USING MORPHOMETRIC ANALYSIS OF HONEY BEE (*APIS MELLIFERA*) COLONIES TO IDENTIFY LINEAGES AND SUBSPECIES ON THE ISOLATED ISLAND OF NEWFOUNDLAND

By

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Abstract

The Island of Newfoundland's honey bees, *Apis mellifera* Linnaeus, are relatively disease free compared to other populations and have an exceptional winter survival record. Perhaps these qualities arose due to the Island's isolation from parasites such as *Varroa destructor* and lower pesticide use compared to the Canadian mainland. Independently, these two environmental qualities alone should make Newfoundland bees highly desirable to apiarists on mainland Canada. In 1980, due to the increase in *Varroa destructor*'s decimation of honey bee populations around the world, the Government of Newfoundland and Labrador put into place legislation that prevents the importation of honey bees into the province. Over the last 40 years, commercial and hobby apiculture has increased drastically in popularity, but without the ability to bring in new honey bee stock there is a greater need to understand what lineages and subspecies are currently available to beekeepers on the Island.

The program IdentiFly was used to determine colony lineages and subspecies by analyzing the species-specific vein patterns and wing shapes of honey bee workers. Analysis of wing vein patterns of worker bees has shown variations of honey bee subspecies currently occuring on the Island. The majority of colonies fell into one of two lineages: lineage C or lineage O; most colonies tested were categorized as a hybrid of these lineages. Further analysis suggests that colony subspecies found on the Island include *Apis mellifera ligustica*, *Apis mellifera carnica*, *Apis mellifera cecropia*, *Apis mellifera armeniaca*, *Apis mellifera meda*, and *Apis mellifera scutellata.* Subspecies population percentages vary across the Island due to local selection, the combination of natural and artificial pressures on colony survivability. A majority of colonies were identified as the Armenian honey bee, *A. m. armeniaca*. This suggests the adaptative advantage this specific honey bee subspecies has in relation to the influences in Newfoundland and mainland Canada.

Acknowledgements

"People underestimate bees.

Granny Weatherwax didn't. She had half a dozen hives of them and knew, for example, there is no such creature as an individual bee. But there is such a creature as a swarm, whose component cells are just a bit more mobile than those of, say, the common whelk. Swarms see everything and sense a lot more, and they can remember things for years, although their memory tends to be external and built out of wax. A honeycomb is a hive's memory – the placement of egg cells, pollen cells, queen cells, honey cells, different *types* of honey, are all part of the memory array.

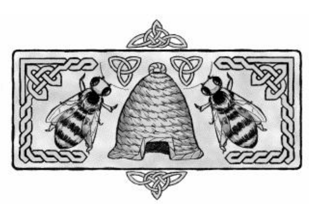
And then there are the big fat drones. People think all they do is hang around the hive all year, waiting for those few brief minutes when the queen even notices their existence, but that doesn't explain why they've got more sense organs than the roof of the CIA building.

Granny didn't really *keep* bees. She took some old wax every year, for candles, and the occasional pound of honey that the hives felt they could spare, but mainly she had them for someone to talk to.

For the first time since she'd returned home, she went to the hives.

And stared…."

-GNU Terry Pratchett, Lords and Ladies



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Chapter One – Introduction

1.1 Colony collapse disorder

As much as 35% of global food production is dependent upon pollinating animals with *Apis mellifera* (honey bees) accounting for 90% of commercial pollination services (Genersch *et al*., 2010), making them an important component of the agricultural ecosystem. Beginning in the mid-2000s, a large number of honey bee colony losses were reported in the United States and around the world (Le Conte *et al*., 2010). The term Colony Collapse Disorder (CCD) was coined to describe the sudden disappearance of bees and the eventual death of colonies. During the initial outbreak of CCD, blame was placed on a wide range of factors such as cell phones, genetically modified crops, microwave towers, and even alien abduction (Jacobsen, 2008). The cause of CCD is still debated today but much research suggests there were multiple factors causing these disastrous losses including *Varroa destructor* mites, high pathogen loads, neonicotinoid exposure, climate change, and the ever-espanding agricultural complex (Di Prisco *et al*., 2016; Le Conte *et al*., 2010; Brandt *et al*., 2016; Francis *et al*., 2013; Jacobsen, 2008). The agricultural importance of honey bees and the looming threat of CCD have made these organisms a prime research subject in the scientific community.

Since the 1990s, the introduction of the pest *Varroa destructor* into commercial honey bee populations has increased the winter mortality by 50% worldwide (Williams *et al*., 2010). *Varroa* mites switched from their natural host (*Apis cerana* Fabricius) when colonies of *Apis mellifera*, the western honey bee, were introduced to areas in Asia where *A. cerana* is endemic (Le Conte *et al*., 2010). *Varroa* mites feed on honey bees' fat body tissues (similar to the mammalian liver) causing physical injury and a weakened immune system; mites are known active biological vectors and are responsible for the introduction of many pathogens into colonies (Le Conte *et al*., 2010; Ramsey *et al*., 2019). Deformed wing virus (DWV), Israeli acute paralysis virus (IAPV), Black queen cell virus (BQCV), and Sacbrood virus (SBV) are only some of the pathogens known to be transferred to honey bees through mites (Francis *et al*., 2013). Due to damage caused by *Varroa*, bees cannot properly develop hypopharyngeal glands that are responsible for the production of royal jelly (Jacobsen, 2008). Without properly functioning hypopharyngeal glands, nurse bees cannot provide adequate nutrition to larvae which produces a malnourished next generation and further exacerbates colony health. Though chemical treatments are available to treat *Varroa* and have been shown to be the most effective means of dealing with mites, no treatment is 100% effective and continued application can lead the evolution of treatment resistance. In fact, mite treatments can negatively affect the honey bees themselves. CheckMite, a previously popular mite treatment, has even been found to cause physical abnormalities in queens, which decreases the likelihood a colony will accept her (Jacobsen, 2008). Coumaphos, the active ingredient in CheckMite, has been observed to cut drones' sperm production in half while limiting the viability of sperm stored in the queen's spermathecae to just six weeks compared to the normal lifespan of several years (Burley, 2007). In 1980, the provincial government of Newfoundland and Labrador enacted an restriction of honey bee imports (Hicks, 2014). This has allowed the honey bee population to remain mite-free thus far. Biological threats only represent a portion of the challenges facing honey bee colonies. Chemicals used for pesticide prevention in agriculture can lead to many ill effects in honey bees.

Pesticide use is widespread, with China and the United States of America as the leaders in chemical application consumption (Pariona, 2017). Canada ranks 8th with 54-million kilograms of pesticides used annually (Pariona, 2017). The effects of commonly-used pesticides have been extensively studied and over 40 years of research has been done looking at the effects on honey bee colonies. Pesticides are an effective method in controlling pests ranging from weeds to insects. Neonicotinoids, a group of currently used pesticides, work systemically by absorbing into plants and spreading to all tissues including pollen and nectar (Ensley, 2018). Unfortunately, this means pesticides can easily be transferred into the hive from foragers bringing back plant products contaminated with the substance (Brandt *et al*., 2016). Neonicotinoids disrupt neuronal signal transduction that can even lead to the death of bees in lethal doses. Sublethal effects on honey bees include impaired learning and memory, higher pathogen impact, lower cellular immune defense, and decrease in antimicrobial activity of hemolymph (Brandt *et al*., 2016). Even in sublethal doses, pesticides cause worker bees to become confused outside of the hive with many unable to make it back to the colony and eventually dying of exposure (Jacobsen, 2008). Pesticides lower the honey bees' immune response to parasites and pathogens, causing colonies to collapse under the increased viral load. Limited research has been conducted on the effects of multiple different pesticides acting together on the health of honey bee colonies. Luckily for Newfoundland honey bees, commercial agriculture is limited on the Island; consequently pesticide use is limited.

As stated above, many different pathogens are prevalent in honey bee populations around the world. Along with the diseases transferred through *Varroa* infestation, many other pathogens can be transferred between colonies. The fungus *Nosema ceranae* moved from its original host, the Eastern honey bee, *Apis cerana* to *A. mellifera* and was first detected in Europe in 2006 (Jacobsen, 2008). Following ingestion by honey bees, the fungus infects the digestive tract and destroys the lining of the gut which stops nutrient absorption and leads to death by starvation (Jacobsen, 2008). *Nosema ceranae* has been reported in Newfoundland in the past, yet reports are rare (Newfoundland and Labrador Beekeeping Association, 2020). American foulbrood (AFB) is a common bacterial infection found in hives around the world. Antibiotic treatment can effectively kill these bacteria but can kill beneficial bacteria found in the guts of honey bees (Moran, 2015). It is clear that both pathogens and their treatments can negatively affect colonies.

In 2006, mapping of the honey bee genome was completed and with it came a flood of new information (Honey Bee Genome project, n.d.). It was found that honey bees have half as many genes devoted to detoxification and immune response compared with most insects (Jacobsen, 2008). This means that honey bees do not do well with new invaders and high pathogen loads. During the initial CCD outbreak, researchers found that many colonies (including those perceived as healthy) had upwards of 14 unique viruses (Cox-Foster *et al*., 2007; Jacobsen, 2008). This intense viral load puts a huge strain on the honey bee immune system and can very easily lead to colony failure.

Increase in demand for specific crops such as corn, soybeans, and almonds has created areas of monoculture in agricultural areas. The resulting low plant diversity provides limited nutrition to honey bee colonies (Jacobsen, 2008), and as demand for pollination services increases, honey bees are being pushed to their breaking point. Exposure to multiple pesticides, trucking of hives over long distances, and poor nutrition from crops grown as monocultures have weakened colonies to the point that many do not survive to pollinate the next year (Jacobsen, 2008). Unlike many areas in the American Midwest and California, honey bees in Newfoundland are able to forage from a variety of crops and wildflowers. Commercial pollination services, though available, are not in as high demand on the Island, which limits the negative impact on colonies.

Human-induced climate change has become one of the leading threats to humanity in the last decade (World Economic Forum, 2019; Ten health issues WHO will tackle this year, n.d.). It has become a major enemy for honey bees. Some taxa such as butterflies have been able to shift their ranges as climate change has progressed (Halsch *et al*., 2021). But some bee species have not been able to make the shift which could account for their decline (Kerr *et al*., 2015). Using long-term data from the last 110 years, researchers found that many bees have failed to migrate closer to the North pole with many populations disapearing in the Southern ends of their range (Kerr *et al*., 2015). Overall, bees have lost up to 320 kilometers of habitat in North America and Europe due to climate change (Kerr *et al*., 2015). Air pollution can destroy the scent molecules of flowering plants and reduce the scent range (Potera, 2008). In turn, fewer pollinators are able to identify these nutrient sources which limits the introduction of pollen and nectar into the hive. Combinations of these factors have contributed to CCD. Newfoundland, with its relatively new beekeeping operations, has the opportunity to learn from the mistakes made around the world and could potentially provide mainland Canada and elsewhere with relatively healthy bees. Further research and understanding of the unique challenges facing beekeepers on the Island of Newfoundland would help to strengthen this blooming apiculture community.

