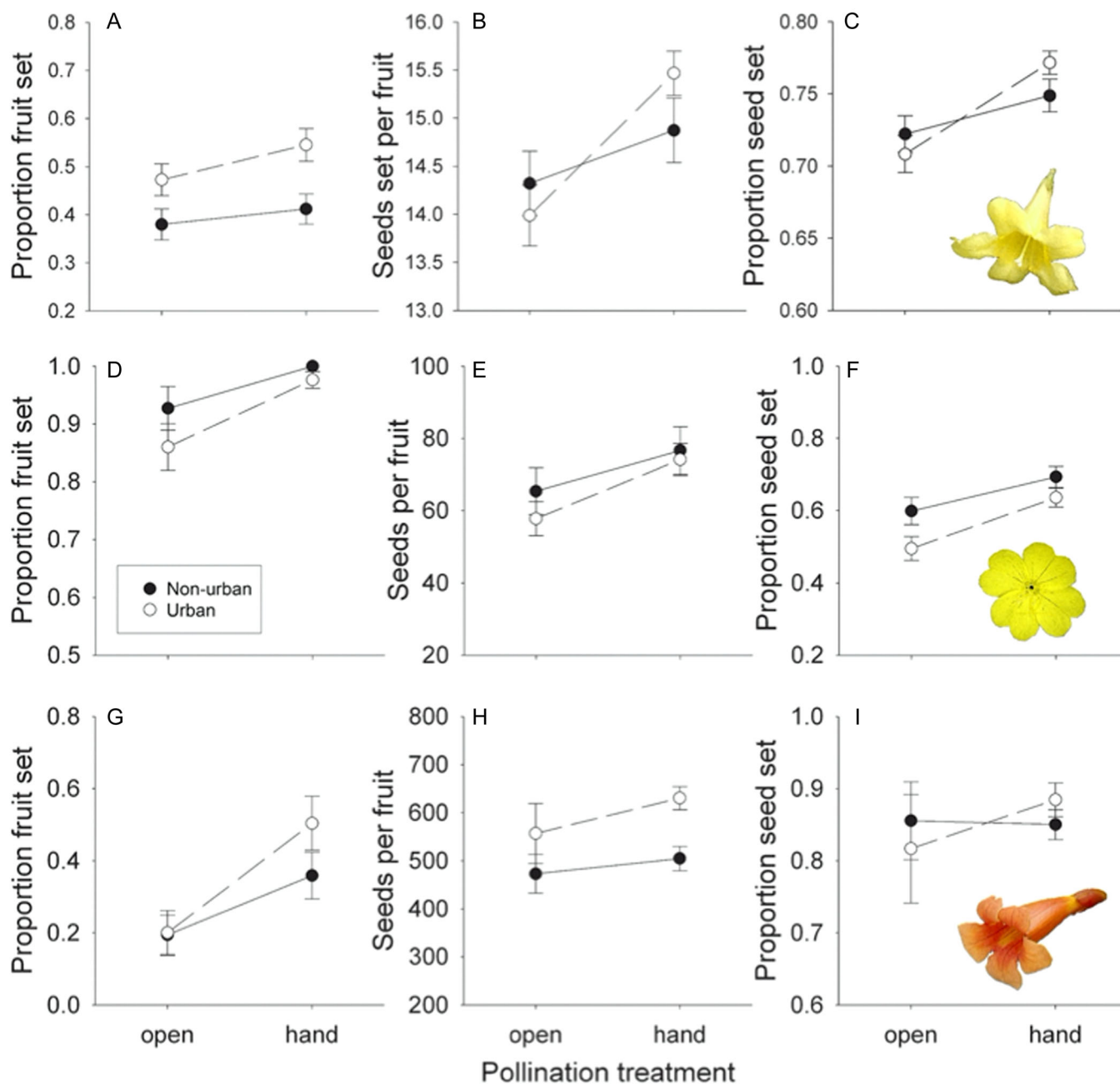
FIGURE 1 Fruit set, seeds per fruit, and proportion seed set per fruit, in paired urban and non-urban sites for *Gelsemium sempervirens* (*n* = 8 site pairs, A–C), *Oenothera fruticosa* (*n* = 4 site pairs, D–F), and *Campsis radicans* (*n* = 3 site pairs, G–I). Black circles and solid lines indicate non-urban sites, and white circles and hatched lines indicate urban sites. Points are means and error bars are SE. *Gelsemium sempervirens* flowers were pollen-limited for all measures of reproduction with the effects of hand-pollination significantly greater in urban relative to non-urban sites for seed set per fruit (B) and proportion seed set per fruit (C). *Oenothera fruticosa* flowers were pollen-limited for all measures of reproduction (D–F), but there was no significant interaction between site type and pollination treatment. *Campsis radicans* flowers were pollen-limited for all measures of reproduction, and the effects of hand-pollination on seeds per fruit (H) and proportion seed set per fruit (I) were significantly greater in urban relative to non-urban sites.

