

Impacts of an urbanization gradient on pollination services to a bee-pollinated plant

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Abstract:

The vast majority of flowering plants rely on pollinators for outcrossed reproduction. Unfortunately, many species of pollinators are in decline. Pollinators face a number of challenges, including shifting land use, climate change and pesticides. In addition, pollinator habitat may be altered or eliminated through urbanization. Fragmented urban landscapes may lengthen the distances among foraging patches, limit resources in a given patch, and reduce the availability of pollinator nesting sites. Here, I examine the effects of urbanization on pollination services by addressing the following questions: (1) Does bee species richness and/or abundance change along an urbanization gradient?; (2) Does the pollen limitation of a focal species vary along the same gradient?; (3) Do plants with a mixed mating system produce more selfed seeds in more urban environments? Using the percentage of impervious land cover in the space immediately surrounding the site as a proxy for site urbanization, 15 study sites were set up across the city of Ottawa to span a range from minimal percent impervious surface (mostly green space) to mostly impervious surface (little green space) at a range of spatial scales. At each site I set up an array of 20 potted *Impatiens capensis* plants as well as six pan traps in order to collect data on pollen limitation, seed production, and the number of selfed progeny, in addition to information about local pollinator species richness and abundance. Plants in the arrays were randomly assigned to either a hand or open pollination treatment in order to assess the level of pollen limitation. Surprisingly, pollinator species richness and abundance were not correlated with urbanization. Pollen limitation declined with urbanization, however, so did overall seed set, making it difficult to detangle the effects of resource limitation and hand pollination treatments. The number of selfed seeds produced by a

plant was also significantly correlated with site urbanization. Further investigation is required to better understand the dynamics of pollination services in urban environments. I recommend that future studies explore how the presence of individual bee species in rural and urban habitats could be influencing pollen limitation and selfing in *I. capensis*.

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Chapter 1: Overview

The world is rapidly urbanizing; the majority of humans now living in cities rather than rural areas (Seto et al. 2012). Urban areas cover only 3–5% of total available land; however, more than half of the Earth’s human population lives in cities, suburbs, and towns (Seto et al. 2010). Urbanization drastically changes the physical environment, ecological communities and the ecosystems it impacts (Seto et al. 2010, Alberti 2015). Decades of research on urban ecology has identified a suite of abiotic and biotic changes associated with urbanization such as higher temperatures (i.e., the heat island effect (Bornstein 1968), higher levels of air pollution (Seinfeld 1989), a lower diversity of biological communities (McKinney 2006) as well as greater habitat fragmentation (Johnson et al. 2015a). Many discoveries of the effects of anthropogenic disturbance came in the early days of the field of urban ecology (Platt et al. 1994, Kearns et al. 1998, Aizen and Feinsinger 2003). Despite these early findings, some recent work contradicts the idea that urbanization would be overwhelmingly negative for species diversity and abundance (Geslin et al. 2013, Baldock et al. 2015, Goulson et al. 2015, Hall et al. 2016, Samuelson et al. 2018). For example, pollinator species richness has been discovered to increase along a sampling transect from the rural outskirts to the urban core of Grand Lyon, France (Fortel et al. 2014), and bumblebee reproductive success has been shown to increase along an urban gradient that ranged from the agricultural outskirts to the urban core of London, England (Samuelson et al. 2018). It is increasingly clear that we know very little about how urbanization affects relationships between plants, pollinators and their environment (Harrison and Winfree 2015). Studying the effects of urbanization on local species will improve our understanding of how well they are adapting to an

increasingly urban landscape. This could in turn provide insight into problems related to conservation within these urban areas (Johnson and Munshi-South 2017).

Urbanization can alter natural habitat in many ways. For example, urban environments have a much higher proportion of impervious surface (e.g., roads and buildings) than natural areas. They also have greater percentage of non-native plant species, as a result of ornamental plantings (Pickett et al. 2011). Urban environments also tend to have a much higher concentration of air pollution as a result of vehicle emissions (Harrison and Winfree 2015). In addition to these impacts, urbanization also typically leads to fragmentation of natural habitat (Cheptou et al. 2017). The increased impervious surface that accompanies urbanization has led to comparisons with the field of fragmentation ecology (Harrison and Winfree 2015, Cheptou et al. 2017).

Anthropogenic fragmentation is considered a major threat for biodiversity and is currently a primary concern for conservation (Pimm and Raven 2000). It has been well documented that habitat fragmentation can result in a loss of species when individual fragments or patches become too small to support viable populations (Laurance et al. 2002). In some cases, species are able to persist in the short term; however, there is a large body of literature suggesting that a prolonged reduction in habitat threatens long-term species success through demographic and genetic effects (Lande 1988, Gilbert and Levine 2013). The impervious surfaces within urban environments may represent an inhospitable matrix for many species, whereas gardens, parks and other green spaces may represent patches of suitable habitat for some wildlife species (Goddard et al. 2010). In much the same way, urbanization may result in species loss if those same hospitable patches are too small and do not contain sufficient resources (Cheptou et al. 2017).

Because of this potential risk for species loss, it is vital that we understand the effects of urbanization on wildlife.

Because fragmentation often restricts animal movement and limits populations to isolated patches, its effects are felt by animals, including pollinators (Rathcke and Jules 1993). Pollinators require floral resources for nourishment and provisioning their young. In natural habitats, they often have access to continuous landscapes with a large diversity of flora to forage. In wild fragmented habitats, these resources can be limited to patches but may still contain sufficient floral abundance and diversity to promote viable populations (Aizen and Feinsinger 1994). In urban fragmented habitats, these isolated patches may be lacking both the diversity and abundance of floral resource to support bee populations (Geslin et al. 2016).

Although urbanization has been shown to have negative impacts on many plant and animal species (Aronson et al. 2014), some groups have been able to successfully adapt to life in anthropogenic ecosystems (Kark et al. 2007). There is some evidence to suggest that bees may be among the groups that are able to successfully adapt to urban life. For example, a recent study has shown that, over the past 80 years, areas that were subject to urban expansion have lost fewer pollinator species than agricultural lands (Senapathi et al. 2015). In comparison to urban areas, agricultural landscapes have been associated with a reduced diversity and abundance of floral resources (Carvell et al. 2006), as well as an increased pesticide load (Desneux et al. 2007). These studies provide evidence that urban environments may act as a potential refuge for bees in an increasingly agricultural world. Given that pollinators are vital to plant reproductive

success (Ollerton et al. 2011) and crop production (Kremen 2018), it is critical that we understand how they are being affected by our increasingly urban world.

We are currently living in an urban age (Elmqvist et al. 2013). The decline of pollinators has become a global concern (Potts et al. 2010a, Thomann et al. 2013, Ollerton 2017) and a high priority for conservation efforts (Gallai et al. 2009). For example, *Bombus* species are in decline across North America (Colla et al. 2012), with similar declines being documented in Europe (Potts et al. 2010b). If urban environments act as a refuge for pollinators (e.g., Samuelson et al. 2018, Fortel et al. 2014), this represents an exciting opportunity for cities and conservation biology. It is paramount to the health of our ecosystems that we discover opportunities to maximize the current use of urban centres for conservation of wildlife diversity and abundance. Currently, our understanding of the impacts of urbanization on pollinators is weak at best. In the next chapters I describe a study that I undertook to improve our understanding of how pollinators and pollination services change over an urban gradient.