1.2. Honey bee life in the colony

In order to understand the genetic variation in honey bees, we need to understand the colony as a whole and how colonies are created. The curious sexual reproduction of the honey bee queen influences the genetic makeup of a colony and in turn influences the characteristics exhibited by the colony. This research focuses on the worker bee caste in a colony which is, in a sense, the most easily studied physical representation of the genes of a colony.

Apis mellifera Linnaeus is the most commonly raised species of honey bees by beekeepers around the world, including in Newfoundland. These bees are eusocial, meaning they live in colonies with one reproductive female (the queen), many non-reproductive female ‘workers’, and a few fertile male ‘drones’ (Marshall, 2006). These types of colonies are described as monogynous where there is only one egg producer (Gullan & Cranston, 2014). A queen honey bee is reared during multiple events within a colony: 1) when the former queen has died, 2) when a queen is no longer producing eggs called supersedure, 3) or in anticipation of colony reproduction called a swarming evet (Langstroth, 1860). Worker bees will begin the process of queen rearing in one of two ways: by constructing a special cup-shaped cell known as a queen cup to house a future egg or by building a larger cell around a young larvae known as a queen cell. Young worker bees, known as nurse bees, secrete a substance called ‘royal jelly’ into the thimble shaped cells holding the young larva (Gullan & Cranston, 2014). Though all honey bee larvae require some amount of royal jelly, only a queen larva is given copious amounts of royal jelly in order to trigger the development of the queen morphology; it takes 16 days for a queen to develop from an egg (Gullan & Cranston, 2014).

A week or two after emerging, the virgin queen will perform a mating flight within 1.6 kilometers of her original colony where she will mate with 15-20 drones in flight (Gullan & Cranston, 2014). The drones, whose main purpose is for reproduction, have been known to fly as far as seven kilometers from their original hive in order to mate with a queen (Ruttner & Ruttner, 1972; Taylor & Rowell, 1988). Drones will also spend most of their time in DCAs while outside of the hive (Ruttner, 1966). During mating, drones will detach their genitals inside of the queen, forming a “mating sign”, before falling to the ground to die (Gullan & Cranston, 2014). The plug is thought to break off inside the queen in order to maximize the amount of semen transferred to the queen by preventing any from flowing out (Oldroyd & Wongsiri, 2006). After removing the mating sign, the queen will proceed to mate with another drone, repeating the process over again. The fertilized queen will ultimately return to the nest where she will stay and begin laying a few days after her mating (Ryde, 2010).

The queen lays eggs throughout her life, and she is able to control the sex of the offspring by controlling whether or not each egg is fertilized. Drones are reared in slightly larger cells and when she encounters an empty cell, the queen will lay an unfertilized egg that develops into a drone. As the egg moves through her reproductive tract, a valvefold covers the median oviduct and can prevent any semen from touching the egg (Gullan & Cranston, 2014). When the queen is laying an egg in a worker cell, this valve opens, allowing a small amount of semen through and producing a female (Gullan & Cranston, 2014). As the queen has mated with multiple different drones during her mating flight, she can produce a genetically diverse colony throughout her lifetime. Workers that share the same queen mother and same drone father will share an average of 75% of their genetic material compared to workers with different drone fathers that share only 25% of their genetic material on average (Sammataro *et al*., 2011).

The majority of the colony is made up of worker bees, the non-reproductive females of the colony. These workers will go through many different jobs throughout their one-to-two-month life span known as age polyethism (Seeley, 1982). After emerging from the capped brood cell, the bee will begin cleaning the cell in preparation for the queen laying another egg or for storing honey or pollen (Gullan & Cranston, 2014). Pheromones released by the worker bee signal to other bees and the queen that the cell is ready to be used again (Winston, 1987). Nurse bees, with their enlarged hypopharyngeal, salivary, and mandibular glands, secrete the food for the larvae and queen bee (Gullan & Cranston, 2014). Young workers will then move on to capping brood cells that are near the end of their larval stage. They close brood cells with a brownish convex cap (Langstroth, 1860). As they age, workers will begin to secrete wax from their developed glands in order to build comb. Workers then move on to receiving and storing nectar and pollen from older bees, cleaning the hive of debris and dead bees, and maintaining hive temperature. Finally they will attend to the most risky job of foraging for pollen, nectar, and water (Marshall, 2006). Male drones, on the other hand, only have one duty: to mate with virgin queens during their mating flight. The larger cells in which drone develop are found along the bottom and edges of the comb or on specifically designed frames (Langstroth, 1860). Several weeks after emerging from their cells, adult bees are sexually mature and can begin their mating responsibilities (Gullan & Cranston, 2014). As cooler weather sets in and autumn approaches, workers will kick their brothers out of the colony to die since their duties are no longer needed.

Honey bees bring two main types of food into a hive: nectar can be turned into honey and pollen is processed into bee bread (Marshall, 2006). Nectar collected by foraging bees is passed onto house bees that mix it with their own saliva containing enzymes and bacteria that help stop fermentation (Winston, 1987). As water evaporates from the nectar, the substance is placed into cells where it is further cured and finally considered honey (their primary source of carbohydrates) (Crane, 1999). Pollen deposited in cells from foraging bees is processed by house bees as they pack the cells tightly. By moistening the pollen with nectar and saliva, the house bees introduce enzymes and bacteria that acidify the pollen to create bee bread (their main source of proteins) (Winston, 1987).

Weather is very important in dictating when honey bees can forage for resources. Honey bees need a minimum temperature of 12.2°C in order to fly (Vicens & Bosch, 2000). They cannot forage in rain or in winds stronger than 24.1 kilometers per hour (Thorp, 1996). There is evidence that rain dilutes the nectar in flowers, making bees' efforts more fruitless (Jones & Sweeney-Lynch, 2010). Strong winds significantly reduce the efficiency of honey bee foraging (Hennessy *et al*., 2020). Cloud cover can also affect flight activity especially if the temperature is close to the lower minimum temperature for flight (Vicens & Bosch, 2000).

Honey bees are able to survive over winter with as few as 10,000 workers and one queen (Marshall, 2006). During spring, a colony can rapidly grow to over 50,000 bees (Marshall, 2006). Environmental cues such as shorter days, dwindling pollen resources, cooler temperatures, and less sunlight begin the process of winter bee creation (Nürnberger *et al*., 2018; Mattila & Otis, 2007). As a direct result of reduced brood rearing and fewer pollen reserves, these bees tend to live longer (as long as 10 months) compared to the summer bees' lifespan of less than 50 days (Gullan & Cranston, 2014). Because brood rearing and foraging behaviors stop during winter in temperate climates, workers at that time of year are responsible for producing heat within the hive. They can maintain a hive temperature of 35°C by rapidly contracting their flight muscles to produce heat (Gullan & Cranston, 2014). During the warmer months, they can maintain this temperature by placing water droplets on the cell walls and fanning their wings at the entrance, allowing evaporation to cool the hive (Winston, 1987).

Honey bees have numerous methods of communication. The queen and worker bees produce pheromones which can communicate different information to other workers. One volatile chemical that is secreted is used to draw others to a location, seen during swarming behaviours (Marshall, 2006). When a colony has outgrown its current hive, multiple queen cups are created. The original queen will follow the worker's pheromones and leave with a fraction of the workers and drones to start a new nest (Marshall, 2006).

The daughter queen will remain behind to inherit her mother's hive. Defensive behaviour, such as stinging, is coordinated in part by using a pheromone found in a worker's stinger. This produces a mixture of chemicals that encourage other bees to sting around the same site (Gullan & Cranston, 2014). This behavior promotes a quick and painful defense against intruders.

Pheromones are only one form of communication found within a colony. Foraging workers will communicate the location of a nectar or pollen source by performing dances (Crane, 1999). The figure eight dance performed for other foraging bees, called the waggle dance, communicates the direction and distance of a new resource through the angle and pace of the wiggle (Gullan & Cranston, 2014). This waggle dance can also be used to communicate a new swarming site to other workers. Other dances have been documented but some still have not been deciphered by researchers. The complexity of honey bee colonies offers researchers many opportunities to learn more about this unique species and how worker bees interact within a colony and with their environment.

1.3. Origin of Island of Newfoundland honey bee population

Humans and honey bees have shared a relationship from as early as 15,000 BC as depicted in honey hunting cave paintings from the Palaeolithic era (Crane, 1999). In the beginning, early humans would hunt for wild colonies in trees and rock alcoves in search of bee products to bring back to their community. By Neolithic times, immovable hives of honey bees were tended. Immovable hives are colonies that cannot be transported because they occur in tree trunks or other natural sites. Natural nesting sites in tree cavities called 'bee wood' were tended to and passed down through a family (Crane, 1999). As civilization progressed, our understanding of the honey bee colony structure progressed. Evidence from hieroglyphics and writing suggests that by 2,400 BC, ancient Egyptians had developed more advanced ways of managing colonies (Crane, 1999). With the introduction of traditional beekeeping, this gave humans the means to maintain multiple colonies without the need to hunt for colonies.

As *A. mellifera* was transported around the world, more and more civilizations adapted beekeeping techniques specific to their region. Honey bees were first introduced into North America around 1622 when colonists in Virginia imported colonies from Europe (Jacobsen, 2008). Further imports to Massachusetts took place in 1639 and soon after, honey bees had expanded to cover the East Coast either by swarms or with human assistance (Jacobsen, 2008). We are not clear on exactly when honey bees were introduced into Newfoundland, but the earliest record of importation was from 1927. Sir Wilfred Grenfell, famous medical missionary responsible for the development of hospitals, orphanages, and schools in Newfoundland and Labrador, wrote to a friend in 1927 that he "made an experiment with bees imported from Canada" and "felt greatly in need of advice" (Crane, 1999). Other occurrences of early beekeeping on the Island were sporadic. By the late 1970s, shipments of colonies from the United States and Nova Scotia were seen around the island with many dying out over the years. The remaining colonies seem to have been sold to experienced beekeeper Wally Skinner, former owner of the Newfoundland Bee Company (Hicks, 2014).

Due to Newfoundland's low temperatures and long winters, honey bees did not originally flourish as they did in other parts of the world. Early beekeeping in

Newfoundland was limited to the summer and little effort was put into overwintering colonies. Beekeepers would purposefully kill colonies and simply import new colonies as "package bees" onto the Island in the following spring. Yet after importation restrictions were put into place, beekeepers began overwintering colonies with the aid of insulated covers or boxes. Today, a majority of beekeepers have obtained their bees directly or indirectly from the Newfoundland Bee Company which was started by Wally Skinner (Hicks, 2014). Mr. Skinner began beekeeping in 1974 with bees he first obtained from the John Bragg Company in Nova Scotia (Hicks, 2014). Skinner and his daughter, Andrea, have continued to raise and sell bee nucs (small honey bee colonies created from a larger colonies, often sold as starter hives) across the Island and the majority of the honey bees in Newfoundland have been linked to the Newfoundland Bee Company (Hicks, 2014).

