THE INFLUENCE OF URBAN GREEN SPACES ON DECLINING BUMBLE BEES (HYMENOPTERA: APIDAE)

Jessica L. Beckham, M.S.

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APPROVED:

Sam Atkinson, Committee Chair
Bruce Hunter, Committee Member
Jeff Johnson, Committee Member
Jim Kennedy, Committee Member
Armin Mikler, Committee Member
Art Goven, Chair of the Department of Biological Sciences
Costas Tsatsoulis, Dean of the Toulouse Graduate School

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Bumble bees (*Bombus* spp.) are adept pollinators of countless cultivated and wild flowering plants, but many species have experienced declines in recent decades. Though urban sprawl has been implicated as a driving force of such losses, urban green spaces hold the potential to serve as habitat islands for bumble bees. North Texas is home to some of the fastest-growing urban areas in the country, including Denton County, as well as at least two declining bumble bee species (*B. pensylvanicus* and *B. fraternus*). Using a combination of field, molecular DNA and GIS methods I evaluated the persistence of historic bumble bee species in Denton county's urban green spaces, determined the importance of local and landscape-scale habitat factors to the use of urban green spaces by bumble bee populations in Denton County, and investigated the genetic structure and connectivity of the populations in these spaces. Field sampling resulted in the discovery of both *B. pensylvancus* and *B. fraternus* in Denton County's urban green spaces. While the relative abundance of *B. fraternus* in these spaces was significantly lower than historic levels gleaned from museum records, that of *B. pensylvanicus* was significantly higher. Statistical analyses found that both bare ground and tree cover surrounding sampling sites were negatively associated with numbers of bumble bee individuals and hives detected in these green spaces. Additionally, limited genetic structuring of bumble bee populations was detected, leading to the conclusion that extensive gene flow is occurring across populations in Denton County.

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CHAPTER 1

INTRODUCTION

1.1 Statement of Problem

Bumble bees (*Bombus* spp.) are declining worldwide. In Texas, five of eight documented bumble bee species have shown evidence of decline in other parts of the United States (Berenbaum et al., 2007; Cameron et al., 2011; Colla et al., 2011; Colla et al., 2012; Colla & Packer, 2008; Grixti et al., 2009; Hatfield et al., 2014), including the two historically most common species in north Texas, *B. pensylvanicus* (DeGeer, 1773) and *B. fraternus* (Smith, 1854). However, the persistence of these declining bumble bee species in Texas has only recently been investigated (Beckham et al., 2015, *in review*). In an effort to assess the status of these declining bumble bee species, this research attempts to detect declines from historic levels present in museum collections in Denton County by studying bumble bee species presence at sampling sites.

The loss of habitat due to anthropogenic activity is one of the leading factors in the decline of bumble bees (Winfree et al., 2009), but there is some evidence suggesting that urban green spaces, such as parks and community gardens, provide suitable habitat for bumble bees and may aid in their conservation (Ahrne et al., 2009; McFrederick & LeBuhn, 2006). The present research examines whether urban green spaces in Denton County are contributing to the conservation of declining bumble bees by evaluating the presence and abundance of these species in parks, community gardens and urban wild spaces across the county. Both the numbers of individuals, as well as the numbers of hives those individuals represent, are used to measure

abundance. Additionally, this research investigates the landscape characteristics that are correlated with bumble bee use of urban habitats.

Because habitat loss and fragmentation is often associated with decreased genetic diversity and increased population structuring, I also investigated the genetic characteristics of the bumble bees in Denton County. Specifically, I quantified genetic diversity and population differentiation based on nine microsatellite loci.

In the following section I outline the objectives achieved and hypotheses tested in this study. Over the course of reaching these objective I have contributed baseline data about bumble bees in urban habitats of Denton County, evaluated landscape factors that influence bumble bee presence in these habitats, and explored the genetic connectivity of the bumble bees found across the county. This fine-scale (~35-km) investigation provides information about declining bumble bee species that will be useful for determining conservation measures that are locally appropriate.

1.2 Objectives and Hypotheses of Study

1.2.1 Objectives

1. Establish baseline data on historic presence of *Bombus* species in Denton County from natural history museum and collection records.
2. Gather baseline data regarding presence and abundance of Denton County *Bombus* populations in green spaces using field and molecular DNA methods.
3. Determine whether landscape-scale factors influence the use of green spaces by *Bombus* populations in Denton County using GIS and remote sensing techniques.

4. Evaluate the genetic structure of *Bombus* populations found in Denton County green spaces using molecular DNA methods.

1.2.2 Null Hypotheses

1. Current relative abundance of *Bombus* spp. in urban green spaces is not different from historic relative abundance of *Bombus* spp.
2. Variation in the numbers of *Bombus* individuals observed in urban green spaces is not explained by habitat characteristics.
3. Variation in the numbers of *Bombus* hives detected in urban green spaces is not explained by habitat characteristics.
4. Variation in the numbers of *Bombus* hives inferred in urban green spaces is not explained by habitat characteristics.
5. Measures of genetic variation within *Bombus* subpopulations found at green spaces do not differ by site.
6. *Bombus* subpopulations found at green spaces are not genetically isolated from each other.

1.3 Scope

To assemble baseline data of historic populations in Denton County, bumble bee specimens from the Elm Fork Natural Heritage Museum were identified; these records were added to records from two other databases that contained records from natural history collections across the United States. Current distributions of Denton County

bumble bees in urban green spaces were assessed by sampling at sites of varying urbanization, including gardens, parks and urban wild spaces. A non–lethal tarsal sample was collected from each individual bumble bee for subsequent microsatellite DNA analysis which allowed investigation into county-wide population structure and illuminated the numbers of hives visiting sites. GIS and remote sensing analyses were used to determine habitat characteristics of the land surrounding sampling sites at multiple scales; these factors were statistically related to sampling and microsatellite data to identify habitat factors contributing to variation in the use of urban spaces by bumble bees.

1.4 Contents of Remaining Chapters

Chapter 2 of this document provides a review of pertinent literature. Chapter 3 defines methodology for the study. Chapter 4 details results of all analyses. Chapter 5 includes a discussion of the results, study implications and future work.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

This chapter begins with a discussion of the natural history of bumble bees (Section 2.1). Section 2.2 gives a brief description of pollination and the importance of pollinator species; the particular effectiveness of bumble bee as pollinators is also detailed. A review of literature regarding worldwide bumble bee declines, including possible causes, follows in Section 2.3 This chapter concludes with a discussion of bumble bee conservation tactics, including a review of those important in urban spaces (Section 2.4).

2.1 Natural History of Bumble Bees

Of the approximately 20,000 known bee species (Hymenoptera: Apiformes), about 250 are considered “bumble bees” (Williams, 1998). Bumble bees (Hymenoptera: Apidae) are classified within the monotypic tribe Bombini in the genus *Bombus* (Latreille 1802) and may be readily distinguished from other bees by their large bodies (9-22 mm in length), head shape with long malar spaces, and pollen-carrying structures (corbiculae) on the hind tibiae of females. As is common in entomological classification, much of the generic and subgeneric classification of bumble bees has historically relied on close examination and categorization of the male genitalia (Michener, 2007). In recent years, DNA analyses have also aided in the classification of bumble bees and the elucidation of evolutionary relationships between subgenera. Cameron et al. (2007) provided a comprehensive analysis of bumble bee relationships using DNA sequence data that upheld most of the morphology-based subgenera; these analyses showed that

most subgenera were arranged into two discrete clades that corresponded to morphological differences in malar space length. However, these analyses do suggest that “splitting” some species and subgenera, while “lumping” others, may be an appropriate route for future *Bombus* classification. For the purposes of this research, subgeneric classification of individual bees follows the morphological classification system in Williams et al. (2008) and Colla et al. (2011).

Depending on the species, bumble bees may lead either cleptoparasitic or eusocial lifestyles. Though the focus of this research is on eusocial bumble bees, the cleptoparasitic lifestyle deserves a brief explanation because cleptoparasites may affect the population dynamics of eusocial species.

Cleptoparasitic bumble bees, also known as cuckoo bees, are currently classified in one small subgenus, *Psithyrus*. Whereas eusocial bumble bees live in hives that consist of individuals of three castes (a detailed explanation of this system follows), cleptoparasitic species consist of just two types of individuals: reproductive males and reproductive females. Males are present for the singular purpose of mating, while mated females parasitize eusocial nests belonging to their bumble bee host species by entering and killing the queen and then laying eggs which are reared by the existing (old queen’s) workers. When these eggs have matured to adulthood, the new cleptoparasites will leave the nest, breed, and then parasitize other eusocial hives (Goulson, 2010). In Texas just one cleptoparasitic species, *B. variabilis,* has been documented, though very rarely. This species parasitizes nests of *B. pensylvanicus*.

The majority of bumble bee species are eusocial, living in hives that contain approximately 50-500 individuals which belong to one of three castes: female queen,

female worker, or male drone. The queen is the largest member of the hive, but is otherwise morphologically indistinguishable from workers; her primary role is to lay eggs, though prior to the maturation of her first workers she builds and maintains her own nest. When mature, sterile female worker bees perform the tasks which allow the hive to subsist, including brood-rearing, foraging, guarding the hive, and constructing new wax cells in which eggs develop and pollen or nectar is stored. Males perform no duties within the hive; they are produced mainly late in the hive's life cycle for the sole purpose of reproducing (Goulson, 2010).

The bumble bee hive typically presents an annual (univoltine) life cycle that begins in the spring when the young queen bumble bee emerges from her winter hibernation site and begins to forage for the nectar and pollen which will give her energy to initiate her nest. Bumble bee queens do not usually dig their own nests, and so search for crevices and holes, often taking up in abandoned rodent nests. Some species (including *B. fraternus*) prefer a subterranean location, while others (such as *B. pensylvanicus*) nest at the ground's surface in substrates like prairie thatch. Once she has found a suitable location, and once her wax glands have developed, the queen constructs the first wax cells of the hive. These cells will hold eggs and pollen, and a special honeypot cell will hold nectar reserves to feed the queen during the night or during bad weather. Having constructed the foundation for her hive, the queen begins to lay fertilized eggs that are destined to become female workers. The first set of brood is typically between 8-14 eggs and the queen herself must keep these warm and feed the larval stages until they emerge as adult workers, about five weeks later. In the early stages of the hive's life, the queen continues to leave the nest to forage for pollen and

nectar. However, once her workforce is strong enough, the queen spends most of her time in the nest laying eggs. Workers usually begin their adult lives performing duties within the nest, and progress to foraging bees, though not always (Goulson, 2010; Kearns & Thomson, 2001).

The hive produces mainly workers for most of its life, but if it reaches sufficient size, it switches to producing reproductive offspring near the end of the cycle, in late summer or fall. Moderate-sized hives produce only males and the largest hives will produce both male and new queens; small hives may never switch to producing reproductives (Schmid-Hempel, 1998). Once the queen begins producing reproductive individuals, she will not revert to worker production and so the workforce diminishes until the old hive eventually dies out (Goulson, 2010; Kearns & Thomson, 2001).

Males leave the hive within about three days of eclosion, never to return. They spend their days foraging on flowers and searching for a mate. Young queens initially come and go from the nest, foraging and consuming pollen and nectar to build up large fat reserves for winter hibernation. After five days or so, the young queen leaves the nest in search of a single mate. After mating, the young queen may return to her mother hive for a brief stay while she further builds up energy stores, but at some point late in the season, the young mated queen finds a hibernation site, burrows underground, and lies dormant until she emerges in the spring (Goulson, 2010; Kearns & Thomson, 2001).

The life cycle of an individual bee is holometabolous, consisting of four distinct stages: egg, larva, pupa, and adult. A few eggs are laid in each wax brood cell within the hive and hatch into larvae within about four days, depending on temperature. The larval stage of bumble bee development consists of four instars and lasts approximately

10-14 days. In this period, the young bumble bees consume fresh pollen, which is provisioned in one of two ways. “Pollen storer” species keep pollen reserves in wax cells separate from the brood clump and the queen or workers feed them directly by regurgitating a mixture of pollen and honey. In the latter stages of development, the pollen storer larvae build loose individual cells out of wax and silk and are fed individually. The “pocket makers” force pollen into pockets under the growing brood clump, and the larvae feed collectively. At the end of larval development, a hole is pierced in the top of the wax cell whereby the brood can be fed regurgitated honey and pollen. Larvae of pocket maker species sometimes display marked differences in worker size due to the competition which exists in the collective brood cell. Pupation commences when the larva secretes silk from its salivary glands and spins itself into a cocoon. After about 14 days in the cocoon, eclosion occurs, at which point the new adult emerges and allows its new wings and exoskeleton to harden and take on the characteristic coloration. Growth from egg to adult takes about five weeks, and adult workers may live as long as two months or more, though foraging bees in the height of the season tend to perish sooner (Goulson, 2010; Kearns & Thomson, 2001).

As in most Hymenopterans, the system of sex determination in bumble bees is haplodiploidy, also known as arrhenotoky. In this system, fertilized eggs develop into diploid females, while unfertilized eggs develop into haploid males. Because bumble bee queens are considered monandrous, this system features sisters that are, on average, 75% related genetically (Goulson, 2010).

However, sex determination in bumble bees is not quite as simple as just described. In some cases diploid males may be produced. A single-locus

complementary sex-determination (CSD) model was first proposed to explain this phenomenon in parasitic Braconids over 70 years ago (Whiting, 1939), and recent evidence of triploid individuals in *B. terrestris* furthers the evidence for such a system in bumble bees (Ayabe et al., 2004). In a single-locus CSD system, multiple alleles at a single locus determine sex. Individuals who are heterozygous at this locus will be female, while hemizygotes (as with haploid individuals) and homozygotes will be male. Because diploid male bumble bees produce diploid sperm, they are effectively sterile; diploid sperm often fail to fertilize eggs or produce low-viability triploid offspring (Cook & Crozier, 1995). As will be discussed further along, this feature of bumble bee sex determination can be detrimental to inbred populations.

2.2 Flowering Plants, Pollination and Bees

2.2.1 Pollination of Flowering Plants

Angiosperms (Phylum Anthophyta), the flowering plants, are the most abundant plants worldwide, holding key roles in virtually every ecosystem and comprising a large proportion of the global human food supply. They are defined by the presence of flowers and the production of fruit, wherein seeds are located. “Flowers” hold the reproductive structures of angiosperms, and pollination is required for them to sexually reproduce and greatly increases fruit and seed yield. Pollination is achieved when pollen is transferred from an anther to a stigma of a conspecific plant. The pollen then germinates and ultimately delivers sperm cells to fertilize egg cells in the receptive plant’s ovary, where seed and fruit production will occur. Many flowering plants are unable to self-fertilize, an evolutionary adaptation against the inbreeding depression that

can result; in these plants pollination will occur only if pollen is dispersed from the mother plant, most often by a pollinator species (Dafni et al., 2005).

The relationship between flowering plants and their pollinators epitomizes a mutualism; the flower depends on its pollinator for reproduction while the pollinator in turn procures necessary dietary requirements. These interactions are both ecologically and economically essential; pollinators are often considered “keystone” species in ecosystems (Bond, 1994), organisms that, according to R.T. Paine (1969), ensure “the integrity of the community and its unaltered persistence through time.” Insects might well be considered the most important of such keystone species, as approximately 80% of the world’s wild plants and 75% of agricultural crops depend on entomophily (insect-pollination) for pollen dispersal and pollination (Klein et al., 2007; Potts et al., 2010). Moreover, Garibaldi et al. 2013 showed that wild insect pollinators universally increased fruit yield in 40 economically important entomophilic crops.

Bees are singular in their significance among insect pollinators. Bees are the most frequent flower visitors in a variety of study systems (Neff & Simpson, 1993), and are the primary pollinators of many agricultural crops (Klein et al., 2007). Most species of bees including all bumble bees, are obligate florivores, obtaining nutrition for all life stages from flowers; floral nectar provides carbohydrates, while pollen provides protein and lipids (Michener, 2007). Flowers have evolved elaborate mechanisms by which to attract bee pollinators, including nectar rewards and “nectar guides,” which take advantage of bees’ visual acuity in the ultraviolet range to channel them towards the location of nectar and, presumably, pollen (Dafni et al., 2005). The chances of pollination are also enhanced by physical means. When flying, bees’ bodies gain a

positive electric charge, while flowers and their pollen grains inherently have a negative potential (Corbet et al., 1982; Vaknin et al., 2000). Thus, pollen virtually leaps on to the many hairs covering a visiting bee's body. Though she may earnestly attempt to sequester aberrant pollen into the appropriate carrying compartments, a few grains are often missed leading to inadvertent pollination when the bee lands on a neighboring plant (Michener, 2007).

According to the fossil record, angiosperms experienced marked adaptive radiation about 100 million years ago in the Cretaceous period, subsequently becoming the dominant plants in most habitats on earth (Hickey & Doyle, 1977). In turn, presumably because of the abundance of new sources of pollen and nectar waiting to be exploited, the florivorous bees appeared in the fossil record about 65 million years ago in the late Cretaceous, having diverged from their omnivorous ancestors, the sphecoid wasps (Hymenoptera: Sphecidae) (Michener & Grimaldi, 1988). Flowering plants and bees have continued to differentiate and today both enjoy incredible diversity across most terrestrial ecosystems on the planet.

2.2.2 Bumble Bees as Pollinators

The chances that a flowering plant will be pollinated by a bumble bee are high. The eusocial structure of most bumble bee species makes them the most abundant wild pollinators found in the majority of ecosystems (Ballantyne et al., 2015; Goulson, 2010) and their long tongues and robust, setae-covered bodies collect large amounts of pollen as they forage. They are also polylectic, foraging on a variety of different flower species and thereby influencing entire communities of flowering plants (Memmott et al., 2004;

Michener, 2007). Bumble bees are not only important to the plants that they pollinate, but are crucial to the persistence of ecosystems of which they are a part; simulations of extinction cascades in plant-pollinator networks by Memmott et al. (2004) showed that plant diversity declined most rapidly when most-linked pollinator species, namely the bumble bees, were removed from ecosystems. Moreover, bumble bees are second only to honey bees in their importance in agricultural systems; they pollinate blueberries, cranberries, and clover, and are the exclusive pollinators of greenhouse tomatoes and peppers (Goulson, 2010; Hatfield et al., 2012; Shipp et al., 1994; Whittington & Winston, 2004).

Also contributing to bumble bees' efficacy as pollinators is their frequent demonstration of floral constancy, wherein individual bees visit just one flower species per foraging trip, and sometimes for multiple trips or even multiple days (Darwin, 1876; Thomson, 1981). This behavior may have evolved because it increases the efficiency in which bumble bees handle flowers, though empirical evidence supporting this hypothesis has not been compelling (Laverty, 1994; Gegear & Laverty, 2004). Regardless of its adaptive benefit to the bee, floral constancy serves to restrict the deposition of pollen from an unrelated species onto a given stigma and aids in successful pollination.

The bumble bee may also be seen flying in more extreme conditions than other pollinators, including the honey bee (Goulson, 2010; Heinrich, 1979; Kearns & Thomson, 2001). This is in part because they exhibit physical and behavioral traits which help them to thermoregulate when necessary, overcoming their innate ectothermic metabolism. In order to fly, the bumble bee must maintain a thoracic

temperature between about 30°C and 40°C, and so raises or lowers its temperature when necessary (Goulson, 2010; Heinrich, 1979; Prys-Jones & Corbet, 1991). In cold weather, the bumble bee can elevate its temperature in preparation for flying by shivering its muscles, and the thick setae covering the bumble bee's body provide insulation against heat loss. In warm weather the flying bumble bee, whose wings beat about 200 times per second, generates a great deal of heat (Heinrich, 1979), and so avoids overheating, by circulating its hemolymph from the thorax to the abdomen, which has a large surface area from which heat may dissipate (Goulson, 2010; Heinrich, 1979).

Finally, bumble bees "buzz pollinate," and so are able to pollinate certain flowers that many other bees, including the honey bee, cannot. While the anthers of most flowering plants dehisce along their lengths to expel readily-accessible pollen grains, approximately 9% of angiosperms, including such economically-important crops as tomatoes and peppers, are poricidally dehiscent, releasing their pollen only through small pores (Buchmann, 1985). The pollen in such flowers is effectively locked away from most pollinators. But bumble bees liberate these flowers' pollen by sonication, grabbing hold of the flower and then audibly vibrating their flight muscles until the pollen is expelled (Buchmann, 1985; Kevan et al., 1991; King, 1993).

2.3 Bumble Bee Declines

2.3.1 Evidence for Declines

Unfortunately, many bee species have experienced precipitous declines in recent years (Buchmann & Nabhan, 1996; Kearns et al., 1998; Potts et al., 2010), raising concerns over an impending global pollinator crisis which could destroy native

ecosystems and reduce worldwide food availability (Kearns et al., 1998; Klein et al., 2007; Memmott et al., 2004). The managed honeybee (*Apis mellifera* Linnaeus), whose value is estimated at $15 billion annually in the United States alone, has suffered losses at alarming rates over the past decade (Johnson, 2010), heightening awareness of human dependence on pollinators for food. Worse yet, many species of native bees, which serve as insurance against honeybee losses and are important pollinators in their own rights, are also deteriorating. Bumble bee species are especially troubled among native pollinators, with losses detected across Europe (Beismeijer et al., 2006; Carvell, 2002; Fitzpatrick et al., 2007; Goulson, 2010; Goulson et al., 2006; Goulson et al., 2008; Kosior et al., 2007; Sarospataki et al., 2005; Williams, 1982; Williams, 1986), Asia (Inoue et al., 2008; Matsumura et al., 2004; Xie et al., 2008; Yang, 1999), and North America (Cameron et al., 2011; Colla et al., 2012; Colla & Packer, 2008; Colla et al., 2011; Grixti et al., 2009; Thorp, 2005; Thorp & Shepherd, 2005). These worldwide losses are particularly disturbing because of the central role of bumble bees as native pollinators in many systems (Memmott et al., 2004).

Declines of North American bumble bee species have been historically difficult to assess due to a lack of long-term monitoring efforts (Berenbaum et al., 2007; Buchmann & Nabhan, 1996) and the practical difficulties of sampling entire species ranges. However, recent investigations into the persistence of bumble bees have documented multiple North American species experiencing reductions, sometimes catastrophic, in abundance and distribution at the regional (Bartomeus et al., 2013; Colla et al., 2012; Colla & Packer, 2008; Colla et al., 2011; Grixti et al., 2009; Thorp, 2005; Thorp & Shepherd, 2005) and national (Cameron et al., 2011) scales.

Cameron et al. (2011) assessed eight bumble bee species across their historical distributions in the United States by comparing data compiled from bumble bee specimens in natural history museums to the results of modern field surveys for target species. Over 73,000 museum records dating to 1900 were compared to the results of field surveys from 382 sites in 40 states that took place from 2007-2009. Their results indicated range contractions of up to 87% for four species (*B. pensylvanicus, B. occidentalis*, *B. affinis,* and *B. vosnesenskii*).

In one of the most extensive analyses of the temporal dynamics of bee taxa in North America, Bartomeus et al. (2013) analyzed 140 years of museum records (30,000 specimens) from the northeastern United States to assess the status of 438 species. In this region it was shown that, while non-*Bombus* species richness losses were modest and statistically insignificant, *Bombus* species richness declined by 30% over the study period, suggesting that bumble bees are much more vulnerable than other bees.

Colla et al. (2012) analyzed historical specimen data compiled from records dating to the late 19th century for 21 eastern North American bumble bee species, finding that 11 of the examined species have experienced populations declines of 50% or greater. Based on International Union for Conservation of Nature (IUCN) red list criteria, the authors ranked eight of the 11 declining bumble bee species as vulnerable to critically endangered and in need of habitat protection efforts.

Grixti et al. (2009) compared historic museum records to current field surveys to assess the change in *Bombus* populations in Illinois from 1900-2007. They found that, despite increased sampling efforts, species richness declined markedly between 1940 and 1960, and that populations have not rebounded. Of the 16 species historically

collected, four species (*B. borealis, B. ternaries, B. terricola,* and *B. variabilis*) were not detected between 2000 and 2007, and four others (*B. affinis, B. fraternus, B. pensylvanicus,* and *B. vagans*) had experienced significant range reductions.

Colla and Packer (2008) provided quantitative evidence for the decline of bumble bee species in eastern North America, comparing the results of surveys performed in 2004-2006 to surveys from 1971-1973. Of the 14 species of bumble bees that were originally documented in the study sites, seven showed signs of significant decrease in abundance (*B. fervidus, B. terricola, B. vagans,* and *B. citrinus*) or were absent altogether (*B. affinis, B. pensylvanicus*, and *B. ashtoni*).

In the United States only Arkansas (Warriner, 2011), Illinois (Grixti et al., 2009), and Nebraska (Golick & Ellis, 2006) possess contemporary re-assessments of their bumble bee faunas. Like most other states in this country, the bumble bee fauna of Texas had gone virtually unstudied until Beckham et al. (2015, in review) performed surveyed a 24-county region of northeastern Texas to determine the persistence of historic bumble bee species. Previously, for nearly one hundred years, Franklin (1913) represented the only published account of species known from the state; a treatment that provided very limited detail on species occurrence. That publication listed seven bumble bee species as occurring in Texas. Then Warriner (2012) mapped bumble bee species distribution based on specimen label data and added two additional species to the state's fauna. Based on that work, the bumble bee fauna of Texas is composed of the following species and subgenera: *B. (Bombias) auricomus* (Robertson, 1903), *B. (Pyrobombus) bimaculatus* Cresson, 1863, *B. (Thoracobombus) fervidus* (Fabricius, 1798), *B. (Cullumanobombus) fraternus* (Smith, 1854), *B. (Cullumanobombus)*

griseocollis (DeGeer, 1773), *B. (Pyrobombus) impatiens* Cresson, 1863*, B. (Thoracobombus) pensylvanicus* (DeGeer, 1773), and *B. (Psithyrus) variabilis* (Cresson, 1872). Specimens identified as *B. sonorus* Say, 1837 were also present in the historic state records, but recent molecular evidence (Cameron et al., 2007; Cameron & Williams, 2003) suggests that *B. sonorus* and B*. pensylvanicus* are conspecific and are treated as such for the purpose of the research presented in this study. The two most common of these were *B. pensylvanicus* and *B. fraternus* (Figures 2.1 and 2.2).

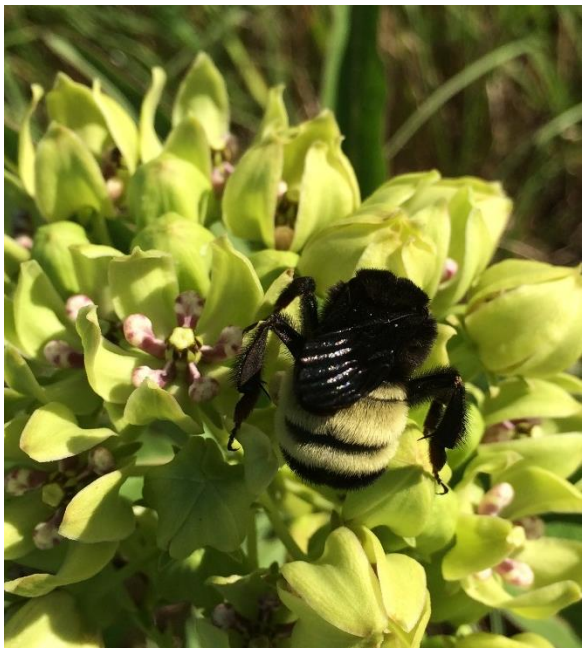

Figure 2.1: Photograph of *B. pensylvanicus*, the most common bumble bee species found historically in Texas.

Figure 2.2: Photograph of *B. fraternus*, the second most common bumble bee species found historically in Texas.

Five of the eight species historically recorded in Texas have been identified as experiencing some degree of decline other regions (Table 2.1), leading the Texas Parks and Wildlife Department to include both *B. pensylvanicus* and *B. variabilis* on their list of species of greatest conservation need for their statewide conservation action plan (Texas Parks &Wildlife Department, 2012). While distributions of these species have been mapped based on historical specimen records, systematic efforts to determine the status of these species in Texas had been lacking until Beckham et al.'s (2015, in review*)* northeastern Texas study. In this research both *B. pensylvanicus* and *B. fraternus*, which are considered declining in other regions, were found to be persisting at similar levels to those historically recorded, suggesting that Texas may be a stronghold for these species and may represent a starting point for their conservation.

Table 2.1: The status of Texas bumble bee species as determined by recent publications and conservation initiatives. Five of the eight historically documented Texas species have been documented as declining (denoted in red). [1]Texas Parks &Wildlife Department 2012; [2]Colla et al. 2011; [3] Berenbaum et al. 2007; [4]Colla & Packer 2008; [5]Grixti et al. 2009; [6]Cameron et al. 2011; [7]- Colla et al. 2012; [8]Hatfield et al. 2014.

Species	Status
B. auricomus	**Vulnerable [7]**
B. bimaculatus	Increasing [4,7], Stable[6]
B. fervidus	**Possibly declining [2], Declining [4], Endangered [7]**
B. fraternus	**Reduced Range [5], Endangered [7], IUCN Red List[8]**
B. griseocollis	Stable [4,7]
B. impatiens	Stable [6], Increasing [4]
B. pensylvanicus	**TX Species of Greatest Conservation Need [1], Possibly declining[2], Declining [3,4,6], Reduced Range [5], Vulnerable [7]**
B. variabilis	**TX Species of Greatest Conservation Need [1], Possibly Extinct [2], Locally Extirpated [5], Critically Endangered [7]**

2.3.2 Factors Involved in Declines

Factors contributing to bumble bee decline on this continent have not been well-defined, but potentially include introduced pathogens (Szabo et al., 2012), pesticides (Gill et al., 2012; Rundlof et al., 2015; Whitehorn et al., 2012), climate change (Kerr et al., 2015), and degradation of suitable habitat (Grixti et al., 2009). Though all of these factors undoubtedly contribute to bee losses, habitat disturbance associated with anthropogenic activity is considered the most detrimental (Aizen & Feinsinger, 2003; Kearns et al., 1998; Winfree et al., 2009). Each of these factors will be described in detail as related to bumble bees in the following section.

2.3.2.1 Ecological Traits

The unique ecological traits of bumble bees may make them especially susceptible to decline and extinction. In particular, bumble bees require not one, but

three, different types of habitats, preferably in close proximity to each other: a hibernation site for the young queen over winter, a nest site, and foraging sites with flowering plants spring through fall (Colla & Packer, 2008; Hatfield et al., 2012). If any of these habitat types are not available, the hive will not survive. Furthermore, the relatively long colony cycle in which reproductive individuals are only produced towards the end causes the hive to be susceptible to compounding effects of adverse circumstances that may begin with reduced numbers of workers and ultimately result in no reproductive progeny or even premature hive death (Colla & Packer, 2008). Colla et al. (2012) cited shared ecological traits such as late-emerging queens and narrow climatic niches as factors in the bumble bee species declines observed in northeastern North America.

2.3.2.2 Disease

Managed bumble bee hives have become widespread due to the demand for pollinators of greenhouse crops such as tomatoes (Whittington & Winston, 2004) and sweet peppers (Shipp et al., 1994), which require buzz pollination and so are not efficiently pollinated by honeybees. In North America, both *B. occidentalis* and *B. impatiens* were historically kept for commercial purposes until managed *B. occidentalis* populations were decimated by the fungal pathogen *Nosema bombi* (Otterstatter & Thompson, 2008; Whittington & Winston, 2003); today only *B. impatiens* is routinely reared for its pollination services in the United States. Still, managed *B. impatiens* hives tend to have higher pathogen loads of *N. bombi*, as well as the intestinal protozoan *Crithidia bombi* and the tracheal mite *Locustacarus buchneri* (Colla et al., 2006).