visits and were more pollen-limited than urban populations (Parker, 1997). A meta-analysis revealed higher pollen limitation of pollinator-reliant plants in urban compared to non-urban areas, but did not explicitly consider studies of the same plant species in urban vs. non-urban habitats (Bennett et al., 2020). We found species-specific effects of experimentally measured pollen limitation, with two species

exhibiting higher pollen limitation in urban sites compared to non-urban ones, and one species showing no difference in the magnitude of pollen limitation between site types. Thus, two of our plant species are consistent with the meta-analysis results, using the same plant species growing in urban and non-urban sites. Even though more specialized plants (those pollinated by one or a few pollinator taxa) tend
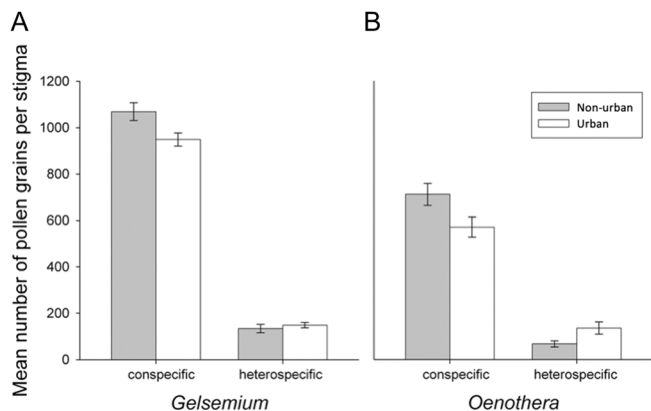
FIGURE 2 (A) *Gelsemium sempervirens* and (B) *Oenothera fruticosa* stigmas received less conspecific and more heterospecific pollen in urban compared to non-urban sites (across eight and four site pairs in 2011, respectively), although the effect of site type on heterospecific pollen receipt was only statistically significant for *Oenothera*. Grey bars indicate non-urban sites and white bars indicate urban sites. Bars are means and error bars are SE.

to be more pollen-limited (Bennett et al., 2020), our results show that even plant species with generalized pollination systems can suffer from pollen limitation in urban habitats. While studies of pollinator abundance and visitation and plant reproduction provide insights into patterns, more studies manipulating pollen limitation of the same plant species in urban and non-urban sites are needed to yield mechanistic insights across urban ecosystems and to confirm the hypothesis that urbanization affects the magnitude of pollen limitation (Wenzel et al., 2020). Moreover, additional mechanistic insight could be achieved if hand-pollination studies included landscape attributes to understand what drives variation in results across systems.

Measurements of pollen receipt by stigmas can mechanistically link changes in pollinator visitation and efficiency to plant reproduction and pollen limitation. In *Gelsemium*, we found that flowers of plants growing in urban sites received significantly less conspecific pollen than those in non-urban sites. While we did not observe pollinators directly, estimates of pollen deposition provide indirect evidence of pollinator visitation and/or behavior (Engel and Irwin, 2003). Lower conspecific pollen deposition in *Gelsemium* in urban sites in 2011 suggested that urban flowers received fewer and/or less efficient pollinator visits than flowers in non-urban sites. These results also match pollen deposition patterns in *Gelsemium* from a similar subset of sites in 2009 in which urban flowers (referred to as 'suburban' in that study) received significantly less pollen than non-urban ones (Irwin et al., 2018), although this pattern may be spatio-temporally variable since pollen receipt between urban and non-urban sites from the same study region did not differ in 2007 (Irwin et al., 2014). One caveat of quantifying pollen deposition was our inability to distinguish between viable out-crossed vs. self-pollen. However, for species such as *Gelsemium*, differences in the