By 1980, the provincial government introduced legislation for Newfoundland that prevents the importation of honey bees from areas of parasite infestation to the Island (Hicks, 2014). This legislation was created in order to protect Newfoundland from the *Varroa* mite and other pests/diseases. Today, Newfoundland is free of not only *Varroa* mites but also tracheal mites, *Tropilaelaps* mites, wax moths, and small hive beetles (Newfoundland and Labrador Beekeeping Association, 2020). Common viruses seen in hives on the mainland such as black queen cell virus (BQCV), Lake Sinai virus (LSV), sacbrood virus (SBV), and deformed wing virus (DWV) as well as some common diseases such as American foulbrood (AFB), chalkbrood (CB), and European foulbrood (EFB) are most likely not present on the Island (Newfoundland and Labrador Beekeeping Association, 2020).

Importation regulations have not only given the Island the title of "*varroa*-free" but have limited the number of honey bee diseases reported in the province. The fungus *N. ceranae* has been detected in Newfoundland and constant vigilance to protect colonies is required by beekeepers (Newfoundland and Labrador Beekeeping Association, 2020). Spores of *Nosema* swallowed by bees germinate within them and penetrate the cells of the stomach lining (Department of Jobs, Precincts and Regions, 2021). Infection by *Nosema* results in the loss of royal jelly production in nurse bees, failure to produce mature larvae, life expectancy is reduced, an increase in dysentery in adult bees, and infected queens stop laying eggs and die within weeks (Department of Jobs, Precincts and Regions, 2021).

Between 2000 to 2014, and with support from the provincial government, the Newfoundland Bee Company imported eggs and queens from Hawaii and Ontario; subspecies included in these imports were Carniolan, "Yellow Russian", "Russian New Blue", and Buckfast (a hybrid race crossed with Anatolians and Italian) (Newfoundland and Labrador Beekeeping Association, 2020). In 2016, with oversight from the provincial government, two beekeepers (Trevor Tuck and Chris Lester) imported 100 nucs and 30 nucs respectively from varroa-free, Western Australia (Newfoundland and Labrador Beekeeping Association, 2020). Tuck's colonies are located at Woody Point in western Newfoundland and Lester's colonies are located at their farm in St. John's on the Avalon Peninsula. As another *Varroa* mite free population, Western Australia is strict about honey bee imports and exports. Today, there are three favored subspecies reared in Australia: *A. m. ligustica*, *A. m. carnica*, and *A. m. caucasica* (Chapman *et al*., 2016). Genetic studies though have only identified European lineages within Australia

(Chapman *et al*., 2008). Present-day Newfoundland bee stocks are a product of this history of importations and the local influences across the Island.

Information on the development of beekeeping in Newfoundland, and related economic data, are sparse. The most recent statistical overview of the honey and bee industry performed by Agriculture and Agri-Food Canada stated "Newfoundland and Labrador is excluded since the province has no honey production to report" (Agriculture and Agri-Food Canada, 2020). These data are incorrect most likely due to no government requirements for beekeepers to report information about their beekeeping activities. The most accurate information obtained comes from the American Bee Journal (Armitage, 2018). Currently, there are seven commercial apiaries, defined by the provincial government as having more than 20 colonies (Armitage, 2018). There are another 130 beekeepers with colony numbers ranging from one to 19 (Armitage, 2018). In total, there are about 800 active colonies across the Island (Newfoundland and Labrador Beekeeping Association, 2020). These honey bee operations provide numerous services such as commercial pollination, the sale of bee products such as honey, beeswax, pollen, and propolis, and value-added products like mead, cosmetics, and honey-based syrups (Armitage, 2018). These products have helped to support numerous other businesses in the province including local restaurants, health food and speciality stores, and farmers' markets (Armitage, 2018). Future beekeeping could have a significant contribution to the provincial economy with increases in pollination services of cranberries (Armitage, 2018). The most current information on the number of colonies in Newfoundland shows a high concentration on the Avalon Peninsula (Figure 1.1). There are natural breaks between groupings of apiaries such as the Isthmus of Avalon, vast boreal forests without human habitation, and areas lacking resources to support feral honey bee colonies (Newfoundland and Labrador Beekeeping Association, 2020). These breaks create areas of isolation where colonies would not naturally mingle and hybridize with each other.

1.4. Lineages and subspecies in Newfoundland & their characteristics

Western honey bee subspecies have been historically divided into four major lineages based on their origin: Lineage A (African), Lineage C (Eastern European), Lineage M (Western European), and Lineage O (Middle Eastern) (Chapman *et al*., 2016). Current research has suggested that there are seven lineages including Lineage Y (Western Asia), Lineage L (Egypt), and Lineage U (Madagascar) (Dogantzis *et al*., 2021). The software, IdentiFly, can only distinguish colonies into the original four lineages.

These six named subspecies/hybrids will be discussed in the following section: Italian honey bee (*Apis mellifera ligustica*), Carniolan honey bee (*Apis mellifera carnica*), Greek honey bee (*Apis mellifera cecropia*), Armenian honey bee (*Apis mellifera armeniaca*), Iranian honey bee (*Apis mellifera meda*), and African honey bee (*Apis mellifera scutellata*). The Island does not have pure lineages due to years of interbreeding between colonies from beekeeper influences. In this text when referring to putative colony subspecies, these are the purest forms that can be found in Newfoundland.

Each of these subspecies are reported to have distinct differences when it comes to aggression, foraging behaviour, honey production, flight abilities, and winter survival. Figure 1.2 displays the distribution and origin of various honey bee subspecies. The

Italian honey bee (*A. m. ligustica*) was the first honey bee stock brought to North America and has been generally the most favoured subspecies ever since. Originally from Italy, colonies of this subspecies thrive in a Mediterranean climate. Known for their extended periods of brood rearing, *A. m. ligustica* colonies are slow to build their numbers in spring but are able to maintain them throughout the summer (Tarpy, 2016). As excellent honey producers with moderate gentleness, these bees have been a favourite of beekeepers (Tarpy, 2016). Yet due to their colony buildup characteristics, *A. m. ligustica* is known to consume surplus honey rapidly and are frequently seen robbing weaker colonies, thus increasing the potential spread of diseases between colonies (Tarpy, 2016). Identified as short-distance foragers, *A. m. ligustica* relies on a resource rich environment which if not available, can lead to a robbing frenzy in apiaries (Sammataro *et al*., 2011).

Originating from central Europe, Carniolan honey bees (*A. m. carnica*) are known for their explosive spring buildup that can take advantage of early spring blooms (Tarpy, 2016). In order to support the brood population, an ample supply of pollen is necessary and build up can be slow if foraging is limited (Sammataro *et al*., 2011). These bees are extremely docile, and beekeepers need little smoke or protective clothing when handling hives (Tarpy, 2016). They are much less prone to robbing compared to *A. m. ligustica,* thus lowering disease transmission between colonies (Tarpy, 2016). Carniolan honey bees are excellent wax comb builders which make them a great subspecies for beekeepers using beeswax for products such as candles, cosmetics, and skin care products (Tarpy, 2016). However, due to their explosive buildup, *A. m. carnica* has a high propensity to swarm which means beekeepers must be vigilant in their colony management (Tarpy, 2016).

Native to Southern Greece, the Greek honey bees (*A. m. cecropia*) are accustomed to the Mediterranean climate and has difficulty surviving in cooler locations in Northern Europe (Crane, 1999). Very similar to *A. m. ligustica,* they are known for being gentle and have a low tendency to swarm. Along with their quick build up during the spring, *A. m. cecropia* makes an ideal beekeeping subspecies but due to their low survival in colder climates, they are not as popular among beekeepers in Canada as *A. m. ligustica* or *A. m. carnica* (Crane, 1999).

The Armenian honey bee (*A. m. armeniaca*) comes from areas of Armenia with long, snowy winters and short, mild summers. With its small population, *A. m. armeniaca* has mostly remained in Armenia and Turkey. During the Soviet Era, colonies were kept under strict conditions with no other colonies allowed into the region (G. Tabakyan, personal communication, August 4, 2021). Coming from an area of high honey bee predation, *A. m. armeniaca* adapted to this threat by responding to differences in reward frequencies and will tend to avoid flower colour morphs with limited resources (Cakmak & Wells, 2001). The foragers division of labor allows this subspecies to maximize their energy efficiency (Cakmak & Wells, 2001).

A closely related subspecies,the Caucasian grey honey bee (*A. m. caucasia*), also occurs in the same areas of Armenia and Georgia. Originating from Central Caucasus, *A. m. caucasia* has one of the longest proboscis of any subspecies of honey bee, which allow them to reach nectar deep within flowers (Gullan & Cranston, 2014). Their ability to out-produce other honey bee subspecies while surviving cold, harsh weather conditions have made them a prime research target. They have been anecdotally noted by beekeepers to forage at lower temperatures and earlier in the day than other subspecies, and even during cool, wet days (Sammataro *et al*., 2011). Around 1905, these bees were introduced into the United States from Russia (Sammataro *et al*., 2011). Soviet-era research was focussed on preserving this profitable line of honey bees and international transportation of *A. m. caucasia* required special permits (Corso, 2013). After the collapse of the Soviet Union, exportation to other countries began again but unfortunately, breeders are difficult to find even today. These bees are not without problems however. They are late starters in spring brood rearing which makes them a poor early spring pollinator and they are more susceptible to *Nosema* disease which requires more medication from beekeepers (Sammataro *et al*., 2011).

Originating in Iran, the Iranian honey bee (*A. m. meda*) has comparatively strong defensive behaviour and swarming tendency compared to other subspecies (Vaziritabar *et al*., 2016). A study of *A. m. meda* colonies found that only 35% of colonies showed hygienic behaviour (Najafgholian *et al*., 2010).

The Africanization of honey bee colonies in the Americas began in the late 1950s when Brazilian scientist, Warwick Kerr, imported *Apis mellifera scutellata* into Brazil (Ellis & Ellis, 2008). European honey bees that were thriving in North America were less successful in tropical regions of Central and South America. Kerr’s hope was to import an African honey bee line that would outperform European bees in Brazil. Unfortunately, colonies that came from these African lines are extremely defensive and tend to swarm upwards of ten times a year (compared to European lines that swarm one to three times a year) (Ellis & Ellis, 2008; Guzmán-Novoa & Page, 1994). Although *A. m. scutellata* was initially quarantined in Brazil, an accidental absconding event allowed their escape and they began to spread. Africanized honey bees are now being recorded in many southern parts of the United States. Luckily, *A. m. scutellata* does not do well in temperate climates and has failed to establish populations above 34° latitude (Ellis & Ellis, 2008).