Various pathogens are often transmitted from managed to wild populations of bumble bees (Colla et al., 2006; Hatfield et al., 2012; Thorp & Shepherd, 2005), contributing to the decline of some species. Colla et al. (2006) found a significantly higher incidence of the pathogens *N. bombi* and *C. bombi* in wild populations of bumble bees located near greenhouses than in those that were not, suggesting that pathogen spillover from commercial populations was infecting natural populations. In their recent study of North American bumble bee populations, Cameron et al. (2011) found that two declining species (*B. pensylvanicus* and *B. occidentalis*) carried higher loads of the fungal pathogen *N. bombi* than stable species. Moreover, just as managed bumble bee hives suffer from an increased risk of pathogens, honey bees also face increased risk from a range of pathogens (Smith et al., 2013). Some of these pathogens have been shown to cross genera and infect wild bumble bees who come into contact with honeybees when foraging, including deformed wing virus (Genersch et al., 2006) and Israeli Acute Paralysis virus (Singh et al., 2010). Additionally, Furst et al. (2014) found significant evidence that the prevalence of deformed wing virus and the parasite *Nosema cerani* is linked between honeybees and nearby bumble bees in the field.

2.3.2.3 Loss of Genetic Variation and Increased Population Fragmentation

The single-locus CSD system of gender determination (reviewed in section 2.1) may be causing bumble bees to experience an “extinction vortex” (Berenbaum et al., 2007; Hatfield et al., 2012) wherein the combined effects of population fragmentation and subsequent inbreeding are driving species into decline. As bumble bee populations become smaller and increasingly fragmented due to habitat loss and other challenges,

genetic homogeneity is amplified, increasing the probability that diploid individuals are homozygous at the gender-determination locus; in other words, the likelihood of a colony producing sterile males is increased (Cook & Crozier, 1995). In a colony that originates from an inbred queen, the sex ratio will be approximately 1:1 due to a higher proportion of diploid males (Ayabe et al., 2004), as opposed to a more efficient hive dominated by female workers. These diploid males are much less likely to reproduce and so represent a dead end for the hive's genes, leading to even less diversity in the population. Stochastic models suggest that diploid male production in Hymenopterans increases the probability of population extinction by an order of magnitude over baseline extinction probabilities (Zayed and Packer, 2005).

Evidence from a variety of studies supports the hypothesis that declining bumble bees suffer from reduced genetic diversity. Two declining North American bumble bee species, *B. pensylvanicus* and *B. occidentalis,* display lower levels of gene diversity (H_E = 0.577 and 0.584, respectively) as compared to stable species at the continental scale (Cameron et al., 2011). Similarly, contemporary populations of *B. pensylvanicus* show reduced genetic diversity (H_E = 0.595) as compared to the stable *B. impatiens* (H_E = 0.62) at a smaller, regional scale (Lozier & Cameron, 2009). In Europe, low genetic diversity has also been exhibited by populations of the declining *B. distinguendus* (HE = 0.391; Charman et al., 2010), *B. sylvarum* (HE = 0.39; Ellis et al., 2006) and *B. muscorum* (HE = 0.443; Darvill et al., 2006).

It is unclear whether genetic structuring of populations is more common in declining species than in stable species; conflicting results indicate that population structuring is highly dependent on the species, the environmental matrix and the scale

of inquiry. However, evidence has shown that some populations of declining bumble bees have displayed increased levels of genetic differentiation at regional scales (100 – 500 km), including *B. muscorum* (Darvill et al., 2010) and *B. sylvarum* (Ellis et al., 2006) in the UK and *B. pensylvanicus* (Lozier & Cameron, 2009) in Illinois. The UK studies attributed reduced gene flow in populations in part to the presence of oceanic (geographic) barriers, while Lozier and Cameron (2009) suggest that *B. pensylvanicus* may not adapt well to increasing levels of row-crop agriculture involving non-insect pollinated crops. By contrast, no structuring of four declining species (including *B. pensylvanicus*) was detected at the continental scale in North America (Cameron et al., 2011), but significant differentiation was detected between various regional populations of *B. bifarius*, a stable species (Cameron et al., 2011; Lozier et al., 2013). Jha and Kremen (2013b) also detected regional (100-km) and fine-scale (<10-km) limitations to gene flow in the stable *B. vosnesenskii* in California, attributing population differentiation to urban land use at the regional scale and limited dispersal abilities and/or queen fidelity to natal colony sites at the fine scale. Further complicating the issue, Dreier et al. (2014) found no fine-scale (<10-km) structuring of stable or declining species in agricultural regions of southern England.

2.3.2.4 Climate Change

Climate change has been loosely referred to as a possible driver of bee declines, in part because of the disruption of the beneficial plant-pollinator mutualisms that could result from phenological changes (Berenbaum et al., 2007; Memmott et al., 2007), though empirical evidence for such shifts has been lacking. However, Miller-Struttmann

et al. (2015) recently demonstrated that two alpine bumble bee species in the central Rocky Mountains have evolved significantly shorter tongue lengths over the past 40 years, arguably in response to the advantage that generalist foraging provides in warmer summers. However, sympatric flower species with deep flower tubes have not evolved shorter corollas in response to their pollinators' shifts in morphology, resulting in a mismatch between some plants and their pollinators.

Kerr et al. (2015) presented evidence that the ranges of several North American and European bumble bee species have experienced a northward movement of their southern limits, but that northern limits have remained unchanged. This has resulted in overall contraction of species ranges due to lagging shifts in northern boundaries and is an unexpected consequence of the warming of regions at lower latitudes associated with global climate change; most terrestrial species who have shifted their ranges have displayed a simultaneous change in northern and southern boundaries, and so have experienced no significant net loss in range area. Changes in plant assemblages at northern boundaries may be hindering similar range shifts in bumble bees.

2.3.2.5 Pesticides

A growing body of evidence has implicated the use of pesticides as a factor in the demise of many species, including butterflies (Gilburn et al., 2015), insectivorous songbirds (Hallmann et al., 2014), honey bees (Cresswell, 2011; Henry et al., 2012), and various species of wild bees (Mallinger et al., 2015; Rundlöf et al., 2015). Bumble bees may encounter pesticides while foraging through direct contact with sprays that have been applied to flowering plants or through uptake of systemic chemicals in

contaminated plant products (Goulson et al., 2008). Bumble bees who have been in contact with neonicotinoids, one commonly used class of systemic pesticide, may show reduced food consumption, increase brood mortality, decreased worker survival rates, and lower rates of foraging activity as compared to those who have not been treated with pesticides (Hopwood et al., 2012). Whitehorn et al. (2012) found that bumble bee colonies treated with neonicotinoids yielded a significantly lower colony growth rate and an 85% reduction in the production of new queens as compared to control colonies. When Gill et al. (2012) treated colonies with a combination of both neonicotinoids and pyrethroids that approximated field concentrations, bumble bee worker mortality increased and foraging behavior was impaired, leading to reduced brood production and frequent hive failure. In contrast, when Baron et al. (2014) treated bumble bee colonies with a common pyrethroid pesticide only worker body size was significantly affected by treatment; similarly Mallinger et al. (2015) found that negative effects on worker body size and production were the only measurable consequences of pesticide use on bumble bee performance.

2.3.2.6 Habitat Disturbance

Anthropogenic activity, including agricultural intensification and urbanization, often disrupts bee habitat and has been identified as one of the primary causes of bee losses (Aizen & Feinsinger, 2003; Kearns et al., 1998; Winfree et al., 2009). Such activity can result in fragmentation of bumble bee habitat and populations, limiting dispersal ability and reducing genetic diversity.

Grasslands typically provide optimal habitat for bumble bees in the form of abundant nest sites and a sequence of flowering plants that are available for the entirety of the bumble bee hive's life cycle (Carvell et al., 2006; Goulson et al., 2006; Hines & Hendrix, 2005; Warriner, 2011). In particular, late-emerging bees are associated with grasslands, as the emergence of new queens tends to coincide with the later-season blooming of prairie flowers (Goulson et al., 2005). Moreover, Hines and Hendrix (2005) found that the best predictor of bumble bee diversity in east-central Iowa was the amount of grassland surrounding bumble bee-occupied habitat patches at the landscape level.

Unfortunately, though grasslands were once valued as rangeland for livestock, modern agricultural practices often involve plowing these natural areas to grow vast monocultures (Goulson et al., 2008). In the United Kingdom, 90% of unimproved grassland was lost to agriculture between 1932 and 1984 (Howard et al., 2003), and many grasslands of the United States (Samson & Knopf, 1994) and Texas (Noss, 2013) have been similarly impacted. This shift in agricultural practice has negatively impacted the land's suitability for bumble bees because the associated plowing disrupts the ground-dwelling bumble bee's nest, and crop monocultures reduce availability of season-long forage (Goulson et al., 2008; Grixti et al., 2009); in a study of 15 bumble bee species in Britain, all late-emerging species were associated with grasslands and were declining, while the species that emerged early and mid-season were stable (Goulson et al., 2005). High levels of agricultural intensification and urban expansion in the state of Illinois were considered the major drivers of population declines observed by Grixti et al. (2009).

While high-quality native grasslands have certainly been lost in northeast Texas, these natural community types have not been fully converted to land uses devoid of resources for native bees. Rather, substantial areas of semi-natural grazing lands remain in the region. These are largely unplowed native grasslands of varying quality that still host some assemblage of native plants and managed for cattle-grazing. For the state overall, native rangelands total nearly 40 million ha or 63% of Texas' non-federal rural lands ((U.S. Department of Agriculture, 2013).

Urbanization represents another method by which human activity can disturb bumble bee habitat. The conversion of land to urban areas very often involves the complete destruction of existing foraging and nesting sites. In a meta-analysis of published literature on bee response to human disturbance, Winfree et al. (2009) report that wild, unmanaged bee abundance and species richness both exhibited overall strong negative responses to "extreme" habitat loss, as is consistent with urbanization; habitat loss was considered extreme when the habitat patch contained little natural habitat (5% cover or less), was at least one kilometer from natural habitat, or was a small (one hectare or less) fragment. In particular, the expansion of impervious surfaces has been shown to negatively affect bumble bees, likely because it eliminates ground-level nest sites (Ahrne et al., 2009). Research has also indicated that impervious surface area inhibits bumble bee dispersal and gene flow through populations (Jha & Kremen, 2013b).

2.4 Bumble Bee Conservation

The conservation of bumble bees relies on the maintenance and/or establishment of the three types that they require: foraging grounds that are available spring – fall, nesting sites (typically underground or at ground's surface) and overwintering sites for queens (also underground). Additionally, some studies have highlighted the importance of the surrounding environmental matrix for bumble bee conservation. Hines and Hendrix (2005) found that bumble bee diversity in tallgrass prairies of Iowa was best predicted by both landscape-scale (500-700-m radii around sites) floral resource availability and availability of floral resources at samples sites; in this study bumble bee abundance was best predicted by the percentage of grassland in the surrounding landscape, as well as the abundance of floral resources at sample sites. Stanley et al. (2013) studied the influence of landscape scale habitat factors (700-m radii around samples sites) on the proportions and nesting densities of bumble bees in oilseed rape fields, finding that the amount of arable land surrounding sample sites negatively affected the proportion of one species (*B. cryptarum*) and the nesting density of another (*B. lucorum*).

The limited foraging and nesting habitat associated with urban areas makes them relatively unfavorable environments for bumble bees. However, a handful of recent studies have suggested that urban green spaces such as parks and gardens can serve as habitat islands within urban areas that support native bumble bee populations. Matteson and Langellotto (2010) found that *B. impatiens* was a common visitor in community gardens in New York City and likely an important pollinator of urban agricultural crops. Similarly, six species of bumble bees, including the most common

non-honeybee species in the study, *B. flavirons,* were encountered in a study of bee species diversity and abundance in community and botanical gardens, flower beds, backyards, and urban wild areas in Vancouver, Canada (Tommasi et al., 2004). Chapman et al. (2003) analyzed polymorphic microsatellite DNA loci in *B. terrestris* and *B. pascuorum* collected from flower patches in London, UK, to extrapolate that urban areas attract workers from a surprisingly large number of colonies. The flower patches used as sampling sites were approximately one hectare in area and attracted an average of 96 colonies of *B. terrestris* and 66 colonies of *B. pascuorum*. It seems that workers from different colonies shared foraging resources within sites.

Studies of bumble bees in urban areas have also helped to identify local and landscape-scale habitat characteristics that affect habitat utilization. Though allotment gardens within urban areas of Stockholm, Sweden were found to support bumble bee assemblages, the degree to which this occurred depended on the surrounding landscape; increasing urbanization, measured in terms of impervious surface area, resulted in decreased bumble bee diversity (Ahrne et al., 2009). McFrederick and LeBuhn (2006) detected four species of bumble bee in urban parks in San Francisco, California. They determined that bumble bee abundance was positively associated with resource availability and, more generally, the surrounding matrix, while species richness was negatively affected by the presence of a dominant species, *B. vosnesenskii*. Jha and Kremen (2013a) investigated the utilization of habitat by *B. vosnesenskii* across regions of varying degrees of urbanization in the Bay and Delta bioregions of California. Microsatellite DNA analysis allowed for the estimation of nest density and foraging distance of bumble bees surrounding sample sites. At the landscape scale, impervious

surface area was found to negatively affect nest density. Meanwhile, local-scale factors affected foraging distance: bumble bees traveled long distances in order to visit species-rich floral patches. They also concluded that a simultaneous expansion of urban areas and reduction in natural woodlands would negatively affect bumble bee nesting densities. A population genetics approach was applied by Goulson et al. (2010) to study the effects of landscape on two species of bumble bee (*B. lapidarius* and *B. pascuorum*) found in arable land of Hertfordshire, United Kingdom. Estimation of nest density surrounding sample sites allowed the researchers to determine that the proximity of gardens and local resources such as clover leys and rough grassland significantly influenced the utilization of habitat by bumble bees, highlighting the importance of gardens for these species.

CHAPTER 3

METHODOLOGY

This chapter includes a description of the study area (3.1) and details methodology for achieving the proposed objectives and testing research hypotheses. Methodology is separated into museum work (3.2), field work (3.3-3.4), molecular genetic analyses (3.5), landscape analyses (3.6), and statistical analyses (3.7).

3.1 Study Area Description

Field surveys were conducted in Denton County, Texas, which is centered at 33°12' north latitude and 97°13' west longitude (Figure 3.1). Denton County includes urban, agricultural, and minimally disturbed land cover and spans approximately 2360 square kilometers (911 square miles) in north central Texas (Odom). Denton County straddles the Eastern Cross Timbers and Blackland Prairies ecoregions (Griffith et al., 2004) and historically consisted of native tallgrass prairies, oak-savannas, oak-hickory slope forests, and bottomland hardwood forests. However, over the past century much of this land has been altered for urban and agricultural uses, and it is estimated that less than one percent of the original prairie vegetation persists in these ecoregions (Smeins & Diamond, 1983).

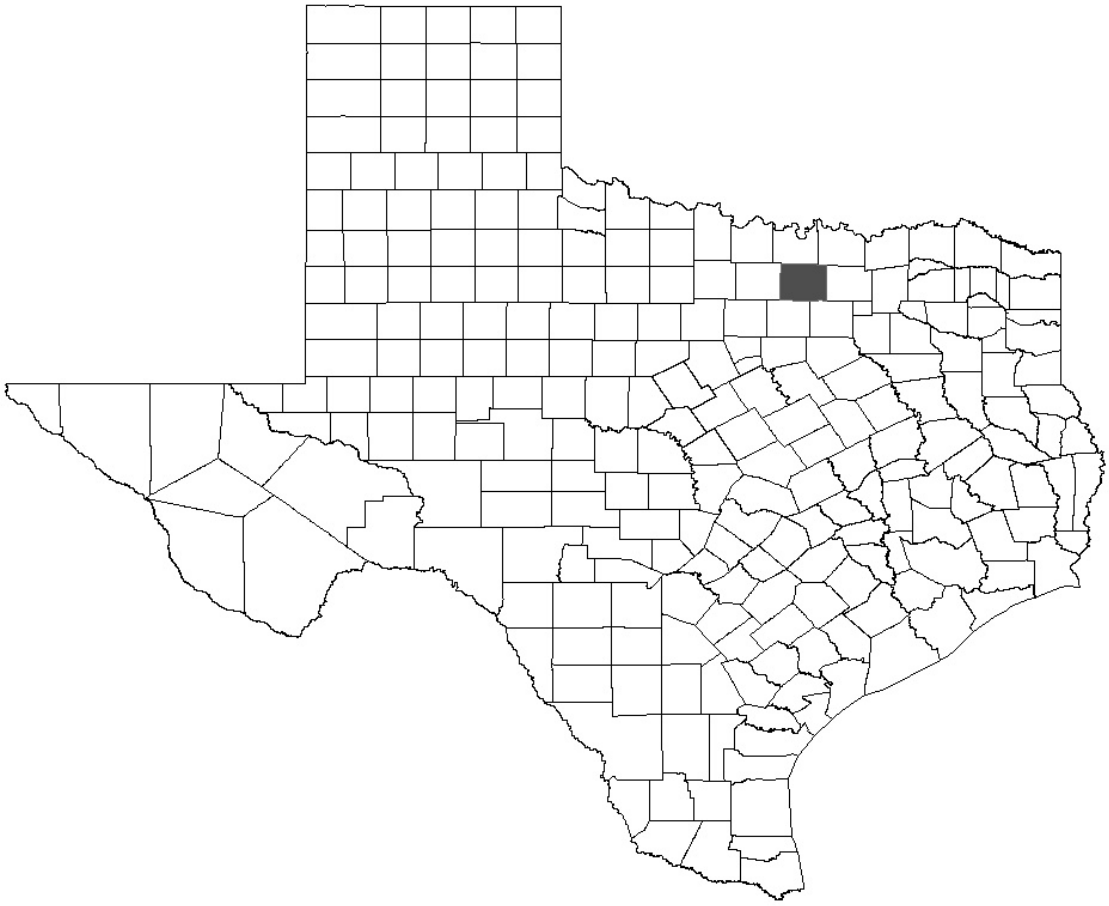

Figure 3.1: Overview map of Texas highlighting Denton County.

3.2 Acquisition of Data from Historic Museum Specimens

Before sampling field sites for current bumble bee presence I established historic presence in the county by assembling data from entomological collections. These data provided baseline presence data and a way to compare historic to current presence in the county. Previously compiled databases were provided by Michael Warriner (Texas Parks & Wildlife Department) and Leif Richardson (University of Vermont); the records in these databases spanned 1905-2012. The Warriner database (Warriner, 2012) included all Texas *Bombus* records from the following collections: Texas A&M

University Insect Collection, Texas Memorial Museum, Illinois Natural History Survey Insect Collection, Cornell University Insect Collection, Florida State University Collection of Arthropods, Mississippi State University Entomological Museum, K.C. Emerson Entomology Museum (Oklahoma State University), Purdue Entomological Research Collection, Smithsonian Natural History Museum, University of Arkansas Arthropod Museum, University of Georgia Collection of Arthropods, University of Michigan Museum of Zoology and University of Minnesota Insect Collection. The Richardson database included additional records from the following: American Museum of Natural History, Canadian National Collection, Essig Museum of Entomology, Los Angeles County Museum, Ohio State University, U.C. Davis Bohart Museum, and the Yale Peabody Museum. Surprisingly, of the over 2,000 statewide records within these databases, only four were from Denton County.

The Elm Fork Natural Heritage Museum (EFNHM) at the University of North Texas houses an entomological collection with roughly 25,000 pinned terrestrial insects dating from the 1950's. Approximately 100 of these specimens, collected between 1952 and 2012, were identified as bumble bees; 80 were from Denton County. Thus, these records were an integral part of the establishment of historic baseline data. Subgeneric classification of these specimens was performed following Williams et al. (2008) and Colla et al. (2011). Classification of select specimens was also verified by Jack Neff of the Central Texas Melittological Institute. Specimen data including date, locality, and collector was obtained from labels attached to pinned specimens. Records were georeferenced (given geographic coordinates) using locality data. Some specimen labels contained specific locations (e.g., North Lakes Park in Denton), whereas others

were labeled more generally (e.g., Denton County). For general localities, the specimen was georeferenced to the center of the city or county provided using Google Earth, version 7.1.5.1557 (Google Inc., Mountain View, California).

3.3 Description of 2013 Sampling Sites

Eight sampling sites of varying size and function were included in the study (Figure 3.2): two urban community gardens (Shiloh Field and Bowling Green Community Garden), one organic farm (Cardo's Farm Project), and five urban wild spaces (Clear Creek Natural Heritage Center and four sites within Lake Lewisville Environmental Learning Area). Distances between sample sites ranged from 0.3 km (between LLELA Buffalo Pen and LLELA Owl Ridge) to 33.9 km (between LLELA Buffalo Pen and Cardo's Farm Project) (Table 3.1). Three other sites (First United Methodist Church garden, the City of Denton landfill garden and the Lake Ray Roberts Greenbelt) were originally planned to be included, but eliminated due to their lack of flowering plants at the time of the study.

Both Shiloh Field and Bowling Green Community Garden are located within the Denton city limits. Shiloh Field is maintained by the Denton Bible Church and covers approximately 14 acres. About half of the land at Shiloh Field contains individual plots that members of the community use to grow food plants and flowers for their own use; the other half of the property contains larger expanses of food plants whose products are donated to various homeless shelters in Denton. Bowling Green Community Garden is owned by the City of Denton and covers about one acre. This site also has plots where individuals in the community may grow their own food. Both Shiloh Field and

Bowling Green Community Garden contain a wide variety of food crops and ornamental flowers.

Cardo's Farm Project is a private organic (pesticide-free) farm that grows a variety of seasonal food crops for distribution through farm shares. The perimeters of the food beds contain volunteer wildflowers. At the time of field surveys, Cardo's was located in Ponder, TX (southwest Denton County) and the area surveyed was approximately 1.65 acres.

Clear Creek Natural Heritage Center is an urban park located in northeast Denton that provides access to more than 2,900 acres of open space that includes bottomland hardwood forest, upland prairie, and various aquatic habitats. This land has been operated and maintained by the City of Denton since 1999. The area surveyed for bumble bees within Clear Creek was approximately eight acres of upland prairie.

Lake Lewisville Environmental Learning Area (LLELA) sits on over 2,000 acres that were reserved for flood control in 1955 when the Lewisville Lake Dam was completed. At that time the land, which had been previously owned by a number of private land owners, was left alone and allowed to return to a wild state. In the 1990s restoration of the native prairies began at LLELA; this effort continues today. LLELA is currently jointly managed by the City of Lewisville, the University of North Texas, and the Lewisville Independent School District in cooperation with the U.S. Army Corps of Engineers. Four separate sites were surveyed for bumble bees within LLELA, including their pocket pollinator garden (0.1 acres), and three separate prairie sites which were 0.6 acres, 2.85 acres and 1 acre, respectively.

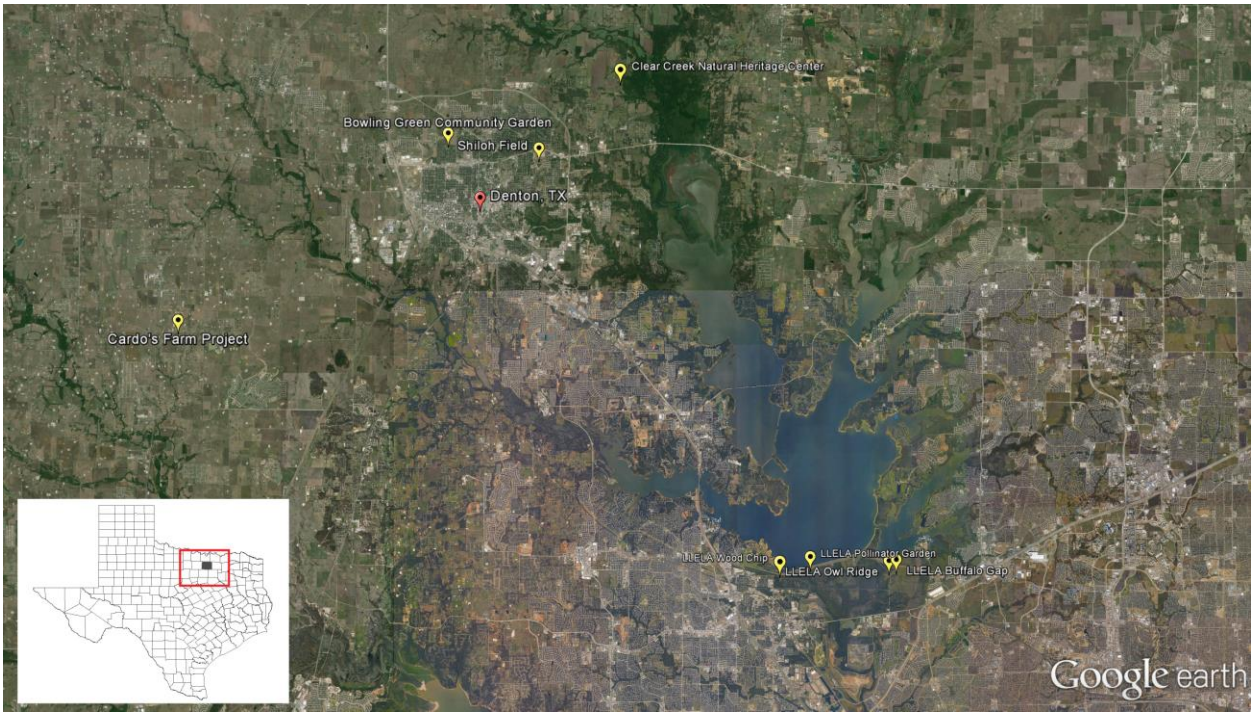

Figure 3.2: 2013 sampling sites in Denton County, Texas. Satellite imagery from Google Earth Pro.

Table 3.1: Distances, in kilometers, between sample sites. Distance ranged from 0.3 km – 33.9 km.

	Bowling Green	Cardo's Farm	Clear Creek	LLELA Buff. Pen	LLELA Owl Rdg.	LLELA Poll. Garden	LLELA Wood Chip	Shiloh
Bowling Green	0	14.8	8.25	27.8	27.5	25.1	24.4	4.2
Cardo's Farm		0	22.8	33.9	33.6	30.2	29	17.9
Clear Creek			0	25.1	25	23.4	23.3	5
LLELA Buff. Pen				0	0.3	3.8	5.2	24.5
LLELA Owl Rdg.					0	3.4	4.8	24.2
LLELA Poll. Garden						0	1.4	22.1
LLELA Wood Chip							0	21.6
Shiloh								0

3.4 Field Sampling of Bumble Bees

Field work was performed during biweekly visits to the study sites during July – August, 2012. Six of the eight sites were visited three times each, but the LLELA Pollinator Garden and the LLELA Buffalo Pen sites were each visited only once. In the case of the Pollinator Garden, very few flowers were available to sample, whereas the Buffalo Pen site was only discovered on my last sampling trip to LLELA. Sampling took place on mostly sunny (<25% cloud cover) days between 7:30 am and 12:30 pm, CST, with ambient temperatures between 21° and 32° C .Dates, times and temperatures for each site visit are shown in Table 3.2.

Table 3.2: Sampling dates and locations of 2013 bumble bee collection trips, with times and temperatures noted.

Date	**Site**	**Time (CST)**	**Temp (°C)**
7/18/2013	Clear Creek Natural Heritage Center	8:15-9:00 am	26.7
7/23/2013	Shiloh Field	8:00-9:00 am	27.2
7/23/2013	Bowling Green	9:20-10:00 am	27.2
7/25/2013	LLELA Owl Ridge	8:00 - 9:00 am	25
7/25/2013	LLELA Wood Chip Pile	9:30-10:30 am	26
7/30/2013	Cardo's Farm Project	8:20-9:00 am	26
8/1/2013	Clear Creek Natural Heritage Center	8:25-9:15	27.2
8/6/2013	Bowling Green	7:35-8:20	26.1
8/6/2013	Shiloh Field	9:10-10:00	27.2
8/13/2013	LLELA Owl Ridge	8-8:45 am	28.9
8/13/2013	LLELA Pollinator Garden	9:05-9:40	28.9
8/13/2013	LLELA Wood Chip Pile	9:50-10:30	29.4
8/15/2013	Cardo's Farm Project	8:10-10:00 am	23.9
8/20/2013	Clear Creek Natural Heritage Center	7:45-9:00 am	22.2
8/20/2013	Shiloh Field	9:15-10:30	26.1
8/20/2013	Bowling Green	11:25-12:30	31.1
8/22/2013	LLELA Owl Ridge	7:45-8:30 am	22.8
8/22/2013	LLELA Buffalo Pen	8:50-10:00 am	27.8
8/22/2013	LLELA Wood Chip Pile	10:15-11:15	31.1
8/27/2013	Cardo's Farm Project	7:45-9:00 am	22.8

The size of the sampling area differed at each site (Table 3.3). At Shiloh Field, Bowling Green Community Garden, the LLELA Pollinator Garden and Cardo’s Farm, we sampled the entire area in which food and flowers were being grown. At the open sites found at Clear Creek Natural Heritage Center and LLELA, sampling areas were

somewhat arbitrarily by fences and flower patches. The exact sampling areas of all sites were calculated by drawing polygons in Google Earth.

Table 3.3: Areas of sites sampled in summer, 2013 (m^2 and acres).

Site	Area (m^2)	Area (acres)
Clear Creek Natural Heritage Center	32375	8
Shiloh Field	54633	13.5
Bowling Green	3673	0.91
LLLELA Owl Ridge	14164	3.5
LLELA Wood Chip	5059	1.25
Cardo's Farm Project	6677	1.65
LLELA Buffalo Pen	5261	1.3
LLLELA Pollinator Garden	607	0.15

During a sampling trip, two to three collectors walked through the designated sampling area, stopping to inspect flower patches for bumble bees. If no bees were detected within one minute, the collector moved on to the next nearest flower patch. Foraging bumble bees were collected from flowers using a 30-cm aerial net fitted with a standard white mesh net bag (mesh size approximately 24 x 20 per inch) and individual plastic collection jars for a total of 30 minutes of collecting time per collector per sampling trip (not to include handling time required to remove bees from net and place in collecting jar). The flower type from which each bee was collected was also noted and effort was made to identify flower species, though not included for analyses.

Once bumble bees had been placed in individual collecting jars, DNA samples were acquired from a non-lethal tarsal clip (Holehouse et al., 2003) in order to minimize damage to populations. Research has shown that this sampling method does not affect worker bumble bee life span or foraging capability (Holehouse et al., 2003). In this

method the terminal 3-4 tarsal segments of each bee's left mid-leg (Figure 3.3) were removed. In order to remove this tissue, each bee (inside its collecting jar) was first placed in a portable cooler that had been filled with ice and ice packs for approximately five minutes. This, in effect, put the bee to sleep and anesthetized it. After an individual bee had been chilled, caste and species was identified in the field and the tarsal segments were removed using a pair of moustache scissors. The segment was placed in a labeled centrifuge tube and stored in 100% EtOH. Once the tarsal segment amputation was complete, the bumble bee was placed in the sun to warm and fly off at its leisure. At least one voucher specimen was taken of each species at each site for verification purposes. These bees were deposited in the Elm Fork Natural Heritage Museum at the University of North Texas or the collection at Texas A&M University.