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Chapter 2: Impacts of an urbanization gradient on pollination services to a bee-pollinated plant.

Introduction:

Pollinators play a crucial role in ecosystem processes by providing pollen transfer among plants. Approximately 87.5% of flowering plants benefit from animal pollinators for outcrossed reproduction (Ollerton et al. 2011). Although many plant species are capable of self-fertilization, most rely, at least to some degree, on pollinators as a means of exchanging genetic material among individuals (Ashman et al. 2004). Many plants are pollinated by multiple species of pollinators who may then visit other species of plants, creating a complex network of plant-pollinator interactions (Waser et al. 1996).

Unfortunately, many species of pollinators are in decline, which is negatively impacting the plant species that rely on them (Biesmeijer et al. 2006).

In 1700, nearly 50% of the terrestrial world was untouched by humans, while the remainder was semi-natural habitat with only minor anthropogenic use (Ellis et al. 2010). Three hundred years later, less than 25% of all terrestrial earth remains untouched, largely due to increases in agriculture and urbanization (Ellis et al. 2010). Urban settings (defined by the development of built up areas within close proximity and presence of a dense human population) can differ from natural settings along many axes. For example, they tend to be characterized by a greater ratio of impervious surface to green space than rural area; they exhibit a greater proportion of non-native species (e.g., ornamental and/or alien plants and weeds) (Harrison and Winfree 2015); and they tend to have lower levels of pesticides (Bertoncini et al. 2012, Botias et al. 2017). The global expansion of urban areas has been accompanied by habitat fragmentation and loss of key habitat for many

pollinator species (Klein et al. 2007). Given the rapid increase in the extent of urbanization globally (Seto et al. 2012) and the importance of intact pollinator communities for plant reproduction (Ollerton et al. 2011), understanding the effects of urbanization on plant-pollinator interactions is critical.

The effects of habitat disturbance on pollinator populations are well studied (Potts et al. 2010a). However, studies that explore the direct effects of urbanization on pollinators are rare. For example, in one of the only studies of its kind, Samuelson et al. (2018) reported the reproductive output of the key European pollinator *Bombus terrestris* along an urbanization gradient near London, U.K. Surprisingly, they found a positive correlation between colony size, reproductive output and site urbanization. This supports a growing theory that urban environments can provide a refuge for key pollinator species (Botias et al. 2017, Fitch 2017, Samuelson et al. 2018). On the other hand, many studies report a decline in the species richness and abundance of bees with increasing levels of disturbance (Winfree et al. 2009). If there are general impacts of urbanization on pollinator species, the trend is currently unclear.

In general, pollinators face a number of challenges, including, shifting land use, climate change and pesticides (Goulson et al. 2015, Harrison and Winfree 2015). These threats may, in some cases, be exacerbated by urbanization (Potts et al. 2010a). Increasing the urbanization of natural and semi-natural habitats is therefore likely to impact pollinators and pollination systems (Harrison and Winfree 2015, Johnson and Munshi-South 2017). Urbanization is expected to increase dramatically over the coming decades (Tilman et al. 2001), making it critical that we better understand how plant-pollinator relationships are affected by land use changes.

The presence of adequate nesting habitat and the distances among foraging areas are two aspects of bee biology that may be especially vulnerable to increasing urbanization (Hernandez et al. 2009). Floral resource availability and diversity are also critical to healthy bee populations (Potts et al. 2003), and increased urbanization may lead to fewer, smaller foraging patches (Matteson et al. 2013). These patches tend to be isolated from one another (Dubois and Cheptou 2016, Cheptou et al. 2017), meaning that bees have to travel further to provide adequate resources to rear their young (Williams and Kremen 2007, Hennig and Ghazoul 2011). It has been documented that the quantity, quality and connectivity of foraging patches can greatly affect bee species richness (Hopfenmüller et al. 2014, Dorchin et al. 2018). If urbanization is altering these aspects of foraging patches, this could impact urban bee communities.

Many bee species nest either in the ground or in cavities (Linsley 1958, Michener 2007). Many ground nesters require soft, sandy substrates to build nests (Cane 1991, Potts et al. 2005) and cavity nesters require logs, dead tree snags or hollow plant stems (Sheffield et al. 2008, Grundel et al. 2010). It has been demonstrated that the availability and spatial distribution of nesting resources can have a strong impact on bee nesting rates in Capay Valley, California (Sardiñas and Kremen 2014). The increased amount of impervious surface cover associated with urbanization could make these necessary habitat requirements more scarce (McFrederick and LeBuhn 2006), although there is currently very little known about how and where bees nest in urban environments.

The insufficient delivery of pollen can negatively impact plant reproductive success (Larson and Barrett 2000, Knight et al. 2005, Harder and Aizen 2010). Pollen limitation is a measure of both quality and quantity of pollen received (Ashman et al.

2004, Aizen and Harder 2007). Knight et al. (2005) surveyed 482 studies and found that 63% of animal-pollinated plant species exhibited pollen limitation in at least some years. Based on this finding, they concluded that, due to inadequate pollinator visitation, many species of plants are unlikely to meet their maximum reproductive potential in most years. These effects on reproductive potential hold significant and diverse consequences for natural populations and their outcrossing success (Chacoff and Aizen 2006, Aizen and Harder 2007).

Many species of plants are capable of both selfed and outcrossed reproduction (Goodwillie et al. 2005). Although selfing in self-compatible species can lead to a loss of fitness via inbreeding depression (Dudash 1990, Husband and Schemske 1996), in many species, selfing serves as a reproductive assurance mechanism (Barrett and Harder 1996). If urbanization leads to a reduction in pollination services, this could in turn lead to pollen limitation and/or increased selfing rates among self-compatible plants in urban settings (Aguilar et al. 2006).

I addressed the following questions using a local native species, Jewelweed (*Impatiens capensis*), in a field experiment performed in and around the city of Ottawa, Ontario: (1) Does bee species richness and/or bee abundance change along an urbanization gradient?; (2) Does the pollen limitation of a focal plant species vary along the same gradient?; (3) Do plants with a mixed mating system produce more selfed seeds in more urban environments?

I hypothesized that: (1) Because habitat fragmentation (as a consequence of urbanization) is likely to limit pollinator foraging and the availability of nesting habitat, wild bee species richness and abundance will be lower at more urban sites; (2) Because

urban areas are expected to have a lower number and diversity of pollinators, pollen limitation should be positively correlated with urbanization, and; (3) If pollen limitation is greater in urban environments, plants that produce more selfed seeds as a reproductive assurance mechanism will have higher fitness.