When identifying the origin of Newfoundland honey bees, we need to consider two hybrids (honey bee lines created from the breeding of two or more honey bee subspecies): Buckfast and Russian. The consensus when talking with numerous beekeepers was that these hybrids were present in the Newfoundland stock. A stock derived from the Italian honey bee stock, Buckfast honey bees are known to thrive in cold, wet weather conditions (Tarpy, 2016). They produce good honey crops and exhibit good hygienic behaviour yet they are moderately defensive (Tarpy, 2016). Unlike the Carniolans, Buckfast honey bee colonies have a slower spring buildup which means they are prevented from taking advantage of early spring nectar and pollen (Tarpy, 2016).

Russian honey bee stocks have evolved a resistance to *Varroa* mite. These colonies have fewer than half the number of mites compared to standard commercial stocks (Tarpy, 2016). They show good hygienic behaviour which may have helped with their mite resistance. A major drawback with this hybrid is that brood rearing only happens during times of nectar and pollen flow thus their success fluctuates with the environment (Tarpy, 2016). They have been shown to perform the best when they are the only subspecies present; uncontrolled crossing with non-Russian bees has led to a decrease in their *Varroa* resistance (Tarpy, 2016). But these traits, though not desired by beekeepers, have actually contributed to their resilience. Russian honey bees keep their population well aligned with what the environment supplies and can survive winters with minimal resources (Jacobsen, 2008). Colonies will often keep multiple queen cells at hand even when they are not prepared to swarm in order to have backups in case of the current queen's death. With their propensity to swarm, Russian honey bee colonies are able to interrupt the reproductive cycle of *Varroa* mites and keep these pests at a minimum (Jacobsen, 2008).

1.5. Local selection across Newfoundland

Local selection, the combination of natural and artificial pressures on colony survivability, can have a drastic influence on the subspecies variety of an region. Newfoundland is a large island (approximately 111,390 square kilometers) off the east coast of North America. Within such a large area, local ecosystems and climate can drastically change, which can affect the survivability of specific honey bee subspecies. Beekeeper activities such as the purposeful splitting of colonies to create new hives, moving of brood between colonies, and killing of unprofitable queens can affect the overall genetic diversity within an apiary.

Annual snowfall in Newfoundland can vary depending on numerous factors such as elevation and location. Widely used in the scientific community and often able to display higher classification accuracy and more detail than previous climate maps, Köppen-Gieger maps can be used to compare the climate and biome distribution of almost any area in the world (Beck *et al*., 2018). Meixner and colleagues (2015) confirmed that subspecies of honey bees do better in their area of origin due to their specific genotype-environment interactions. It is thus likely that specific subspecies mixes will do better in areas similar in climate and biodiversity as their country of origin. Much of Newfoundland is divided into two climate zones: Subarctic (Dfc) and Warm-summer humid continental (Dfb). Subarctic areas have light snowfall but long periods of cold temperatures, with snow on the ground for several months (Beck *et al*., 2018). In the summer, temperatures can sometimes exceed 26°C. Areas classified as Dfb typically have long, cool summers with relatively low humidity. Winter temperatures can go below -18°C for long periods of time (Beck *et al*., 2018).

Depending on the location, Newfoundland can experience harsh climates. Strong, cold winds that blow off the Gulf of St. Lawrence and Labrador cause a climate that is almost arctic-like on the western coast of Newfoundland (Boland, 2017). Honey bees require a minimum temperature of ~12°C in order to achieve flight (Vicens & Bosch, 2000). The average monthly high and low temperatures of locations in Newfoundland are shown in Figure 1.3. Differences in average temperatures throughout the Island which can affect the length of the foraging season and thus the success of a colony.

The Grand Banks, located just south of Newfoundland, are considered one of the foggiest places in the world (Clarke & Drinkwater, 2015). The Gulf Stream's warm air masses move over the colder Labrador current, producing heavy fog. In spring, when the air-sea temperature difference is at its greatest, especially dense fog is produced (Clarke & Drinkwater, 2015). Though honey bees are able to fly in fog due to their hydrophobic wings (Liang *et al*., 2017), fog can still disrupt the performance of a colony. Under cold temperatures, bees first cluster more closely within their nest, then begin to generate heat with their flight muscles to regulate the internal nest temperature. Accumulation of water droplets on the bodies of foraging bees can weigh them down and disrupt their ability to fly (Liang *et al*., 2017).

Weather not only affects honey bees directly, it will affect the plant diversity in any given area. Plant diversity on the Island varies due to factors such as proximity to the coast, elevation, soil pH, and precipitation (Boland, 2017). A majority of Newfoundland is made up of sandstone, siltstone, and granite which create acidic soil (low pH) which not all plants can tolerate (Boland, 2017). Areas of acidic and sub-acidic soils support larger plants and emit pollen earlier than plants located in neutral pH soil (Gentili *et al*., 2018). Micronutrients are more available to plants in acidic soil compared to neutral-alkaline soil and tend to favor plant growth (Lončarić *et al*., 2008). In Western Newfoundland we find areas of limestone which causes an increase in soil pH. Plants that grow in these areas have a restricted distribution due to their specialized requirements and traits (Boland, 2017). Serpentine rock outcrops are rare, but some notable barrens can be found near Corner Brook (Blomidon Mountains), Gros Morne National Park (Tablelands), and St. Anthony (White Hill). Soil derived from serpentine rock is highly toxic to most plants which means only a few specialists can survive in this environment (Boland, 2017). Pollen and nectar quality and diversity can alter honey bee physiology (Pasquale *et al*., 2013). Higher quality pollen has been shown to improve colony resistance to pesticides (Schmehl *et al*., 2014) while high quality nectar can increase pathogen resilience of bees (Mao *et al*., 2013). Table 1.1 lists some common flowering plants in Newfoundland that are often visited by honey bees. Western Newfoundland has the largest flowering plant diversity compared to Central Newfoundland and the Avalon Peninsula. Newfoundland's elevation ranges from sea level to the highest point of 815 meters above sea level. These changes in elevation can affect plant diversity

Körner (2000) evaluated species diversity in mountain ranges and found that plant species richness decreases with increase in elevation. Ecosystem properties change along an elevation gradient which in turn affects plant diversity (Lomolino, 2001). In areas of Western Newfoundland, elevation can reach 600-800 kilometers above sea level; these areas have little to no tree growth and are dominated by sub-alpine barrens (Boland, 2017). Similar environments occur in the Southern Avalon and the south coast of Newfoundland. Temperatures can drop by 5.5°C per vertical kilometer, affecting water vapor and air pressure (Körner, 2007). Naturally occurring plant diversity accounts for a portion of pollen and nectar sources available to honey bees. In urban areas, home gardening and farming can offer even more diversity in flowering plants.

Backyard gardening, especially in urban areas, can offer honey bees with a wider range of pollen and nectar sources which could include non-native flowering plants. Common garden plants listed in table 1.2 show just how diverse urban gardens can be. In these areas there is relatively high application of pesticides use for lawn care, both on personal property and public property. Carbaryl pesticides, commonly used to control aphids, fleas, ticks, spiders, and other pests, was the most frequently detected insecticide in pollen and wax comb (Ostiguy *et al*., 2019). This pesticide is known to kill gut microbes in honey bees, disrupt enzymatic processes, and decrease bee health (Nogrado *et al*., 2019). Even in sublethal doses, carbaryl pesticides shorten honey bee lifespans and decrease immune related gene expression (Tarek *et al*., 2018).

Beekeeping in Newfoundland ranges from the backyard beekeeper with only a couple of colonies to the commercial apiary with hundreds of colonies. In any scenario, beekeepers can greatly influence the population genetics of their apiary by creating new colonies from existing ones, moving brood frames between hives, and killing off highly defensive or unprofitable colonies. All of these actions are common practices in apiculture and beekeepers have learned to read the language of the colony. Splitting strong colonies is done to create a new colony and can influence the genetic makeup of an apiary. How a beekeeper interacts with their colonies can have a great influence on the future colony traits exhibit in their specific region. Newfoundland contains a unique environment influenced by the climate, plant diversity, and human activities throughout its history. Understanding how these influences affect honey bee colonies is important to understand subspecies variation across the Island.

1.6. IdentiFly program

The software program IdentiFly (*http://drawwing.org/identifly*) was developed to easily identify the lineages and subspecies of honey bee colonies based on morphological differences of the forewing vein patterns of worker bees. Tofilski (2008) provides an example of the differences in wing patterns that the program uses to differentiate subspecies (Figure 1.6). Wing size, shape, and placement of vein intersections are all used to identify colony lineages and subspecies. Currently, the software is able to identify 19 subspecies and four evolutionary lineages of honey bees shown in Table 1.4 (Nawrocka *et* *al*., 2018). IdentiFly has a relatively high accuracy rate in identification of lineages (99.5%) and subspecies (88.4%) [Kandemir *et al*., 2017; Nawrocka *et al*., 2018]. Images of forewings of at least 10 separate workers are needed to accurately identify groups and a total of 19 landmarks are used (Figure 1.5).

After landmarks are labeled for each wing image for bees in a sample from a colony, colony classification can be done automatically by the program using a database of wing images of known subspecies. IdentiFly interprets the data and classifies the colony and includes a probability value ranging from zero to one; the high probability of assignment to a particular group indicates high similarity of the classified colony to reference samples. If classification probability is relatively high in two or more subspecies then the colony should be considered a hybrid between the subspecies with high probability. If the classification probability is low in all subspecies, then this can mean (1) the colony is very different from all reference samples used or (2) there is an error in measurements. This can happen if the colony belongs to a species other than *A. mellifera* or a subspecies not included in the classification model.

IdentiFly uses reference images obtained through the Morphometric Bee Data Bank in Oberursel, Germany (Nawrocka *et al*., 2018). Collecting of samples for this database was initiated by Freidrich Ruttner in the 1950s and the database continues to expand today. Care is taken in collecting samples from regions without known importation and hybridization (M. Meixner, personal communication, August 4, 2021). Analysis and interpretation of honey bee genetic data has built strongly on the knowledge gained by morphometric analysis used in programs such as IdentiFly (Meixner *et al*., 2013). The reference data for each lineage and subspecies varies in the number of colonies sampled. Analyses of wing veins were based on the average values calculated for each colony dataset (Nawrocka *et al*., 2018). High precision can be obtained because the program uses the mean values of all workers from a colony to predict classifications. Once analysed, the software provides a 'probability of classification' for each lineage and subspecies.

Identifying lineages and subspecies of honey bees using morphological data such as wing vein patterns is a fast, easy, and less expensive means of classifying colonies. Other methods, such as genetic analysis, requires specialized equipment and training. Additionally, the IdentiFly program has the added benefits of accessibility to both scientists and beekeepers. Using this morphological classification program rather than a more specialized method would allow continuous monitoring of changes in the composition of the bee population in Newfoundland by untrained researchers such as beekeepers. The use of this approach has allowed the creation of a foundation of classification data that can be accessed and used long after the completion of this research. As more wing images are added to the database, IdentiFly will only become more accurate over time.