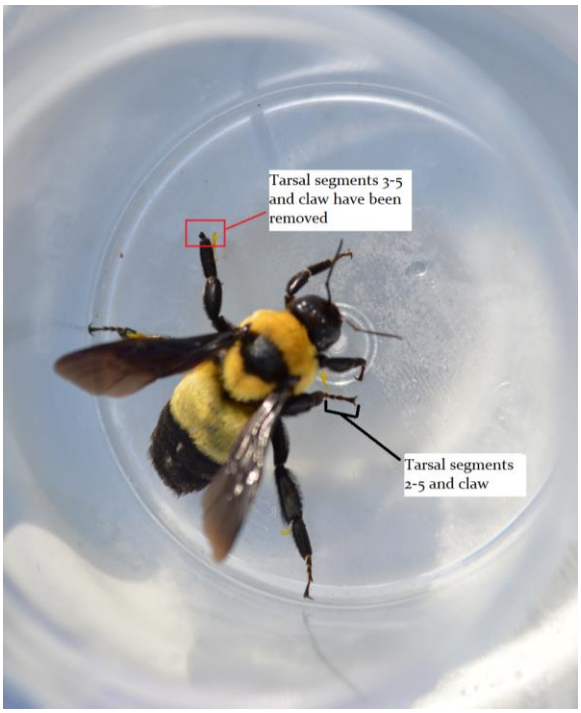

Figure 3.3: B. fraternus with tarsal segments delineated on right mid-leg. Tarsal segments have already been removed from left mid-leg for DNA analysis.

3.5 Molecular Genetic Methods

DNA was extracted, amplified and genotyped from the tarsal clip samples of all *B. pensylvanicus* individuals sampled in the field. Though 15 *B. fraternus* individuals had also been sampled, amplification of DNA at study loci was not achieved and so they were omitted from the DNA analyses.

3.5.1 DNA Extraction

DNA was extracted from each tarsal sample using a modified HotSHOT protocol (Truett et al., 2000). First the tarsal sample was mechanically broken apart into small pieces using razor blade. In order to prevent loss of pieces of sample, the entire sample was tucked in a piece of folded weigh paper and then chopped through the paper. The razor blade was sterilized after each use with bleach solution in order to prevent contamination between samples. The tarsal sample was then placed into a 200 µl PCR tube. Then 25 µl lysis buffer was added to the PCR tube containing sample. Lysis buffer consists of 25 mM NaOH and 0.2 mM disodium EDTA (Table 3.4). Samples were incubated in PCR machine for 30 minutes at 95°C (hot start) and then cooled to 4°C (accomplished with a 4°C hold in the PCR machine). Samples were removed from the PCR machine and 25 µl neutralization buffer was added to each tube (Table 3.5). Samples were vortexed briefly to mix liquids and then stored in -20 C freezer until ready for PCR.

Table 3.4: Reagents required for HotSHOT lysis buffer (12.5 ml final volume, or approximately 500 DNA extractions).

HotSHOT Lysis Buffer Reagent	Volume of reagent for 12.5 ml final volume
1 M NaOH	312.5 µL
0.5 M EDTA	5 µL
DNAse free RNAse free H_20	to 12.5 ml final volume

Table 3.5: Reagents required for HotSHOT neutralization buffer (12.5 ml final volume, or approximately 500 DNA extractions).

HotSHOT Neutralization Buffer Reagent	Volume of reagent for 12.5 ml final volume
1 M Tris-HCl	0.5 ml
DNAse free RNAse free H_20	to 12.5 ml final volume

3.5.2 DNA Amplification

After extracting DNA from each sample, DNA was amplified at nine microsatellite loci in *B. pensylvanicus* individuals using a multiplex approach to the polymerase chain reaction (PCR) (Table 3.6). Though not specifically developed for my study species, these loci had previously been successfully amplified in *B. pensylvanicus* by Lozier and Cameron (2009). Dr. Jeffrey Lozier, who has worked extensively with these microsatellite loci in *B. pensylvanicus* from around the United States, provided allele size ranges for all loci from his Texas bumble bee samples, as well as his multiplex protocols, to aid in multiplex design (personal communication). The B10 locus was also included in PCRs, but eliminated from analyses due to insufficient amplification in multiple trials. Loci were initially amplified in two different multiplexes (multiplex A and multiplex B). Individuals who amplified poorly in their initial PCRs were reamplified one to two times. Later, a third multiplex (multiplex C) was also designed for problem loci and samples that had amplified poorly at one or more of those loci were reamplified

(Tables 3.7-3.9). This process resulted in some individuals being scored at individual loci more than once, allowing for calculation of scoring and allelic drop-out error rates.

PCRs were carried out in 10-µl volumes within 200-µl strip PCR tubes with 2 µl of DNA extract, 0.1 µl of Go*Taq* DNA polymerase (Promega), 2 µl of 1x Go*Taq* reaction buffer (Promega), 0.65 µl of 1.875 mM $MgCl_2$, 0.2 µl of 0.2 mM dNTP, 0.08 – 0.5 µl of each 20 µM primer (forward labeled with NED, 6-FAM or HEX dyes, Invitrogen or Applied Biosystems; reverse unlabeled); ddH_20 was added to reach the final reaction volume. Thermal cycling conditions were: 94°C for 3 minutes; 30-40 cycles of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 30 seconds; followed by a final extension of 72°C for 30 minutes.

In my protocol PCR was carried out as follows. First the PCR “mastermix” was produced with all PCR reagents. Mastermix consisted of the total volume of each PCR reagent (other than DNA sample) required for the number of samples that were being amplified. All reagents were thawed and placed on ice while the mastermix was being made. Prior to adding to mastermix, following reagents were vortexed: $MgCl_2$ and Go*Taq* reaction buffer. The dNTPs, primers and Go*Taq* polymerase were not vortexed. Next the thawed DNA samples were vortexed and 2 µl of each sample was aliquoted into the corresponding labeled PCR tube. Then 8 µl of mastermix was added into each PCR tube. Tubes were spun to assure all liquid was in the bottom of each tube and then placed in the thermocycler, where the appropriate PCR program was implemented. Finally, PCR tubes were removed from the thermocycler and storied in the -20 C freezer until genotyping.

Table 3.6: Microsatellite loci used for *B. pensylvanicus* population analyses. Size ranges, repeat structure and original references for marker descriptions are given.

Locus	Size Range (bp)	Repeat Structure	Source
BT10	117-159	dinucleotide	Reber Funk et al., 2006
BT28	185-191	trinucleotide	Reber Funk et al., 2006
BT30	192-210	trinucleotide	Reber Funk et al., 2006
BTERN01	91-113	dinucleotide	Reber Funk et al., 2006
BL15	153-185	dinucleotide	Reber Funk et al., 2006
B121	134-178	dinucleotide	Estoup et al., 1995
B124	238-262	dinucleotide	Estoup et al., 1995
B126	138-144	dinucleotide	Estoup et al., 1995
B10	Insufficient data	dinucleotide	Estoup et al., 1995
B96	232-266	dinucleotide	Estoup et al., 1996

Table 3.7: Loci combinations for multiplex protocol A. Fluorescent labels for forward primers and volumes per sample of forward and reverse primers are given.

Locus	Dye	Volume per sample in mastermix of forward / reverse primer (µl)
BTERN01	Blue (6-FAM)	0.15
BT30	Blue (6-FAM)	0.3
BL15	Yellow (NED)	0.3
B124	Yellow (NED)	0.4
B10	Green (HEX)	0.5

Table 3.8: Loci combinations for multiplex protocol B. Fluorescent labels for forward primers and volumes per sample of forward and reverse primers are given.

Locus	Dye	Volume in mastermix of forward / reverse primer (µl)
BT10	Blue (6-FAM)	0.15
BT28	Blue (6-FAM)	0.08
B121	Green (HEX)	0.5
B96	Green (HEX)	0.4
B126	Yellow (NED)	0.3

Table 3.9: Loci combinations for multiplex protocol C. Fluorescent labels for forward primers and volumes per sample of forward and reverse primers are given.

Locus	Dye	Volume in mastermix of forward / reverse primer (µl)
BL15	Yellow (NED)	0.3
B96	Green (HEX)	0.4
B124	Yellow (NED)	0.4
B121	Green (HEX)	0.5

3.5.3 Genotyping

Final PCR products were prepared for genotyping and delivered to a technician at the University of North Texas DNA Core Facility for genotyping on ABI 3130xl Genetic Analyzer capillary sequencer (Applied Biosystems) using the following protocol, adapted from instructions provided by Sarah Schulwitz, UNT DNA Core Facility technician. First all PCR tubes were thawed and centrifuged to ensure samples were at bottoms of tubes. PCR products were then diluted in strip tubes to 1/3 original concentration with ddH_20 using a multichannel micropipette. In this case, 20 µl ddH_20 was added to the 10 µl of PCR product. This step was skipped if the sample had been previously genotyped. Next strip tubes were vortexed briefly and spun to bring liquid to the bottom of tube. Then 1 µl of diluted PCR product from each sample was transferred

to the corresponding well in a 96-well intermediate plate. A second dilution, wherein 19 µl ddH_20 was added to each well (20 µl final volume), was then carried out in the intermediate plate using a multichannel micropipette. The entire plate was then sealed with an adhesive foil plate cover, vortexed briefly and centrifuged. The plate cover was then removed and 1 µl of the contents of each well in the intermediate plate was transferred to the corresponding well in a 96-well genotype plate (MicroAmp Optical 96-Well Reaction Plate). For a full plate (i.e., all 96 wells are occupied), 4.12 µl of standard DNA ladder was added to 1240 µl of formamide in a separate tube and vortexed to mix. 12 µl of the standard ladder / formamide mix was then added to each reaction tube using a single-channel micropipette and the plate was covered with a clean septa and centrifuged. The plate was then refrigerated in a light-safe container in refrigerator for no longer than 24 hours before genotyping.

Genotypes were examined manually using GeneMarker (SoftGenetics, State College, PA). Alleles for each locus for each individual were scored by inspecting the electropherograms, which display the fluorescence pattern detected by the genotyping machine. Because each microsatellite locus portrays a unique pattern of peaks in its electropherograms, a standard convention for reading peaks was determined in pilot runs for each locus (Table 3.10). An example of an electropherogram and its scores is shown in Figure 3.4. Raw scores were recorded from GeneMarker and then converted to integer values according to locus repeat structure for analyses purposes. All data were maintained in Excel. In cases where more than four loci could not be scored for an individual the sample was excluded from analyses because the data were considered unreliable. Males were also excluded from population analyses.

Table 3.10: Conventions for scoring peaks for each microsatellite locus.

Locus	Scoring Convention
BT10	Last peak (of 2-3 peaks)
BT28	First peak
BT30	Second peak (of 2 peaks)
BTERN01	Last peak (typically just one peak, but if 2 present, call second)
BL15	Last peak
B121	Last peak (of 2-3 peaks)
B124	Second peak
B126	First peak
B96	Last peak (of 2-3 peaks)

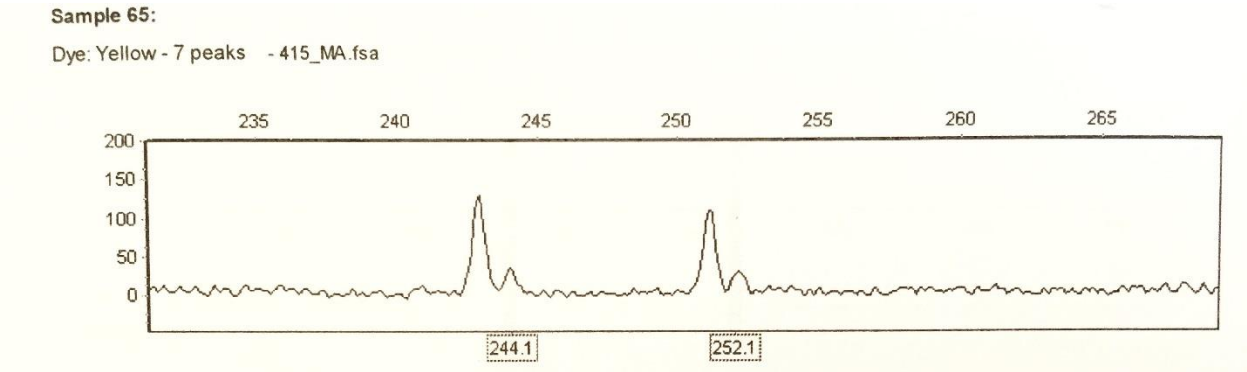

Figure 3.4: Sample electropherogram and scores. Electropherogram for individual 415 for locus B124. This was read as a heterozygote with scores (allele sizes in base pairs) of 244.1 and 252.1. These were scored for analyses as 244 and 252.

3.5.4 Estimation of Marker Characteristics

Descriptive statistics across loci, including observed heterozygosity and expected heterozygosity, were calculated in Arlequin, version 3.5.2.2 (Excoffier & Lischer, 2010) for each population with one individual per hive included; an allowed level of missing data of 0.25 was used for these calculations. Mean expected heterozygosity (H_E) ranges from 0 to 1 and refers to the expected proportion of loci to be heterozygous in an individual; expected heterozygosity was also calculated by locus for each sampling site and translates to the proportion of haplotypes expected to be heterozygous at a given

locus. Mean observed heterozygosity (H_O) also ranges from 0 to 1 and is the actual proportion of loci that are heterozygous in an individual; this was also calculated on a per-locus basis. FSTAT, version 2.9.3 (Goudet, 1995), was used to calculate numbers of alleles and to estimate allelic richness (which measures the number of alleles independent of sample size) for each locus at each site and across sites. All of these statistics give an idea of the genetic variation within and between populations. To compare measures of mean allelic richness, mean observed heterozygosity and mean expected heterozygosity across sampling sites, and test research hypothesis 5, ANOVA was implemented in SAS (PROC ANOVA, $\alpha = 0.05$). ANOVA tests whether mean values of a given dependent variable differ between groups. For these analyses, each dependent variable was blocked by locus.

Loci were tested for the presence of null alleles, which could result in inaccurate estimations of within-population genetic diversity, using the program MICRO-CHECKER, version 2.2.3 (Van Oosterhout et al., 2004). This program estimates the frequency of null alleles from differences between observed and expected numbers of homozygotes. The program was run on a per-site basis and included one randomly-selected individual per hive for analyses, with 95% confidence intervals and 1000 randomizations.

Microsatellite genotypes were tested for linkage disequilibrium (LD) and deviations from Hardy-Weinberg Equilibrium (HWE) in GENEPOP, version 4.4.3 (Raymond & Rousset, 1995; Rousset, 2008). Key modeling assumptions for assigning genetic structure to populations are that there is both linkage and Hardy-Weinberg equilibrium. LD, which is the non-random association of alleles at different loci, was

tested for each pair of loci in each population with one individual per hive included for analyses. Departures from HWE, wherein allele frequencies in a population differ from those expected, were detected using GENEPOP's probability test, which is the exact Hardy-Weinberg test of Haldane (1954) and others. This was run on a per-site basis using one individual per hive and parameters of 10000 dememorizations, 100 batches, and 5000 iterations per batch.

3.5.5 Colony Assignment and Estimation

Once genotypes were determined, COLONY 2.0.5.9 software (Wang 2004) was used to estimate sibling relationships and infer the number of nests that were directly detected from microsatellite data. This program implements a maximum likelihood sibship reconstruction method to assign individuals to unique colonies using genotype data. Because bumble bees are haplodiploid and monoandrous, full sisters share, on average, 75% of their genetic information. In the analysis implemented by COLONY, sisters are distinguished from unrelated individuals on this basis. The numbers of colonies detected per site was equal to the number of sister groups identified.

COLONY was run with the settings indicated in Table 3.11. The marker parameter file indicated that all markers were codominant and included two error rates for each marker. These error rates were calculated from individuals who had been amplified and scored more than once at individual loci using PEDANT 1.0 software (Johnson & Haydon, 2007); in this software maximum likelihood error rates were determined using 1000 search steps. The first error rate required to run COLONY is the allelic dropout rate, which occurs when PCR fails to amplify one of an individual's two

homologous alleles at a locus; this type of error in a heterozygote results in the individual appearing to be a homozygote. The second error rate includes stochastic errors; in this case, only false allele error rates (from polymerase enzyme errors that resulted in incorrect allele calls) were considered because all effort was made to eliminate other sources of stochastic error such as contaminant DNA and data entry errors. The error rates for each marker, as calculated in PEDANT, are include in Table 3.12.

Table 3.11: Parameter settings for COLONY runs.

Parameter	Run Setting
Mating System I	Female Monogamy, Male Monogamy
Mating System II	Without Inbreeding, Without Clone
Species	Dioecious, Haplodiploid
Length of Run	Medium
Analysis Method	Full-Likelihood
Run Specifications	No – Update Allele Frequency No – Sibship Scaling One run Unique random number seed generated for each run
Sibship Prior	No Prior

Table 3.12: Marker error rates, as calculated in PEDANT 1.0 (Johnson and Haydon 2007), used for COLONY analysis.

Locus	Allelic Dropout Rate	False Allele Error Rate
BT10	0.034737	0.012864
BT28	0	0
BT30	0	0
BTERN01	0.035705	0
BL15	0.048036	0.084487
B121	0.005797	0.005646
B124	0.067341	0.024078
B126	0	0.000001
B96	0.038007	0.059142

By assigning hive siblings, mark-recapture data were in effect created; these "recaptures" were identified when multiple individuals were sampled from a unique colony. Because field samples only provide a snapshot of the individuals visiting a site at any given time, it was useful to also estimate the numbers of hives not sampled. In order to do this, data regarding the numbers of nests represented by 1, 2, 3, … *k* workers were fitted to a truncated Poisson, allowing for the numbers of unsampled nests (i.e., zero sampled workers) to be estimated. This was achieved by fitting the data to a curve similar to a truncated Poisson distribution using CAPWIRE software (Miller et al., 2005). This program was run using the Even Capture Model, which assumes equal chances of sampling bees from a given colony. Models were run with the following parameter settings: 0.1 search increments, capturability ratios between 1 -20, 95% confidence intervals for population size estimates based on 1000 bootstrap replicates, 1000 replicates to estimate to approximate distribution for likelihood ratio test, and a likelihood ratio rejection region of 0.1.

3.6 Landscape Analyses

Remote sensing and G.I.S. techniques were used to define habitat characteristics within 0.25-km, 0.5-km, 1-km and 2-km buffers around the center of each sampling site; i.e., circular buffers with radii of the given lengths were constructed around each site to analyze land use characteristics. The largest buffer of 2-km was chosen to reflect a reasonable maximum foraging distance for bumble bees within urban landscapes (Greenleaf et al., 2007; Hagen et al., 2011; Jha & Kremen, 2013a; Osborne et al., 2008). In order to complete these analyses, high-resolution satellite

imagery which was acquired on May 7, 2011 (2-m resolution) of the parcels of land surrounding the study sites were purchased from DigitalGlobe (Figure 3.5-3.8).

Figure 3.5: Satellite imagery of land parcel surrounding Cardo's Farm sampling site.

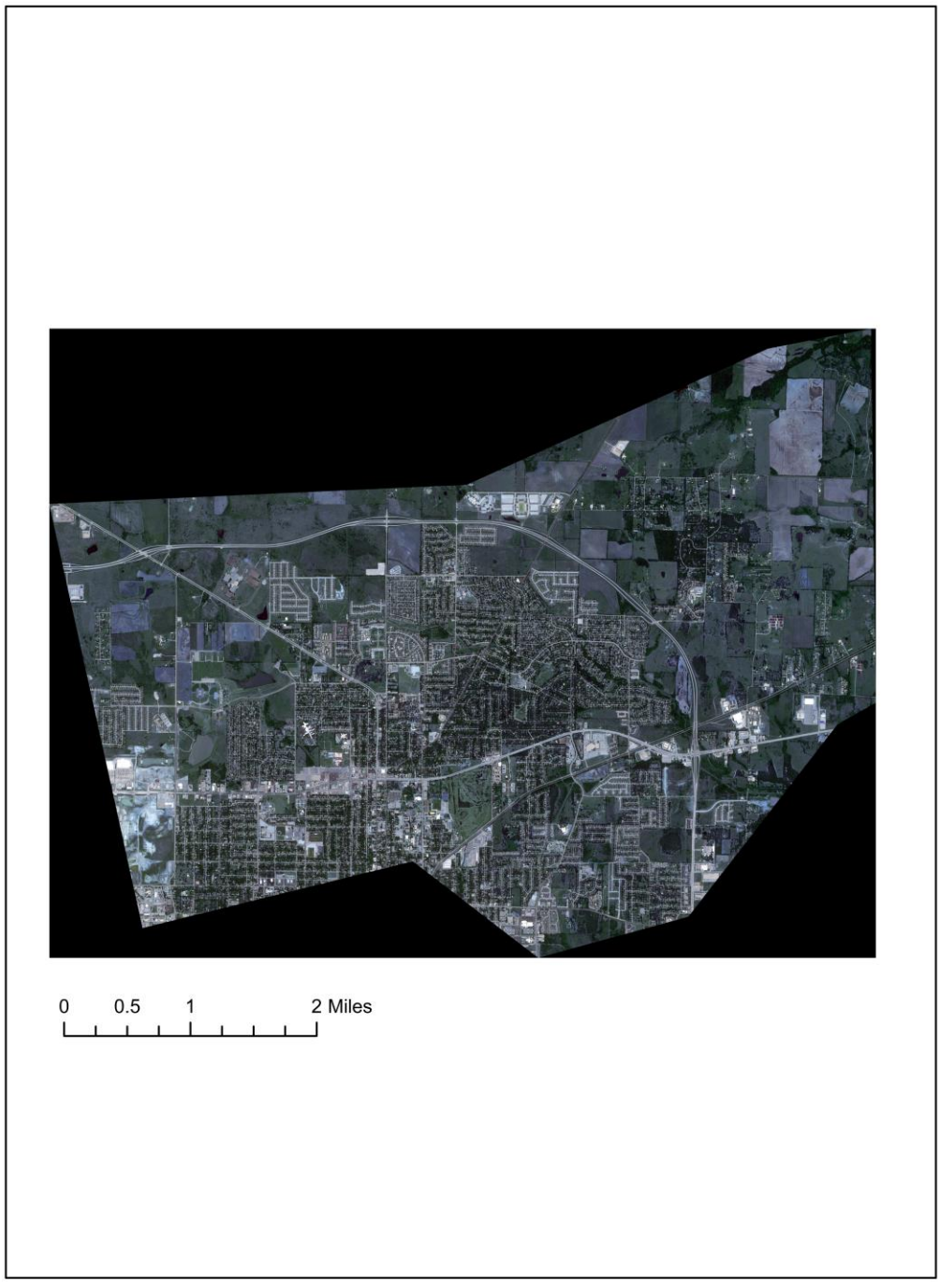

Figure 3.6: Satellite imagery of land parcel surrounding Shiloh Field, Bowling Green Community Garden and western portion of Clear Creek Natural Heritage Center sampling sites.

Figure 3.7: Satellite Imagery of land parcel surrounding eastern portion of Clear Creek Natural Heritage Center sampling site.

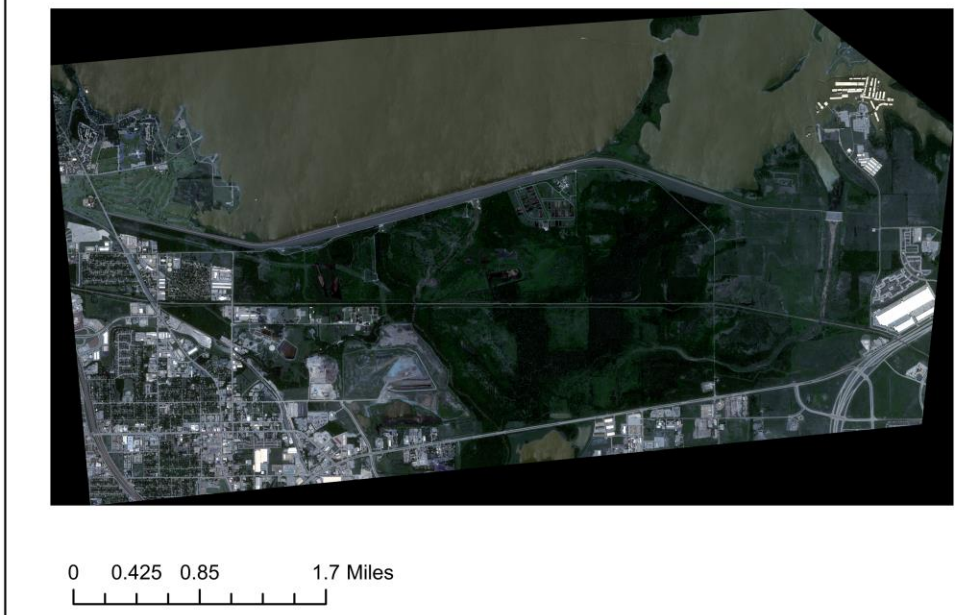

Figure 3.8: Satellite imagery of land parcel surrounding LLELA sampling sites.

Image pixels were classified into one of five land cover types (water, impervious surface, tree cover, bare ground, or vegetation) in ERDAS Imagine 2015 (Hexagon Geospatial, 2015) using the unsupervised classification procedure, followed by a cluster busting process. The initial unsupervised classification was executed using the K Means method to classify the image into a maximum of 100 clusters; processing options were left at default. The output clusters from this step were then classified manually into one of the five classes by comparing the classified ERDAS output to satellite imagery in Google Earth. However, many output clusters contained a mixture of land types, and so could not be placed into a single class. Therefore a cluster busting procedure, modified from that described in Jensen (1996), was next employed to extract more information from the images.

In the cluster busting process, ERDAS Imagine was used to recode clusters that could be easily classified to a new class value of “1”; those that were difficult to classify were recoded to a value of “0.” This in effect created a binary file that was then used to mask the original remote sensing image. An unsupervised classification of the masked image was then performed to classify the new image into up to 50 classes. If necessary, multiple iterations of these steps were performed in order to break up as many clusters as possible. Thus, multiple classified images were created for each land parcel. Ultimately, these were stitched back together using the MosaicPro feature in ERDAS Imagine, and areas that overlapped in land parcels were removed. A step-by-step guide to this process is detailed in Appendix A.

Buffers of diameters 0.25-km, 0.5-km, 1-km and 2-km were then created around sampling points using the buffer tool within geoprocessing tools in ArcMap 10.2.2 (Esri,

2014). Classified images were then clipped to include only buffered areas using the clip tool in the Raster Processing section of ArcToolbox. The histogram values, which correspond to the numbers of pixels in each land class, of each clipped image were then viewed in the corresponding attribute tables and exported into Microsoft Excel, where total areas of each land class were calculated in m^2.

3.7 Statistical Analyses

3.7.1 Species Persistence

A *z*-test of independent proportions was used to evaluate research hypothesis 1 and test whether the current relative abundance of each bumble bee species sampled in Denton County green spaces in 2013 differed significantly from the historic (pre-2000) relative abundance gleaned from museum records; a similar approach was used by Colla and Packer (2008) to assess the persistence of bumble bee species across northeastern North America. Species relative abundances were calculated as the number of individuals of each species sampled during each time period (either pre-2000 or 2013) divided by the total number of individuals sampled in that time period. The *z* test statistic was calculated as

$$z = \frac{p_h - p_c}{\sqrt{\frac{p_h(1-p_h)}{n_h} + \frac{p_c(1-p_c)}{n_c}}}$$

Where p_h = estimated historic relative abundance, p_c = estimated current relative abundance, n_h = total number of individuals sampled in historic time period, and n_c =

total number of individuals sampled in current time period. Two-tailed tests were performed with α level of 0.05.

3.7.2 Relationships Between Landscape Characteristics and Bumble Bee Abundance

In order to test research hypotheses 2-4 and identify the best predictors of variation in the numbers of *Bombus* individuals, hives directly detected and hives inferred, stepwise multiple linear regression analyses were performed at each scale of interest (0.25-km buffer, 0.5-km buffer, 1-km buffer and 2-km buffer). Prior to statistical analyses, dependent variables were averaged across sampling trips for each site to attain a “per sampling trip” value for each dependent variable (i.e., number of individuals sampled per trip, number of colonies detected per trip and number of colonies inferred per trip). Additionally, independent variables were natural log transformed to attain normal distributions. Six of the eight sampling sites were then randomly chosen to include in the modeling phase of analyses. The remaining two sites were used to test the applicability of significant models in the validation phase of analyses.

Pearson’s correlation analyses were performed in SAS 9.4 (SAS Institute) (PROC CORR PEARSON) on all variables to check for collinearity between independent variables and to identify the most important independent variables to be included in each regression analysis. At each scale of interest, the four independent variables that were most correlated with each dependent variable were chosen for inclusion. Additionally, if any of the independent variables chosen for inclusion showed significant signs of collinearity ($\alpha = 0.05$), the variable of the pair that was least

correlated with the dependent variable of interest was omitted from the regression analysis.

A maximum r^2 improvement approach to multiple linear regression modeling (PROC REG / selection = maxr) was implemented in SAS to identify significant models for each independent variable. This method finds the model with the largest r^2 value for 1, 2 … *n* numbers of independent variables by testing each possible combination of *n* variables until no improvement in r^2 is possible. In addition, regressions between most correlated variables, as identified in Pearson's correlation analyses, were also run directly. Models were deemed significant when the model and all regression coefficients for independent variables were significant at the $\alpha = 0.05$ level. Inherently, multiple regression modeling requires the data to meet certain assumptions, including a linear relationship between dependent and independent variables, normally distributed data and no collinearity between independent variables. These assumptions were tested prior to modeling through visual inspection of plots (dependent vs. independent variables), Shapiro-Wilk normality tests (on ln-transformed data) and Pearson's correlation tests (on ln-transformed data). Once significant models had been identified, plots of observed versus expected values from all sites (including validation sites) were created with data from all in Microsoft Excel 2013 for visual inspection of model validity.

A bootstrapping (resampling) step was then performed to investigate the variability of r^2 and adjusted r^2 values for each model that was found to be significant and allow for further investigation of the validity of each model. In this process a dataset of 100 randomly selected combinations (replicates) of six (of eight) sample sites was first created in SAS (PROC SURVEYSELECT). Then a linear regression analysis was

run for each replicate for each regression model that had previously been identified as significant. The r^2 and adjusted r^2 values for each replicate's model were stored in a dataset. Then mean values were calculated for the r^2 and adjusted r^2 datasets for each model in SAS (PROC UNIVARIATE).

Examples of SAS programs are included in Appendix B.

3.7.3 Genetic Structure of Bumble Bee Populations

I investigated whether bumble bees sampled from different sites were genetically isolated from each other, and evaluated research hypothesis 6, using two methods: by calculating pairwise F_{ST} values between sites in Arlequin, version 3.5.2.2 (Excoffier & Lischer, 2010) and by identifying the most likely genetic clusters in STRUCTURE, version 2.3.4 (Pritchard et al., 2000).

F-statistics, first developed by Wright (1951) are commonly used to describe population structure. F_{ST} values range from 0 to 1 and can be used to describe the genetic diversity due to allele frequency differences among populations. Small values of F_{ST} can be interpreted to mean that allele frequencies between the populations being compared are similar. The method of F_{ST} calculation implemented in Arlequin follows that defined by Weir and Cockerham (1984). I performed pairwise comparisons of allele frequencies between sites across all loci using 100 permutations and a significance level of 0.05; one individual per colony was included for the analysis.

STRUCTURE is a program that assigns individuals to population clusters (K) using a Bayesian approach to minimizing linkage and maximizing HWE within unique clusters. Each population is assumed to be typified by a unique set of allele frequencies

at each locus. The software outputs probabilities associated with each value of K that is tested; these probabilities can be interpreted by the researcher to determine the most likely population configuration, or K. The program was run with one individual per colony included, with sampling sites as an *a priori* structure and allowing for admixture; each simulation for K = 1 – 8 was performed five times with a burnin period of 100000 and 500000 MCMC repetitions.