relative position of anthers and stigmas (distyly) should promote disassortative pollen movement (Barrett et al., 2000) and thus reduce self-pollination. The anthers are at the end of long filaments in *Gelsemium* thrum morphs and deposit pollen primarily on the mesosoma of bees, which in turn deposit it on the stigmas at the end of long styles of pin morphs. Conversely, pollen from the short stamens of pin morphs is primarily deposited on the heads of bees and transferred to the short stigmas of thrum morphs (A. Carper, personal observation). Thus, pollen deposition on different locations of pollinator bodies reduced intra-morph (including self) pollen transfer. More detailed observations in future studies will help determine which components of pollinator visitation and behavior differ in urban vs. non-urban sites and how those translate into pollen deposition and plant reproduction.

Any time plants co-flower and share potential pollinators, they are at risk of receiving heterospecific pollen (Morales and Traveset, 2008). In a prior study with *Gelsemium*, we found that flowers in some of the same urban sites as this study received 6 times more heterospecific pollen than in non-urban sites (Irwin et al., 2014), although this pattern may be spatio-temporally variable (Irwin et al., 2018). In our current study for *Gelsemium* and *Oenothera*, we found a higher proportion of heterospecific pollen receipt in urban compared to non-urban sites (akin to Irwin et al., 2014). Other studies also highlight the potential for greater heterospecific pollen transfer in urban compared to non-urban sites. For example, studies of networks of plant-pollinator interactions suggest that insect species visit more plant species on average in urban compared to non-urban sites, but a lower proportion of total available plant species because of higher flowering plant species richness in urban sites (Baldock et al., 2015; Martins et al., 2017); these patterns could lead to higher heterospecific pollen transfer. While the species and origin of the heterospecific pollen in our study remain unknown, we suspect the higher amounts in urban habitats came from urban garden plantings and pollinators switching foraging among horticultural and wild species. This pollinator switching can lead to mixed pollen loads on pollinator bodies, heterospecific pollen deposition, and a loss of conspecific pollen to heterospecific flowers, which could exacerbate pollen limitation of plant reproduction and reduce male components of plant fitness (Morales and Traveset, 2008). Heterospecific pollen receipt can have a range of effects on female components of plant reproduction, depending on the amount, species, composition, and timing of heterospecific pollen deposition relative to conspecific pollen (e.g., Arceo-Gómez and Ashman, 2011; Lanuza et al., 2021). Moreover, the effects of heterospecific pollen deposition on seed production as a function of these factors may be non-linear, hampering predictive insight. Why *Gelsemium* and *Oenothera* reproduction responded differently to the amount or proportion of heterospecific pollen received is unknown and warrants further investigation, as does variation in the degree of pollen limitation.

More generally, given the observed patterns of increased heterospecific pollen receipt by urban plants (here and Irwin et al., 2014), data on the floral abundance of co-flowering plant species and community-level pollinator visitation data will be useful in clarifying the role of the larger plant community in driving pollen deposition differences in plants in urban vs. non-urban sites.

One interesting outcome of this study is that even though urban sites harbored more bee pollinators in recently preceding years (Carper et al., 2014), *Gelsemium* and *Oenothera* growing in those urban sites received significantly less conspecific pollen than those growing in non-urban sites. This result was unexpected, as it suggests that increased bee abundance may not translate into increased pollination to particular focal plants. At least five non-mutually exclusive hypotheses could account for a decoupling of bee abundance and pollen limitation. First, the availability of alternate floral resources may lure pollinators away from focal plant species. In a prior study using some of the same field sites, we found that urban sites had higher floral density and species richness than non-urban sites, but patterns were temporally variable and often not statistically significant (Carper et al., 2014). The presence of diverse flowering species can facilitate pollination of co-flowering species (Ghazoul, 2006), and flower-rich urban areas could promote pollination of individual species through facilitation. However, we cannot rule out the possibility that more diverse or abundant floral resources could increase competition for pollination. For example, pollinator visitation to *Trifolium pratense* L. (Fabaceae) in Zurich, Switzerland declined with increasing abundance and diversity of co-flowering species (Hennig and Ghazoul, 2011). Second, if floral abundance increases faster than bee abundance in urban areas, this could reduce the ratio of bees to flowers. Reduced bee-to-flower ratios can result in pollinator dilution (Holzschuh et al., 2011), reducing per-flower visitation rates and per-flower pollen receipt, leading to higher pollen limitation. Third, estimates of higher bee abundance in urban vs. non-urban sites were collected by pan trap and netting survey-based methods (Carper et al., 2014). These methods are designed to sample the entire bee community and not specific focal plant-pollinator interactions, and so bee abundance and survey methods may not accurately predict pollinator visitation to and pollination success of focal plant species (Boyer et al., 2020). Fourth, we were logistically unable to survey pollinator communities concurrently with pollination treatments in this study. The magnitude of differences in bee abundance (nearly twice) were consistent across multiple recently preceding years (Carper et al., 2014). However, we acknowledge that bee abundance and diversity can vary among years and so differences in bee abundance between urban and non-urban sites observed in years prior may not be applicable to the year of study. Fifth, differences in plant interactions with other consumers in urban vs. non-urban sites could affect pollinator visitation. Prior studies have shown that