Methodology:

Study Species

Impatiens capensis (Meerb.) (Balsaminaceae) is an annual plant, native to North America and commonly found in moist forested areas around Ottawa. It produces multiple bright orange flowers with dark spots located on the labellum (Rust 1977). Plants produce both cleistogamous (obligately selfing) and chasmogamous (predominately outcrossing) flowers. Cleistogamous flowers, which appear first, do not open at all and appear only as small white buds; these eventually turn into seedpods without pollinator visitation. Chasmogamous flowers appear sequentially on the raceme after cleistogamous flowers, and contain two upper sepals and a single front facing one (Mitchell-Olds and Waller 1985). These flowers attract pollinators (mainly bees in the genera *Bombus* and *Apis*) through a combination of highly nutritious nectar and a brightly coloured display (Rust 1977, Zimmerman and Cook 1985). Being an annual plant, *I. capensis*' lifetime reproductive success is achieved in a single flowering season.

Study Sites

Using the community garden network managed by *Just Food Ottawa* (www.justfood.ca), I located 25 potential field sites that varied in terms of their location in the city, from the downtown core to the rural areas surrounding Ottawa. By focusing solely on community gardens, I was able to standardize many variables that would have otherwise been difficult to control, such as: the type and abundance of floral resources, habitat type, site size, the surrounding plant community and level of human/animal activity. Community garden managers were happy to co-operate with the project and each site also had a source of water. The 25 potential sites were all located within 50 km of downtown

Ottawa. I visited each site, spoke to the garden coordinator, and took photos and notes to describe sites. At each site, I located a 1.5 m x 7 m plot where I could place the study plants in shade (to prevent plant desiccation) and minimize disturbance. Sites were chosen to be a minimum of 750 metres apart, which is near the maximum foraging distance for many native bee species (Osborne et al. 1999, Zurbuchen et al. 2010). Once the study sites were documented, I selected a stratified random sample of 15 actual sites to be included in the study (as described below).

Urbanization Gradient

Using Geographical Information System (GIS) ground cover data obtained from the City of Ottawa (originally developed by Natural Resources Canada), I conducted a spatial analysis in order to assign a level of urbanization to each of the 15 study sites. The GIS data contained distinct layers for all impervious surfaces: roads, streets, driveways, buildings, sidewalks and laneways. Since our main metric of interest was impervious surfaces, non-impervious surfaces were not included in calculations. One pixel on the GIS data is equivalent to 20cm², providing high resolution. Using Arc GIS I combined these impervious surface files into one dataset. I merged all the individual data files into one layer (impervious surface), which is used as a proxy for urbanization throughout the study (Geslin et al. 2016). Each study site was then imported into ArcGIS using its geographical coordinates and converted into a shape file. I then created a radius of 25, 100, 300, 500, and 1000 metres around each study site. This allowed me to calculate the percent cover of impervious surface at a variety of spatial scales. I was then able to calculate the area within each radius that was impervious land cover. Knowing the area of

the circle at each spatial scale, I calculated the percent cover of impervious surface for each study site, at each of the five scales (Table 2.1).

To narrow the original 25 possible sites down to 15, three strata of sites were created based on their percent cover of impervious surfaces: low (0-32%, N = 9), medium (33-65%, N = 8) and high (66-100%, N= 8). Five sites per stratum were selected randomly from the pool of 25 total possible sites. This ensured that the sites included in the study spanned the entire range of urbanization and met the other site criteria (as described above).

On June 12th 2017, 380 2-gallon greenhouse pots and 1.5 cubic metres of Promix© potting soil were purchased from a local nursery. Each pot was filled with soil, watered and stored behind a building on the University of Ottawa campus. On June 14th, with permission from the city, our team collected three hundred and eighty *I. capensis* seedlings from two natural areas within the Ottawa region. Specifically, 300 seedlings were collected from Stony Swamp (4897 Old Richmond Rd, Nepean, ON) and 80 from Shirley's Bay (3701 Carling Ave, Nepean, ON, a site about 10 km away to minimize relatedness). The 300 plants were used as the focal plants in the study and other 80 were kept as spare plants and pollen donors for the hand-pollination treatments. A subsequent trip to both collection sites was required, as some seedlings did not survive the initial collection. Plants were put into 25 L totes while in the field and watered immediately. Back at the university, the seedlings were separated and planted individually into the prepared pots. Upon initial collection, plants were watered daily; over time this was adapted to a two-day watering schedule. Once the plants had fully established in the pots, a random number generator was used to assign 20 plants to each site; plants were also

assigned via a random number to the open or hand pollination treatments and labelled accordingly. Over the following two days, plants were transported to their respective field sites. The spare and donor plants had their natural light conditions replicated as closely as possible in a walled courtyard area on the University of Ottawa campus until pollination treatments began on July 20th.

At each site, 20 plants were arranged in an array. Plants were placed in a shady area on site, in two rows with 10 cm between plants and 10 cm between rows. Hand and open pollination treatments (HP and OP respectively) were interspersed in the array in order to randomize any spatial effects of the array. A HOBO data logger (Model: UA-002) was placed next to the array at each field site to gather environmental data (temperature and light intensity) between July 20th and October 2nd 2017. The data loggers recorded temperature and light every 15 minutes. Light measurements were taken between the hours of 7:00AM and 8:00PM. Six plastic pan traps (2 yellow, 2 white and 2 blue) were also set up in a sunny location at the site where disturbance was unlikely. The bowls were set up in an interspersed manner such that bowls of the same colour were not immediately next to each other.

Data Collection

Sites were visited every other day for watering, maintenance, hand pollination, and eventually, seed collection. Pan traps were filled with soapy water and left for 24 hours, after which bees were removed and placed in ethanol filled Whirlpack© bags for storage at -18°C in the lab freezer. Pan traps were set up a total of 12 times per site on sunny days between July 20th and August 31st. In late July 2017, I visited each study site to collect data on the floral resources that were present. The number of animal-pollinated plant

species (hereafter: plant species richness) in the garden was recorded along with the number of individual plants, and the number of floral heads (on plants with umbel arrangements, individual flowers in an umbel were counted) that were in bloom at the time of visit (hereafter: floral units). This provided information on what and how many floral resources were present at each site. The area of each garden was also recorded using the measurement functions in Google maps (www.google.ca/maps).

During the flowering season, all study plants were monitored for the timing of flower and seed production. Once chasmogamous flowers were open, hand pollination treatments were performed using pollen collected from the 80 non-experimental plants from a separate source population (Shirley's Bay) and stored at the university. To hand pollinate, donor flower anthers were cut off each morning, stored in Eppendorf tubes and brought to the field sites in a cooler. Pollen was collected from the flower using toothpicks and rubbed directly on the recipient stigma until it was visibly saturated (Beans and Roach 2015, Thomsen and Sargent 2017). Since the flowers of *I. capensis* are protandrous, recipient plants were examined for flowers at the appropriate stage. If a stigma was present, pollen was applied to the flower. This procedure was repeated every other day along with regular site visits, ensuring that the stigmas of as many chasmogamous flowers as possible on HP plants were saturated with pollen within a day or two of receptivity.