CHAPTER 4

RESULTS

This chapter details study results in the following sections: 4.1 describes historical records compiled from Denton County; 4.2 details results from summer, 2013, field sampling of urban green spaces; 4.3 contains information about marker characteristics and 4.4 covers colony assignment and inference of unsampled colonies; 4.5 describes land use analyses; and 4.6 details all statistical analyses and hypothesis testing. Abbreviations used to identify each sampling site in this chapter are identified in Table 4.1.

Table 4.1: Abbreviations identifying the eight study sample sites.

Site	**Abbreviation**
Bowling Green	BG
Cardo's	CA
Clear Creek	CC
LLELA Buffalo Pen	LBP
LLELA Owl Ridge	LOR
LLELA Pollinator Garden	LPG
LLELA Wood Chip	LWC
Shiloh	SH

4.1 Historic Records of Bumble Bees in Denton County

Prior to this study just four bumble bee specimens consisting of one species (*B. pensylvanicus*) had been documented from Denton County (Warriner, 2012; database from Leif Richardson). By formally documenting specimens from the Elm Fork Natural Heritage Museum I was able to add 79 new records and accounts of three new species: *B. fraternus*, *B. bimaculatus*, and *B. impatiens*. These records spanned from 1952-2012. Overall, 66 of the specimens were identified as *B. pensylvanicus*, 15 were *B. fraternus*, and one each were *B. bimaculatus* and *B. impatiens* (Figure 4.1). Of the two "common" species within the historic records, *B. pensylvanicus* was consistently found in the records across collecting years, but the last specimen of *B. fraternus* was from 1998. This raised the concern that *B. fraternus* was declining in the area, or possibly had experienced local extirpation.

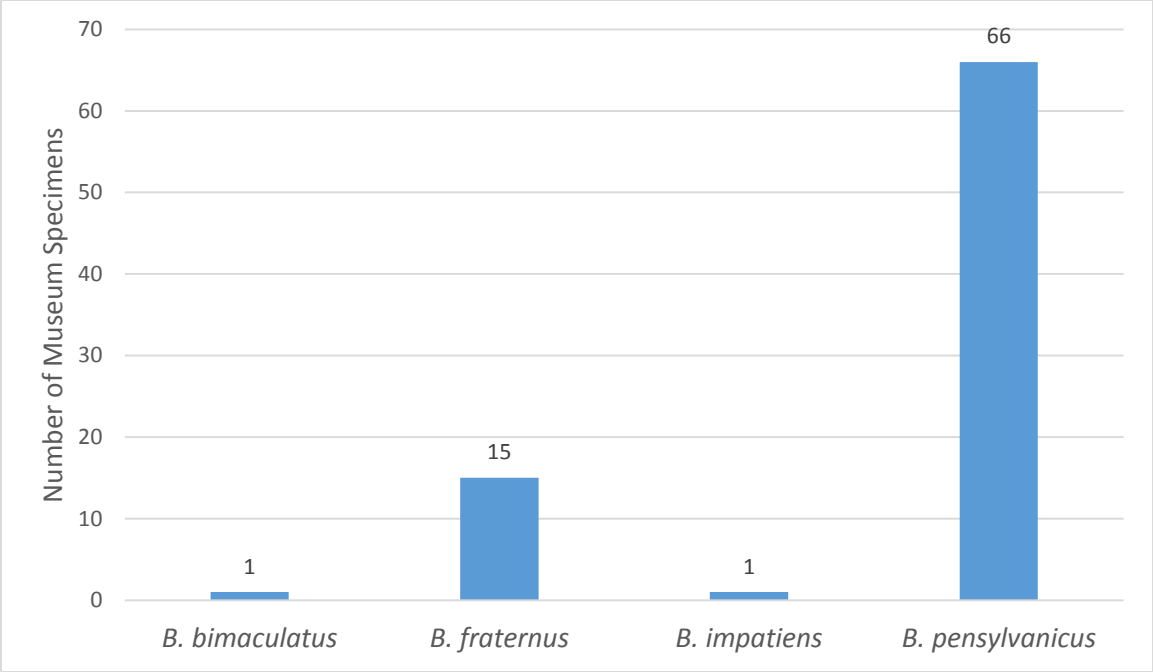

Figure 4.1: Historic numbers of bumble bee individuals of each species collected from Denton County present in museum records (1952-2012). Exact numbers of individuals are listed above columns.

4.2 Results from 2013 Field Sampling

During the course of the summer, 2013, sampling efforts, 450 unique individuals from two species were catalogued. This included a total of 435 individuals identified as *B. pensylvanicus* and 15 identified as *B. fraternus*. These specimens included the first formal records of *B. fraternus* in Denton County since 1998. While *B. pensylvanicus* was encountered at all sample sites, *B. fraternus* was found only at Clear Creek Natural Heritage Center and Bowling Green Community Garden (Figure 4.2). Interestingly, of the sites sampled three times, the two sites that contained two species also had the fewest numbers of bees sampled overall: Clear Creek Natural Heritage Center had the fewest with a total of 25 individuals, followed by Bowling Green with a total of 30 individuals. By contrast, the largest number of individuals sampled at a single site was

141 at Cardo's Farm Project.

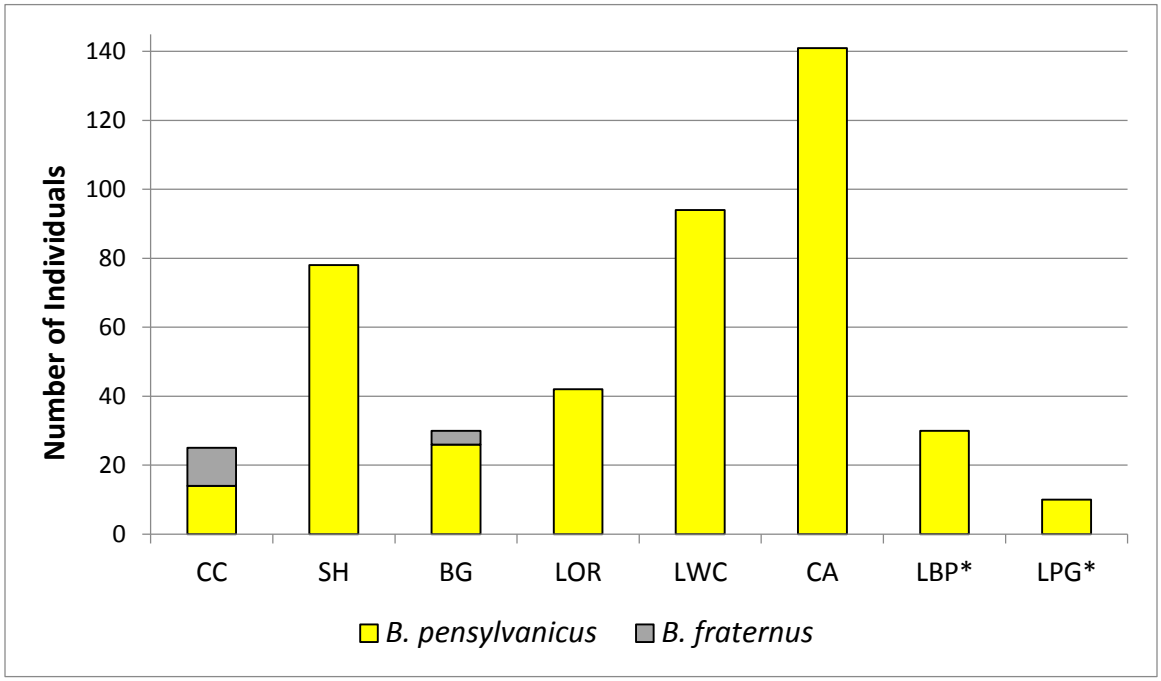

Figure 4.2: Total numbers of bumble bees collected by site during summer 2013 sampling effort. All sites were visited three times except those indicated with *, which were each visited once. Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

The average numbers of *B. pensylvanicus* individuals sampled per trip are reported in Figure 4.3. Like total numbers of individuals, these numbers were also lowest at the sites containing two species; on average, 4.7 ± 0.58 individuals were sampled per trip at Clear Creek Natural Heritage Center and 8.7 ± 6.5 individuals at Bowling Green Community Garden. The highest average number of *B. pensylvanicus* individuals sampled per trip (47 ± 12.3) was at Cardo's Farm Project. These values were used for subsequent statistical analyses (Section 4.5.2).

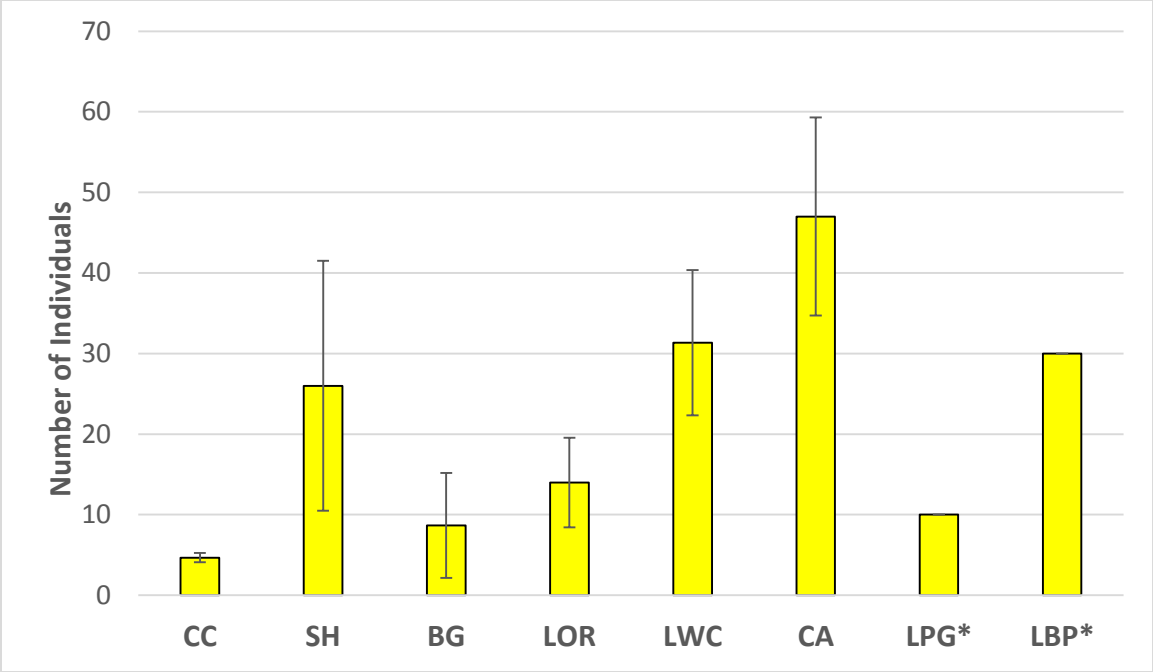

Figure 4.3: Average numbers of *B. pensylvanicus* individuals (mean ± standard deviation) collected per sampling trip, by site, in summer 2013 sampling effort. All sites were visited three times except those indicated with *, which were each visited once. Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

4.3 Microsatellite Marker Characteristics

Of the 402 unique female *B. pensylvanicus* individuals sampled in the field, 342 (or approximately 85%) were successfully genotyped at five or more loci and so were included in the DNA analysis portion of this study. The numbers of individuals sampled and genotyped for each site are shown in Table 4.2. When a scatterplot was produced of the numbers of individuals sampled versus genotyped (Figure 4.4), there was an almost perfect linear relationship ($r^2 = 0.8898$) between the two and so I infer that the individuals who were unable to be genotyped were proportional to total numbers of individuals at each sample site.

Table 4.2: Numbers of *B. pensylvanicus* individuals sampled and successfully genotyped at five or more loci, by sampling site. Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

Site	Number of individuals (sampled)	Number of Individuals (genotyped)
CC	14	13
SH	78	72
BG	26	21
LOR	42	35
LWC	94	87
CA	141	84
LBP*	30	23
LPG*	10	7

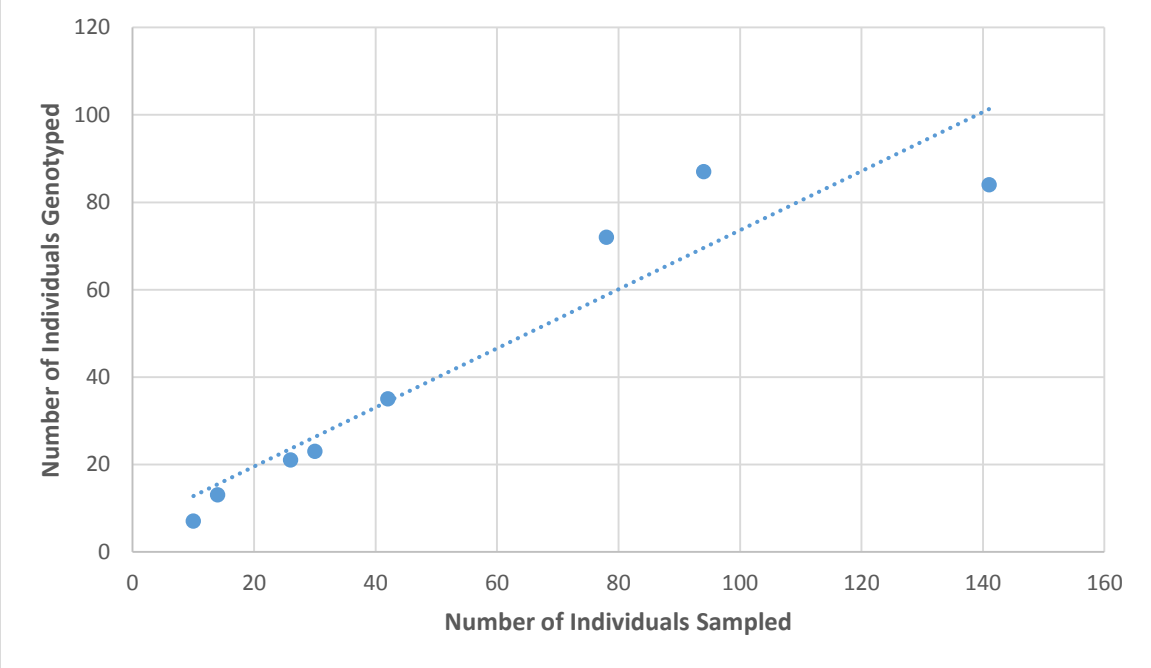

Figure 4.4: Scatterplot of numbers of *B. pensylvanicus* individuals sampled versus genotyped at five or more loci, by sampling site. The r^2 value for the linear relationship ($y = 0.6764x + 5.971$) is $r^2 = 0.8898$, suggesting very little bias in the individuals who were unable to be genotyped.

No markers showed consistent evidence of null alleles across populations from MICRO-CHECKER analyses, though certain loci did show some evidence of null alleles at some sites (Table 4.3). Similarly, no markers showed consistent deviation from HWE as calculated in Genepop across all sampling sites, though a few loci did show evidence at some sites (Table 4.4). There was no evidence for linkage between any two loci (Table 4.5), also calculated in Genepop. Based on these data all nine markers were retained in subsequent population analyses.

Table 4.3: Estimated null allele frequencies for each locus at each sampling site, as calculated in MICRO-CHECKER. Loci that show some evidence of null alleles according to MICRO-CHECKER at a sampling site are indicated in red. No loci show consistent evidence of null alleles. Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

	BG	CA	CC	LBP	LOR	LPG	LWC	SH
BTERN01	-0.0122	0.1509	0.1224	0.0385	0.0717	0.0868	0.1713	0.0336
BL15	0.0681	-0.001	-0.0985	0.0511	0.1093	-0.0719	-0.0171	-0.0112
BT30	0.023	-0.0613	0.0343	0.1215	0.0296	0.1489	-0.0806	-0.0784
B124	0.1722	-0.0854	0.0107	-0.0858	0.112	0.0006	0.1014	-0.0265
BT10	0.1891	0.0351	-0.0719	0.0158	0.0652	-0.0053	0.0061	-0.0281
B126	0	0.1124	0.1892	0.1892	0.095	0.069	-0.0198	-0.1106
B121	0.2546	0.0702	0.1554	0.0511	0.0341	0.0904	0.1187	0.059
B96	0.107	0.0578	-0.1128	-0.0867	0.1205	0.1495	0.0126	-0.0271
BT28	0	0	0	0	0	0	-0.0392	0

Table 4.4: p-values for tests for deviations from HWE for each locus at each sampling site; Hardy-Weinberg exact tests calculated in GENEPOP. Significant departures are noted in red. No loci showed consistent departures across all sample sites. Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

	BTERN01	**BL15**	**BT30**	**B124**	**BT10**	**B126**	**B121**	**B96**	**BT28**
BG	0.9379	0.1902	0.1116	**0.0132**	**0.0018**	1.0000	**0.0001**	0.1342	-
CA	**0.0006**	0.1622	0.4791	0.3391	**0.0071**	0.0977	**0.0098**	**0.0023**	-
CC	0.0848	1	0.1795	1.0000	0.9780	0.4406	**0.0166**	1	-
LBP	0.4003	0.3503	0.3344	0.6875	0.1099	0.1324	0.1669	0.4544	-
LOR	0.0598	0.1608	0.3697	0.3639	0.3721	0.3786	0.6753	0.1379	-
LPG	0.3109	0.9221	0.4406	0.6619	0.5760	1	0.0858	**0.0403**	-
LWC	**0.0053**	0.4970	0.8797	0.2258	0.4747	1	**0.0017**	0.6053	1
SH	0.2506	0.5644	0.1755	0.6281	0.2085	0.7976	0.4052	0.3614	-

Table 4.5: p-values for tests for linkage disequilibrium between each locus pair across all sampling sites (Fisher's method, calculated in GENEPOP). No linkage disequilibrium was detected between any pairs of loci.

Locus pair			X^2	df	p-Value
BTERN01	&	BL15	15.102991	12	0.235852
BTERN01	&	BT30	4.979752	16	0.995853
BL15	&	BT30	6.742148	12	0.874173
BTERN01	&	B124	6.903615	14	0.938364
BL15	&	B124	7.343537	12	0.834102
BT30	&	B124	14.809004	14	0.391334
BTERN01	&	BT10	12.169861	10	0.273849
BL15	&	BT10	10.355878	10	0.409845
BT30	&	BT10	7.435514	10	0.683789
B124	&	BT10	8.158323	10	0.613375
BTERN01	&	B126	10.396898	16	0.845096
BL15	&	B126	14.59076	12	0.264583
BT30	&	B126	5.411445	16	0.993299
B124	&	B126	9.819123	14	0.775284
BT10	&	B126	7.224312	10	0.704112
BTERN01	&	B121	10.397063	10	0.406375
BL15	&	B121	19.388165	10	0.035601
BT30	&	B121	1.976605	10	0.996516
B124	&	B121	6.383468	10	0.782083
BT10	&	B121	1.912029	8	0.983591
B126	&	B121	2.981896	10	0.981847
BTERN01	&	B96	13.013351	14	0.525473
BL15	&	B96	22.179919	12	0.035552
BT30	&	B96	12.409334	14	0.573466
B124	&	B96	11.876456	14	0.616224
BT10	&	B96	12.389801	10	0.259815
B126	&	B96	10.854395	14	0.697435
B121	&	B96	14.23944	10	0.162347
BTERN01	&	BT28	2.856184	2	0.239766
BL15	&	BT28	0.709582	2	0.70132
BT30	&	BT28	1.874048	2	0.391792
B124	&	BT28	0.286139	2	0.866694
BT10	&	BT28	0.623217	2	0.732268
B126	&	BT28	2.53499	2	0.281536
B121	&	BT28	0.499812	2	0.778874
B96	&	BT28	0.634985	2	0.727972

To understand the genetic diversity associated with each locus, loci were individually examined for diversity at each sampling site and across sites in terms of number of alleles (Figure 4.5 / Table 4.6), allelic richness (Figure 4.6 / Table 4.7), observed heterozygosity (Figure 4.7 / Table 4.8) and expected heterozygosity (Figure 4.8 / Table 4.9). BT28 showed the least diversity in all categories because it was monomorphic at all sampling sites except LWC, where there were two alleles. BT10 and B121 were highly polymorphic, with 21 and 19 alleles detected across sites, respectively. Overall, loci displayed 2-21 alleles total, with BT28 being the only one that was monomorphic at any sites. Allelic richness varied from 1.043 ± 0.12 alleles for BT28 to 6.934 ± 0.705 for B121. BT28 also showed the lowest mean observed heterozygosity (0.010 ± 0.03) and mean expected heterozygosity (0.009 ± 0.027). BT10 displayed the highest mean observed heterozygosity (0.814± 0.13) and B121 had the highest expected heterozygosity (0.912± 0.033).

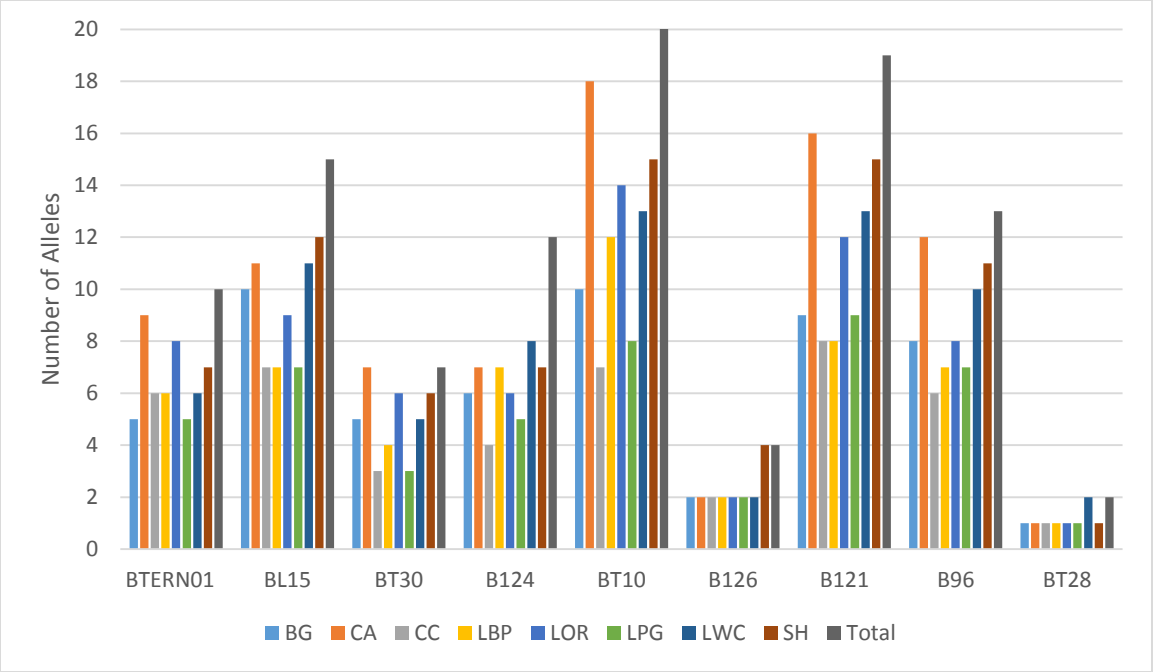

Figure 4.5: Comparison of numbers of alleles per locus at each site and overall, as calculated in FSTAT. Locus BT28 had the lowest number of alleles (2 total) and was monomorphic at all sites except LWC. BT10 had the largest number of alleles overall (21 total). Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

Table 4.6: Numbers of alleles per locus at each site and overall.

	BG	CA	CC	LBP	LOR	LPG	LWC	SH	Total
BTERN01	5	9	6	6	8	5	6	7	10
BL15	10	11	7	7	9	7	11	12	15
BT30	5	7	3	4	6	3	5	6	7
B124	6	7	4	7	6	5	8	7	12
BT10	10	18	7	12	14	8	13	15	21
B126	2	2	2	2	2	2	2	4	4
B121	9	16	8	8	12	9	13	15	19
B96	8	12	6	7	8	7	10	11	13
BT28	1	1	1	1	1	1	2	1	2

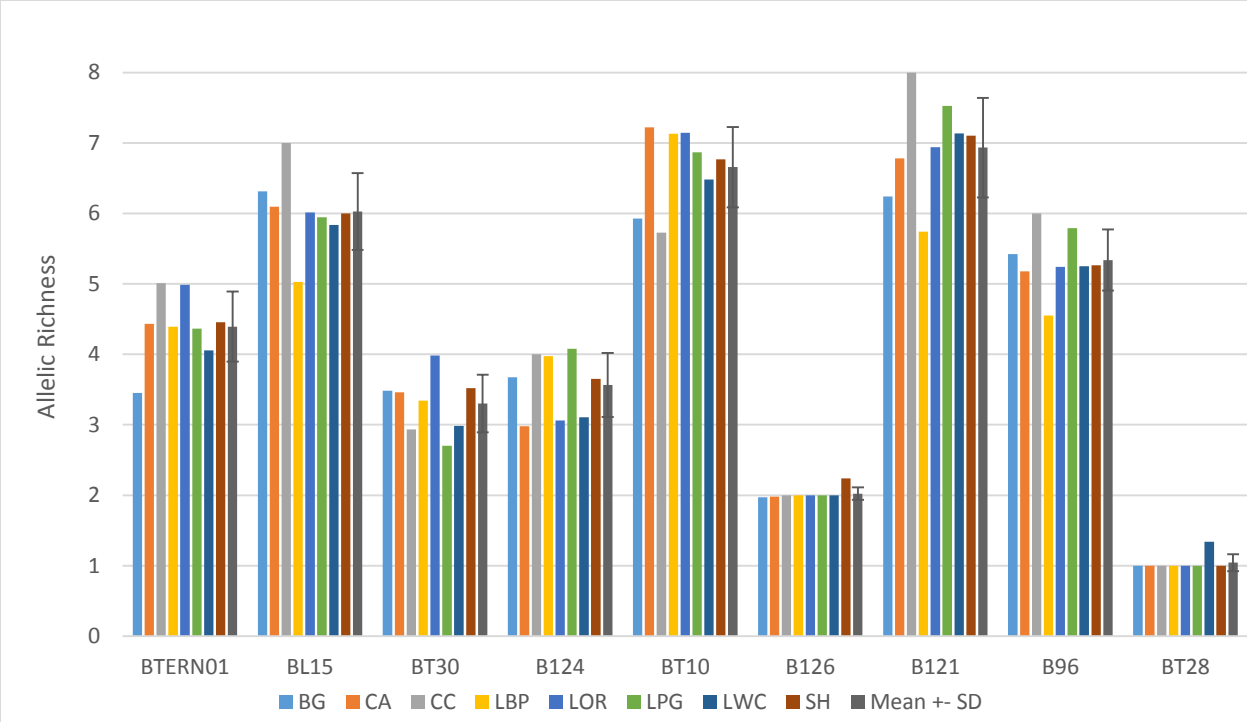

Figure 4.6: Allelic richness, by locus, at each sampling site, as calculated in FSTAT. Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

Table 4.7: Allelic richness for each locus at each sampling site.

	BG	CA	CC	LBP	LOR	LPG	LWC	SH	Mean ± SD
BTERN01	3.454	4.434	5.011	4.39	4.985	4.363	4.054	4.454	4.393± 0.498
BL15	6.315	6.095	7	5.028	6.011	5.945	5.836	5.998	6.029± 0.545
BT30	3.484	3.463	2.933	3.344	3.983	2.703	2.984	3.521	3.301875± 0.408589
B124	3.676	2.981	4	3.975	3.059	4.077	3.105	3.653	3.56575± 0.45485
BT10	5.925	7.219	5.725	7.129	7.146	6.868	6.48	6.769	6.657625± 0.569535
B126	1.97	1.982	2	1.999	1.999	2	1.998	2.24	2.0235± 0.088165
B121	6.242	6.782	8	5.741	6.941	7.527	7.133	7.103	6.933625± 0.705229
B96	5.421	5.179	6	4.551	5.239	5.789	5.252	5.263	5.33675± 0.433589
BT28	1	1	1	1	1	1	1.341	1	1.042625± 0.120562

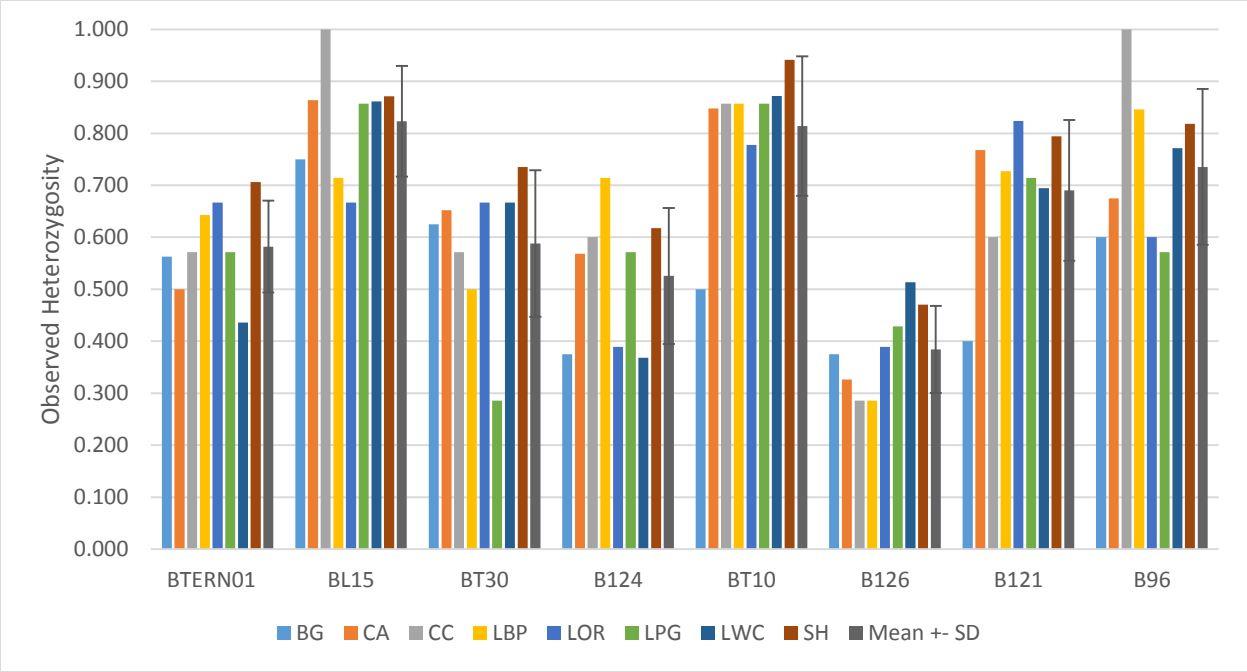

Figure 4.7: Observed heterozygosity, by locus, at each sampling site. BT28 not included because it was monomorphic at all sample sites except LWC, where its observed heterozygosity was 0.077. Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

Table 4.8: Observed heterozygosity for each locus at each sampling site.