1.7. Objectives

With limited research of the honey bee populations of the Island of Newfoundland, a foundation of understanding is needed in order to continue pollinator research. Due to limitations of honey bee importations, knowing what lineages and subspecies are already available to beekeepers will help determine the focus of future research on these uniquely healthy bees. Variation in subspecies numbers can give insight into the natural and artificial pressures across the Island. My thesis focuses on identifying lineage and subspecies variation between the Avalon Peninsula, Central, and Western Newfoundland. I address two main questions: (1) are there multiple lineages and subspecies present in Newfoundland and (2) are there differences in colony numbers between lineages and subspecies across the Island? These two questions were addressed using morphological data obtained through the collection of worker honey bee samples and subsequent analysis of their wing shapes. Harpur *et al*. (2014) found that "novel genes play a disproportionately large role in adaptive evolution of eusocial insects" and "worker-biased proteins have higher signatures of adaptive evolution relative to queen-biased proteins". Different vein patterns on the forewings of honey bee workers are distinctively different between subspecies (Kandemir *et al*., 2017), therefore focusing the current research on the worker caste is the most accurate and easiest way of analysing a colony's traits.

Further in this thesis, I will address possible explanations for lineage and subspecies variation. This will mostly focus on two factors: natural and artificial selection. Factors such as climate, foraging opportunities, and beekeeper actions will be considered. These factors together constitute what I will refer to as "local selection" which dictate the survival of honey bee colonies and their propagation across the Island. I will outline potential opportunities for future research and discuss some of the avenues of questioning researchers can take.

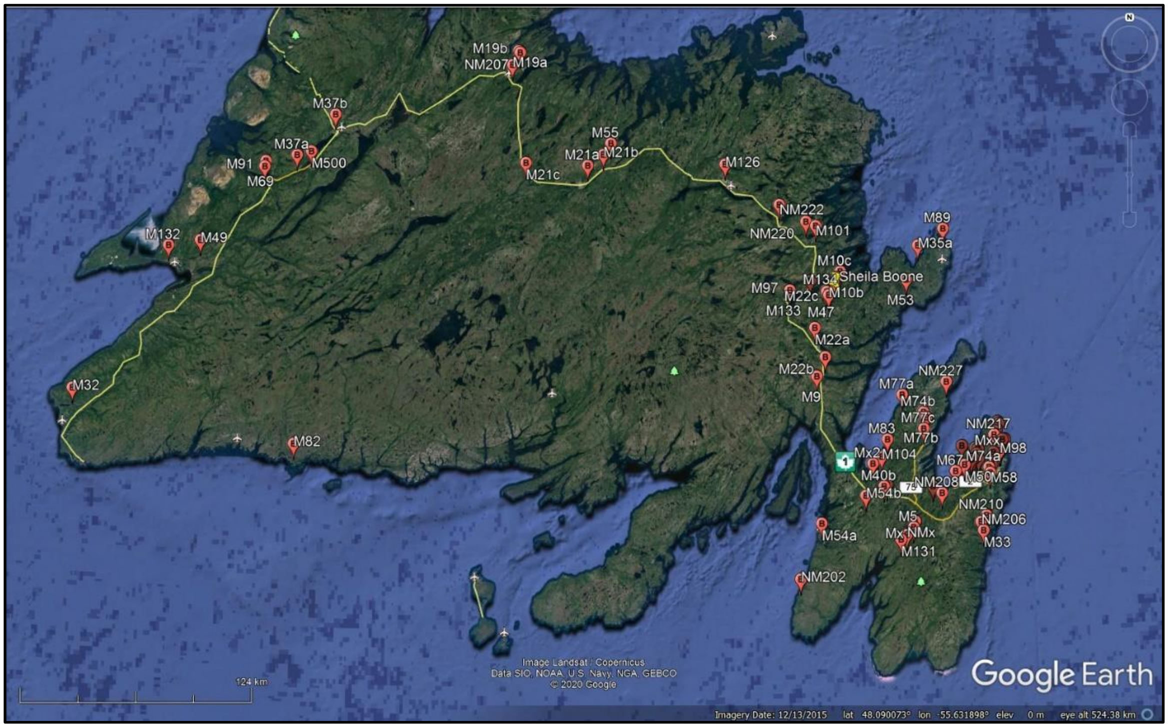


Figure 1.1. Map of known locations of hives in Newfoundland as of September 2018 (Newfoundland and Labrador Beekeeping Association, 2020). Some information for this map was gathered by asking beekeepers to name other beekeepers they know of, especially those that are not registered with the NLBKA.

Figure 1.2. Map of the distribution of honey bee subspecies in Europe (Gerth, 2011). These are the areas of origin of the major European honey bee subspecies.

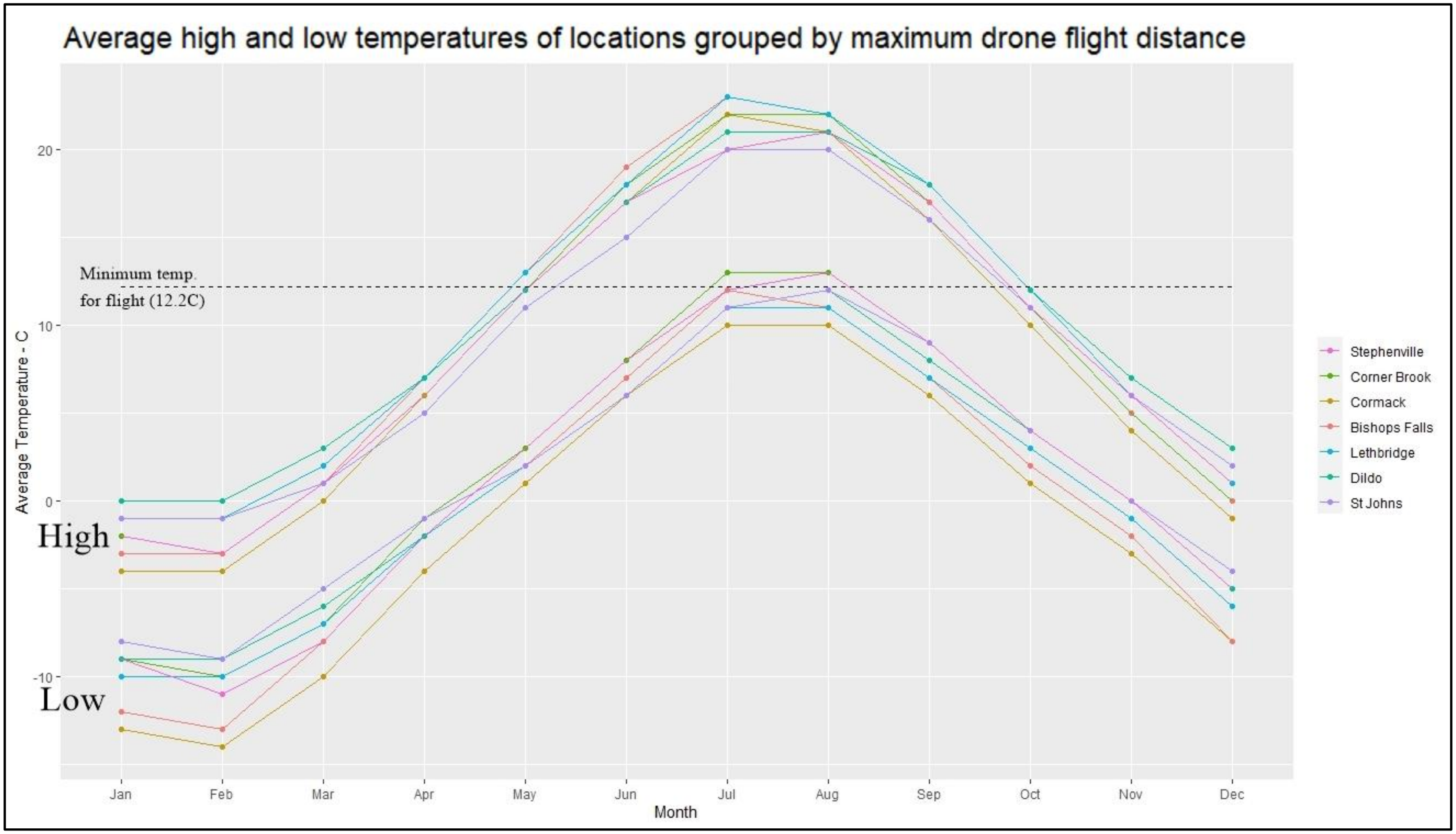


Figure 1.3. Graph showing the average monthly high and low temperatures (°C) for each location as well as the minimum temperature for honey bee flight (12.2°C). Retrieved through Environment Canada (2010).

Table 1.1. Common wild flowering plants in Newfoundland that are known pollen and nectar sources for honey bees (*A. mellifera*). Each entry lists where in Newfoundland these plants can be found. W represents Western, C represents Central, and A represents the Avalon Peninsula. (Boland, 2017; Pollinator Partnership Canada, 2017)

Family	**Scientific/Common name**	**Bloom period**	**Location**
Asclepiadaceae	*Asclepias syriaca* "Common milkweed"	July	W, C, A
Asteraceae	*Achillea milfefolium* "Common yarrow"	July-September	W, C, A
Asteraceae	*Erigeron hyssopifolius* "Hyssop-leaved fleabane"	June-July	W & C
Asteraceae	*Erigeron philadelphicus* "Philadelphia fleabane"	June-August	W
Asteraceae	*Erigeron strigosus* "Rough fleabane"	July-September	W, C, A
Asteraceae	*Euthamia graminifolia* "Grass-leaved goldenrod"	August- September	W, C, A
Asteraceae	*Euthochium maculatum* "Spotted Joe-Pye weed"	July-September	W, C, A
Asteraceae	*Rudbeckia hirta* "Black-eyed Susan"	July-August	W & C
Asteraceae	*Symphyotrichum punniceum* "Purple-stemmed aster"	July-September	W, C, A
Fabaceae	*Medicago satira* "Alfalfa"	June-August	W & C
Fabaceae	*Meliolotus albus* "White sweet clover"	June-August	W, C, A
Fabaceae	*Trifolium repens* "White clover"	June-September	W, C, A
Fabaceae	*Trifolium prantense* "Red clover"	June-September	W, C, A
Fabaceae	*Vicia cracca* "Tufted vetch"	June-August	W, C, A
Onagraceae	*Chamerion angustifolium* "Fireweed"	July-August	W, C, A
Rosaceae	*Rubus arcticus* subsp. *acaulis* "Arctic raspberry"	June-July	W
Rosaceae	*Rubus chamaemorus* "Baked apple/Cloudberry"	June-July	W, C, A
Rosaceae	*Rubus pubescens* "Dewberry"	June	W, C, A

Table 1.2. Common garden plants found in North America and Europe that honey bees are known to visit. Includes the family, scientific and common names, and the blooming period. This is not a complete list, but it does provide many popular flowering plants found in home gardens (Boland, 2017).