urban areas can have increased herbivory compared to natural habitats (Christie and Hochuli, 2005). While we did not estimate herbivory on experimental plants in this study, other *Gelsemium* in the same urban ecosystem experience higher levels of floral herbivory (florivory) compared to *Gelsemium* growing in non-urban sites (Irwin et al., 2014, 2018). In other flowering plant species, florivory can reduce pollinator visitation, pollination, and plant reproduction (McCall and Irwin, 2006). In a *Gelsemium* experimental array, florivory reduced the number of pollinator visits and increased time spent per flower, but florivory had no effect on estimates of female plant reproduction, likely because plants in that experiment were not pollen-limited (Carper et al., 2016). Understanding how foliar and floral herbivory affect *Gelsemium* and other focal species reproduction in urban vs. non-urban sites under pollen-limited conditions requires additional experimentation.

An additional surprising result in this study was that *Gelsemium* and *Campsis* reproduction were often higher in urban relative to non-urban sites when plants were released from pollen limitation. One hypothesis is that abiotic factors that promote plant reproduction differed among the sites. In general, urban landscapes tend to be warmer (heat-island effect), have greater rates of atmospheric mineral deposition, and altered hydrology relative to rural environments (Pickett et al., 2001). However, total soil nutrients, temperature, relative humidity, and light availability did not differ between urban and non-urban sites (Appendix S2; Irwin et al., 2018). It is unknown whether we measured the abiotic factors that most limit plant reproduction in these habitats. We also cannot rule out the possibility that the higher measures of some plant reproduction estimates in urban *Gelsemium* and *Campsis* were due to gene flow and introgression between horticultural and wild plants. Both species can be purchased commercially, and *Gelsemium* in particular is a common horticultural vine. While we assumed that the plants we were studying in urban sites were wild genotypes, we cannot rule out that they were the product of seed that escaped from horticultural plantings or introgression between horticultural and wild species. The growth and fitness of crop-wild hybrids has been extensively studied, in some cases under various ecological conditions (Mercer et al., 2014). However, gene flow and introgression between horticultural and wild species has been less well-studied, likely in part because horticultural species are grown at lower densities compared to crop plants (Johnson and Galloway, 2008). Whether there are genotype and phenotype differences between horticultural and wild *Gelsemium* and *Campsis* is unknown. However, if there has been selection for increased growth, flowering, and reproductive capacity in horticultural plants with subsequent introgression with wild relatives, it could explain higher reproductive output in the absence of pollen limitation. Given the differences in reproductive potential of plants in urban vs. non-urban sites, an important next step is to assess the degree to which differences in seed

production have demographic consequences in the site types.

CONCLUSIONS

We found that two of the three plant species we studied exhibited higher pollen limitation in urban relative to non-urban sites. Moreover, conspecific pollen deposition was generally lower in urban sites, and heterospecific pollen was generally higher in urban sites, suggesting an important role of pollinator switching among plant species driving the patterns observed. Mechanistic, multi-species research across a broader range of urban and non-urban sites will help identify the more proximate and ultimate factors driving the patterns observed, including detailed observations of pollinator foraging behavior, the abiotic and human-built environment, and plant species composition. Results from such mechanistic research may inform conservation and management of pollinators and pollination in urban landscapes.

DATA AVAILABILITY STATEMENT
Data are archived with and available from Dryad Digital Repository: https://doi.org/10.5061/dryad.5mkkwh78r (Irwin et al., 2022).