	BG	CA	CC	LBP	LOR	LPG	LWC	SH	Mean ± SD
BTERN01	0.563	0.5	0.57143	0.64286	0.66667	0.57143	0.4359	0.70588	0.582± 0.09
BL15	0.750	0.86364	1	0.71429	0.66667	0.85714	0.86111	0.87097	0.823± 0.11
BT30	0.625	0.65217	0.57143	0.5	0.66667	0.28571	0.66667	0.73529	0.588± 0.14
B124	0.375	0.56818	0.6	0.71429	0.38889	0.57143	0.36842	0.61765	0.525± 0.13
BT10	0.500	0.84783	0.85714	0.85714	0.77778	0.85714	0.87179	0.94118	0.814± 0.13
B126	0.375	0.32609	0.28571	0.28571	0.38889	0.42857	0.51351	0.47059	0.384± 0.08
B121	0.400	0.76744	0.6	0.72727	0.82353	0.71429	0.69444	0.79412	0.690± 0.14
B96	0.600	0.675	1	0.84615	0.6	0.57143	0.77143	0.81818	0.735± 0.15
BT28	0.000	0.000	0.000	0.000	0.000	0.000	0.07692	0.000	0.010±0.03

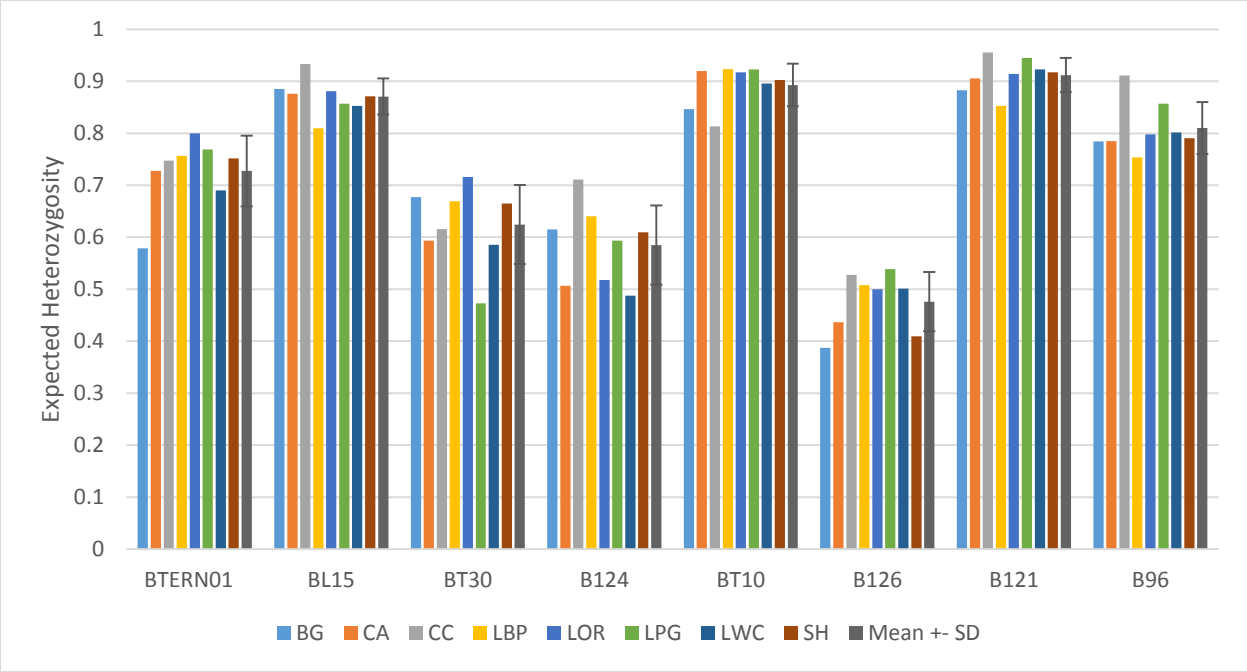

Figure 4.8: Expected heterozygosity, by locus, at each sampling site, as calculated in Arlequin. BT28 not included because it was monomorphic at all sample sites except LWC, where its expected heterozygosity was 0.075. Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

Table 4.9: Expected heterozygosity for each locus at each sampling site.

	BG	CA	CC	LBP	LOR	LPG	LWC	SH	Mean ± SD
BTERN01	0.57863	0.7279	0.74725	0.75661	0.8	0.76923	0.68998	0.75154	0.72764± 0.06802
BL15	0.88508	0.87591	0.93333	0.80952	0.88095	0.85714	0.8529	0.87097	0.87073± 0.03486
BT30	0.67742	0.59365	0.61538	0.66931	0.71587	0.47253	0.58575	0.66506	0.62437± 0.076
B124	0.61492	0.50653	0.71111	0.64021	0.51746	0.59341	0.48737	0.60975	0.58509± 0.07633
BT10	0.84677	0.91973	0.81319	0.92328	0.91746	0.92308	0.89577	0.90255	0.89273± 0.04095
B126	0.3871	0.43645	0.52747	0.50794	0.5	0.53846	0.50093	0.40957	0.47599± 0.05688
B121	0.88276	0.90561	0.95556	0.85281	0.91444	0.94505	0.92254	0.91703	0.91197± 0.0328
B96	0.78421	0.78481	0.91111	0.75385	0.7977	0.85714	0.80166	0.79068	0.81014± 0.04999
BT28	0	0	0	0	0	0	0.07493	0	0.00937± 0.02649

Average values of numbers of alleles, allelic richness, observed heterozygosity and expected heterozygosity were also calculated for each site across loci (Table 4.10). These values provided one means by which sites could be compared in terms of genetic diversity. ANOVA was used to statistically compare mean allelic richness, mean observed heterozygosity and mean expected heterozygosity values across sampling sites and to test research hypothesis 5: “Measures of genetic variation within *Bombus* subpopulations found at greens spaces do not differ by site.” ANOVA was implemented in SAS to specifically test whether the mean values of each dependent variable differed by site; in these analyses, sites were blocked by loci and mean values were taken across polymorphic loci. Mean allelic richness was not significantly different across sites (df = 7; *F*-value = 0.22; p = 0.9794) and the distributions of these means are shown in Figure 4.9. Similarly, neither mean observed heterozygosity (df = 7; *F*-value = 0.94; p = 0.4841) or mean expected heterozygosity (df = 7; *F*-value = 0.39; p = 0.9051) significantly differed across sites. The distributions of these means for each site are shown in Figures 4.9-4.11. These analyses suggest that levels of genetic diversity in *B. pensylvanicus* were similar across all sites and caused me to accept null hypothesis 5.

Table 4.10: Mean values ± standard deviation of microsatellite descriptive statistics across 9 loci for each sampling site (N = sample size, which corresponds to one individual per colony; A = mean number of alleles; AR = mean allelic richness; H_O = mean observed heterozygosity; H_E = mean expected heterozygosity). All mean values were calculated across loci in FSTAT (AR) or Arlequin (A, H_O, H_E). Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

Site	**N**	**A ± SD**	**AR ± SD**	**H_O ± SD**	**H_E ± SD**
BG	16	6.875 ± 2.85	4.165±1.925	0.523 ± 0.136	0.707 ± 0.176
CA	46	10.25 ± 5.175	4.348±2.161	0.65 ± 0.183	0.719 ± 0.187
CC	7	5.375 ± 2.134	4.63±2.334	0.686 ± 0.247	0.777 ± 0.155
LBP	14	6.625± 2.925	4.129±1.858	0.661 ± 0.189	0.74 ± 0.131
LOR	18	8.125 ± 3.72	4.485±2.147	0.622± 0.160	0.755 ± 0.167
LPG	7	5.75 ± 2.435	4.475±2.248	0.607 ± 0.198	0.745 ± 0.2
LWC	39	7.778 ± 4.123	4.243±2.04	0.585 ± 0.261	0.646 ± 0.27
SH	34	9.625± 4.207	4.445±2.054	0.744 ± 0.149	0.74 ± 0.173
Mean	22.625	7.55±3.446	4.365±2.096	0.635 ± 0.19	0.728 ± 0.18

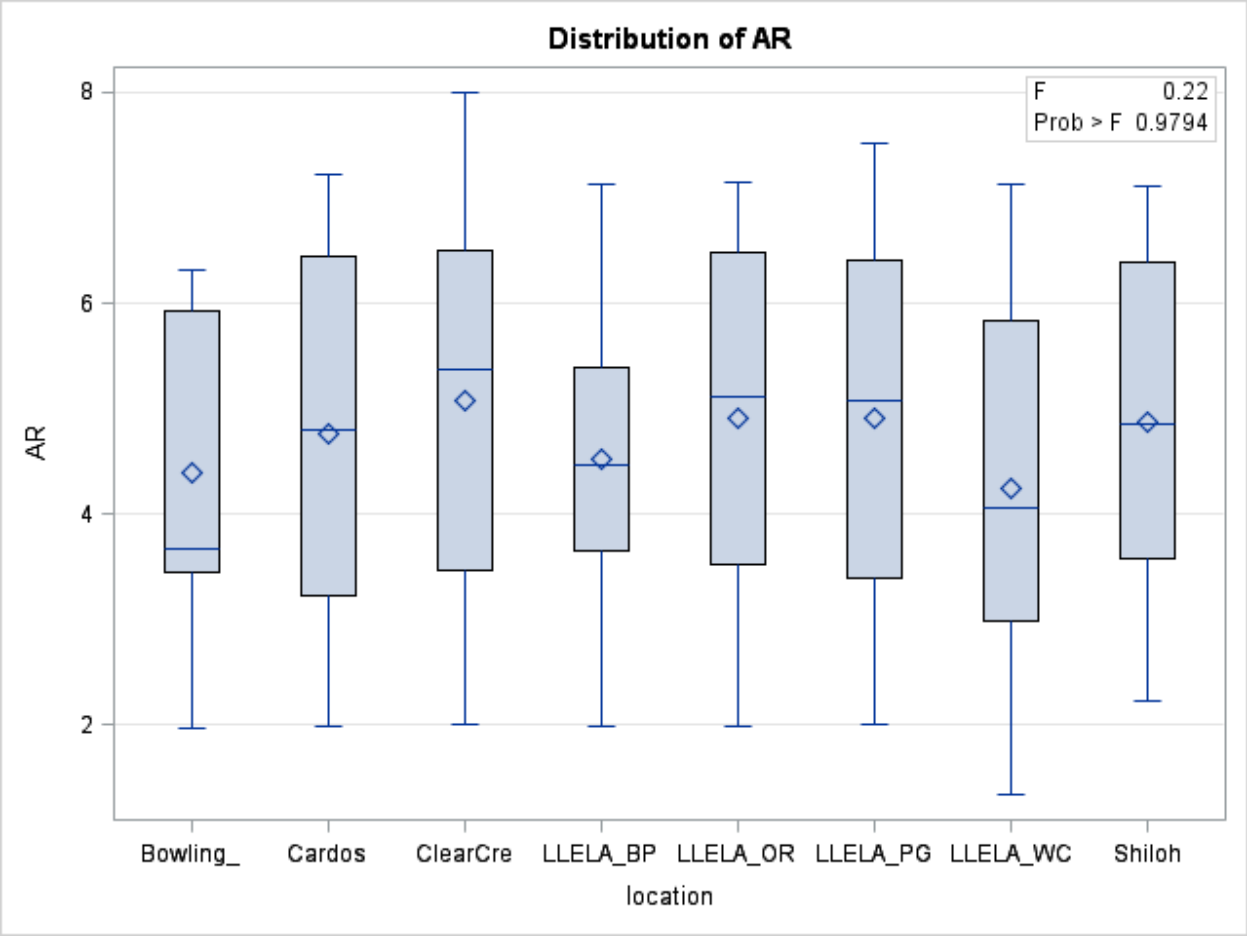

Figure 4.9: Box plot diagrams showing distributions (minimum, quartiles 1-3, and maximum values) of allelic richness across sampling sites. Mean values are indicated with diamonds. ANOVA indicated that mean allelic richness values did not significantly differ across sites (df = 7; *F*-value = 0.22; p = 0.9794).

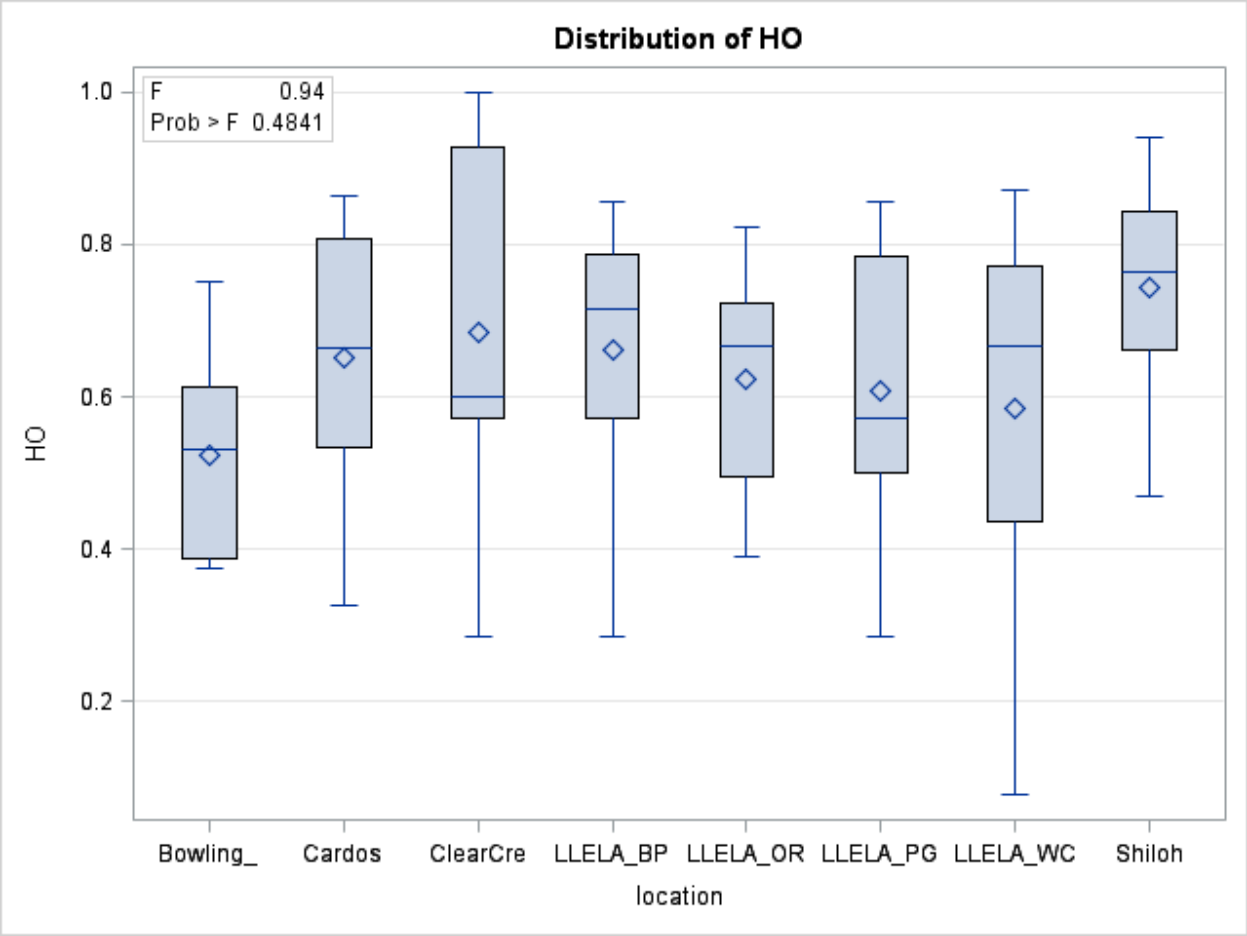

Figure 4.10: Box plot diagrams showing distributions (minimum, quartiles 1-3, and maximum values) of observed heterozygosity across sampling sites. Mean values are indicated with diamonds. ANOVA indicated that mean observed heterozygosity values did not significantly differ across sites (df = 7; *F*-value = 0.94; p = 0.4841).

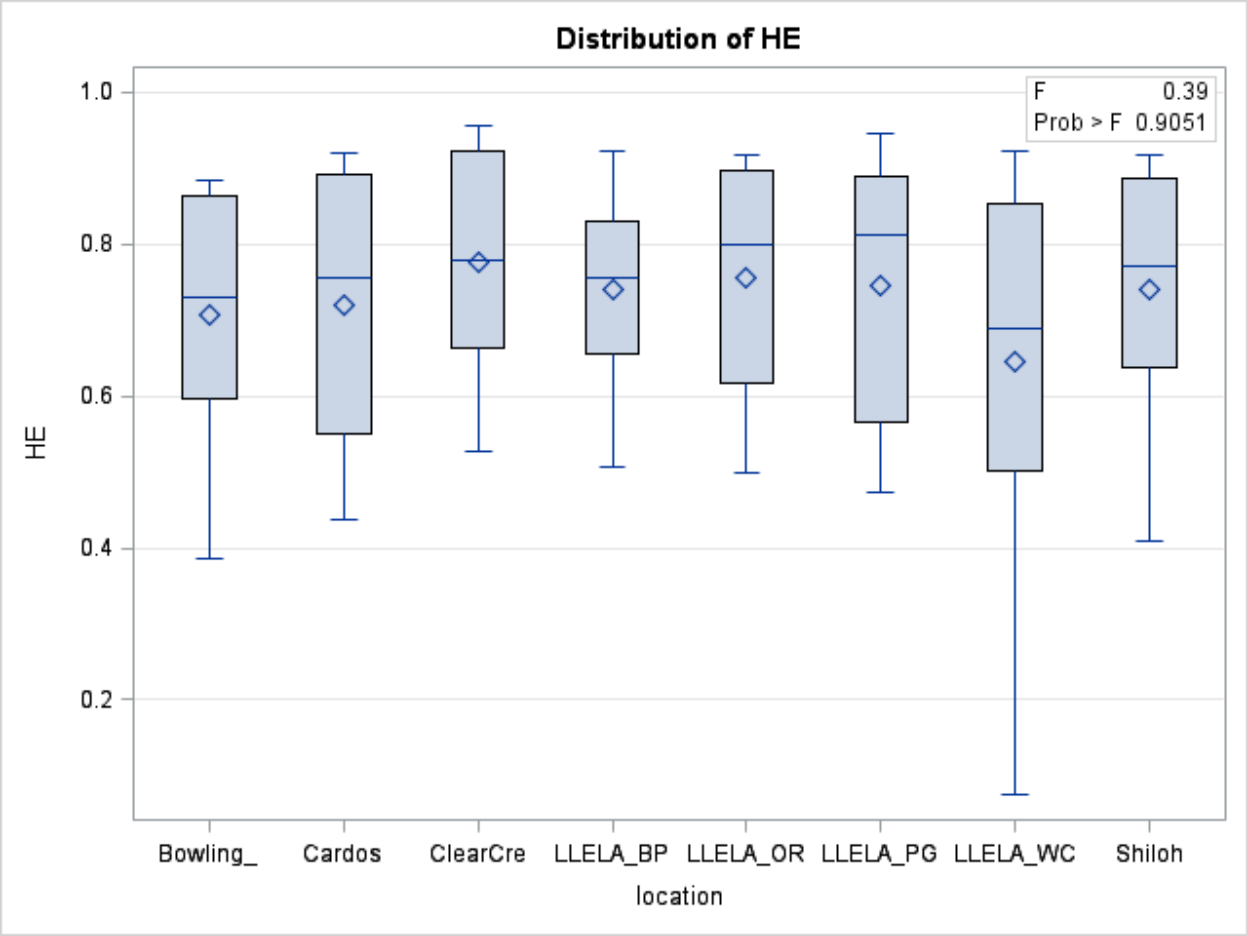

Figure 4.11: Box plot diagrams showing distributions (minimum, quartiles 1-3, and maximum values) of expected heterozygosity across sampling sites. Mean values are indicated with diamonds. ANOVA indicated that mean expected heterozygosity values did not significantly differ across sites (df = 7; *F*-value = 0.39; p = 0.9051).

4.4 Colony Assignment and Abundance Estimation

All 342 *B. pensylvanicus* individuals that were successfully genotyped at five or more loci were included in sibship analyses in COLONY 2.0.5.9 to determine which individuals were sisters and the total numbers of hives sampled at each sampling site. Figure 4.12 details the numbers of individuals sampled per colony across all sites as assigned by COLONY. The majority of these colonies (103) were identified from just one individual; the most individuals identified from one hive was 14 at LOR. In all, 181 unique colonies were detected across all sites; CCNHC and LPG were found to have the least number of hives, with 7 each, while CA had the greatest number of hives directly sampled, 46. The two community gardens, BG and SH, included 16 and 34 hives, respectively. The remaining LLELA sites, LBP, LOR and LWC, had 14, 18 and 39 hives directly detected, respectively. Note that LPG and LBP were each only visited once and so their numbers may be inherently smaller than the other sites due to smaller sampling efforts. Figure 4.13 includes the numbers of hives estimated by COLONY at each site. These data reiterate the importance of urban spaces to bumble bees for foraging.

Using the data shown in Figure 4.12, CAPWIRE was implemented to infer the numbers of hives at each site that had not been sampled (i.e., the numbers of hives with 0 individuals sampled). With these data I was able to reach an inferred total number of hives (and 95% confidence intervals on these estimates) visiting each site (Figures 4.12 and 4.13). These calculations suggest that approximately 253 hives were frequented the eight sampling sites; when considering the 95% confidence intervals, the total number of hives across sites is between 188 – 361 hives. CA was inferred to have the

greatest numbers of *B. pensylvanicus* hives visiting, while CC had the least number.

The data generated from COLONY and CAPWIRE analyses are summarized in Table 4.11.

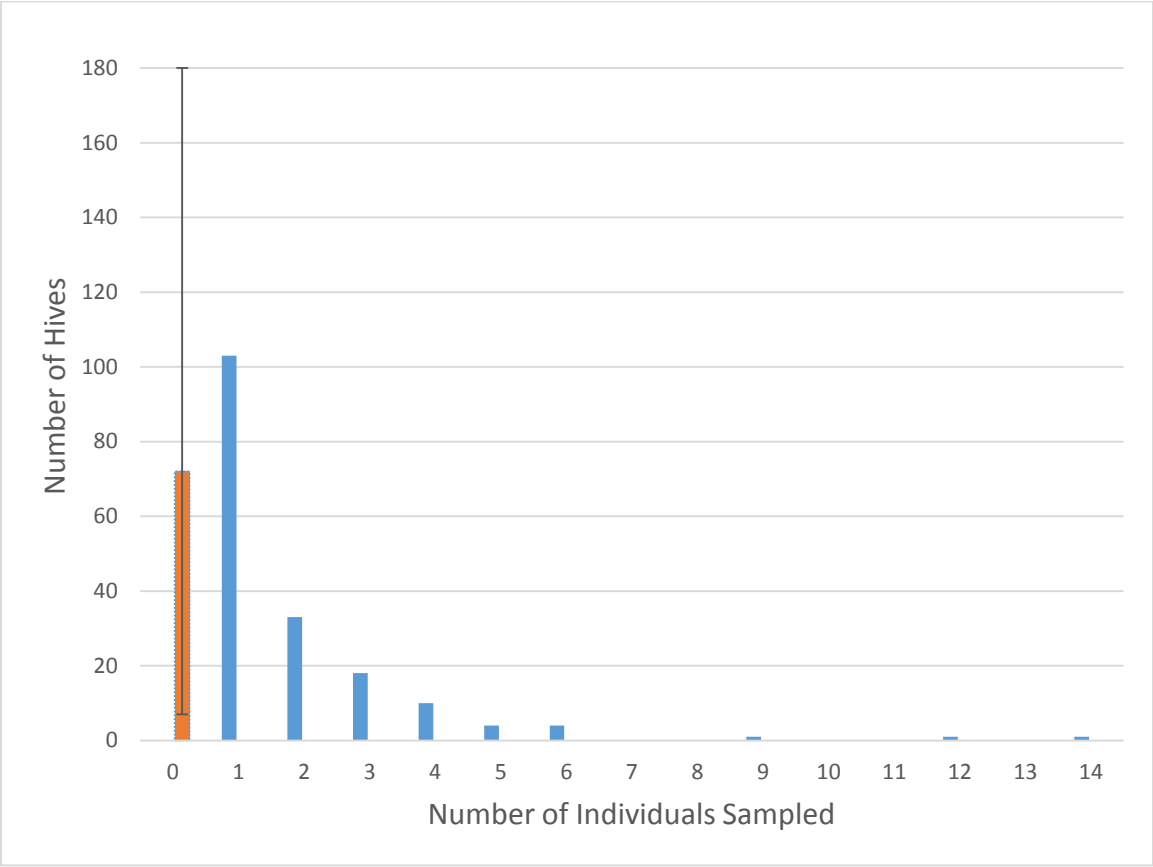

Figure 4.12: Numbers of individuals sampled from *B. pensylvanicus* hives and corresponding numbers of hives, as determined in COLONY. The majority of hives were detected from just one individual (103 hives). The zero point shows the numbers of hives inferred by CAPWIRE, including 95% confidence intervals.

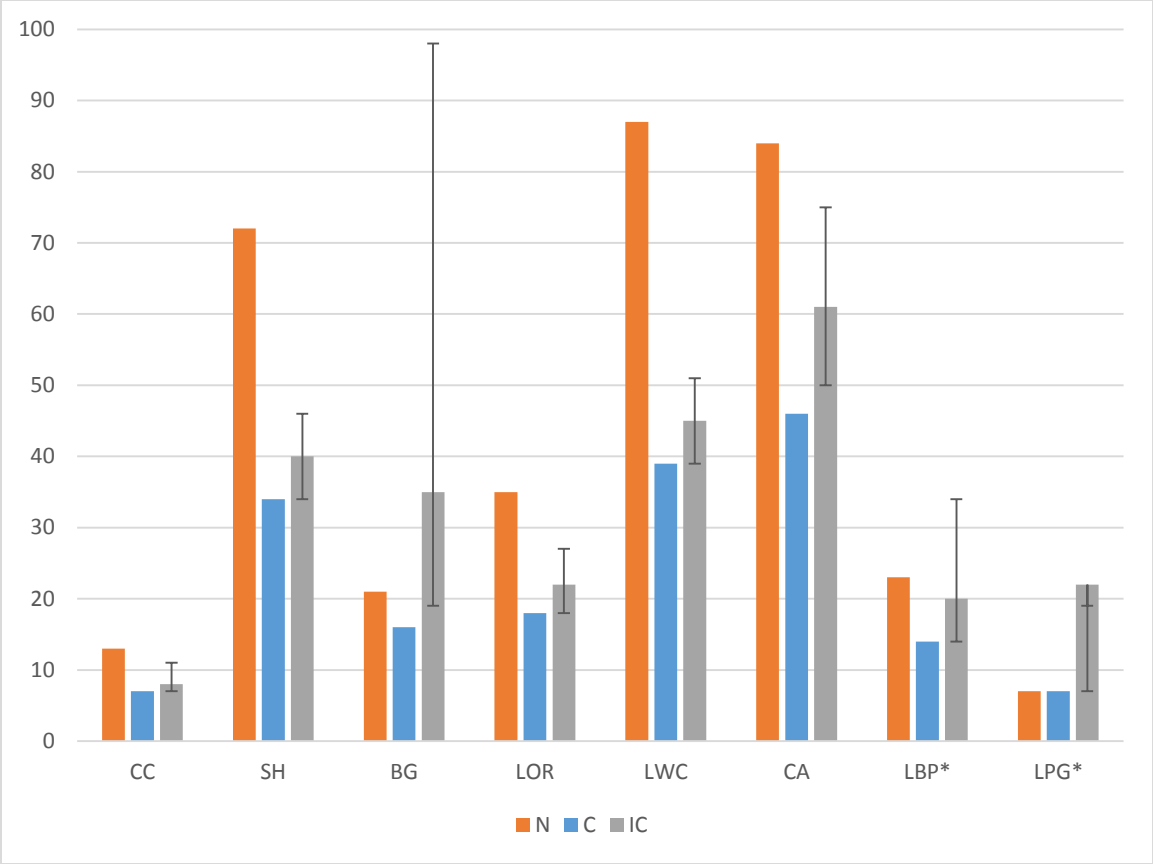

Figure 4.13: Numbers of *B. pensylvanicus* individuals genotyped (N) and numbers of colonies estimated from microsatellite data (C) and inferred (IC), by site. IC includes 95% confidence intervals, as calculated in CAPWIRE. LBP and LPG were each sampled once only; other sites were sampled three times. Site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

Table 4.11: Summary of data generated from COLONY and CAPWIRE for each sampling site from genotyped *B. pensylvanicus* individuals. CA included the most number of hives directly sampled and inferred, while CC had the least of both. LBP and LPG were only visited once each (versus three times for every other site), and so numbers might be smaller due to lower sampling efforts. Site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

Site	**Number of Individuals Genotyped**	**Number of Colonies Detected (COLONY)**	**Number of Colonies Inferred (CAPWIRE)**	**Lower 95% CI**	**Upper 95% CI**
CC	13	7	8	7	11
SH	72	34	40	34	46
BG	21	16	35	19	98
LOR	35	18	22	18	27
LWC	87	39	45	39	51
CA	84	46	61	50	75
LBP*	23	14	20	14	34
LPG*	7	7	22	7	19
Total	342	181	253	188	361

4.5 Results of Landscape Analyses

Satellite image pixels were classified into one of five land cover classes: impervious surface, vegetation (not trees), tree cover, bare ground or water. Table 4.12 details abbreviations used for variables in landscape analyses and subsequent statistics in this chapter.

The total areas of each land cover types was calculated within buffers (circles) with radii of 0.25-km, 0.5-km, 1-km and 2-km surrounding each sampling site. The total areas for each of these buffers, in ascending order, were: 196,350 m^2, 785,398 m^2, 3,141,590 m^2, and 12,566,400 m^2. Figures 4.14 - 4.16 show classified maps of land parcels surrounding each sample site with buffers included.

Table 4.12: Abbreviations for dependent (DV) and independent (IV) variables used in land use analyses. By convention, numbers following abbreviations for land use variables will correspond to areas within buffer levels (25 = 250 m buffer, 5 = 500 m buffer, 1 = 1 km buffer, and 2 = 2 km buffer). For example, IS2 refers to impervious surface area within the 2-km buffer. When IVs include an "L" in front of them, this means the value has been natural log transformed; this will be common in the statistical analyses section.

Variable	Abbreviation	DV or IV?
Individuals per trip (average number of individual bees sampled per sampling trip)	IPT	DV
Colonies per trip (average number of individual hives sampled per sampling trip, as estimated in COLONY software)	CPT	DV
Inferred colonies per trip (average number of individual hives inferred per sampling trip, as estimated in CAPWIRE software)	ICPT	DV
Sample site area	A	IV
Impervious surface	IS	IV
Vegetation, not trees	V	IV
Tree Cover	T	IV
Bare ground	BG	IV
Water	W	IV

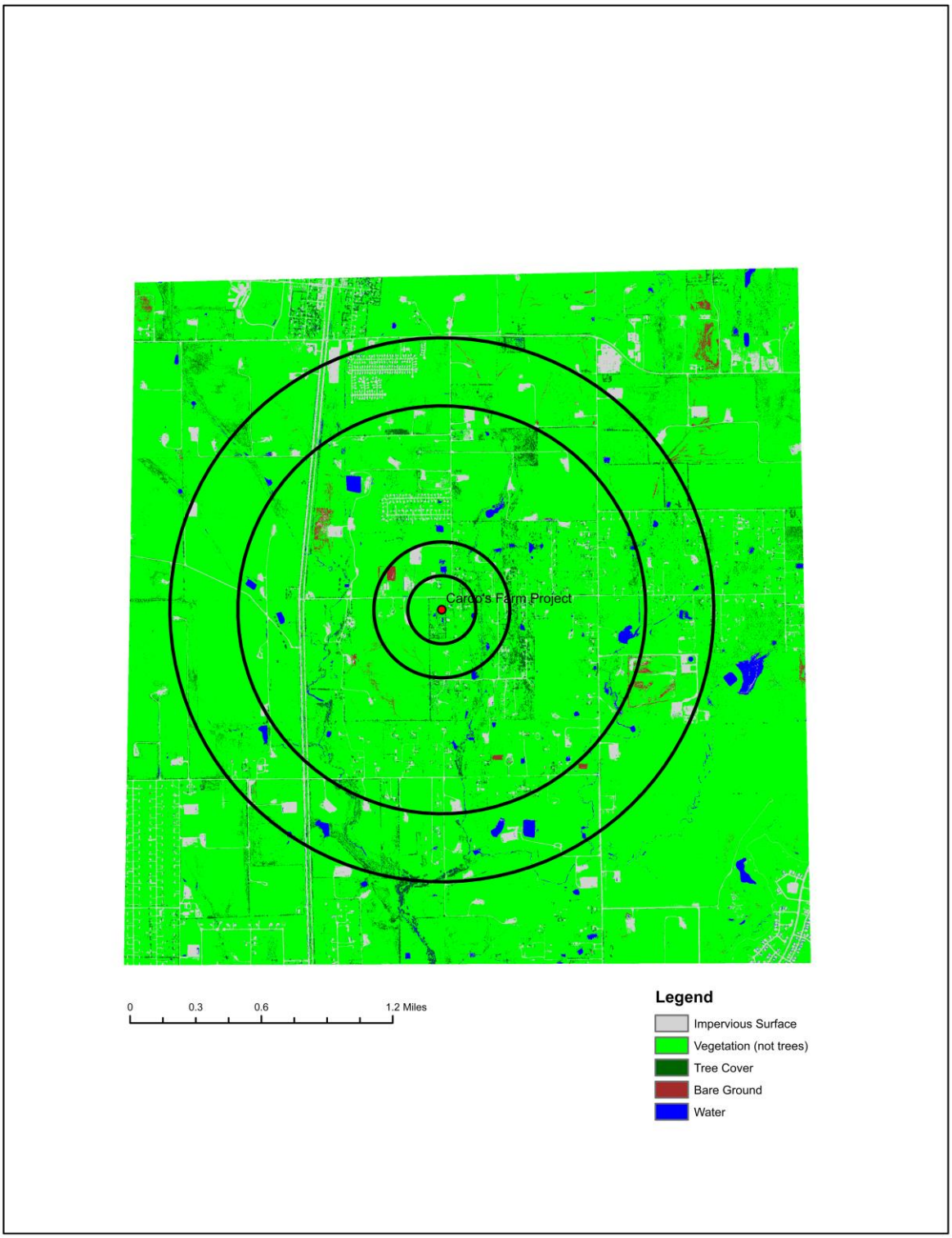

Figure 4.14: Classified map of area surrounding CA, with buffers shown.