Family	**Scientific/Common name**	**Bloom period**
Apiacea	*Angelica archangelica* "Angelica"	June to August
Apiacea	*Levisticum officinale* "Lovage"	May to June
Asteraceae	*Cosmea bipinnatus* "Cosmos"	July to October
Asteraceae	*Cichorium intybus* "Chicory"	June to October
Asteraceae	*Erigeron speciosus* "Fleabane"	June to October
Asteraceae	*Echinops ritro* "Globe thistle"	July to August
Asteraceae	*Leucanthemum vulgaris* "Ox-eye"	June to October
Asteraceae	*Chrysanthemum maximum* "Shasta daisy"	June to October
Asteraceae	*Helianthus annuus* "Sunflower"	July to October
Asteraceae	*Achillea filipendulina* "Yarrow"	June to September
Boraginaceae	*Anchusa azurea* "Alkanet"	July to August
Boraginaceae	*Borage officinalis* "Borage"	June to July
Boraginaceae	*Echium vulgare* "Viper's bugloss"	July to October
Brassicaceae	*Iberis matronalis* "Candytuft"	May to July
Brassicaceae	*Sinapis arvensis* "Charlock"	May to June
Brassicaceae	*Alyssum saxatile* "Golden alyssum"	April to May
Brassicaceae	*Lunaria biennis* "Honesty"	April to May
Brassicaceae	*Reseda odorata* "Mignonette"	June to October

Brassicaceae	*Aubrieta deltoides* "Rockcress"	May to June
Brassicaceae	*Hesperis matronalis* "Sweet rocket/Dame's violet"	March to June
Brassicaceae	*Cheiranthus cheiri* "Wallflower"	April to June
Campanulaceae	*Campanula* sp. "Bellflowers"	May to October
Cannabaceae	*Humulus lupulus* "Hop"	September to October
Dipsacaceae	*Scabiosa* sp. "Scabious"	April to October
Dipsacaceae	*Dipsacus fullonum* "Teasel"	July to September
Elaeagnaceae	*Elaeagnus argentea* "Oleaster"	May to July
Fabaceae	*Cytisus* sp. "Broom"	March to May
Grossulariaceae	*Ribes* sp. "Currant"	March to April
Lamiaceae	*Origanum vulgare* "Marjorum"	July to September
Lamiaceae	*Mentha* sp. "Mint"	June to August
Liliaceae	*Muscari botryoides* "Grape hyacinth"	April to May
Limnanthaceae	*Limnanthes douglasii* "Poached egg plant"	March to May
Lythraceae	*Lythrum* sp. "Loosestrife"	May to September
Malvaceae	*Alcea rosea* "Hollyhock"	June to August
Malvaceae	*Malva* & *Lavatera* sp. "Mallow"	June to September
Onagraceae	*Oenothera biennis* "Evening primrose"	June to September
Polemoniaceae	*Phlox paniculata* "Phlox"	July to September
Primulaceae	*Primula* sp. "Polyanthus"	May to June
Rosaceae	*Cotoneaster vulgaris* "Cotoneaster"	May to June

Rutaceae	*Monarda didyma* "Bergamot"	May to October
Valerianaceae	*Centranthus ruber* "Valerian"	May to June

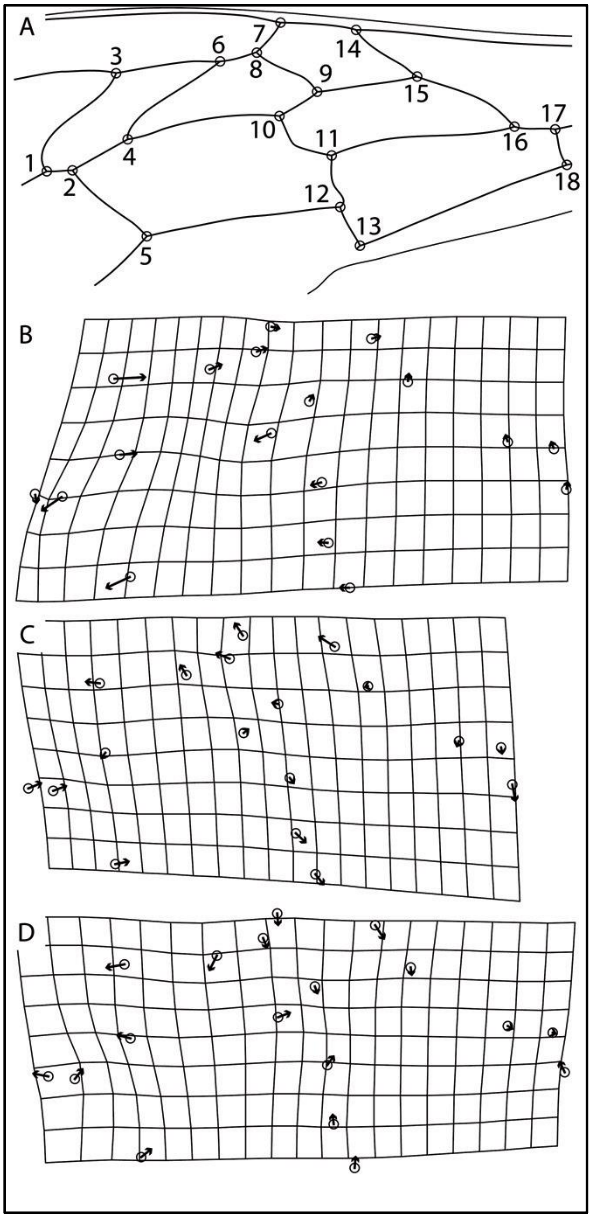


Figure 1.4. Forewing diagram (A) comparing vein intersects between *A. m. carnica* (B), *A. m. caucasica* (C), and *A. m. mellifera* (D). Distortion of the grid indicates differences between the average wing shape of each subspecies. It should be noted that differences between the subspecies were exaggerated 5 times in order to be visible (Tofilski, 2008).

Table 1.3. Lineages and subspecies/hybrids of *Apis mellifera* currently identifiable through the software IdentiFly and their common names. Lineages are categorized by location of origin.

Lineages	Subspecies/hybrids	Common name
A (African)	*A. m. adansonii*	African honey bee
	A. m. intermissa	Tunisian honey bee
	A.m. jemenitica	Arabian honey bee
	A. m. lamarckii	Egyptian honey bee
	A. m. litorea	East African honey bee
	A. m. monticola	East African mountain honey bee
	A. m. ruttneri	Maltese honey bee
	A. m. scutellata	East African lowland honey bee
	A. m. unicolor	Madagascan honey bee
C (Eastern European)	*A. m. carnica*	Carniolan honey bee
	A. m. cecropia	Greek honey bee
	A. m. ligustica	Italian honey bee
O (Middle Eastern)	*A. m. adami*	Cretan honey bee
	A. m. anatoliaca	Anatolian honey bee
	A. m. armeniaca	Armenian honey bee
	A. m. caucasia	Caucasian honey bee
	A. m. meda	Persian honey bee
	A. m. syriaca	Syrian honey bee
M (Western European)	*A. m. mellifera*	German honey bee

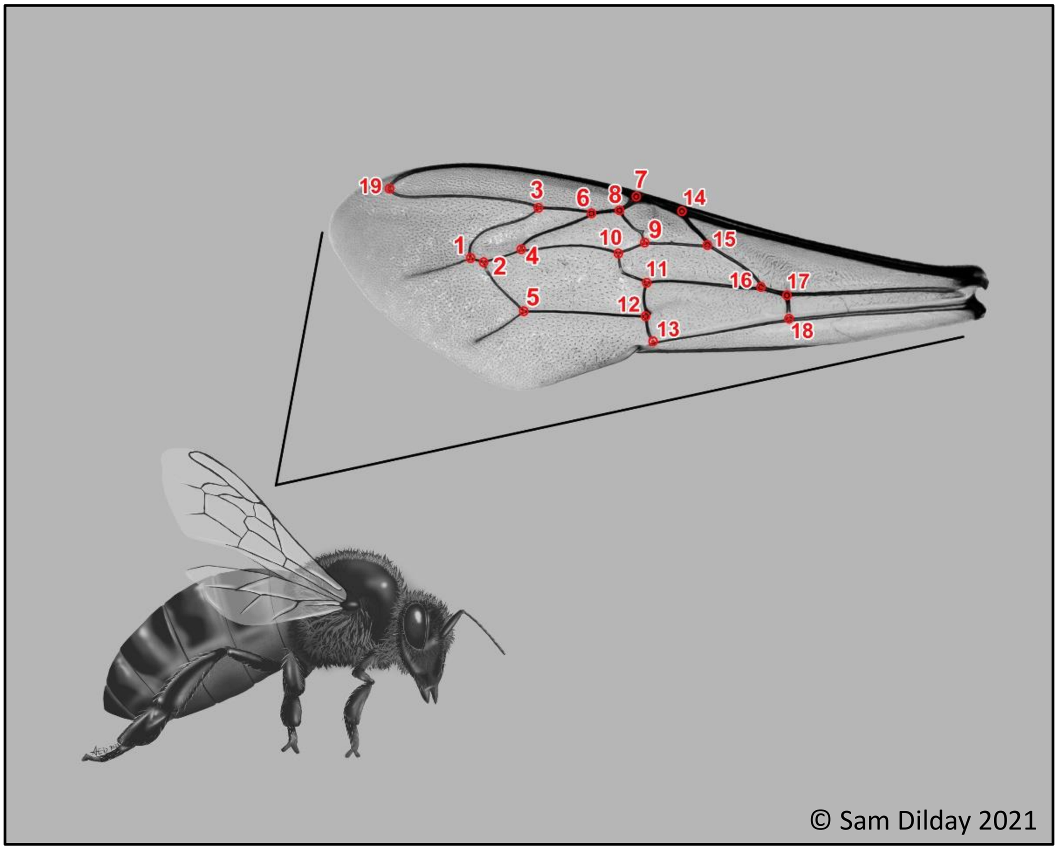


Figure 1.5. The 19 landmarks on the forewing of a honey bee used to determine the lineage and subspecies.

Chapter 2 – Methods

2.1. Collecting and processing worker samples

Honey bee workers were collected from a total of 83 colonies in 14 apiaries in Newfoundland during the summer of 2020 and 2021 (about 10% of the total honey bee population of Newfoundland). Due to constraints from COVID-19 regulations, summer 2020 research locations were limited to the Avalon Peninsula. Honey bee worker samples were taken from 25 colonies in eight different locations in the Avalon Peninsula (Figure 2.1). During summer 2021, a total of 58 colonies in six different locations were sampled in Central and Western Newfoundland (Figure 2.1).