ORCID
Adrian L. Carper http://orcid.org/0000-0002-8657-3613
Paige S. Warren http://orcid.org/0000-0003-0787-4635
Lynn S. Adler http://orcid.org/0000-0003-2125-5582
Rebecca E. Irwin http://orcid.org/0000-0002-1394-4946

REFERENCES

Adler, L. S., and R. E. Irwin. 2005. Ecological costs and benefits of defenses in nectar. *Ecology* 86: 2968–2978.

Adler, L. S., and R. E. Irwin. 2012. Nectar alkaloids decrease pollination and reproduction in a native plant. *Oecologia* 168: 1033–1041.

Arceo-Gómez, G., and T. L. Ashman. 2011. Heterospecific pollen deposition: Does diversity alter the consequences? *New Phytologist* 192: 738–746.

Ashman, T. L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, et al. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology* 85: 2408–2421.

Baldock, K. C. R., M. A. Goddard, D. M. Hicks, W. E. Kunin, N. Mitschunas, H. Morse, L. M. Osgathorpe, et al. 2019. A systems approach reveals urban pollinator hotspots and conservation opportunities. *Nature Ecology and Evolution* 3: 363–373.

Baldock, K. C. R., M. A. Goddard, D. M. Hicks, W. E. Kunin, N. Mitschunas, L. M. Osgathorpe, S. G. Potts, et al. 2015. Where is the UK's pollinator biodiversity? The importance of urban areas for flower-visiting insects. *Proceedings of the Royal Society of London, B, Biological Sciences* 282: 20142846.

Barker, C. A., and R. D. Sargent. 2020. Pollination services to *Impatiens capensis* (Balsaminaceae) are maintained across an urbanization gradient. *International Journal of Plant Sciences* 181: 937–944.

Barrett, S. C. H., L. K. Jesson, and A. M. Baker. 2000. The evolution and function of stylar polymorphisms in flowering plants. *Annals of Botany* 85: 253–265.

Bennett, J. M., J. A. Steets, J. H. Burns, L. A. Burkle, J. C. Vamosi, M. Wolowski, G. Arceo-Gómez, et al. 2020. Land use and pollinator dependency drives global patterns of pollen limitation in the Anthropocene. *Nature Communications* 11: Article 3999.

Bertin, R. I. 1982a. Floral biology, hummingbird pollination and fruit production of trumpet creeper (*Campsis radicans*, Bignoniaceae). *American Journal of Botany* 69: 122–134.

Bertin, R. I. 1982b. Paternity and fruit production in trumpet creeper (*Campsis radicans*). *American Naturalist* 119: 694–709.

Bertin, R. I., and M. Sullivan. 1988. Pollen interference and cryptic self-fertility in *Campsis radicans*. *American Journal of Botany* 75: 1140–1147.

Biesmeijer, J. C., S. P. Roberts, M. Reemer, R. Ohlemueller, M. Edwards, T. Peeters, A. Schaffers, et al. 2006. Parallel declines in pollinators and insect-pollinated plants in northwest Europe, Britain, and the Netherlands. *Science* 313: 351–354.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology and Evolution* 24: 127–135.

Boyer, K. J., F. P. Fragoso, M. E. D. Mabin, and J. Brunet. 2020. Netting and pan traps fail to identify the pollinator guild of an agricultural crop. *Scientific Reports* 10: 13819.

Brosi, B. J., and H. M. Briggs. 2013. Single pollinator species losses reduce floral fidelity and plant reproductive function. *Proceedings of the National Academy of Sciences, USA* 110: 13044–13048.

Burkle, L. A., J. C. Marlin, and T. M. Knight. 2013. Plant-pollinator interactions over 120 years: Loss of species, co-occurrence, and function. *Science* 339: 1611–1615.

Campbell, D. R., and K. J. Halama. 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* 74: 1043–1051.

Carper, A. L., L. S. Adler, and R. E. Irwin. 2016. Effects of florivory on plant-pollinator interactions: Implications for male and female components of plant reproduction. *American Journal of Botany* 103: 1061–1070.

Carper, A. L., L. S. Adler, P. S. Warren, and R. E. Irwin. 2014. Effects of suburbanization on forest bee communities. *Environmental Entomology* 43: 253–262.

Christie, F. J., and D. F. Hochuli. 2005. Elevated levels of herbivory in urban landscapes: Are declines in tree health more than an edge effect? *Ecology and Society* 10: Article 10.