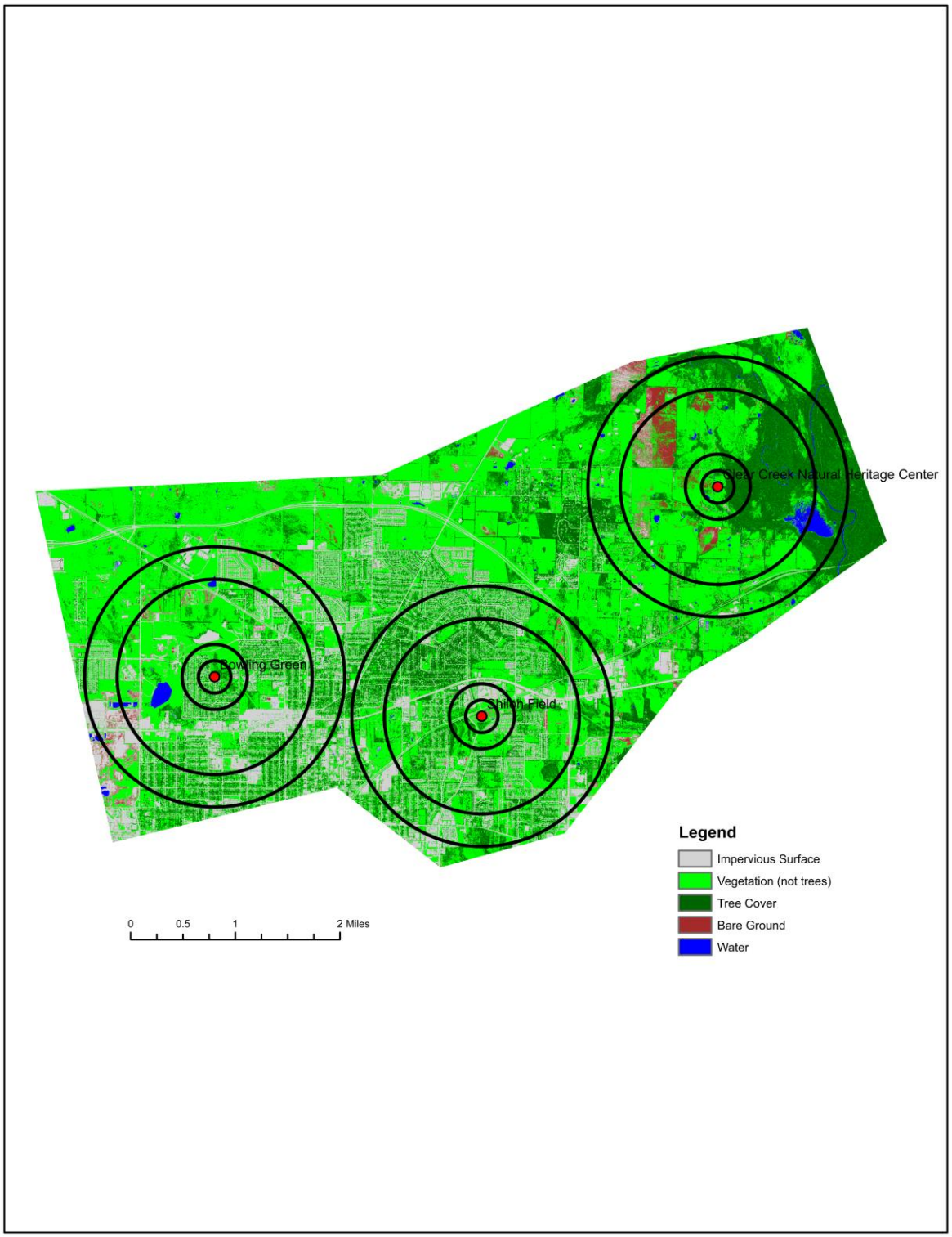

Figure 4.15: Classified map of area surrounding BG, SH and CC, with buffers shown.

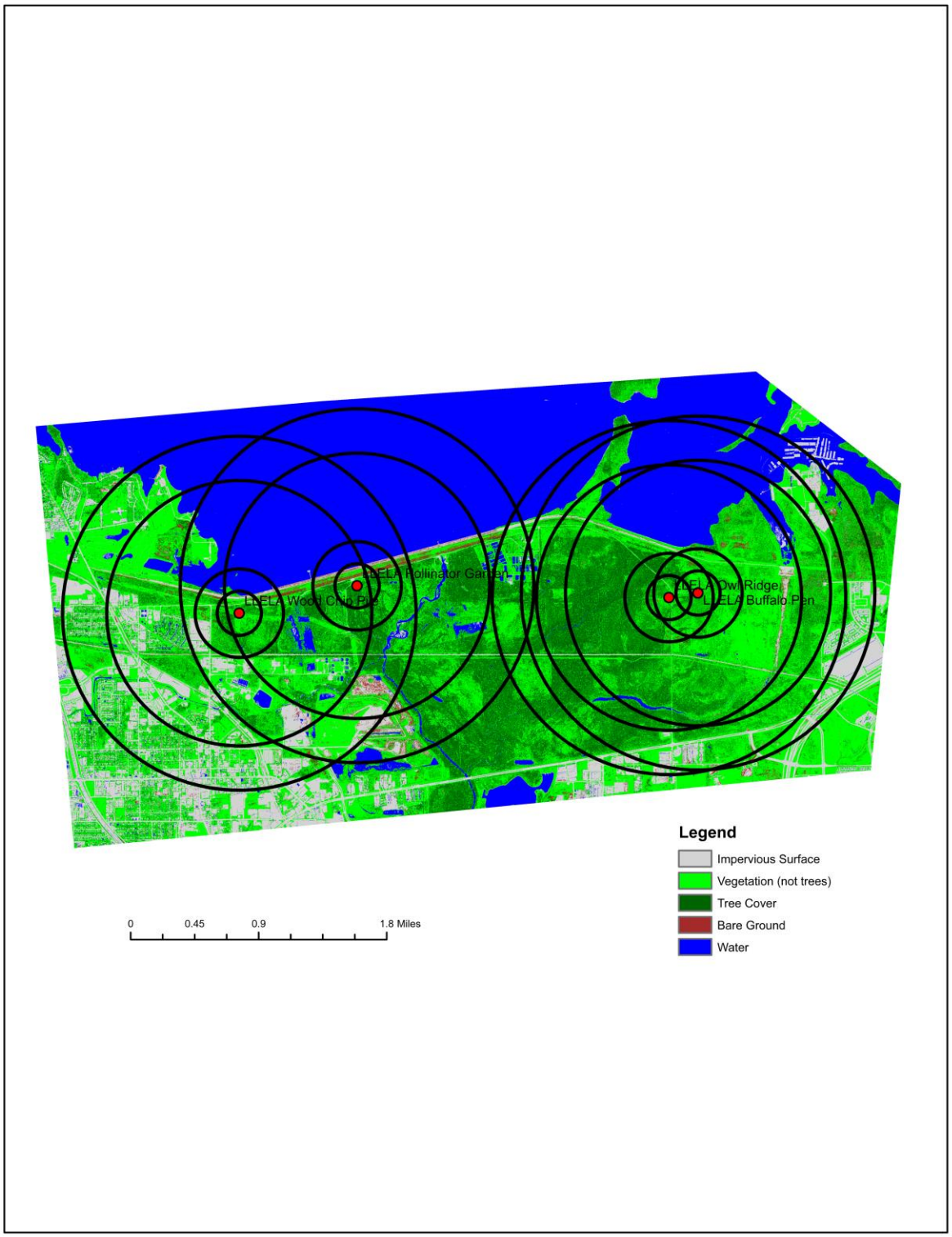

Figure 4.16: Classified map of area surrounding LBP, LOR, LPG and LWC, with buffers shown.

Within all buffers, vegetation other than trees was the dominant land cover type surrounding each site, and Cardo's Farm had the highest proportion of vegetation surrounding it at every buffer since it was located within an area that is predominately agricultural land. Impervious surface was most abundant around the two community gardens (Shiloh Field and Bowling Green Community Garden), as they are small parcels of land located within urban areas of the City of Denton. LLELA sites generally had more water surrounding them than other sites due to their proximity to Lake Lewisville. Areas of each type of land cover surrounding sites at each buffer level are shown in Figures 4.17- 4.20.

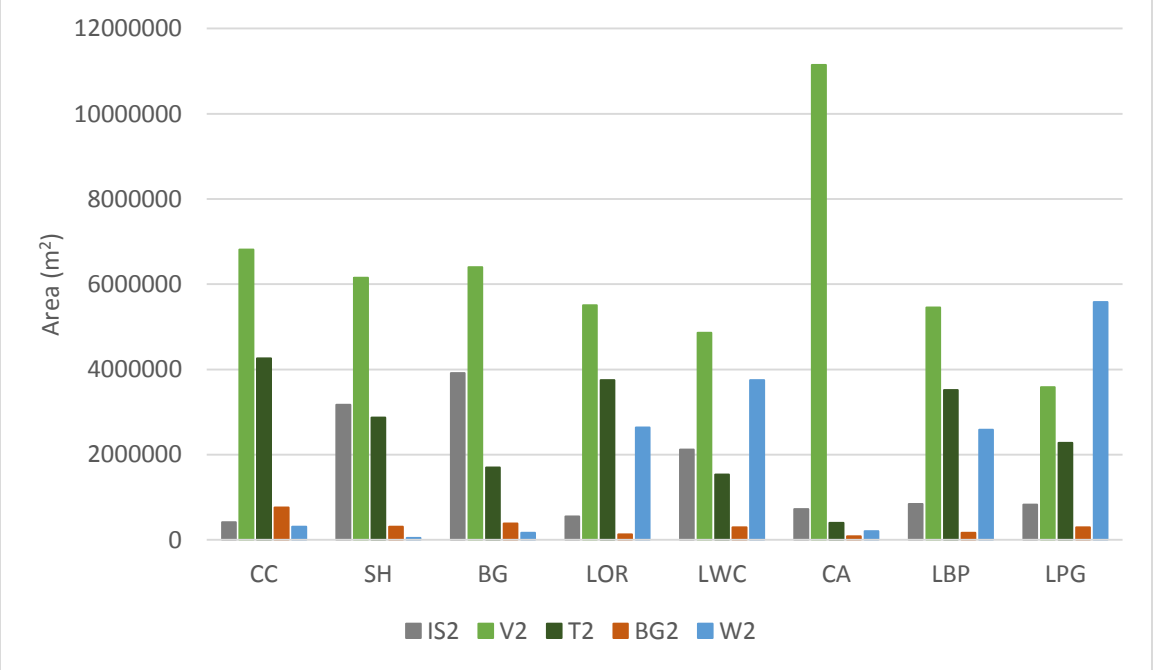

Figure 4.17: Land cover (m^2) surrounding each sampling site within the 2-km buffer. The total area surrounding each site within this buffer was 12,566,400 m^2. Site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden. Land use abbreviations are: IS – impervious surface, V – vegetation, T – tree cover, BG – bare ground, and W – water.

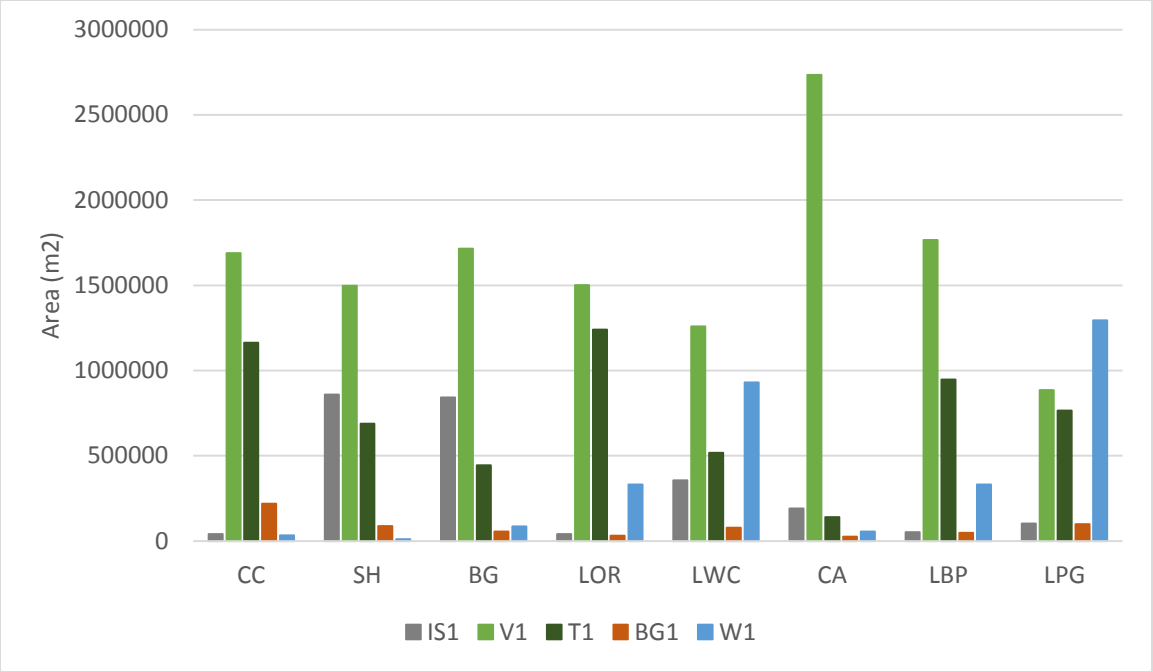

Figure 4.18: Land cover (m^2) surrounding each sampling site within the 1-km buffer. The total area surrounding each site within this buffer was 3,141,590 m^2. Site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden. Land use abbreviations are: IS – impervious surface, V – vegetation, T – tree cover, BG – bare ground, and W – water.

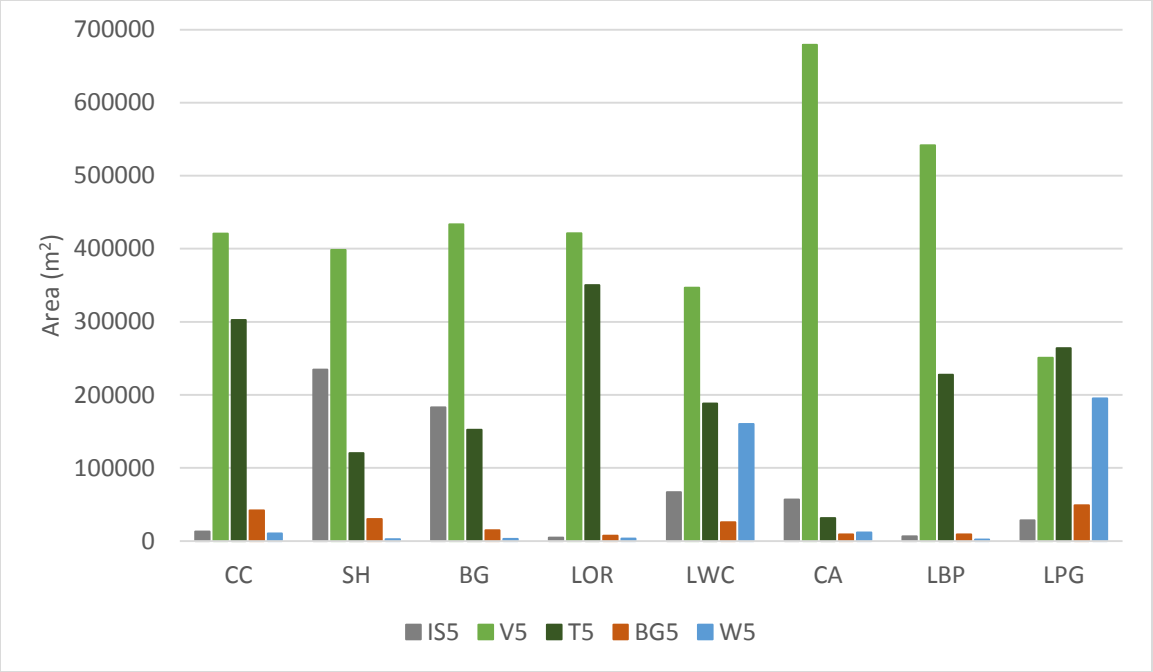

Figure 4.19: Land cover (m^2) surrounding each site within the 0.5-km buffer. The total area surrounding each site within this buffer was 785,398 m^2. Site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden. Land use abbreviations are: IS – impervious surface, V – vegetation, T – tree cover, BG – bare ground, and W – water.

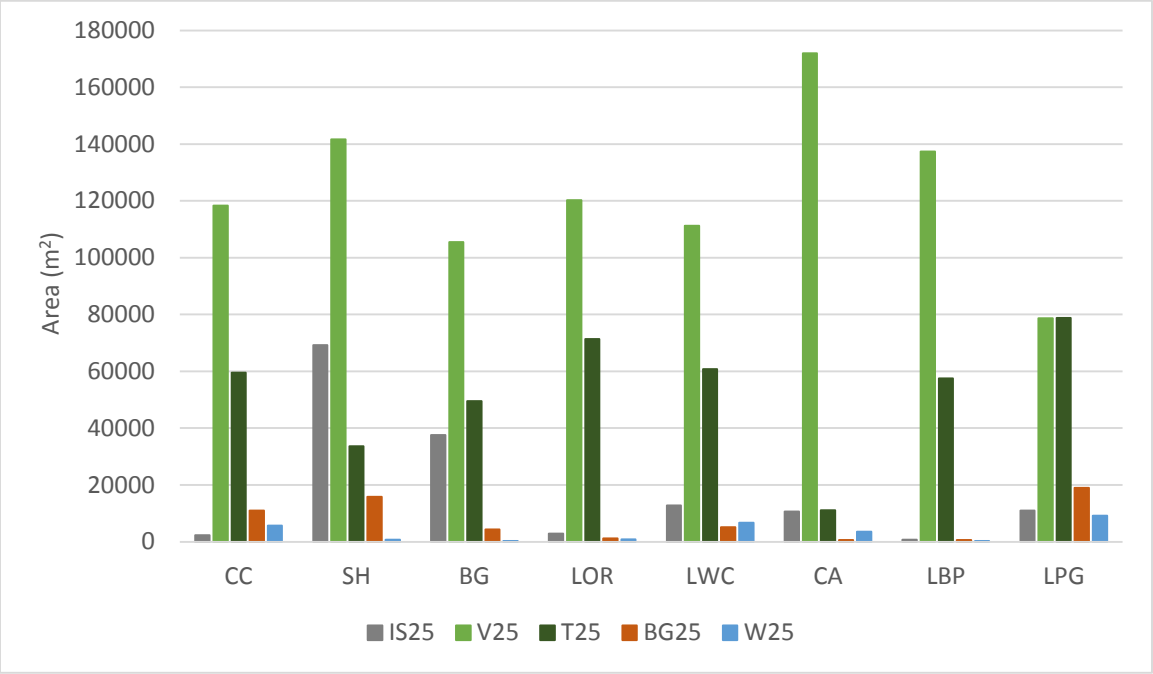

Figure 4.20: Land cover (m^2) surrounding each site within the 0.25-km buffer. The total area surrounding each site within this buffer was 196,350 m^2. Site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden. Land use abbreviations are: IS – impervious surface, V – vegetation, T – tree cover, BG – bare ground, and W – water.

4.6 Results of Statistical Analyses

4.6.1 Analysis of Species Persistence – z-Test of Independent Proportions

To investigate hypothesis 1, which stated that "Current relative abundance of *Bombus* spp. in urban green spaces is not different from historic relative abundance of *Bombus* spp.", the data generated from museum and field records were compared with a *z*-test of independent proportions. Historic specimens were defined as having been collected before 2000; in all 60 historic specimens were identified from museum records. Of those, 45 (~75%) were identified as *B. pensylvanicus* and 15 (~25%) were

B. fraternus. One historic specimen of *B. bimaculatus* (from 1970) was excluded from analyses because there were no current data for comparison. All 450 specimens sampled from urban spaces in this study were included in current abundance values; as stated earlier, 435 (96.7%) of these were *B. pensylvanicus* and 15 (3.3%) were *B. fraternus*.

The results of the *z*-tests for both *B. pensylvanicus* and *B. fraternus* were highly significant ($p << 0.05$), indicating that the current relative abundances of both species, at least in Denton's urban green spaces, are significantly different from historic relative abundances (Table 4.13). The relative abundance of *B. pensylvanicus* is much higher currently than historically, whereas that of *B. fraternus* is lower, and so I reject null hypothesis 1.

Table 4.13: Values for *z*-test of independent proportions for *B. pensylvanicus* and *B. fraternus* between historic and current datasets. The null hypothesis states that there is no difference between historic and current proportions.

Species	**Historic Proportion (p_h), where** $n_h =$ 60	**Current Proportion (p_c), where** $n_c =$ 450	***z*-score**	**p-value**
B. pensylvanicus	0.75	0.967	-6.7	0*
B. fraternus	0.25	0.033	6.7	0*

4.6.2 Analyses of the Relationships Between Landscape Characteristics and Bumble Bee Abundance – Pearson's Correlation Analyses

In this section, I report the results of the investigation of research hypotheses 2-4, wherein I explained variations in the numbers of *B. pensylvanicus* individuals, *B. pensylvanicus* hives and inferred numbers of *B. pensylvanicus* hives in terms of

variations in landscape characteristics at each buffer level and thus rejected the null hypotheses that each variable could not be explained by land use characteristics. Table 4.12 will be useful to aid in deciphering abbreviations used in this section. Also note that all values for independent variables were natural log transformed prior to statistical tests being employed. This allowed for the assumption of normally distributed data to be met.

Pearson's correlation analyses were first used to investigate the relationships between independent and dependent variables, check for independent variable collinearity, and eliminate least important independent variables for subsequent multiple linear regression analyses (Tables 4.14-4.17).

At the 2-km buffer level (Table 4.14), no significant correlations between any independent and dependent variable combinations were found. Variations in both CPT and IPT were best explained by variations in the areas of bare ground surrounding sample sites ($p = 0.0756$ and 0.0505, respectively). Variation in ICPT was best explained was by variation in sample site area ($p = 0.0723$). All of these relationships were inversely proportional. No independent variables showed signs of collinearity at this buffer level. The least correlated independent variables were removed from further analyses as follows: LA and LW2 were removed from CPT analyses, LIS2 and LV2 were removed from ICPT analyses, and LA and LIS2 were removed from IPT analyses.

Table 4.14: Pearson's correlation analysis for 2 km buffer. For each relationship, two lines are present: the top line contains the value of the Pearson's correlation coefficient and the bottom line contains the associated p-value. No significant collinearity was identified between independent variables. Variables least correlated with each independent variable (in blue) were removed from subsequent regression analysis. Dependent variables are CPT (colonies per trip), ICPT (inferred colonies per trip) and IPT (individuals per trip). Independent variables are land use characteristics that were log-transformed (L); abbreviations for independent variables are IS – impervious surface, V – vegetation, T – tree cover, BG – bare ground, and W – water.

Pearson Correlation Coefficients, N = 8									
Prob > |r| under H0: Rho=0									
	CPT	**ICPT**	**IPT**	**LA**	**LIS2**	**LV2**	**LT2**	**LBG2**	**LW2**
CPT	1	0.71705	0.95361	-0.07489	0.19791	0.26933	-0.56406	-0.65882	0.03071
		0.0453	0.0002	0.8601	0.6385	0.5189	0.1453	0.0756	0.9425
ICPT		1	0.57933	-0.66443	0.15601	-0.1321	-0.51556	-0.52947	0.28497
			0.1323	0.0723	0.7122	0.7552	0.191	0.1772	0.4939
IPT			1	0.05484	0.07953	0.50595	-0.6949	-0.70563	-0.09643
				0.8974	0.8515	0.2008	0.0557	0.0505	0.8203
LA				1	-0.01065	0.46807	0.28211	0.1681	-0.62384
					0.98	0.2421	0.4984	0.6907	0.0983
LIS2					1	-0.09758	-0.15591	0.19241	-0.39339
						0.8182	0.7124	0.648	0.335
LV2						1	-0.587	-0.3241	-0.6732
							0.1261	0.4335	0.0673
LT2							1	0.51312	0.21491
								0.1934	0.6093
LBG2								1	-0.1868
									0.6578
LW2									1

At the 1-km buffer level (Table 4.15), no individual independent variables were significantly correlated with any dependent variables; variations in CPT and IPT were best explained by variation in tree cover (p=0.1405and 0.0566, respectively) and ICPT variation was best explained by variation in sample site area (p=0.0723). All of these relationships were inverse. LA and LW1 exhibited significant collinearity (p = 0.0209) within the 1-km buffer. Both of these independent variables were removed from subsequent analyses of CPT and IPT variations since they were also the independent variables least correlated with these dependent variables. In the case of ICPT, LIS1 and LV1 were removed due to low correlation, and LW1 was also removed because it was less correlated than LA with variations in ICPT.

Table 4.15: Pearson's correlation analysis for 1 km buffer. For each relationship, two lines are present: the top line contains the value of the Pearson's correlation coefficient and the bottom line contains the associated p-value. Variables least correlated with each independent variable were removed from subsequent regression analysis. Significant collinearity was identified between water and sampling site area (in red). Both of these variables were removed from CPT and IPT analyses based on grounds that they were least correlated with DV (highlighted in blue). A third variable, LW1, was removed from ICPT analyses (highlighted in orange), due to collinearity. Dependent variables are CPT (colonies per trip), ICPT (inferred colonies per trip) and IPT (individuals per trip). Independent variables are land use characteristics that were log-transformed (L); abbreviations for independent variables are IS – impervious surface, V – vegetation, T – tree cover, BG – bare ground, and W – water.

Pearson Correlation Coefficients, N = 8									
Prob > |r| under H0: Rho=0									
	CPT	**ICPT**	**IPT**	**LA**	**LIS1**	**LV1**	**LT1**	**LBG1**	**LW1**
CPT	1	0.71705	0.95361	-0.07489	0.24744	0.33932	-0.56965	-0.53406	0.0731
		0.0453	0.0002	0.8601	0.5546	0.4109	0.1405	0.1728	0.8634
ICPT		1	0.57933	-0.66443	0.1824	-0.0854	-0.493	-0.39545	0.40433
			0.1323	0.0723	0.6655	0.8406	0.2145	0.3322	0.3205
IPT			1	0.05484	0.20892	0.53314	-0.6933	-0.59031	-0.0639
				0.8974	0.6195	0.1736	0.0566	0.1234	0.8805
LA				1	0.03854	0.43052	0.1994	0.15925	-0.7854
					0.9278	0.287	0.6359	0.7064	0.0209
LIS1					1	0.02535	-0.5032	-0.04053	-0.3352
						0.9525	0.2037	0.9241	0.417
LV1						1	-0.5551	-0.48476	-0.5674
							0.1532	0.2234	0.1424
LT1							1	0.49383	0.17042
								0.2136	0.6866
LBG1								1	-0.144
									0.7338
LW1									1

At the 0.5-km buffer level (Table 4.16), variation in tree cover was significantly correlated with variation in IPT (p=0.0261); no other independent variables were found to be significantly correlated with any dependent variables. The best predictor of CPT variation was also tree cover (p=0.0913) and of ICPT was sample site area (p=0.0723). These variables were all inversely related. LV5 and LBG5 exhibited significant collinearity (p = 0.0417) within the 0.5-km buffer. Both of these independent variables were removed from subsequent analyses of ICPT variations since they were also the independent variables least correlated with this dependent variable. LA and LW5 were least correlated with CPT and IPT and so were removed from subsequent analyses, along with LBG5 which was less correlated with these dependent variables than LT5.

Table 4.16: Pearson's correlation analysis for 0.5 km buffer. For each relationship, two lines are present: the top line contains the value of the Pearson's correlation coefficient and the bottom line contains the associated p-value. IPT was found to be significantly correlated with LT5 (in bold). Variables least correlated with each independent variable (highlighted in blue) were removed from subsequent regression analysis. Significant collinearity was identified between bare ground and vegetation (in red). Third variables removed from analyses due to collinearity are highlighted in orange. LBG5 was removed from ICPT analyses due to low correlation. LA, LW5 and LBG5 were all removed from CPT and IPT analyses. Dependent variables are CPT (colonies per trip), ICPT (inferred colonies per trip) and IPT (individuals per trip). Independent variables are land use characteristics that were log-transformed (L); abbreviations for independent variables are IS – impervious surface, V – vegetation, T – tree cover, BG – bare ground, and W – water.

Pearson Correlation Coefficients, N = 8									
Prob > |r| under H0: Rho=0									
	CPT	**ICPT**	**IPT**	**LA**	**LIS5**	**LV5**	**LT5**	**LBG5**	**LW5**
CPT	1	0.71705	0.95361	-0.0749	0.17677	0.44447	-0.6341	-0.3669	0.03393
		0.0453	0.0002	0.8601	0.6754	0.2699	0.0913	0.3713	0.9364
ICPT		1	0.57933	-0.6644	0.16105	0.01287	-0.4455	-0.0896	0.3224
			0.1323	0.0723	0.7032	0.9759	0.2686	0.8329	0.4361
IPT			1	0.05484	0.17239	0.5961	***-0.7679***	-0.4342	0.0039
				0.8974	0.6831	0.1189	***0.0261***	0.2824	0.9927
LA				1	0.04992	0.38471	-0.04807	-0.06972	-0.5809
					0.9066	0.3467	0.91	0.8697	0.131
LIS5					1	-0.0904	-0.5347	0.32489	0.07794
						0.8314	0.1722	0.4323	0.8544
LV5						1	-0.613	-0.7253	-0.6214
							0.1061	0.0417	0.1
LT5							1	0.26883	0.06607
								0.5197	0.8765
LBG5								1	0.57158
									0.1388
LW5									1

At the 0.25-km buffer level (Table 4.17), variations in tree cover and vegetation were significantly correlated with variation in IPT (p=0.0345 and 0.0421, respectively); IPT was positively related to vegetation cover and negatively related to tree cover. No other independent variables were found to be significantly correlated with any dependent variables. The best predictor of CPT variation was vegetation (p=0.1334) and of ICPT was sample site area (p=0.0723); both of these were negative correlations. LV25 and LT25 exhibited significant collinearity (p = 0.0242) within the 0.5-km buffer. For IPT, LIS25 and LW25 were removed from further analyses because of low correlation; in addition, even though it was significantly correlated with IPT, LT25 was less correlated with IPT than LV25 and so was removed due to significant collinearity with LV25. LIS25, LW25 and LT25 were removed from CPT analyses and LW25 and LV25 were removed from ICPT analyses.