Worker samples from the Avalon Peninsula were collected between June 21, 2020 and August 7, 2020 from GPS locations listed in Table 2.1. Worker samples from Central and Western Newfoundland were collected between June 4, 2021 and June 15, 2021 from GPS locations listed in Table 2.2. Extra worker samples were collected in April 2021 for colonies B.3, C.1, E.3, and E.6 due to low sample numbers collected from the previous summer (less than 20 individuals). This was done in order to more accurately represent these colonies. Unfortunately, colonies C.2 and G.1 did not survive the winter and extra worker samples could not be collected during spring 2021. Collection of honey bee workers was done using the standard method many beekeepers and researchers follow. First, removal of a brood frame from the hive and a scan for the queen was done to ensure the queen was not removed during sampling. A jar was then gently dragged across the vertical frame so that workers fell into the receptacle. To label the samples, a scrap paper was added to the jars which included the hive number, the date the sample was taken, and the location of the colony (apiary address). Each colony sample was then placed in a freezer (-18°C) for at least 24-hours to kill the specimen in accordance with current federal regulations.

When ready to mount the bee wings, jars were taken out of the freezer to thaw at room temperature for several minutes after which one forewing from each worker was gently removed, placed on a clean glass slide and covered with a cover slip in order to keep the wings from blowing away. Each slide was labeled with an alphanumeric designation for each location and appropriate hive number. Slides were then photographed using a light table and a digital single-lens reflex camera (DSLR) with a 35 mm image sensor format. The honey bee worker bodies were placed into labeled tubes for long-term storage in -80° freezer to be used for future genetic research.

2.2. IdentiFly program

Images of wings were uploaded into the free morphometric identification program, IdentiFly. Nineteen landmarks were individually placed onto each wing (Figure 1.7). Each image was reviewed and adjustments made to ensure the position of each landmark was as accurate as possible. The positions of the landmarks were saved within the file and could be reassessed at any time. Classifications can be set to determine either the colony lineage or subspecies. For each colony, both the lineage and the subspecies classification were measured.

An example of classification results for evolutionary lineages and subspecies of a colony is shown in Figure 2.2. and Figure 2.3. respectively. It is important that probabilities be compared between all lineages and subspecies. If the classified lineage or subspecies probability is drastically higher than all others, then it is safe to conclude that the colony belongs to the identified lineage/subspecies. If classification probability is relatively high in two or more subspecies, then that colony is considered a hybrid of those subspecies. As Nawrocka and colleagues (2018) stated in their discussion of the accuracy of the identification software IdentiFly, lineage classifications are more reliable than subspecies classification. There is more room for wing shape variation between workers of the same colony when classifying lineages compared to the more specific vein patterns observed to identify subspecies.

Along with lineage and subspecies classifications and supporting probabilities, a graphical representation is presented (Figures 2.2 and 2.3). These CV points are calculated through IdentiFly using a multivariate statistical analysis. CV1 represents size variation across wings while CV2 represents the shape differences between wings. These coordinates can pin-point the likelihood of a colony belonging to the appropriate lineage or subspecies. A point closer to the centre of the ellipses will have a higher similarity to that particular lineage or subspecies than a point farther away from the centre.

2.3. Analysis of population percentage variation

Data were compiled into Excel and imported into the opensource programs R and RStudio. The codes used to produce all graphs are included in Appendix A. Descriptive analysis of lineage and subspecies variation was conducted using grouped CVA graphs and colony numbers across Newfoundland. Identification probabilities were considered when labeling subspecies and lineage designations of colonies.

To relate annual snowfall to subspecies variation across the Island, snowfall data were taken from the Environment Canada (2010) for each bee sample location, with location determined based on maximum drone flight, the maximum distance a drone has been recorded to fly (Ruttner & Ruttner, 1972). A list of the most common honey bee flowering resources found on the Island of Newfoundland was compiled, based on Boland (2017) and Pollinator Partnership Canada (2017).

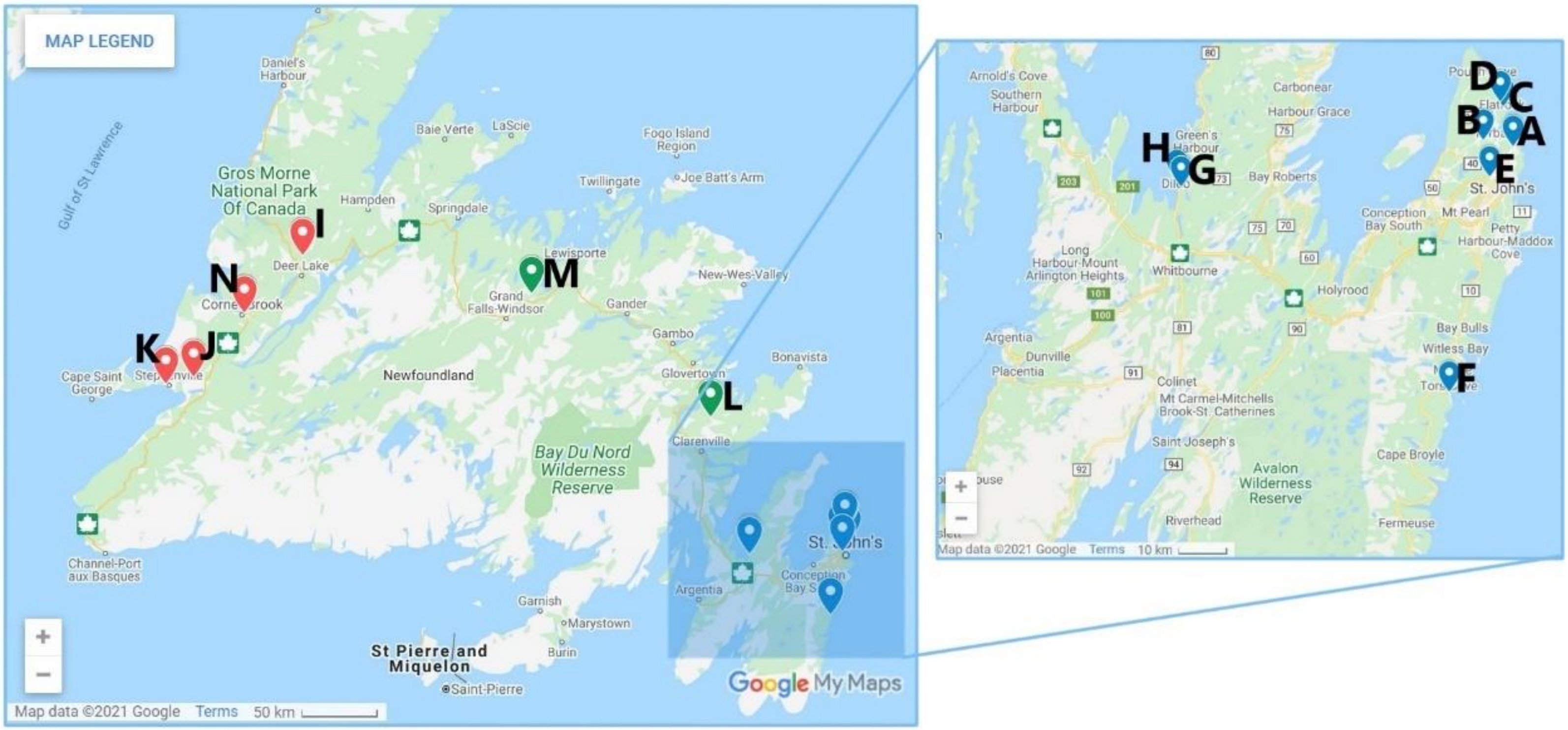


Figure 2.1. Map of apiary locations in Newfoundland. A total of 83 colonies were sampled across the Island. Twenty-three colonies from four locations in Western Newfoundland (red), 35 colonies from two locations in Central (green), and 25 colonies from eight locations in the Avalon Peninsula (blue).

Table 2.1. GPS coordinates of the eight locations in the Avalon Peninsula sampled during summer 2020.

Location ID	**GPS coordinates**
A	47.632N, 52.685W
B	47.644N, 52.761W
C	47.701N, 52.709W
D	47.709N, 52.719W
E	47.578N, 52.745W
F	47.203N, 52.849W
G	47.563N, 53.542W
H	47.572N, 53.551W

Table 2.2. GPS coordinates of the six locations in Central and Western Newfoundland sampled during summer 2021.

Location ID	**GPS coordinates**
I	49.325N, 57.361W
J	48.579N, 58.374W
K	48.549N, 58.608W
L	48.379N, 53.841W
M	49.062N, 55.437W
N	48.953N, 57.932W

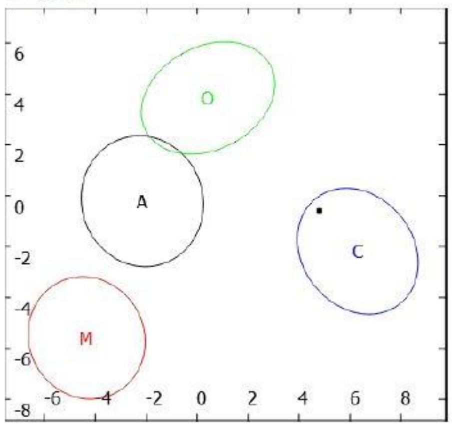
Most similar class:

id	most similar	probability
A.1 Colony	**C**	**0.147725**

CVA probabilities:

id	A	C	M	O
A.1 Colony	5.7534023e-13	0.14772508	1.5612511e-17	3.9194269e-12

CVA graph:



CVA scores:

id	CV1	CV2	CV3
A.1 Colony	4.7974514	-0.59136367	1.2897756

Figure 2.2. An example of evolutionary lineage assignment for coloney A.1 obtained through the morphometric identification program, IdentiFly. Analysis of data includes the most likely lineage with appropriate probability and a graphical representation of the colony lineage along with the CV coordinates. Points closer to the centre of an ellipses will have a higher similarity to that lineage than points farther away.

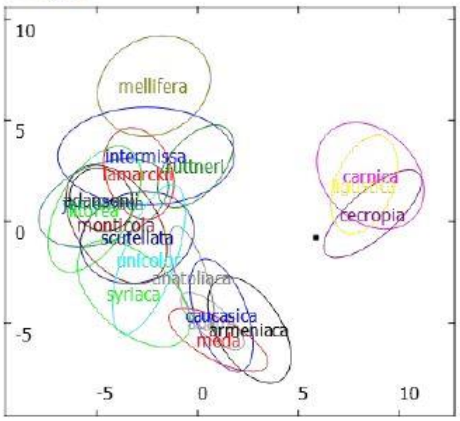
Most similar class:

id	most similar	probability
A.1 Colony	**ligustica**	**0.00539358**

CVA probabilities:

id	adansonii	intermissa	lamarckii	litorea	monticola	ruttneri	scutellata	unicolor	jemenitica	carnica	cecropia	ligustica	mellifera	adami	anatoliaca	armeniaca	caucasica	meda	syriaca
A.1 Colony	4.1199683e-17	4.0982842e-17	4.1904414e-17	4.0332321e-17	0	4.0982842e-17	5.3541752e-14	0	4.1199683e-17	0.00029645758	2.51747e-05	0.0053935787	4.1091262e-17	0	0	4.6458063e-17	4.1416523e-17	0	4.0657581e-17

CVA graph:



CVA scores:

id	CV1	CV2	CV3	CV4
A.1 Colony	5.8595156	-0.79055857	0.23247059	0.49451003

Figure 2.3. An example of subspecies assignment for colony A.1 obtained through the morphometric identification program, IdentiFly. Analysis of data includes the most likely subspecies with appropriate probability, various subspecies and their probabilities, and a graphical representation of the colony subspecies along with the CV coordinates. Points closer to the centre of the ellipses will have a higher similarity to that subspecies than points farther away.