To assess pollen limitation and the number of cleistogamous and chasmogamous seeds, the total number of seeds produced by each flower on every plant had to be determined. I therefore collected all fruits as they began to ripen. At each site visit, all cleistogamous fruits for a given plant were placed in a single coin envelope while

chasmogamous fruits were placed in another. The envelopes were then labelled according to fruit type (cleistogamous vs. chasmogamous), the number of fruits collected that day, treatment, pot number, site number and date. Since the fruits explode upon contact after ripening, ripe fruits were cut and placed in an envelope to be counted later. This minimized the potential for premature fruit eruption and loss of data.

Once the study plants had finished flowering in late September, they were harvested (on October 2nd 2017) and brought to the lab. During the collection phase, pots were emptied and plants were carefully separated from the soil, saving as much of the root mass as possible. Once the harvest was complete, the plants were placed in a paper bag that was labelled with treatment, pot number, site and the date. Back in the lab, the plants were dried for 72 hours at 65 degrees Celsius in a drying oven (Beans & Roach 2015). After drying, the plants were removed from the bag, placed on a weigh boat and their dry biomass was recorded. In the fall of 2017, seed envelopes were removed from the freezer and counted so that we could obtain a full seed count for each study plant. In January, 2018 the pan trap samples were organized and sorted according to site. Since flies are not pollinators of *I. capensis*, only the bees were pinned and identified. After being pinned, bees were identified to the genus level using identification keys. The genus was later verified by a local expert at the University of Ottawa (Dr. Jessica Forrest). We recorded the identification and number of each bee type in a spreadsheet.

Statistical analysis

All data analyses were carried out in *R*, version 3.4.3 (R Core Team 2017). Figures were made using the *ggplot2* package.

Determining Scale — To examine how bee species richness and abundance, pollen limitation and the production of cleistogamous and chasmogamous seeds varied with site urbanization, I first needed to select which spatial scale to use for the analyses. For each study site, the percentage-cover of impervious surface (hereafter urbanization) was calculated at five different spatial scales: 25, 100, 300, 500, and 1000 metres (as described above). Since the primary pollinators of *I. capensis* are bees, I determined which of the five scales best described the bee data and then used this scale for all subsequent analyses (Fitch 2017). Using the total number of bee species present at each site (hereafter bee species richness) and total number of individual bees (hereafter bee abundance) as response variables, I created 10 separate linear models. A mixed model was not necessary as all variables were at the site-level. The sole predictor in these models was urbanization; this was substituted in each model for a different scale. For each linear model, the Akaike information criterion (AIC) was used to estimate the quality of the model fit (Johnson and Omland 2004, Fitch 2017). After obtaining AIC values for all 10 models, it was determined that the 100-meter spatial scale had the lowest AIC value for both response variables (Table 2.2). Both models were tested and met the assumptions of linear models (Crawley 2007). Because it was the best scale at which to explain the variation in bee species richness and abundance, hypothesized to be major drivers of differences in pollen limitation, urbanization at the 100-meter spatial scale was used for all subsequent data analysis.

Bee species richness and abundance — In order to examine the factors influencing bee species richness and abundance, I examined two separate linear models. A mixed model was not necessary as all variables were at the site-level. The two linear models were fit

using the 'lm' function (Crawley 2007). The predictor variables included in these models were chosen based on their likelihood of influencing bee activity and behaviour. These were: urbanization (100 m), plant species richness observed over the season, the estimated number of floral units at each site, site area (m²), the average daily light intensity (lux) at each site (from July 20th- October 2rd), and the number of years the site had been actively used as a community garden. As a means of selecting the model with the best fit, these two global models were then put through a model selection process using the 'dredge' function in the MuMIN package (Thomas et al. 2013). A total of 64 models were produced and sorted from lowest to highest AIC. The top six models produced had lower AIC values than any other combination of predictor variables; therefore the remaining combinations were left out of the analysis. Six individual linear models were then created for each response variable (bee species richness and bee abundance); these six models contained the same predictor variables as listed above but were run one at a time instead of in combination. The results of this analysis were interpreted based on the individual univariate models chosen using the 'dredge' function rather than the two global models.

Pollen Limitation — The relationship between the level of site urbanization and pollen limitation (i.e., (Total HP Seeds – Total OP Seeds)/ Total HP Seeds) was assessed using a generalized linear mixed effect model (GLMM) analysis with a negative binomial distribution. A mixed model was fit because the data were organized at the plant level, and therefore site number was included as a random variable in order to account for non-independence of plants at a site. The models were fit using the 'glmer' function in R from the 'lme4' package (Crawley 2007, Bolker et al. 2009). The predictor variables included

in the model were: site urbanization, treatment and total fruits per plant while the response variable was the number of chasmogamous seeds. The interaction term (pollination treatment*site urbanization) allowed me to examine whether the degree of pollen limitation varied according to the degree of site urbanization. The same question was tested using a second model that examined the total number of seeds produced per plant as the response variable. This second model contained the same predictor variables (urbanization, treatment and total fruits); however, in this case, the number of cleistogamous seeds was included in the seed total for each plant. The two models allowed me to contrast how pollen limitation is affected by urbanization while accounting for differences in how study plants invested in selfing vs. outcrossing. The total number of fruits was included to account for differences in plant size. Both of these models met the assumptions of a generalized linear model (Bolker et al. 2009).

Mating system — To assess the relationship between the number of selfed vs. outcrossed seeds and site urbanization, a linear mixed effect model analysis was performed. The model was fit using the ‘lmer’ function from the ‘lme4’ package in R (Crawley 2007, Bates et al. 2014). The response variable in the model was the proportion of total seeds produced by each plant that were from cleistogamous flowers. The predictor variables were urbanization, light intensity (lux), total plant biomass and the total number of cleistogamous fruits produced. Despite the response variable being in the form of a proportion, all models were tested and met the assumptions of a linear model (Crawley 2007).

Results:

Site Description:

The mean site area was $886 \pm 386 \text{ m}^2$ (95% CI, Table 2.3). The total number of flowering heads on plants (floral units) varied among sites, with a mean of $44,662 \pm 21,900$ flowering units (95% CI, Table 2.3). Light intensity also varied among sites, with a mean of $11,486 \pm 4010$ lux units (95% CI, Table 2.3) per day. Unsurprisingly, temperature was similar among sites; the daily mean temperature was $20.1^\circ\text{C} \pm 0.3^\circ\text{C}$ (95% CI, Table 2.3).

I collected a total of 219 bees from 23 different genera over the course of the study (Table 2.3). Any empty pan traps were recorded as zeroes and included in the analysis while any pan traps that were tipped over were noted and excluded. Bee species richness (measured as the number of genera present), varied with a mean of 7.1 ± 2.2 genera per site (95% CI, Table 2.3). Bee abundance also varied with a mean of 14.6 ± 5.57 (95% CI, Table 2.3) total bee specimens collected per site over the course of the study.