Table 4.17: Pearson's correlation analysis for 0.25 km buffer. For each relationship, two lines are present: the top line contains the value of the Pearson's correlation coefficient and the bottom line contains the associated p-value. Variables least correlated with each independent variable (in blue) were removed from subsequent regression analysis. Significant collinearity was identified between tree cover and vegetation (in red). Third variables removed from analyses due to collinearity are highlighted in orange. LV25 was removed from ICPT analyses due to low correlation. LIS25, LW25 and LT25 were all removed from CPT. LIS25, LV25 and LW25 were removed from IPT analyses. Dependent variables are CPT (colonies per trip), ICPT (inferred colonies per trip) and IPT (individuals per trip). Independent variables are land use characteristics that were log-transformed (L); abbreviations for independent variables are IS – impervious surface, V – vegetation, T – tree cover, BG – bare ground, and W – water.

Pearson Correlation Coefficients, N = 8									
Prob > |r| under H0: Rho=0									
	CPT	**ICPT**	**IPT**	**LA**	**LIS25**	**LV25**	**LT25**	**LBG25**	**LW25**
CPT	1	0.71705	0.95361	-0.0749	0.02811	0.57807	-0.5683	-0.4921	-0.0748
		0.0453	0.0002	0.8601	0.9473	0.1334	0.1416	0.2155	0.8603
ICPT		1	0.57933	-0.6644	0.08169	-0.0021	-0.2974	-0.2134	0.06765
			0.1323	0.0723	0.8475	0.9961	0.4743	0.6118	0.8735
IPT			1	0.05484	0.03663	***0.72432***	***-0.7434***	-0.562	0.00852
				0.8974	0.9314	***0.0421***	***0.0345***	0.1471	0.984
LA				1	0.03934	0.61991	-0.2252	0.01545	-0.2368
					0.9263	0.1011	0.5918	0.971	0.5723
LIS25					1	-0.0912	-0.2956	0.53187	0.03574
						0.8299	0.4772	0.1749	0.9331
LV25						1	-0.7741	-0.6062	-0.315
							0.0242	0.1111	0.4473
LT25							1	0.37716	-0.0009
								0.357	0.9983
LBG25								1	0.42153
									0.2983
LW25									1

4.6.3 Analyses of the Relationships Between Landscape Characteristics and Bumble Abundance – Multiple Linear Regression

Once Pearson's analyses had been completed and independent variables had been parsed, the maximum r^2 improvement approach was implemented in SAS to identify significant multiple linear regression models. Bowling Green Community Garden and LLELA Owl Ridge were randomly selected to be omitted from initial regression analyses to be used as subsequent validation sites. Each dependent variable (CPT, ICPT and IPT) was investigated at each buffer level; these analyses resulted in the identification of five significant regression models (Table 4.18). Additionally, most correlated dependent and independent variables from the Pearson's correlation analyses were run against each other to identify any other significant models that were not identified using the maximum r^2 improvement approach. One model (Model D) was detected in this way. No significant models were detected at the 0.25-km buffer level, but one was detected at the 0.5-km buffer level, two at the 2-km buffer level, and three at the 1-km buffer level.

Within the 0.5-km buffer, a one-variable model, "Model A," explained variation in IPT (IPT = 207.307-15.283 * LT5; $p = 0.0402$, $r^2 = 0.6142$; adjusted $r^2 = 0.6142$); in Model A tree cover was negatively related to IPT (individuals per trip). No models were significant for CPT (colonies per trip) or ICPT (inferred colonies per trip) at the 0.5-km buffer level.

Within both the 1 and 2-km buffers, significant one-variable models containing bare ground as the independent variable explained variations in both CPT (colonies per trip) and IPT (individuals per trip). The complete models for CPT (Models B and E) are as follows: CPT = 79.85374 -6.17679 * LBG1 ($p = 0.0119$; $r^2 = 0.8272$; adjusted r^2 =

0.7840) and CPT = 84.90184 - 5.96977 * LBG2 ($p = 0.0158$; $r^2 = 0.7632$; adjusted $r^2 = 0.70403$). The complete models for IPT (Models D and F) are: IPT = 245.273 -19.633 *LBG1 ($p=0.0084$; $r^2 = 0.8544$; adjusted $r^2 =0.8180$) and IPT = 258.591 - 18.756 * LBG2 ($p = 0.0215$; $r^2 = 0.7703$; adjusted $r^2 = 0.7128$). In all of these models, increased bare ground resulted in decreases in the dependent variable. No significant models were identified to explain variation in ICPT (inferred colonies per trip) at the 1 or 2-km buffers. An additional significant one-variable model (Model D) that described an inverse relationship between variations in IPT and tree cover at the 1-km buffer level was also generated: IPT = 246.281-16.666 *LT1 ($p = 0.047$; $r^2 = 0.6681$; adjusted $r^2 = 0.5851$).

Table 4.18: Summary of significant linear regression models identified by maxr^2 approach (DV = dependent variable, IV = independent variable(s). Included are p-values associated with IVs and models, model r^2 and adjusted r^2 values, and mean ± SD values for r^2 and adjusted r^2 from bootstrapping. Dependent variables included in models are CPT (colonies per trip) and IPT (individuals per trip). Independent variables included in models are land use characteristics that were log-transformed (L); abbreviations for independent variables are T – tree cover and BG – bare ground.

Model ID	**Buffer (km)**	**DV**	**IV(s) and (p value(s))**	**Model**	**Model p value**	**r^2**	**mean r^2 ± SD**	**Adj r^2**	**mean adj r^2 ± SD**
A	0.5	IPT	LT5 (0.0402)	IPT = 207.307-15.283 (LT5)	0.0402	0.6913	0.55539 ± 0.21365	0.6142	0.44423 ± 0.26706
B	1	CPT	LBG1 (0.0119)	CPT = 79.8537 - 6.177 (LBG1)	0.0119	0.8272	0.28842 ± 0.20175	0.7840	0.11053 ± 0.25218
C	1	IPT	LT1 (0.047)	IPT = 246.281-16.666 (LT1)	0.047	0.6681	0.43494 ± 0.27008	0.5851	0.29368 ± 0.33761
D	1	IPT	LBG1 (0.00837)	IPT = 245.273 - 19.633 (LBG1)	0.0084	0.8544	0.3511 ± 0.22044	0.8180	0.18887 ± 0.27555
E	2	CPT	LBG2 (0.0229)	CPT = 84.902 - 5.97 (LBG2)	0.0229	0.7632	0.4244± 0.2405	0.7040	0.2805± 0.3006
F	2	IPT	LBG2 (0.0215)	IPT = 258.591 - 18.756 (LBG2)	0.0215	0.7703	0.4722 ± 0.21754	0.7128	0.34025 ± 0.27192

I investigated the validity of the regression models by first visually inspecting the plots of observed and expected values of dependent variables, including those of the two validation sites (Bowling Green and LLELA Owl Ridge) (Figures 4.21-4.26). In most cases, Bowling Green Community Garden's observed values were quite different from the expected values. The expected values for LLELA Owl Ridge were relatively similar to observed at the 0.5-km scale, but diverged from observed values at the 1-km and 2-km scales.

Bootstrapping of the significant models, wherein random combinations of six sites were included for regression analyses, revealed a great degree of variability in the applicability of these models depending on what sites were included in the regression analysis. Mean values of r^2 and adjusted r^2 (Table 4.18) from 100 replicates for each regression analysis, as well as the range of values, as indicated by the five-number summaries (minimum, quartile 1, median, quartile 3 and maximum values) for both (Tables 4.19-4.20) were used to interpret model validity. Model A had an original r^2 value of 0.6913; bootstrapping showed a range of 0.078 – 0.836 and a mean of 0.55539 ± 0.21365. Model B first showed an r^2 of 0.865, which was shown to be the maximum value of r^2 possible from bootstrapping; the range of r^2 for model B was 0.005-0.865 and the mean was 0.31383 ± 0.23248. Model C had an original r^2 of 0.6681; bootstrapping showed the range of this value to be 0.002-0.8403 and the mean to be 0.43494 ± 0.27008. Model D's original r^2 value was also equal to the maximum value attained from bootstrapping, 0.854; the range was 0.018-0.854 and the mean was 0.3511 ± 0.22044. Model E had an original r^2 value of 0.802, which was on the high end of the range indicated from bootstrapping of 0.007-0.870; the mean value was 0.44003 ± 0.24268.

Finally, Model F had an original r^2 of 0.77; the range was 0.024-0.848 and the mean was 0.4722 ± 0.21754. Adjusted r^2 values were similarly varied (Table 4.20). Overall, Model A had the highest mean r^2 value, but a very low mean adjusted r^2 (as this statistic takes into account sample size). Model A had a similiar mean r^2 value, and much higher mean adjusted r^2. However, because of high degrees of variation in r^2 and adjusted r^2 values, I do not suggest that they be used as predictive models. Instead, these models provide insight into the relationships between habitat characteristics and *B. pensylvanicus* abundance at sample sites that may be generalized to other areas. Generally speaking, as bare ground and tree cover surrounding urban green spaces increase, bumble bee abundance decreases.

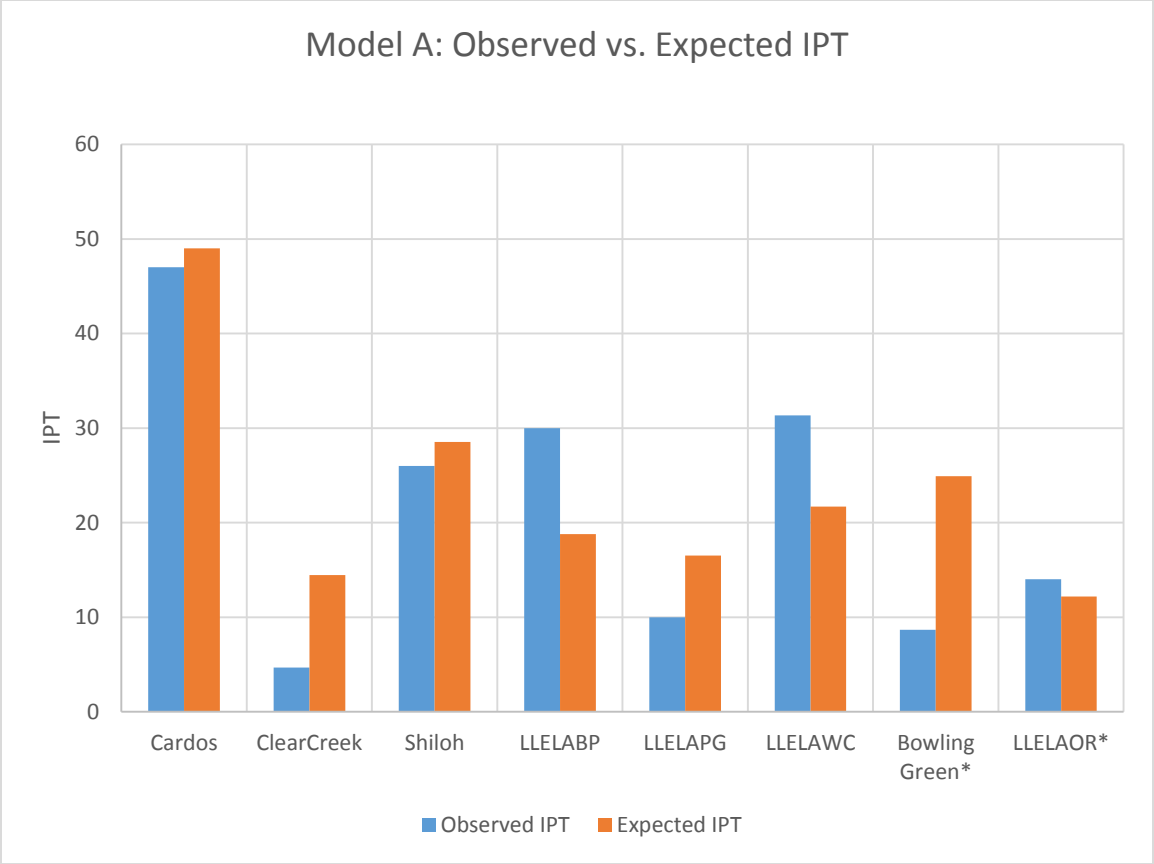

Figure 4.21: Observed versus expected values of IPT given significant model A: **IPT = 207.307-15.283 (LT5).** All sites are included in plot. Bowling Green and LLELA OR were the two validation sites. The expected value for Bowling Green was somewhat different from the observed, but LLELA OR was very close to the expected value.

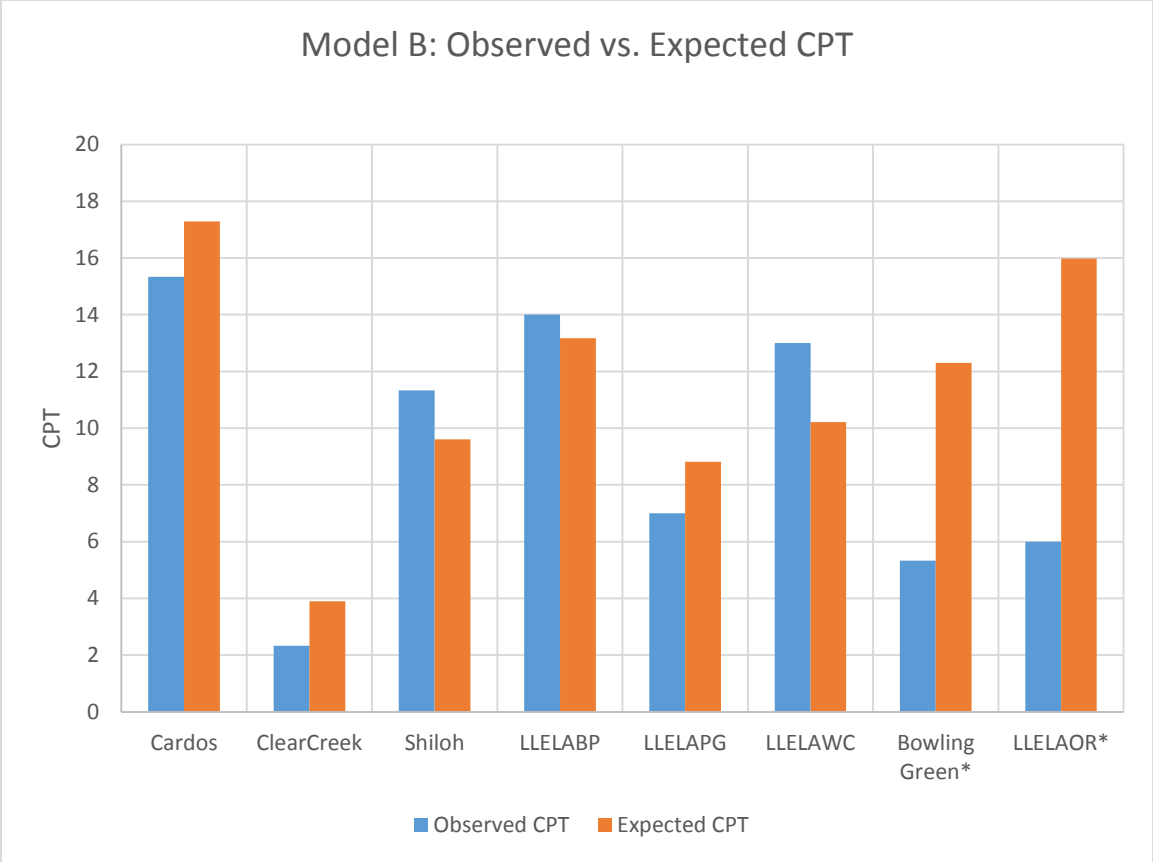

Figure 4.22: Observed versus expected values of CPT given significant model B: **CPT = 79.854 – 6.177 (LBG1).** All sites are included in plot. Bowling Green and LLELA OR were the two validation sites. The expected values for both validation sites are far from the observed values.

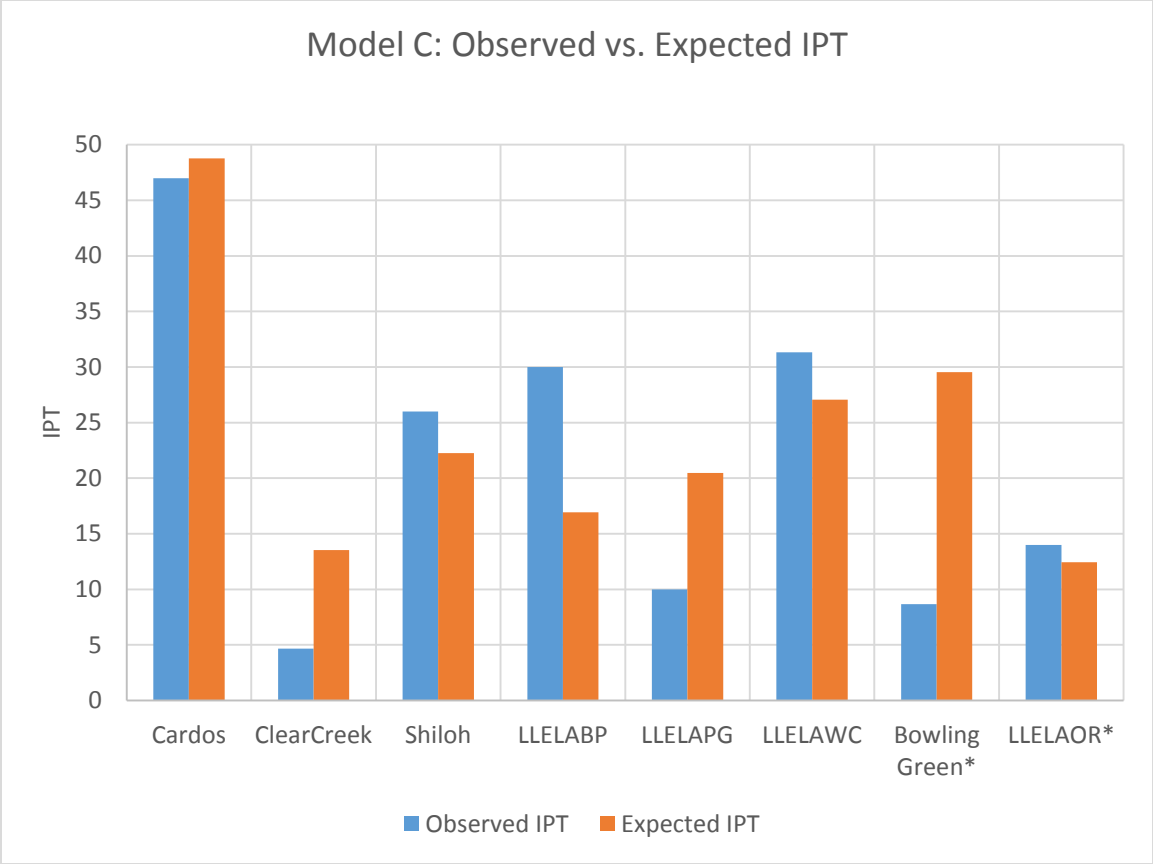

Figure 4.23: Observed versus expected values of IPT given significant model C**: IPT = 246.281-16.666 (LT1).** All sites are included in plot. Bowling Green and LLELA OR were the two validation sites. The expected values for validation site Bowling Green is relatively far from the observed.

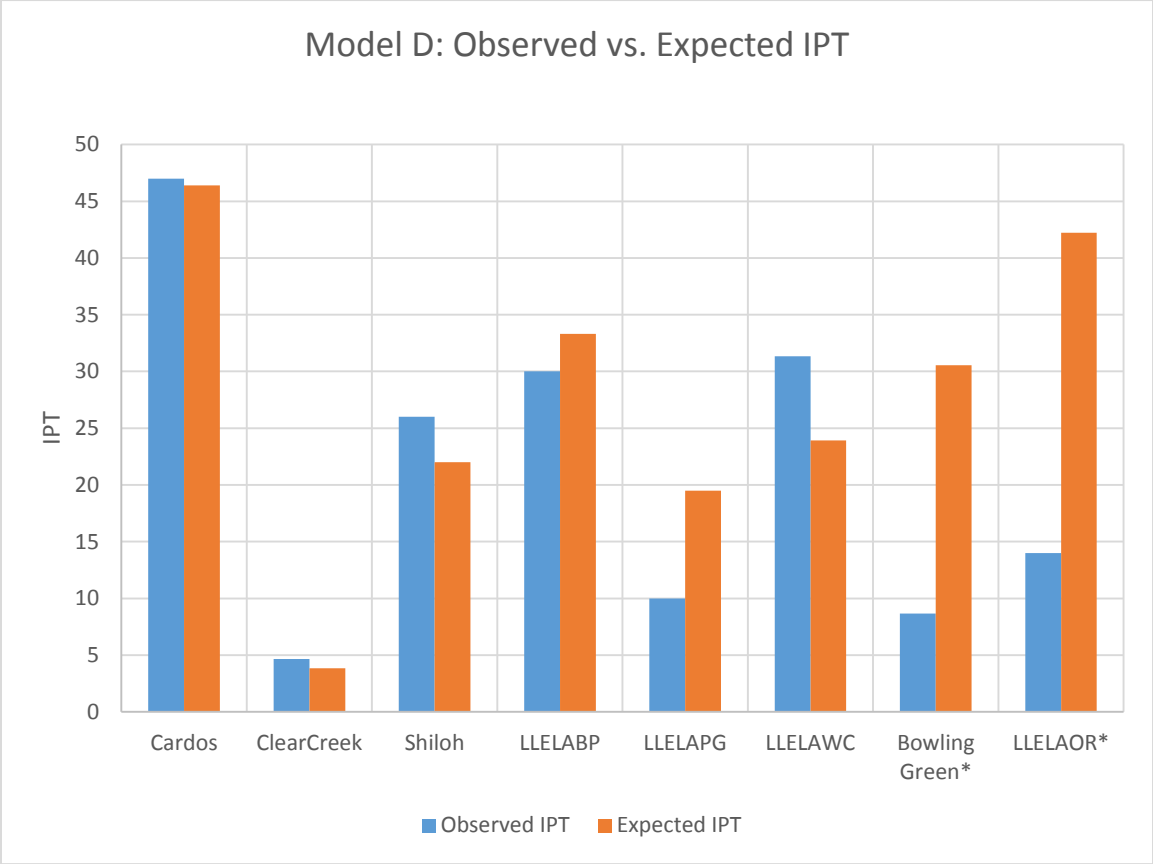

Figure 4.24: Observed versus expected values of IPT given significant model D: **IPT = 245.273-19.633 (LBG1).** All sites are included in plot. Bowling Green and LLELA OR were the two validation sites. The expected values for both validation sites are far from the observed values.

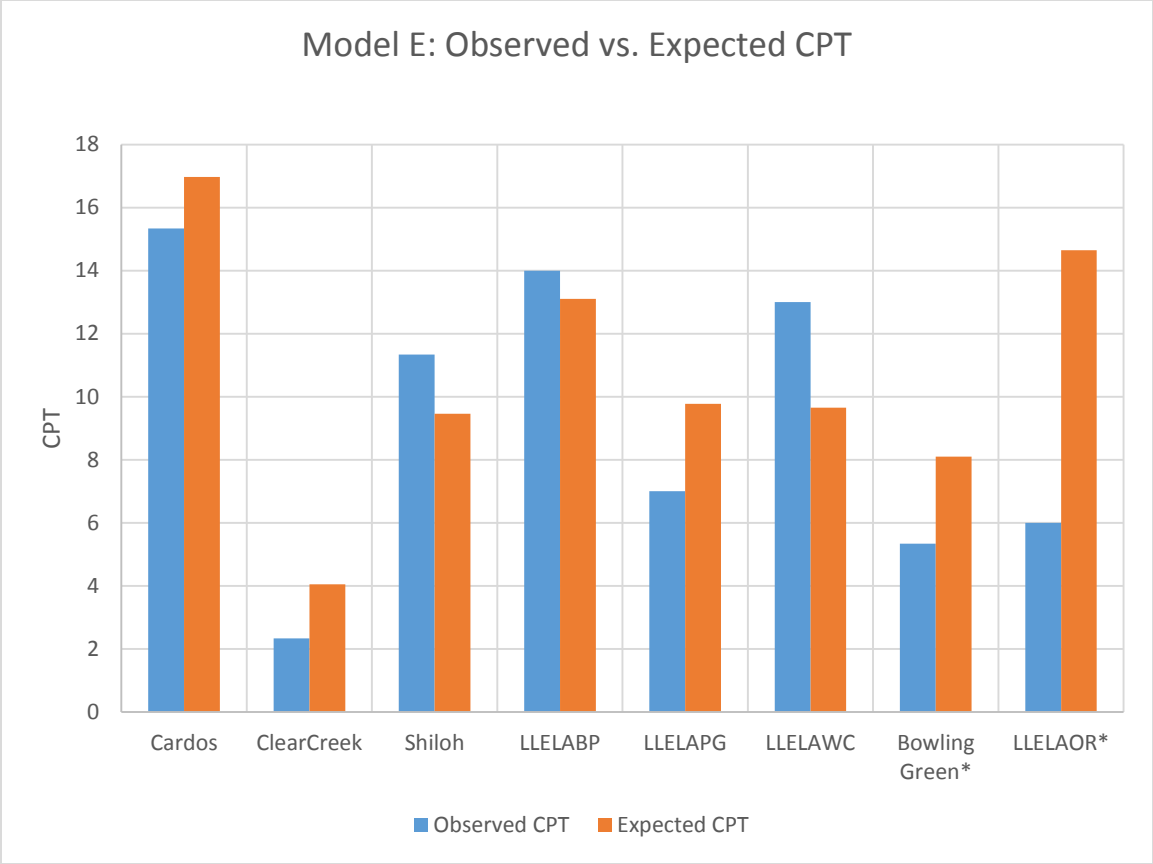

Figure 4.25: Observed versus expected values of IPT given significant model E: **CPT = 84.90 – 5.97 (LBG2).** All sites are included in plot. Bowling Green and LLELA OR were the two validation sites. In this case, the expected value for LLELA OR differed more from its observed value than Bowling Green's.

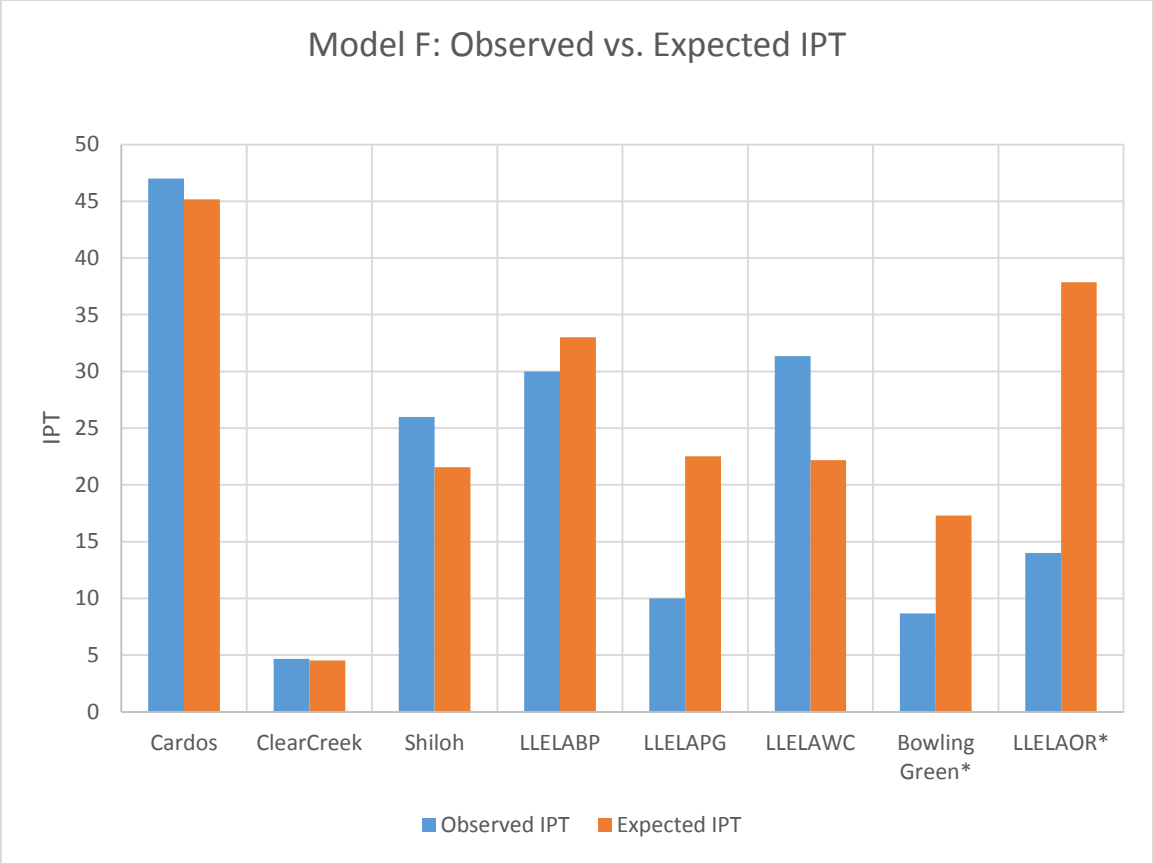

Figure 4.26: Observed versus expected values of IPT given significant model F: **IPT = 258.591 -18.756 (LBG2).** All sites are included in plot. Bowling Green and LLELA OR were the two validation sites. Both validation sites had observed values that were quite different from expected values.

Table 4.19: Range of r^2 values attained from bootstrapping of significant regression models. Included are the r^2 values attained from the original regression analysis, along with the five-number summaries from bootstrapping 100 replicates.

Model	**A**	**B**	**C**	**D**	**E**	**F**
Original value	0.691	0.827	0.668	0.854	0.763	0.770
Minimum	0.078	0.0006	0.0002	0.018	0.008	0.024
Q1	0.504	0.0842	0.163	0.178	0.248	0.326
Median	0.644	0.329	0.492	0.328	0.445	0.496
Q3	0.704	0.4337	0.674	0.477	0.587	0.600
Maximum	0.836	0.8272	0.84	0.854	0.861	0.848

Table 4.20: Range of adjusted r^2 values attained from bootstrapping of significant regression models. Included are the adjusted r^2 values attained from the original regression analysis, along with the five-number summaries from bootstrapping 100 replicates.