Engel, E. C., and R. E. Irwin. 2003. Linking pollinator-visitation rate and pollen receipt. *American Journal of Botany* 90: 1612–1618.

Galen, C., and T. Gregory. 1989. Interspecific pollen transfer as a mechanism of competition: Consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia* 81: 120–123.

Gaston, K. J., T. M. Blackburn, and K. K. Goldewijk. 2003. Habitat conversion and global avian biodiversity loss. *Proceedings of the Royal Society of London, B, Biological Sciences* 270: 1293–1300.

Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. *Journal of Ecology* 94: 295–304.

Greenleaf, S. S., N. M. Williams, R. Winfree, and C. Kremen. 2007. Bee foraging ranges and their relationship to body size. *Oecologia* 153: 589–596.

Hall, D. M., G. R. Camilo, R. K. Tonietto, J. Ollerton, K. Ahrne, M. Arduser, J. S. Ascher, et al. 2017. The city as a refuge for insect pollinators. *Conservation Biology* 31: 24–29.

Hamblin, A. L., E. Youngsteadt, and S. D. Frank. 2018. Wild bee abundance declines with urban warming, regardless of floral density. *Urban Ecosystems* 21: 419–428.

Harrison, T., J. Gibbs, and R. Winfree. 2019. Anthropogenic landscapes support fewer rare bee species. *Landscape Ecology* 34: 967–978.

Hennig, E. I., and J. Ghazoul. 2011. Plant-pollinator interactions within the urban environment. *Perspectives in Ecology, Evolution and Systematics* 13: 137–150.

Holzschuh, A., C. F. Dormann, T. Tscharntke, and I. Steffan-Dewenter. 2011. Expansion of mass-flowering crops leads to transient pollinator dilution and reduced wild plant pollination. *Proceedings of the Royal Society of London, B, Biological Sciences* 278: 3444–3451.

Hostetler, N. E., and M. E. McIntyre. 2001. Effects of urban land use on pollinator (Hymenoptera: Apoidea) communities in a desert metropolis. *Basic and Applied Ecology* 2: 209–218.

Irwin, R., A. Carper, L. Adler, and P. Warren. 2022. Pollen limitation of native plant reproduction in an urban landscape. *Dryad, Dataset*, website: https://doi.org/10.5061/dryad.5mkkwh78r

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 of London, B, Biological Sciences* 285: 20181239.

Irwin, R. E., P. S. Warren, A. L. Carper, and L. S. Adler. 2014. Plant-animal interactions in suburban environments: Implications for floral evolution. *Oecologia* 174: 803–815.

Irwin, R. E., E. Youngsteadt, P. S. Warren, and J. L. Bronstein. 2020. The evolutionary ecology of mutualisms in urban landscapes. *In* M. Szulkin, J. Munshi-South, and A. Charmantier [eds.], Urban evolutionary biology, 112–129. Oxford University Press, Oxford, UK.

Johnson, L. M. K., and L. F. Galloway. 2008. From horticultural plants to wild populations: Movement of pollen and genes in *Lobelia cardinalis*. *Plant Ecology* 197: 55–67.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado, USA.

Knight, T. M., J. A. Steets, J. C. Vamosi, S. J. Mazer, M. Burd, D. R. Campbell, M. R. Dudash, et al. 2005. Pollen limitation of plant reproduction: Pattern and process. *Annual Review of Ecology, Evolution, and Systematics* 36: 467–497.

Lanuza, J. B., I. Bartomeus, T. L. Ashman, G. Bible, and R. Radar. 2021. Recipient and donor characteristics govern the hierarchical structure of heterospecific pollen competition networks. *Journal of Ecology* 109: 2329–2341.

Martins, K. T., A. Gonzalez, and M. J. Lechowicz. 2017. Patterns of pollinator turnover and increasing diversity associated with urban habitats. *Urban Ecosystems* 20: 1359–1371.

McCall, A. C., and R. E. Irwin. 2006. Florivory: The intersection of pollination and herbivory. *Ecology Letters* 9: 1351–1365.

Mercer, K. L., D. J. Emry, A. A. Snow, M. A. Kost, B. A. Pace, and H. M. Alexander. 2014. Fitness of crop-wild hybrid sunflower under competition conditions: Implications for crop-to-wild introgression. *PLoS One* 9: e109001.