Total seed number varied widely across sites with a mean of 62.6 ± 9.4 (95% CI) seeds produced per plant. Chasmogamous seed production was relatively variable, with a mean of 26 ± 7.5 (95% CI) seeds produced per plant across all sites. Cleistogamous seed production on the other hand exhibited little variability, with a mean of 36.6 ± 3.7 (95% CI) seeds produced per pot.

Relationship between pollinators and urbanization

In order to estimate the relationship between urbanization and bee species richness as well as bee abundance, I ran two separate global linear models. The first and second linear models were identical with the exception of the response variable. The response

variable in the first model was bee species richness at each site while the response variable in the second model was bee abundance at each site. The predictor variables for both models were: urbanization, plant species richness, number of floral units, site area (m^2), mean daily light intensity (lux) and the number of years the garden had been active in the community garden network. Using a model selection technique and AIC (See Methods), these models were then sorted to find the one that could explain the most variance in the response variables (Table 2.4). It was determined that univariate models had the lowest AIC and a total of 12 were created; 6 with bee species richness as the response variable and 6 with bee abundance. Univariate model predictors used were the same as listed above (See Methods). In all cases, the slopes were indistinguishable from zero for all of the predictor variables (Table 2.4).

Pollen Limitation:

Ten plants (across 4 sites) died during the field season and were excluded from the analyses. Seeds were categorized as having originated from chasmogamous (open-pollinated) or cleistogamous (self-pollinated) flowers. Two separate generalized linear mixed models were used to assess pollen limitation. One modeled the total number of chasmogamous seeds per plant as the response variable and a second modeled total seeds (cleistogamous + chasmogamous) as the response variable. The second model (total seeds) was run to examine whether the inclusion of selfed seeds altered the results. Overall, in the first model, plants at sites with greater urbanization produced fewer chasmogamous seeds ($p = 0.0221$, Table 2.5; Figure 2.5) and in the second model, fewer total seeds ($p = 3.86 \times 10^{-4}$, Table 2.5) than those at less urban sites. The significant interaction between hand pollination and urbanization in both the chasmogamous seed

($p = 1.88 \times 10^{-9}$, Table 2.5; Figure 2.1) and the total seed ($p = 1.26 \times 10^{-11}$, Table 2.5) models indicates a significant decline in pollen limitation with increasing site urbanization.

Relationship between selfing and urbanization

A linear mixed effect model was used to assess the relationship between plant investment in selfing and urbanization. The proportion of total seeds that were cleistogamous was modeled as the response variable. The predictor variables included in the model were site urbanization, daily mean light levels, plant biomass and the total number of cleistogamous fruits collected. The proportion of cleistogamous seeds produced was negatively associated with a site's daily level of light intensity (lux), ($p = 0.0041$). The proportion of cleistogamous seeds was also negatively correlated with total plant biomass ($p = 2 \times 10^{-16}$) and positively correlated with the number of cleistogamous fruits produced per plant ($p = 0.0178$). There was no relationship between urbanization and cleistogamous fruit production ($p = 0.1399$).

Discussion

One of the major conservation issues associated with urbanization is loss of habitat for a variety of organisms, including those that provide key ecosystem services such as pollination (Harrison and Winfree 2015). For pollinators, increased urbanization is expected to lead to a reduction in nesting and foraging habitat, and, as a consequence, an increase in the distance between foraging patches (Matteson et al. 2013, Johnson et al. 2015b). These factors are predicted to drive declines in pollinator abundance and diversity in urban settings (Harrison and Winfree 2015, Cheptou et al. 2017). In order to test this, I collected data on pollinator species richness and abundance, and measured pollen limitation of a native plant, at sites within the limits of a major Canadian city that spanned a gradient in terms of their degree of urbanization.

Somewhat surprisingly, I found no relationship between urbanization and either pollinator species richness or abundance at a site (Figure 2.2). In addition, contrary to my original expectation, pollen limitation tended to decline with site urbanization. The average daily light intensity at a site was a significant predictor of the proportion of selfed seeds produced by a plant. Previous research has shown that the focal plant species, *I. capensis*, tends to produce more selfed seeds in shadier environments (Waller 1980, Simpson et al. 1985), which is consistent with this finding. Surprisingly, the total number of seeds produced by a plant declined over the urbanization gradient; suggesting that plants at the most urban sites were more resource limited. This was a surprise, and it makes it more difficult to assess the impacts of the pollination treatment.

There are many potential drivers of pollinator species decline, but habitat loss and fragmentation are at the top of the list (Potts et al. 2010a). Although there are good

reasons to suspect that urbanization and associated land use changes (e.g., road and building construction, removal of native vegetation, replacement of natural areas) negatively impact pollinator population dynamics, there may also be some aspects of urban areas that are beneficial to pollinators. For example, recent studies have shown that urban areas tend to have lower levels of pesticides than the agricultural areas that surround them, which could positively impact pollinators (Bertoncini et al. 2012, Botias et al. 2017). Similarly, while a loss of native vegetation may impact the pollinators that specialize on them; urban areas typically offer a much wider variety of plant species diversity than the surrounding areas, due to the introduction of horticultural specimens that are not normally found in the region (Harrison and Winfree 2015, Johnson and Munshi-South 2017). Additionally, urban areas tend to host an array of gardens, parks, recreational and even natural spaces that may operate as a refuge for pollinators (McFrederick and LeBuhn 2006, Baldock et al. 2015, Fitch 2017). In other words, the relationship between pollinator species richness and abundance along an urban gradient is not necessarily clear, and there are reasons to expect a variety of outcomes.

I had initially hypothesized that due to the documented negative effects of anthropogenic disturbance on pollinators (Winfree et al. 2009, Potts et al. 2010a, Desaezgher et al. 2017), pollination services would decline as urbanization increased. However, I found the opposite; plants in the most urban locations were less pollen limited than those in more rural locations, suggesting adequate pollination services. However, this finding is confounded by the finding that seed set also declined across the urbanization gradient, suggesting that plants in the most urban environments suffered higher resource limitation than those in the more rural parts of the city. Resource

limitation, similarly to pollen limitation, is expected to limit the number of seeds available. Studies that have set out to test the impacts of pollen and resource limitation are rare. Campbell and Halama (1993), tested the impacts of each in a fully factorial design and found that both resource and pollen additions lead to an increase in the number of seeds produced in *Ipomopsis aggregata*. In the case of the present study, it's difficult to determine whether the lack of impact of the hand pollination treatment in the most urban sites is attributable to a lack of pollen limitation, or the presence of resource limitation. If plants are too resource limited to produce more seeds, then no amount of supplemental pollen would lead to additional seed production. However, the effect is still strongly significant when a proxy for resources, total fruit number, is included in the model, suggesting that there is some increase in pollen services to *I. capensis* along our urban gradient.