Model	**A**	**B**	**C**	**D**	**E**	**F**
Original value	0.614	0.784	0.585	0.818	0.704	0.713
Minimum	-0.153	-0.249	-0.25	-0.228	-0.24	-0.221
Q1	0.380	-0.145	-0.047	-0.028	0.06	0.157
Median	0.554	0.161	0.365	0.160	0.306	0.369
Q3	0.630	0.292	0.592	0.346	0.483	0.500
Maximum	0.795	0.784	0.8	0.818	0.826	0.811

4.6.4 Genetic Structure of Populations

Pairwise comparisons of allele frequencies between sites were used to calculate F_{ST} values for each set of sites. These analyses suggested population differentiation between LBP and CA (p = 0.03604±0.0201), BG (p = 0.03604±0.0148) and SH (p = 0.02703±0.0139), as well as between SH and LWC (p = 0.03604±0.0201). No other pairwise comparisons showed evidence of population differentiation (p-values all > 0.05) (Table 4.21). Upon further analysis with STRUCTURE, however there was no evidence of genetic clustering of individuals, even when the most conservative analyses, which included *a priori* population structure, were implemented. The most likely arrangement of individuals was into one large genetic population (Table 4.22). The results from F_{ST} and STRUCTURE analyses suggest that there is considerable gene flow between most sampling sites across Denton County and resulted in acceptance of null hypothesis 6.

Table 4.21: Pairwise F_{ST} values and p-values from comparisons of allele frequencies of nine loci between sampling sites, as calculated in Arlequin. Pairwise comparisons between LBP and BG, CA and SH suggest significant differentiation. Additionally, SH and LWC show significant differentiation. * indicates F_{ST} was < 0 due to algorithm implemented in Arlequin and so was changed to 0. Sampling site abbreviations are as follows: CC – Clear Creek Natural Heritage Center, SH – Shiloh Field, BG – Bowling Green Community Garden, LOR – LLELA Owl Ridge, LWC – LLELA Wood Chip, CA – Cardo's, LBP – LLELA Buffalo Pen, LPG – LLELA Pollinator Garden.

	BG	CA	CC	LBP	LOR	LPG	LWC	SH
BG	0							
CA	0.00107 (0.52252±0.0297)	0						
CC	0.00425 (0.60360±0.0430)	0.00277 (0.43243±0.0297)	0					
LBP	**0.02879 (0.03604±0.0148)**	**0.01419 (0.03604±0.0201)**	0* (0.73874±0.0379)	0				
LOR	0.00435 (0.47748±0.0471)	0* (0.78378±0.0385)	0* (0.81982±0.0439)	0* (0.72973±0.0497)	0			
LPG	0.0147 (0.39640±0.0454)	0* (0.66667±0.0454)	0* (0.87387±0.0141)	0* (0.77477±0.0433)	0* (0.84685±0.0365)	0		
LWC	0.00442 (0.35135±0.0566)	0.00323 (0.26126±0.0459)	0* (0.78378±0.0305)	0.00177 (0.46847±0.0402)	0.00092 (0.54955±0.0515)	0* (0.85586±0.0340)	0	
SH	0* (0.97297±0.0125)	0* (0.62162±0.0710)	0* (0.40541±0.0493)	**0.01152 (0.02703±0.0139)**	0* (0.90991±0.0214)	0.00727 (0.28829±0.0402)	**0.00566 (0.03604±0.0201**)	0

Table 4.22: Summary of results from STRUCTURE analysis of population differentiation. K=1 is most likely configuration.

K	LnP(D)	Var[LnP(D)]	Fst_1	Fst_2	Fst_3	Fst_4	Fst_5	Fst_6	Fst_7	Fst_8
1	-4949.4	47.3	0.0015	-	-	-	-	-	-	-
1	-4949.2	47.1	0.0008	-	-	-	-	-	-	-
1	-4949.1	47	0.0001	-	-	-	-	-	-	-
1	-4949	46.4	0.0014	-	-	-	-	-	-	-
1	-4949.3	47.1	0.0003	-	-	-	-	-	-	-
2	-5048.2	421.4	0.1412	0.0012	-	-	-	-	-	-
2	-5028.6	375.8	0.0016	0.179	-	-	-	-	-	-
2	-5122.9	482.3	0.0982	0.1018	-	-	-	-	-	-
2	-4993.8	315.1	0.1618	0.0001	-	-	-	-	-	-
2	-4992.7	320.3	0.1342	0.0013	-	-	-	-	-	-
3	-5086.5	594.3	0.154	0	0.1719	-	-	-	-	-
3	-5139.8	665.1	0.1577	0.0106	0.21	-	-	-	-	-
3	-5049.9	329.6	0.233	0.0004	0.199	-	-	-	-	-
3	-5140.6	656.6	0.0009	0.1778	0.2499	-	-	-	-	-
3	-5192.9	766.2	0.0009	0.2357	0.1641	-	-	-	-	-
4	-6006.1	2425.4	0.0478	0.1832	0.2237	0.13	-	-	-	-
4	-5365.4	1239	0.0684	0.1806	0.0972	0.1706	-	-	-	-
4	-5956.5	2342.3	0.2034	0.0012	0.2107	0.2046	-	-	-	-
4	-5304.2	1088.3	0.1764	0.1819	0.0348	0.1859	-	-	-	-
4	-5679.3	1821.7	0.0192	0.221	0.1764	0.1233	-	-	-	-
5	-6506.8	3382.3	0.1115	0.1921	0.0803	0.1995	0.158	-	-	-
5	-5409	1288.7	0.1409	0.1007	0.1238	0.1956	0.2432	-	-	-
5	-5634.9	1778.6	0.1899	0.1894	0.0001	0.1739	0.2059	-	-	-
5	-5471.1	1237.5	0.1002	0.0803	0.2062	0.1136	0.154	-	-	-
5	-5167.9	672.4	0.2036	0.0339	0.1833	0.0903	0.0942	-	-	-
6	-5453.5	1286.1	0.2038	0.1248	0.0543	0.1475	0.1374	0.2411	-	-
6	-5816.7	2061.1	0.1624	0.1916	0.0572	0.176	0.3006	0.2033	-	-
6	-6585.9	3511.7	0.1376	0.1776	0.2137	0.1739	0.0888	0.0913	-	-
6	-5457.4	1155.3	0.0361	0.1553	0.0964	0.1527	0.0394	0.0428	-	-
6	-5967.4	2220.4	0.1419	0.0821	0.2117	0.1572	0.0456	0.0796	-	-
7	-7455.2	5215.9	0.1408	0.1544	0.0767	0.0851	0.1347	0.1087	0.1387	-
7	-6459.6	3427.6	0.1779	0.2211	0.2008	0.1653	0.0315	0.161	0.1631	-
7	-5883.9	2319.4	0.0391	0.1432	0.1834	0.1812	0.2449	0.1425	0.1922	-
7	-5978	2508.4	0.1608	0.1713	0.1913	0.1103	0.1227	0.1565	0.2432	-
7	-6034.8	2513.7	0.1695	0.0685	0.1904	0.1052	0.2037	0.2038	0.1905	-
8	-5238	722.4	0.0562	0.1613	0.0701	0.1204	0.1799	0.096	0.0627	0.179
8	-6151.4	2732.7	0.1481	0.1586	0.0053	0.2217	0.1835	0.1623	0.0894	0.1895
8	-5417.1	1081.9	0.0874	0.1129	0.1116	0.1705	0.0787	0.1255	0.1298	0.0005
8	-6519.1	3588.2	0.1807	0.1823	0.2154	0.1765	0.1544	0.1938	0.1876	0.0919
8	-5213.3	694.8	0.1478	0.0528	0.0316	0.0513	0.2452	0.1282	0.0001	0.0421

CHAPTER 5

DISCUSSION AND IMPLICATIONS

Bumble bee declines have been documented worldwide; the alterations and losses of habitat caused by rapid human population growth and associated urban sprawl comprise a main factor in these losses. Though the solutions for ecological questions are often best identified when these problems are studied at multiple scales, only a handful of studies have explored bumble bee conservation at regional or finer (<100 km) scales, bearing mixed conclusions that tend to be dependent on the species and the local environment. In order to conserve declining bumble bee species in north Texas it is crucial that we assess local declines and identify best practices for the particular species present in urban areas. This fine-scale (~35-km) interdisciplinary study has presented the first effort to quantify bumble bee abundance and diversity in the urban green spaces of Denton County and to document landscape characteristics that are important to the use of these spaces by a declining species, *B. pensylvanicus*. In the course of this study I have formally documented three new historic bumble bee species records (*B. fraternus*, *B. bimaculatus* and *B. impatiens*), presented evidence of *B. fraternus* decline in Denton urban areas, provided the first-ever study of the genetic diversity of bumble bees in north Texas, and related local and landscape-scale habitat characteristics to differences in *B. pensylvanicus* abundance at sample sites. The results of this study provide baseline data for future studies of bumble bees in north Texas, clarify the current status of two declining species in the area, and present considerations for conservation measures that are locally appropriate.

5.1 Persistence of Bumble Bee Species in Urban Spaces of Denton County

Evidence for range reductions and local extirpations in other parts of the United States has led both *B. pensylvanicus* and *B. fraternus* to be placed on various conservation watch lists. The examination of museum records that spanned the years 1952-2012 indicated that these two species historically have been the most common bumble bees in Denton County. (One individual each of *B. bimaculatus* and *B. impatiens* were also present in the records, but not included in persistence analyses. These species are considered stable across their ranges, but Denton County is on the western limit of their ranges and so they are, not surprisingly, rare in this area and were not detected in the present study.) Interestingly, though *B. pensylvanicus* remained consistently common across collecting years in the museum records, the last historic specimen recorded of *B. fraternus* was collected in 1998, leading to concern regarding the current status of the species; on the other hand, *B. pensylvanicus* remained consistently common in the historic records. However, because the museum records available for study had not originated from targeted bumble bee surveys, but instead mainly came from student collections or general insect surveys, the absence of *B. fraternus* from the more current records was interpreted cautiously.

When field surveys were performed at urban green spaces in 2013, I found that *B. pensylvanicus* was present at every single site, and comprised ~97% of the individuals sampled. Though not nearly as abundant, *B. fraternus* was discovered at two sites and comprised ~3% of the individuals sampled, eliminating the concern of local extirpation. Still, when I compared the current relative abundance of each species to their historic proportions (85% *B.pensylvanicus* and 15% *B. fraternus*), I found that *B.*

fraternus is significantly less abundant currently than historically, at least in urban areas. On the other hand, *B. pensylvanicus* is considerably more abundant.

The fact that *B. pensylvanicus* persists locally in north Texas contrasts with results of studies at the national scale (Cameron et al., 2011), as well as in other regions (Colla et al., 2012; Colla & Packer, 2008; Colla et al., 2011; Grixti et al., 2009; Lozier & Cameron, 2009), where declines have been documented. However, this finding agrees with the assertion in Cameron et al. (2011) that *B. pensylvanicus* continues to remain abundant in some southeastern states, including Texas, as well as the findings presented in Beckham et al. (2015, in review*)*, where 2010-2014 *B. pensylvanicus* abundance across a 24-county region of north Texas was not different from historic levels. On the other hand, the decline detected in *B. fraternus* is on par with findings in other regions of the United States (Colla et al., 2012; Grixti et al., 2009), but contrasts with the results of Beckham et al. (2015, in review*)*, which showed no difference between current and historic relative abundance of the species across the north Texas region.

It is well-documented that bumble bee declines often coincide with loss of native grasslands (Carvell et al., 2006; Grixti et al., 2008), which provide ideal foraging and nesting habitat. Modern row-crop agriculture, as well as urban sprawl, typically result in the complete destruction of such habitat. Though many of North America's grasslands have suffered this fate (Samson & Knopf, 1994), many Texas grasslands have been converted to rangeland (Texas Land Trends, 2009) wherein substantial habitat is still available for bumble bees. This may be one factor allowing for *B. pensylvanicus* to continue to thrive across the region.

However, it would seem that both *B. pensylvanicus* and *B. fraternus* would benefit from the retention of rangeland in Texas, and so it is unclear why *B. pensylvanicus* would be performing better in urban areas than *B. fraternus*, as is suggested by the data from Denton County urban green spaces. It is possible that these two species directly compete with each other, and that *B. pensylvanicus* typically comes out ahead. Much that is known about these species' natural history is similar between the two, but one major difference is tongue length: *B. pensyvlanicus* is considered a "long-tongued" species, while *B. fraternus* is a "short-tongued" species (Williams et al., 2014). Because of this difference, *B. pensylvanicus* may be able to exploit a wider range of flowers, to include those with very deep corollas, than *B. fraternus*. Though data are lacking regarding competition between these two species, I found that the two sampling sites in my study where both species were present, Bowling Green Community Garden and Clear Creek Natural Heritage Center, exhibited the overall fewest numbers of bumble bee individuals of all sites, suggesting that competition could indeed play a role in relative abundance. Further research into this phenomenon in the form of expansion of sample sites in urban areas across the region is warranted.

The decline of *B. pensylvanicus* in other parts of the United States has also been linked to diminished genetic diversity at both the continental scale (Cameron et al., 2011) and regional scale (Lozier & Cameron, 2009). As compared to these populations, the Denton County *B. pensylvanicus* population displayed elevated gene diversity; total H_E was 0.577 at the continental scale and 0.584 within the Illinois populations, versus 0.725 for my population, possibly aiding in the persistence of this species in the study area. At this point no data exist regarding the genetic diversity of any populations of *B.*

fraternus and so I cannot say whether this may be contributing to its decline here or elsewhere; I was unable to amplify *B. fraternus* DNA at any of the loci that were used for *B. pensylvanicus*, and so future studies should involve identifying appropriate markers and increasing the sample size of *B. fraternus*.

5.2 Importance of Urban Green Spaces for *B. pensylvanicus*

Urban sprawl and the simultaneous expansion of impervious surface results in reduction of the three main habitats required by bumble bees: foraging grounds, underground nest sites, and underground overwintering sites for queens. As such, it would seem that human development would result in the eradication of bumble bees in urban areas. However, the present study has shown that this is not always the case, especially when green spaces are retained within urban areas, reinforcing the findings of others.

The abundance of *B. pensylvanicus* individuals at urban green spaces in Denton County was measured both in terms of individuals, as well as in terms of the numbers of colonies represented by those individuals. Overall I sampled 435 unique individuals across the eight sample sites and subsequently used microsatellite DNA data to estimate that these individuals arose from between 188 - 361 hives. From these numbers it is clear that the urban green spaces of Denton County are providing foraging habitat to a large number of *B. pensylvanicus* individuals and hives. These findings are in line with those from studies of other bumble bee species in urban areas wherein large numbers of individuals (Ahrne et al., 2009; Matteson & Langellotto, 2010; McFrederick

& LeBuhn, 2006; Tommasi et al., 2004) and nests (Chapman et al., 2003; Jha & Kremen, 2013b) have been detected visiting urban sites.

This is not to say that urban green spaces are ideal habitat for bumble bees. Instead, it is likely that urban green spaces serve as a sort of “watering hole” where bumble bees from the surrounding areas converge and are forced to share scarce resources. These findings are particularly important as Texas experiences rapid population growth and associated expansion of urban areas (from 1997 to 1012 Texas grew from 19 million to 26 million residents). By including green spaces in city plans and expanding existing spaces we may be able to mitigate the habitat loss associated with urbanization and conserve bumble bees.

5.3 Importance of Landscape Characteristics

A variety of studies have highlighted the importance of landscape-scale habitat factors for pollinator conservation (Cariveau & Winfree, 2015; Hines & Hendrix, 2005; Scheper et al., 2013; Stanley et al., 2013). This study investigated whether landscape characteristics at four different scales could be used to explain variations in *B. pensylvanicus* abundance at urban green spaces in Denton County in order to ultimately provide insight into urban habitat management for bumble bee conservation purposes.

Tree cover surrounding sampling sites was found to be significantly negatively associated with the numbers of individuals collected per sampling trip at the 0.25-km and 0.5-km scales (and was almost significant at the 1-km and 2-km buffers); tree cover was also included in significant models at both the 0.5-km and 1-km buffer levels. In

contrast, Jha and Kremen (2013a) found that natural woodland cover was positively associated with *B. vosnesenskii* nesting densities at a 2-km scale. We suggest that these findings are a product of *B. pensylvanicus* nesting preferences; the limited data on this, as well as anecdotal evidence, suggest that *B. pensylvanicus* prefers open grasslands and the thatch therein for nests. Perhaps increased tree cover, which is not typically associated with grassland habitat (FGDC, 2008) is indicative of less available nesting habitat for this species.

At the 1-km and 2-km scales, bare ground was found to be strongly negatively correlated with both the numbers of individuals collected per sampling trip and the numbers of colonies detected per trip, and was included in significant linear regression models for both at these scales. In my land use analyses bare ground pixels often consisted of either bare soil associated with arable land or construction zones that were indicative of urban sprawl. Thus, the present study upholds previous findings that both agricultural and urban expansion are negative for bumble bee populations.

Of note was the high degree of variability in r^2 and adjusted r^2 values associated with significant explanatory models that was revealed in the model validation bootstrapping step. This was likely due to the small number of sample sites (eight) involved in the study and leads me to exercise caution in stating that significant models can be broadly applied for prediction purposes. However, the relationships between dependent and independent variables may be viewed with confidence. I conclude that tree cover at both local and landscape scales, as well as bare ground cover at landscape scales, are likely to be negative drivers of *B. pensylvanicus* abundance in urban green spaces across regions. Expanded studies involving more sites across the

north Texas region, and even across the state, will be important for determining whether these conjectures are accurate.

5.4 Genetic Diversity and Structure of *B. pensylvanicus* in Denton County

Gene flow between populations is an important factor in the maintenance of genetic variation and the ability for species to adapt to changing environments. Generally, the fragmented landscapes associated with urban areas can result in restriction of biological dispersal and gene flow; specifically, urban land use has been shown to limit gene flow in at least one bumble bee species (Jha & Kremen, 2013b). However, the contribution of population structuring to bumble bee declines has been ambiguous. Some declining species have shown significant population differentiation at regional scales (Darvill et al., 2010; Ellis et al., 2006), to include *B pensylvanicus* in Illinois (Lozier & Cameron, 2009), but some stable species have also shown significant structuring at this scale (Jha & Kremen, 2013b; Lozier et al., 2013). Fine-scale studies of genetic differentiation in bumble bees have been scarce, but Dreier et al. (2014) found no structuring of stable or declining species in their UK study. Cameron et al.'s (2011) study of four declining and four stable species across North America showed significant population structure in just one stable species; *B. pensylvanicus* showed no differentiation at that scale.

The microsatellite DNA analyses in this study showed some evidence of fine-scale (~35-km) genetic differentiation between sites that were geographically far from each other when F-statistics were calculated. However, STRUCTURE analyses found no evidence of genetic clustering of *B. pensylvanicus* subpopulations the Denton County

urban matrix, despite high levels of genetic diversity. These results imply that barriers to gene flow and dispersal (i.e., movement of new queens and males) across the area are very limited, likely promoting the continued presence of *B. pensylvanicus* in Denton County urban spaces. Expanded population genetics studies across the region should be useful in determining whether this factor could help explain the persistence of *B. pensylvanicus* in Texas while it declines across its northern and eastern ranges.

5.5 Final Conclusions

This research has presented evidence that urban green spaces in Denton County provide important foraging habitat that is helping to conserve two declining bumble bee species, *B. fraternus* and *B. pensylvanicus*. Additionally, these spaces are supporting a surprisingly large number of *B. pensylvanicus* individuals and hives. Competition between *B. fraternus* and *B. pensylvanicus* may drive the numbers of both down at shared sites, as evidenced by the fact that sites housing both species had the lowest numbers of individuals over all. The decline in *B. fraternus* relative abundance at urban green spaces, as compared to historic levels, is likely in part due to competition from *B. pensylvanicus* for these increasingly scarce resources, along with other unknown factors. It is possible that *B. fraternus* simply has not adapted well to living in close proximity to humans, while *B. pensylvanicus* is more amenable to the situation.

Across sample sites, *B. pensylvanicus* displayed high levels of genetic diversity as compared to declining populations of bumble bees elsewhere; moreover, no population structuring is evident, suggesting high levels of dispersal and gene flow. I was unable to investigate the population genetics of *B. fraternus* due to a low sample

size, as well as an inability to amplify this species' DNA at the chosen markers, and so I cannot say whether population differentiation or low genetic diversity are contributing to *B. fraternus* losses in urban areas.

The finding that tree cover is a negative driver of *B. pensylvanicus* abundance at all scales studied is likely due to this species' propensity for nesting habitat associated with grasslands that inherently have few trees. Additionally, the evidence that bare ground negatively affects *B. pensylvanicus* can be attributed to the association of bare ground with arable (plowed) land and construction zones across the study area, both of which provide little to no habitat for bumble bees. These findings highlight the importance of maintaining nesting habitat, as well as foraging habitat, for bumble bees as we disturb more land for human uses. The continued conversion of grasslands in the study region to agricultural land and, possibly more importantly, urban spaces will likely be negative for bumble bees in the future. However, this study has shown that mitigation of these practices in the form of providing nest sites and green spaces with ample floral resources will be important to the continued conservation of local bumble bee species in urban areas.

APPENDIX A

CLUSTER BUSTING PROCEDURE

Unsupervised Classification and Cluster Busting Procedure for Satellite Imagery in ERDAS Imagine 2015; addendum to Remote Sensing Manual produced by Bruce Hunter and Sam Atkinson (2010):

1. Open ERDAS Imagine and load satellite imagery file. This file will be called the “satellite image” from here forward. (Open → Raster Layer → choose imagery file from appropriate directory)
2. Perform an unsupervised classification of the satellite image from the Raster menu (tab). (Raster → Unsupervised → Unsupervised Classification; input raster file is the satellite image; choose clustering option and number of classes; other options may be left at default or changed, depending on your preferences.)
3. Manually classify clusters of output file into appropriate land use categories where possible. I suggest opening Google Earth and displaying it beside your classified image for reference. Open the attribute table of the image by right-clicking on the image name. Change the color of the class that you are evaluating to red (or another color that stands out to you) and determine if it should be assigned a land class or not, i.e. it contains multiple land class types. If it can be classified, assign the correct name and give it an appropriate color (e.g., green for vegetation). If it contains a mixture of land types, designate it as such. Do this for all clusters and stay consistent with names and colors.

4. Once you have determined which classes need further evaluation, save your (partially classified) file.
5. Recode the partially classified file, changing obvious classes to a new value of “0” and problematic classes to a new value of “1.” (Raster → Thematic → Recode; your input file should be the output file from step 2, i.e. the product of the unsupervised classification; designate an ouput file; leave other options default; press “setup recode” to change class values.)
6. Mask your satellite image with recoded output file from step 5. (Raster → Subset & Chip → Mask; input file is satellite image, input mask file is recoded image from Step 5, designate output file; leave all other options default.)
7. Perform unsupervised classification of masked image (output from step 6), repeating steps 2-3 above.
8. If there are still problematic classes, then repeat entire process (steps 2-7) as many times as needed.
9. Stitch classified images together. (Toolbox → Mosaic → MosaicPro → Edit → Add Images; choose images that should be added back together; then Process → Run Mosaic and designate output file.)

APPENDIX B

SAS PROGRAM EXAMPLES

MaxR Process:

```
DATA BUMBLE_Random6_LN;
*Jessica Beckham, Urban Denton Bumble Data;
*Landscape data for 6 random locations;
INPUT SITE Location $ CPT ICPT IPT LA LIS2 LIS1 LIS5 LIS25 LV2 LV1 LV5     LV25 LT2          LT1
          LT5      LT25 LBG2       LBG1    LBG5 LBG25 LW2         LW1      LW5 LW25;
*SITE = SITE NUMBER, Location = Location ID,CPT = average number of colonies detected per trip,
ICPT = average number of inferred colonies per trip,
IPT = average number of individuals sampled per trip, (L) in front of each independent variable indicates
ln transformation applied: A=site area in m2, IS2/IS1/IS5 = impervious surface area (m2) in 2km, 1km,
0.5km buffers respectively,
V2/V1/V5 = vegetation area (m2) in corresponding buffers, T2/T1/T5 = tree cover area (m2) in
corresponding buffers, BG2/BG1/BG5 = bare ground area (m2) in corresponding buffers,
W2/W1/W5 = area water (m2) in corresponding buffers;
CARDS;

2         Cardos 14       17.33333333 47        8.806424064     13.48777286     12.16180723
          10.94362291     9.26823185      16.22688082     14.82130702     13.42877103     12.05515673
          12.90481415     11.85148124     10.3573618      9.311451873     11.37884535     10.12918783
          9.115150185     6.498282149     12.22383398     10.91675979     9.365975664     8.166216269
3         ClearCreek      2       2       4.666666667     10.3851418      12.93349564     10.62871463
          9.460320577     7.756195344     15.73476692     14.33988399     12.94933292     11.68080997
          15.26435897     13.96679198     12.61917518     10.99359713     13.54509194     12.29699138
          10.64323202     9.304194947     12.64617104     10.3870242      9.245707516     8.655214489
4         Shiloh  10      11      26      10.90839337     14.97081567     13.66426432     12.36490378
          11.1451029      15.63235411     14.21920343     12.89471972     11.86087445     14.87107198
          13.44202656     11.69834223     10.4237089      12.63715352     11.37274343     10.31453704
          9.670293665     10.82201578     9.174920427     7.756195344     6.674561392
5         LLELABP         12      15      30      8.568076402     13.64519188     10.83918868
          8.740656692     6.527957918     15.51189651     14.38412799     13.20221653     11.83001099
          15.0730791      13.76190723     12.33451863     10.95995753     12.02689813     10.79556756
          9.124782484     6.423246964     14.76677762     12.71042939     7.508787171     5.730099783
7         LLELAPG         6       19      10      6.408528791     13.63163099     11.53851524
          10.2429553      9.302372457     15.09202353     13.69306718     12.4320761      11.2735509
          14.63953511     13.54871288     12.48373468     11.27416053     12.58579592     11.5002049
          10.79573146     9.848820151     15.53463195     14.07399963     12.18159551     9.123474439
8         LLELAWC         11      12      31.33333333     8.528924114     14.56644729     12.78404464
          11.10688019     9.45469732      15.39601799     14.04643178     12.75622667     11.61933743
          14.24229871     13.15473763     12.1444738      11.01409429     12.60486219     11.27405895
          10.15517969     8.540909718     15.13783264     13.74308609     11.98237894     8.817001444

PROC REG;
MODEL IPT = LA LT25 LBG25 / selection = maxr;
*output results of maxr regression process for IPT vs natural log transformed independent variables at
0.25 km buffer. LIS25 and
LW25 removed because least correlated IVs. LV25 removed because of collinearity with LT25;
RUN;
```

Pearson's Correlation Process:

```
PROC CORR PEARSON data=bumble_ln;
VAR CPT ICPT IPT LA LIS2 LV2 LT2 LBG2 LW2;
*correlation analysis for parametric data - testing for a significant correlation between variables for ALL SITES;

PROC CORR PEARSON data=bumble_ln;
VAR CPT ICPT IPT LA LIS1 LV1 LT1 LBG1 LW1;

PROC CORR PEARSON data=bumble_ln;
VAR CPT ICPT IPT LA LIS5 LV5 LT5 LBG5 LW5;

PROC CORR PEARSON data=bumble_ln;
VAR CPT ICPT IPT LA LIS25 LV25 LT25 LBG25 LW25;

RUN;
```

Bootstrapping – Survey Select Process:

```
proc surveyselect data=bumble_ln
   method = srs
          n = 6
reps = 100
out= bootstrap100;
*creates dataset of randomly selected samples (i.e., sites) from bumble_ln.
method srs requests simple random sampling (units selected with equal probability and without
replacement).
n = -- specifies number of samples to be included in each replicate.
reps = number of replicates;

RUN;

PROC PRINT;
run;
```

Bootstrapping – regression by replicate

```
proc reg data = bootstrap100 outest = bootstrapCPT_LBG1 tableout rsquare adjrsq alpha = 0.05;
by Replicate;
model CPT = LBG1 ;
*running regression model on each replicate generated from previous bootstrapping step (proc
surveyselect).
output dataset created with rsquare and adjrsq values included
model DV = IV is stated;
run;
 proc print data = bootstrapCPT_LBG1;
 run;

 PROC EXPORT
DATA=bootstrapCPT_LBG1
DBMS=TAB
LABEL
OUTFILE='C:\Users\Jessica\Desktop\Dissertation Files\SAS Output\Six sites random\ln
transformed\bootstrapCPT_LBG1.txt'
REPLACE;
RUN;
*exports dataset to a tab-delimited text file in named file location;
```

Bootstrapping – summarizing regression by replicate in PROC UNIVARIATE

```
PROC UNIVARIATE data = bootstrapCPT_LBG1 normal plot outtable = bootstrapCPT_LBG1_CI
CIBASIC alpha = 0.05;
var _rsq_ _adjrsq_;
*construct 95% confidence intervals around the mean r2 and adj r2 values from the bootstrap procedure;
run;

proc print data = bootstrapCPT_LBG1_CI   ;
run;

PROC EXPORT
DATA=bootstrapCPT_LBG1_CI
DBMS=TAB
LABEL
OUTFILE='C:\Users\Jessica\Desktop\Dissertation Files\SAS Output\Six sites random\ln
transformed\bootstrapCPT_LBG1_CI .txt'
REPLACE;
RUN;
*exports dataset to a tab-delimited text file in named file location;
```

ANOVA for genetic diversity comparisons

```
DATA EXPHETEROZYGOSITY_BUMBLE;
*Jessica Beckham, allelic richness data per locus per sampling site;

INPUT location $ HE @@;
*Location = Location ID, followed by 8 values of expected heterozygosity for each locus (in this order:
BTERN01AR    BL15AR          BT30AR
B124AR          BT10AR          B126AR          B121AR          B96AR  ;
CARDS;


Bowling_green  0.57863 Bowling_green 0.88508 Bowling_green 0.67742 Bowling_green 0.61492
Bowling_green  0.84677 Bowling_green 0.3871 Bowling_green   0.88276 Bowling_green 0.78421
Cardos 0.7279 Cardos  0.87591 Cardos 0.59365 Cardos 0.50653
Cardos 0.91973 Cardos 0.43645 Cardos 0.90561 Cardos 0.78481
ClearCreek 0.74725 ClearCreek 0.93333 ClearCreek 0.61538 ClearCreek 0.71111
ClearCreek 0.81319 ClearCreek 0.52747 ClearCreek 0.95556 ClearCreek 0.91111
LLELA_BP       0.75661 LLELA_BP       0.80952 LLELA_BP       0.66931 LLELA_BP       0.64021
LLELA_BP       0.92328 LLELA_BP       0.50794 LLELA_BP       0.85281 LLELA_BP       0.75385
LLELA_OR       0.8 LLELA_OR  0.88095 LLELA_OR       0.71587 LLELA_OR       0.51746
LLELA_OR       0.91746 LLELA_OR       0.5 LLELA_OR  0.91444 LLELA_OR       0.7977
LLELA_PG       0.76923 LLELA_PG       0.85714 LLELA_PG       0.47253 LLELA_PG       0.59341
LLELA_PG       0.92308 LLELA_PG       0.53846 LLELA_PG       0.94505 LLELA_PG       0.85714
LLELA_WC       0.68998 LLELA_WC       0.8529 LLELA_WC        0.58575 LLELA_WC       0.48737
LLELA_WC       0.89577 LLELA_WC       0.50093 LLELA_WC       0.92254 LLELA_WC       0.80166
LLELA_WC       0.07493 Shiloh  0.75154 Shiloh  0.87097 Shiloh  0.66506
Shiloh   0.60975 Shiloh  0.90255 Shiloh  0.40957 Shiloh  0.91703
Shiloh   0.79068

proc anova data = EXPHETEROZYGOSITY_BUMBLE OUTSTAT=ANOVA_HE;
class location;
*class statement to identify independent variable;
model HE = location;
*model statement to define dependent = independent variable - in this case location is independent
variable and we are
testing hypothesis that mean HO is same across sites;

means location /bon ;

run;

PROC print;
run;

PROC EXPORT
DATA=ANOVA_HO
DBMS=TAB
LABEL
OUTFILE='C:\Users\Jessica\Desktop\Dissertation Files\ANOVA_HE.txt'
REPLACE;
RUN;
```

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