Minnefors, A. 2016. Pollen limitation in the city: Measuring floral traits and pollination services in urban *Chamerion angustifolium*. M. S. thesis, Uppsala University, Uppsala, Sweden.

Morales, C. L., and A. Traveset. 2008. Interspecific pollen transfer: Magnitude, prevalence and consequences for plant fitness. *Critical Reviews in Plant Sciences* 27: 221–238.

Ollerton, J., R. Winfree, and S. Tarrant. 2011. How many flowering plants are pollinated by animals? *Oikos* 120: 321–326.

Ornduff, R. 1970. Systematics and breeding system of *Gelsemium* (Loganiaceae). *Journal of the Arnold Arboretum* 51: 1–17.

Ornduff, R. 1979. Features of pollen flow in *Gelsemium sempervirens* (Loganiaceae). *Journal of the Arnold Arboretum* 60: 377–381.

Ornduff, R. 1980. The probable genetics of distyly in *Gelsemium sempervirens* (Loganiaceae). *Canadian Journal of Genetics and Cytology* 22: 303–304.

Parker, I. M. 1997. Pollinator limitation of *Cytisus scoparius* (Scotch broom), an invasive exotic shrub. *Ecology* 78: 1457–1470.

Pickett, S. T. A., M. L. Cadenasso, J. M. Grove, C. H. Nilon, R. V. Pouyat, W. C. Zipperer, and R. Costanza. 2001. Urban ecological systems: Linking terrestrial ecological, physical, and socioeconomic components of metropolitan areas. *Annual Review of Ecology and Systematics* 32: 127–157.

Primack, R. B., and J. A. Silander. 1975. Measuring the relative importance of different pollinators to plants. *Nature* 255: 143–144.

Rivkin, L. R., V. J. Nhan, A. E. Weis, and M. T. J. Johnson. 2020. Variation in pollinator-mediated plant reproduction across an urbanization gradient. *Oecologia* 192: 1073–1083.

Shochat, E., S. B. Lerman, J. M. Anderies, P. S. Warren, S. H. Faeth, and C. H. Nilon. 2010. Invasion, competition, and biodiversity loss in urban ecosystems. *Bioscience* 60: 199–208.

Silander, J. A., and R. B. Primack. 1978. Pollination intensity and seed set in the evening primrose (*Oenothera fruticosa*). *American Midland Naturalist* 100: 213–216.

Steffan-Dewenter, I., and C. Westphal. 2008. The interplay of pollinator diversity, pollination services and landscape change. *Journal of Applied Ecology* 45: 737–741.

Terando, A. J., J. Costanza, C. Belyea, R. R. Dunn, A. McKerrow, and J. A. Collazo. 2014. The southern megalopolis: Using the past to predict the future of urban sprawl in the southeastern U.S. *PLoS One* 9: e102261.

Theodorou, P., R. Radzevičiūtė, G. Lentendu, B. Kahnt, M. Husemann, C. Bleidorn, J. Settele, et al. 2020. Urban areas as hotspots for bees and pollination but not a panacea for all insects. *Nature Communications* 11: Article 576.

Verboven, H. A. F., R. Brys, and M. Hermy. 2012. Sex in the city: Reproductive success of *Digitalis purpurea* in a gradient from urban to rural sites. *Landscape and Urban Planning* 106: 158–164.

Waser, N. M., and M. V. Price. 1989. Optimal outcrossing in *Ipomopsis aggregata*: Seed set and offspring fitness. *Evolution* 43: 1097–1109.

Wenzel, A., I. Grass, V. V. Belavadi, and T. Tscharntke. 2020. How urbanization is driving pollinator diversity and pollination—A systematic review. *Biological Conservation* 241: 108321.

Winfree, R., R. Aguilar, D. P. Vazquez, G. LeBuhn, and M. A. Aizen. 2009. A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 90: 2068–2076.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Table documenting non-urban and urban sites in Wake, Durham, and Chatham Counties, NC, USA studied to assess pollen limitation of plant reproduction, and two figures (site map and estimates of impervious surface cover).

Appendix S2. Text and table describing measurements and results of abiotic factors in non-urban and urban sites.

Appendix S3. Model ranking tables for reproductive responses to supplemental pollination in non-urban and urban sites.

Appendix S4. Tables showing means and standard errors for measures of reproduction in the non-urban and urban sites.