I had also hypothesized that plants in low pollination environments would produce more seeds through selfing. This hypothesis was linked to my prediction that pollinators, and hence pollination services, would decline with increased urbanization. Geslin et al. (2013) examined plant-pollinator networks along an urbanization gradient and discovered significantly fewer interactions in more urban environments than semi-natural and agricultural ones. A recent empirical study demonstrated that both bee abundance and species richness in urban environments were negatively associated with greater proportion of impervious surface in urban centres (Geslin et al. 2016). A decline in availability of pollination services may leave plants with lower reproductive success; plants with mixed mating systems may rely more on selfing to compensate. Although there are some interesting correlations in my data between pollen limitation, selfing rates

and urbanization, more data are required on the pollinator side of the equation in order to determine the source of these results.

My results indicate that, for *I. capensis*, light intensity was negatively correlated with the number of selfed seeds produced by a plant (Figure 2.2). *Impatiens capensis* is known to display plasticity in terms of its mating system (i.e., production of cleistogamous vs. chasmogamous flowers) in response to light intensity (Waller 1980, Simpson et al. 1985) and plant density (Schmitt et al. 1987, Schmitt 1993). Specifically, plants grown in high light environments tend to produce more chasmogamous flowers than plants grown in shady environments. I had attempted to control for differences in light by placing plants in similarly shaded environments at each study site, as well as controlling density through the use of identical plant arrangements across sites. In addition, plants were collected from their native habitat at the seedling stage, and it is possible that allocation to cleistogamous and chasmogamous flowers is determined early in development.

Unexpectedly, total plant seed set declined as urbanization increased (Figure 2.1), simultaneously, the most urban sites produced a greater proportion of selfed seeds than less urban sites. It's possible that due to the plasticity of *I. capensis*, in the most urban sites, shading by tall buildings or other structures limited resource availability (e.g., light) to the arrays, thereby decreasing their resources (and ultimately their seed set); the most urban sites were frequently next to high-rise buildings, which could have impacted the number of hours a day that the site received direct sunlight (although we had HOBO recorders at each site and no correlation was detected between light and urbanization). Indeed, it has previously been documented that large urban buildings can reduce

photosynthetic radiation by up to 50% when compared to fully exposed locations (Tan et al. 2014), although it is surprisingly difficult to find data on this topic. It has also been demonstrated that reduced light levels caused by urban infrastructure can negatively affect plant growth (Song and Li 2016). More research on the impact of urban buildings on shading of gardens and other pollinator resources is required.

Although pan traps are a widely used tool in pollination ecology (Roulston et al. 2007), there are several known issues to consider (Campbell and Hanula 2007, Popic et al. 2013). First, pan traps may underestimate the number of bee species present because larger bodied bees may be able to escape the traps at a disproportionate rate (Cane 1991). On the other hand, especially in areas with low floral resources, pan traps may be prone to overestimating pollinator abundance, because the traps themselves are attractants (Kearns and Inouye 1993). Similarly, in areas with abundant floral resources, pan traps may under sample because, in the presence of real flowers, they receive lower visitation (Wilson et al. 2008, Baum and Wallen 2011). For all these reasons, sweep netting and/or observation may provide a more accurate assessment of which species of, and how many, pollinators are actually present in an area. In addition to the possible obfuscation of patterns due to the collection methods, I also offer that the sampling locations could have obscured potential connections between an urban gradient and pollination/pollinator communities. Community gardens, while a good study system for many reasons, may act as refuges for pollinators (Baldock et al. 2015, Samuelson et al. 2018), due to their high floral diversity and abundance, and their potential to offer nesting habitat (e.g., many community gardens purposely plant bee attracting species of plants; they may also supplement nesting habitat by adding mason bee houses, etc.). As such, community

gardens may harbour a more diverse pollinator community relative to their urban gradient; potentially making it less likely I would detect any pattern of pollinator decline associated with percent impervious surface in the surrounding area. For these reasons, I caution that the bee species richness and abundance data presented here may not be an entirely accurate reflection of the impacts of urbanization. Additional study is clearly needed.

Overall, I found no correlation between site urbanization and bee species richness and abundance. I found that, at the most urban sites, plants produced more selfed seeds, and exhibited lower pollen limitation. It seems likely that the finding that plants at the most urban sites produced fewer seeds, and a greater proportion of selfed seeds, is at least partially explained by differences in resource availability, because of the strong negative correlation between fruit number and site urbanization. Additional data on the specific pollinators of *I. capensis* at my study sites would be useful to better understand the relationship between pollen limitation and urbanization. For example, in the future, using sweep netting or measuring pollinator visitation to focal *I. capensis* plants instead of using a passive collection method could improve my ability to pinpoint the availability of the most relevant pollinators of my focal species.

My data point to a few possible patterns that are not currently recognized. First, the lack of relationship between bee species richness as well as bee abundance and urbanization suggests that some urban environments, such as community gardens, may provide resources that support bee populations and pollination services (Fortel et al. 2014, Geslin et al. 2015). Second, the impact of building shade on plants and pollination in urban settings, and its impact on resource limitation, is underexplored. Overall, my

study makes clear that some of the existing assumptions about the impacts of urban environments on pollination biology may not hold in all cases, and points to new study directions, such as the impact of building shade on urban plant resource availability. How urbanization impacts pollinators and pollination services is clearly an important field with many remaining questions.

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Chapter 3: General Conclusions and Future Directions

Due to their important role in plant reproduction, and their potential to act as a bellwether for ecosystem decline, pollinators and their population dynamics are currently the focus of a large number of ecological studies (Potts et al. 2010a, Kremen 2018). Urbanization, one of the most common types of land use changes, may lead to the fragmentation of pollinator habitats, reducing access to nesting habitat and potentially leading to increased distances between foraging patches (Tschardtke and Brandl 2004, Harrison and Winfree 2015). I had hypothesized that these impacts would drive a decline in pollinator populations along an urbanization gradient. I used a native annual plant to test the impact of an urbanization gradient on pollination services.

Surprisingly, I found no correlation between urbanization and bee species richness or bee abundance in my study sites. Contradictory to my expectations, plants at the most urban sites were less pollen limited than those at the most rural sites. I also discovered that my study plants produced more selfed seeds in the most urban study sites. *I. capensis* has been shown to produce more obligately selfing flowers in shady environments, which is consistent with my finding that more urban sites tended to have lower average light intensity. An alternative explanation for my finding of greater pollen limitation in urban environments is that these environments (community gardens in particular) could offer a refuge for pollinators. This is an exciting possibility that is backed up by studies reporting higher pollinator visitation to urban gardens compared to similar urban areas outside of gardens (Fitch 2017) as well as the finding that bumblebee colony reproductive success can be greater in urban vs. rural areas (Samuelson et al. 2018).

Additional data on the specific pollinators visiting *I. capensis* at my study sites would be important in further understanding the relationship between urbanization and pollen limitation. For example, it's possible that, although I found no general change in total bee abundance or bee richness across my sites, a study that focused on those pollinators that specifically visit *I. capensis* could reveal more detail about changes in pollination service.

Future research should focus on shade effects of urbanization created through the construction of high-rise buildings and how they may impact light availability to ground level plants. This is a factor that may influence plant growth and pollinator behaviour; however, it is largely unexplored. Another avenue for future work is to explore the hypothesis that urban gardens are acting as a refuge for pollinators. This could be done by using a paired experimental design and comparing pollinator communities inside and outside of urban gardens along an urbanization gradient. While my study was unable to directly identify all of the ways in which urbanization impacts plant-pollinator relationships in *I. capensis*, it does highlight the importance of examining other variables such as light when investigating the impacts of urbanization. The field of urban ecology remains an important and exciting area of research with many unanswered questions.

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Tables and Figures:

Table 2.1 Percent area of impervious surface at each spatial scale for all 15 study sites.

Values in table are listed as a percentage and were calculated using the *Zonal statistics as table* tool in ArcGIS version 10.5.

Site Number	Spatial Scale				
	25 Meters (%)	100 Meters (%)	300 Meters (%)	500 Meters (%)	1000 Meters (%)
1	45.7	40.7	40.9	40.5	32.7
2	42.2	11.5	4.4	2.4	3.7
3	33.1	35.8	29.0	26.9	18.8
4	47.8	31.9	30.7	32.2	38.7
5	42.6	31.4	38.1	38.6	34.3
6	26.9	30.4	34.4	34.2	34.9
7	66.7	48.8	56.7	56.2	37.4
8	72.8	27.3	27.7	31.6	39.6
9	35.7	38.7	33.6	30.9	31.0
10	20.0	10.6	21.0	30.1	29.8
11	0.0	4.6	13.1	15.1	16.5
12	85.4	74.5	55.6	64.9	79.0
13	35.3	65.5	57.7	45.3	69.3
14	79.3	85.3	75.2	68.9	76.3
15	67.9	50.3	32.9	26.1	56.9

Table 2.2. List of AIC values obtained from two linear models created to determine the scale at which the response variables (bee species richness or bee abundance at a site) are most correlated with site urbanization.

Spatial Scale	AIC Values	
	Bee Species Richness	Bee Abundance
25 Meters	92.9	120.4
100 Meters	92.1 ¹	120.1 ¹
300 Meters	92.6	120.5
500 Meters	92.8	120.5
1000 Meters	92.7	120.4

¹Model with the lowest AIC values.

Table 2.3. Description of site-wide variables for each study site, including bee bowl collection data.

Site Number	Site Address	Percent Urban Cover (100m)	Site Area (m ²)	Total Floral Units ¹	Light (lux) ²	Temperature (°C) ²	Bee Species Richness	Total Bee Abundance
1	151 Brewer Way, Ottawa	40.7	1752.8	17684	7483.7	21.2	1	1
2	27 Weir Rd. Gloucester	11.5	549.4	45942	13645.7	18.6	2	4
3	2410 Orient Park Dr. Gloucester	35.8	903.9	10932	33097.6	19.8	9	19
4	235 Viewmount Dr. Nepean	31.9	378.1	19087	10114.5	19.5	14	30
5	35 Castlefrank Dr. Kanata	31.4	405.2	159814	5770.9	19.5	13	33
6	12 Dayton Cr. Ottawa	31.4	1417.9	123355	19333.1	20.3	11	18
7	556 Laurier Ave, Ottawa	48.8	3008.8	41692	7482	20.5	11	32
8	300 Peres-Blancs Ave. Ottawa	27.3	598.3	57369	4018.4	19.8	8	13
9	39 Dufferin Rd. Ottawa	38.7	157.9	5855	4784.5	20.1	2	3
10	430 Wiggins Prv. Ottawa	10.6	1879.4	24121	20097.7	20.5	7	14
11	1825 Trim Rd. Orleans	4.6	524.4	59120	19703.4	19.7	7	11
12	31 Rochester St. Ottawa	74.5	248.2	20117	8661.4	20.9	5	11
13	10 Blackburn Ave, Ottawa	65.4	476.4	69204	5530.1	20.5	1	1
14	125 McLeod St. Ottawa	85.3	526	7530	6769.2	20.7	3	3
15	448 Lisgar St. Ottawa	50.3	472.4	8120	5791.5	20.4	12	26

¹Total Floral Units were counted as the total number of flowering heads at each study site.

²Light and temperature were both calculated as a mean daily value from July 20th until October 2nd, 2017.

Table 2.4. Results from the linear models of bee species richness and total bee abundance against a variety of predictors. A total of twelve models were run, six using bee species richness as the response variable and six using bee abundance as the response variable. The predictor variables were: urbanization, plant species richness, number of floral units, the garden area, the mean daily light intensity and the age of the community garden. Each predictor variable was run in a separate model. All variables in this analysis are site-wide. The associated AIC values for each model are listed as well.

Response Variable	Predictor Variables	Estimated Slope	p-value	R squared	AIC Values²
Bee Species Richness	Urbanization ¹	-0.456	0.407	0.053	94.3
	Plant Species Richness	2.27	0.529	0.031	94.6
	Number of Floral Units	1.56	0.212	0.12	93.3
	Garden Area	0.646	0.613	0.020	94.8
	Mean Light	0.747	0.558	0.027	94.7
	Garden Age	0.0489	0.646	0.016	94.9
	Urbanization ¹	0.0791	0.570	0.025	122.3
Bee Abundance	Plant Species Richness	9.50	0.286	0.087	121.4
	Number of Floral Units	3.67	0.242	0.104	121.1
	Garden Area	2.89	0.379	0.059	121.8
	Mean Light	0.813	0.801	0.005	122.6
	Garden Age	0.177	0.507	0.034	122.2
	Urbanization ¹	0.0791	0.570	0.025	122.3

¹Urbanization values from the 100m spatial scale were used.

²AIC values were obtained using the dredge function from the MuMIN package in R.

Table 2.5. Results from the generalized linear mixed models (negative binomial distribution). The response variables were the number of chasmogamous seeds produced per plants and the total number of seeds produced per plant. The predictor variables in the first model were urbanization, treatment, the total number of fruits produced, and the interaction between urbanization and treatment. The predictor variables in the second model were urbanization, treatment, the total number of fruits produced, the number of cleistogamous seeds and the interaction between urbanization and treatment.

Response Variable	Predictor Variables	Estimated Slope	p-value
Total number of chasmogamous seeds per plant ²	Urbanization ¹	-0.613	0.0221 *
	Hand Pollination	0.641	< 3.41x10 ⁻⁹ ***
	Total Fruits	0.508	< 2x10 ⁻¹⁶ ***
	Urbanization * Hand Pollination	0.154	1.88x10 ⁻⁹ ***
Total number of seeds per plant ²	Urbanization ¹	-0.152	3.86x10 ⁻⁴ ***
	Hand Pollination	0.205	< 2x10 ⁻¹⁶ ***
	Total Fruits	0.564	< 2x10 ⁻¹⁶ ***
	Cleistogamous Seeds	-0.101	2.40x10 ⁻¹⁰ ***
	Urbanization * Hand Pollination	0.111	1.26x10 ⁻¹¹ ***

¹Urbanization values from the 100m spatial scale were used.

²Site was included as a random effect in both models.

Table 2.6. Results from the linear mixed effect model used to evaluate the relationship between plant investment in selfing and urbanization. Proportion of total seeds that were cleistogamous was the response variable.

Response Variable	Predictor Variables	Estimated Slope	p-value
Proportion of Cleistogamous Seeds	Urbanization ¹	0.00184	0.1399
	Light	-0.0802	0.0041 *
	Plant Biomass	-0.0352	2 x10 ⁻¹⁶ ***
	Total Cleistogamous Fruits	0.002	0.0178 *

¹Urbanization values from the 100m spatial scale were used.

²Site was included as a random effect in both models.

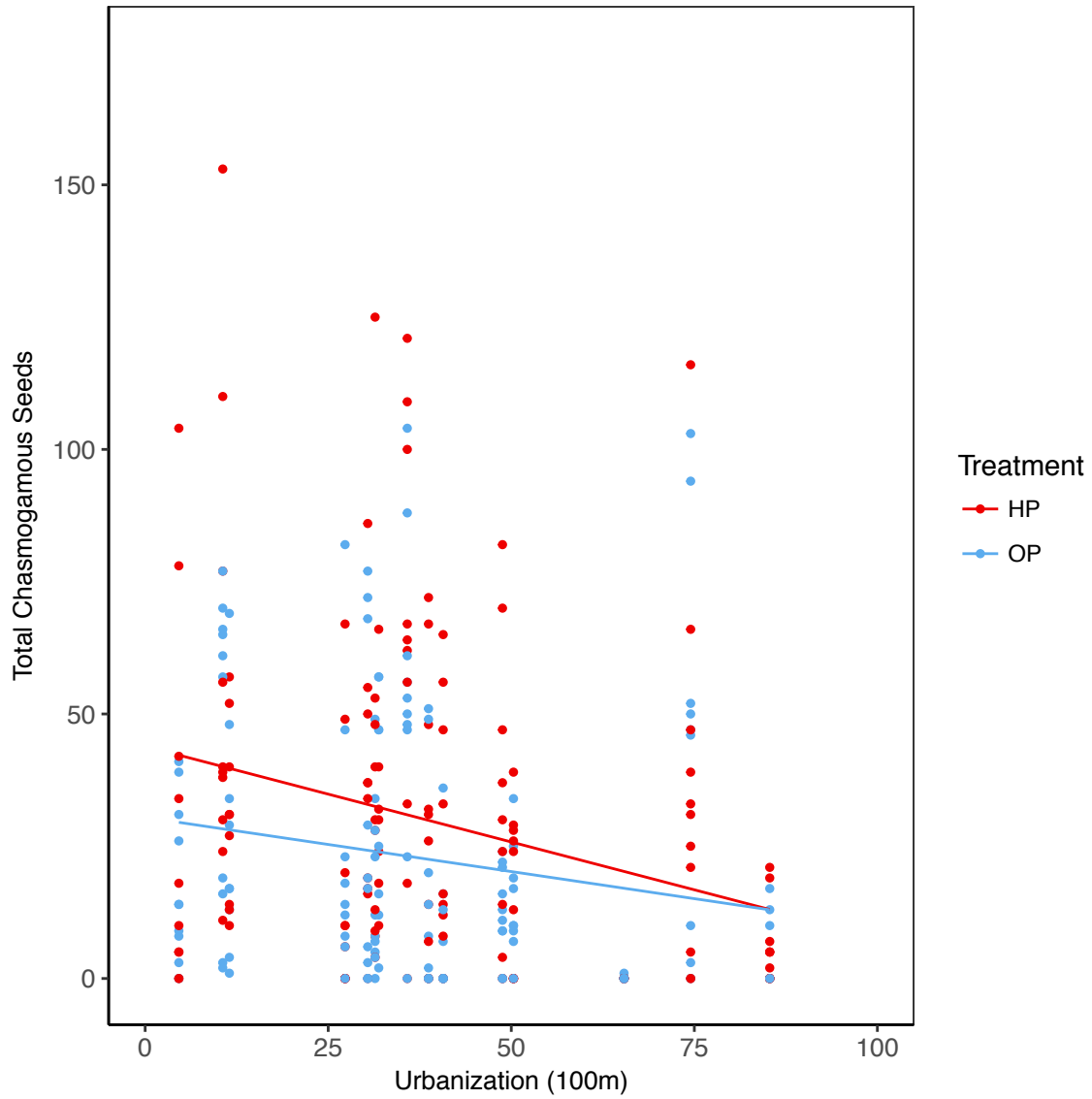


Figure 2.1. Raw data showing the relationship between chasmogamous seed production and urbanization at the 100-metre spatial scale. Data are split by treatment, HP = hand pollination (red), OP = open pollination (blue).

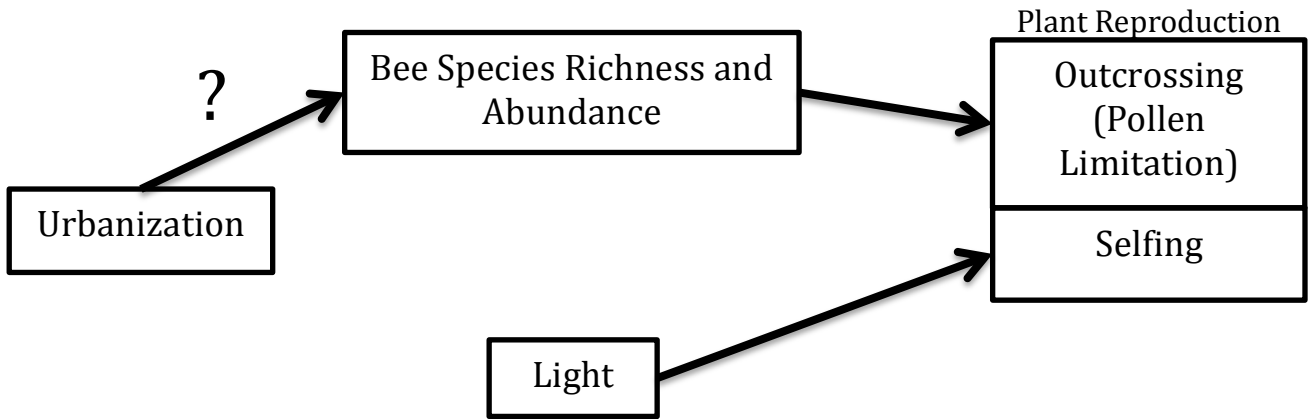


Figure 2.2. Path diagram showing the relationships discovered between urbanization, bee species richness and abundance and plant reproduction. These variables are shown to have the potential to explain pollen limitation and plant investment in selfing. The question mark indicates relationships that were inconclusive.