Chapter 3 – Results

3.1. Lineage identification

All colony samples were identified as originating from Lineage C (Eastern European) or Lineage O (Middle Eastern). Of the 83 colonies, 20 (24.1%) colonies were identified as Lineage C, 34 (41.0%) colonies were identified as Lineage O, and 29 (34.9%) colonies were hybrids of the two lineages. Figure 3.1 displays the lineage population percentages in Newfoundland.

CV scores, calculated through IdentiFly, show the differences in morphology of lineages across Newfoundland (Figure 3.2). Figure 3.3 displays the number of colonies from each geographic location coming from Lineage C and Lineage O along with hybrids of the two. Visualizing potential interbreeding of colonies is done by grouping apiaries within seven kilometers of each other; this represents the maximum distance a drone has been recorded to fly (Ruttner & Ruttner, 1972). Figure 3.4 displays the CV scores of each colony divided into seven locations based on the maximum recorded flight distance of honey bee drones (from west to east): Stephenville, Corner Brook, Cormack, Bishop's Falls, Lethbridge, Dildo, and St. John's. The number of colonies from Lineage C, Lineage O, and hybrids of the two grouped by maximum drone flight distance is shown in Figure 3.5.

3.2. Subspecies/hybrid identification

Of the 83 colonies sampled across Newfoundland, 42 (50.6%) were identified as *A. m. armeniaca* or a hybrid of this subspecies. Twenty-one (25.3%) colonies were identified as *A. m. ligustica* or a hybrid of this subspecies. Thirteen (15.7%) colonies were identified as *A. m. cecropia* or a hybrid of this subspecies. The remaining seven colonies were identified as an *A. m. carnica* hybrid (three colonies, 3.6%), an *A. m. scutellata* hybrid (three colonies, 3.6%), and an *A. m. meda* hybrid (one colony, 1.2%). Figure 3.1 displays the subspecies population percentages of Newfoundland.

Figure 3.6 displays the number of colonies from each subspecies or hybrid based on geographic location within Newfoundland. Colonies for which the probability of more than one subspecies is relatively high are considered a hybrid of those subspecies (Tofilski, 2008). The higher probability is considered the dominant lineage or subspecies of the colony. Figure 3.7 groups apiaries by maximum drone flight distance and shows the subspecies and hybrid numbers. Figure 3.8 groups apiaries by maximum drone flight distance and displays the CV scores, calculated through IdentiFly, showing the differences in morphology of subspecies across Newfoundland.

3.3. Local selection

Many factors can contribute to the selection pressure on specific traits of honey bee colonies within an area. Average temperatures, annual snowfall, and resource availability are considered natural selection factors. On the other hand, beekeeper interactions and the movement of colonies across the Island are considered artificial selection factors. Combined, these factors will be referred to as “local selection” and this can vary across Newfoundland.

A huge obstacle many beekeepers face in Newfoundland is successfully over-wintering colonies. The intensity of winters on the Island can drastically affect the survival of colonies. The most commonly identified subspecies in Newfoundland were *A. m. armeniaca* (50.6%) and *A. m. ligustica* (25.3%). Figure 3.9 correlates the average annual snowfall and the percentage of colonies identified as *A. m. armeniaca* per location. I also correlated the average annual snowfall and the percentage of colonies identified as *A. m. ligustica* per location (Figure 3.10).

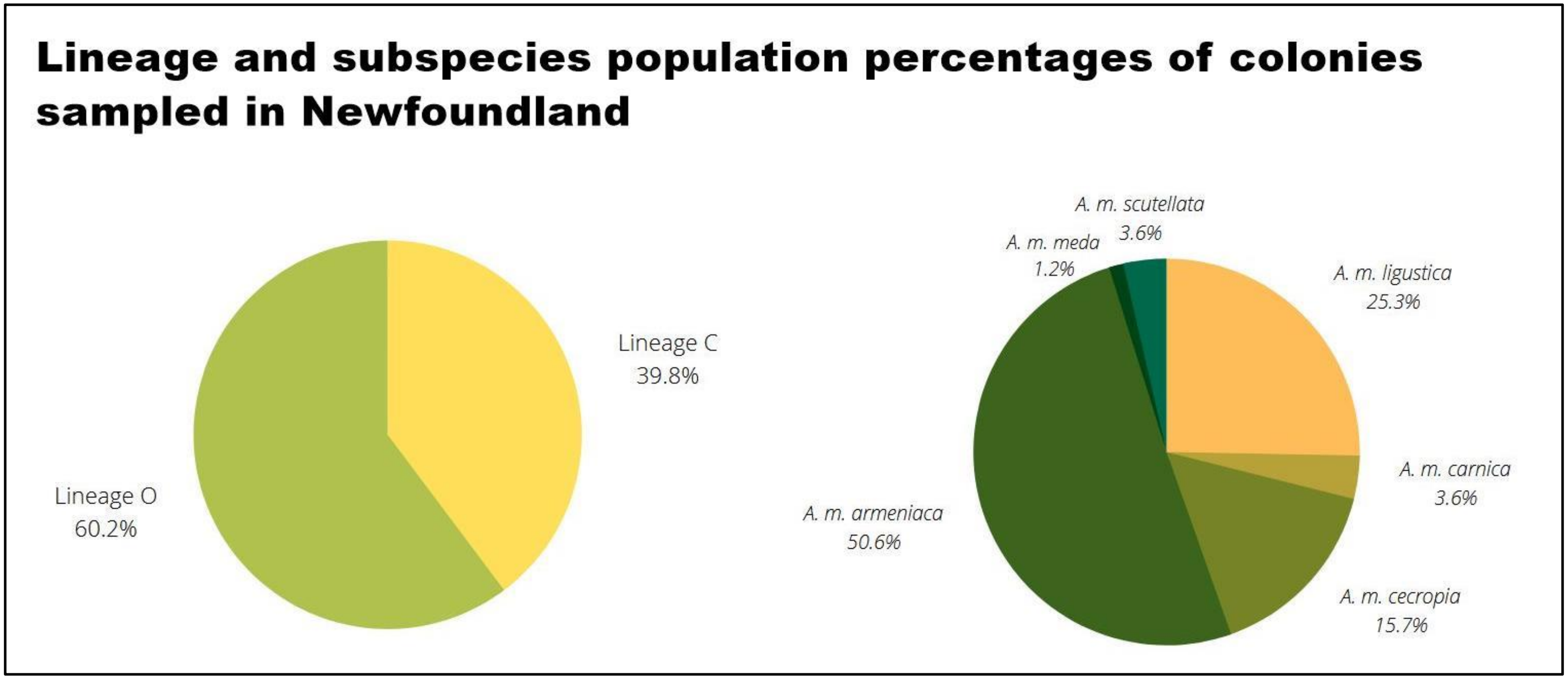


Figure 3.1. Lineages and subspecies identified from the 83 colonies sampled in Newfoundland and the population percentages of lineages and corresponding subspecies for the Island.

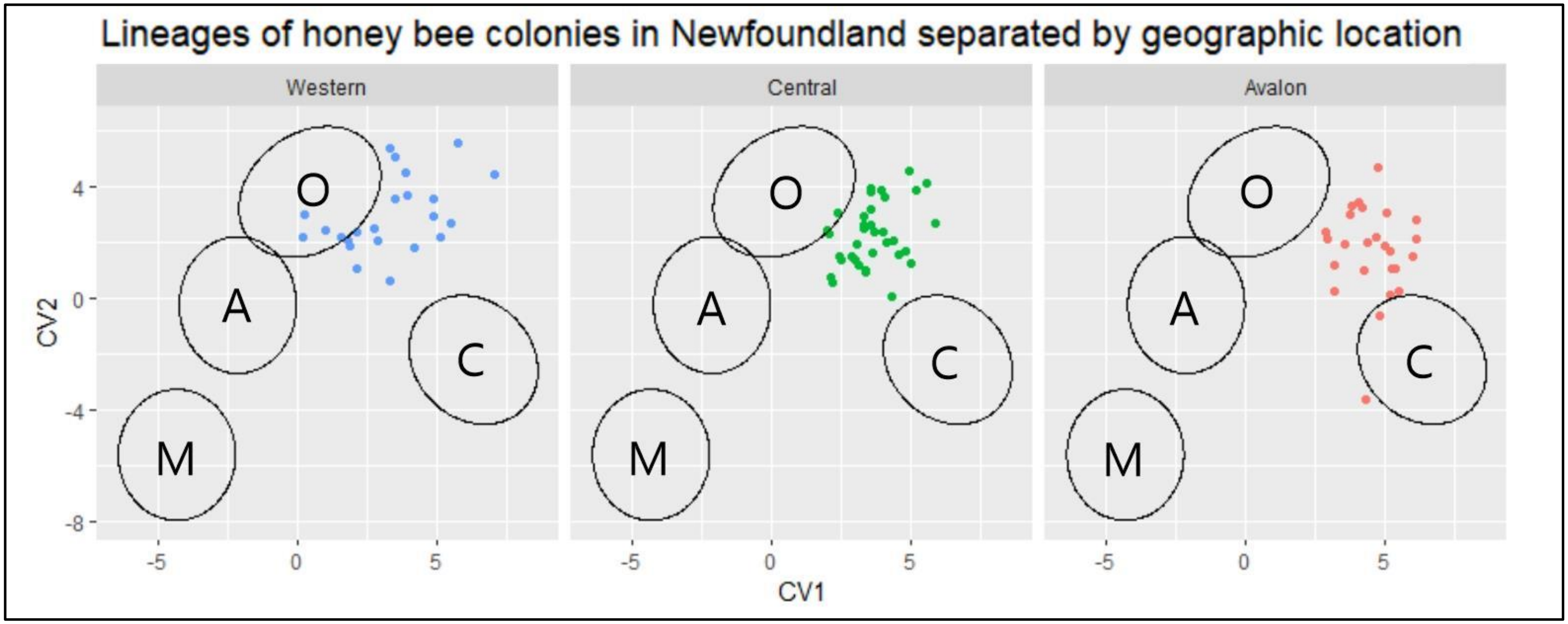


Figure 3.2. A graphical representation of evolutionary lineages identified using IdentiFly and multivariant statistical analysis. CV scores calculated through the program create points representing individual colonies within the Avalon Peninsula (blue data points), Central (green data points), and Western (red data points) Newfoundland. Each lineage is represented as an ellipse and the closer a point is to the center of the ellipse the higher similarity the colony has to the lineage. Letters represent the four honey bee lineages: A (African), C (Eastern European), M (Western European), and O (Middle Eastern) (Chapman *et al*., 2016).

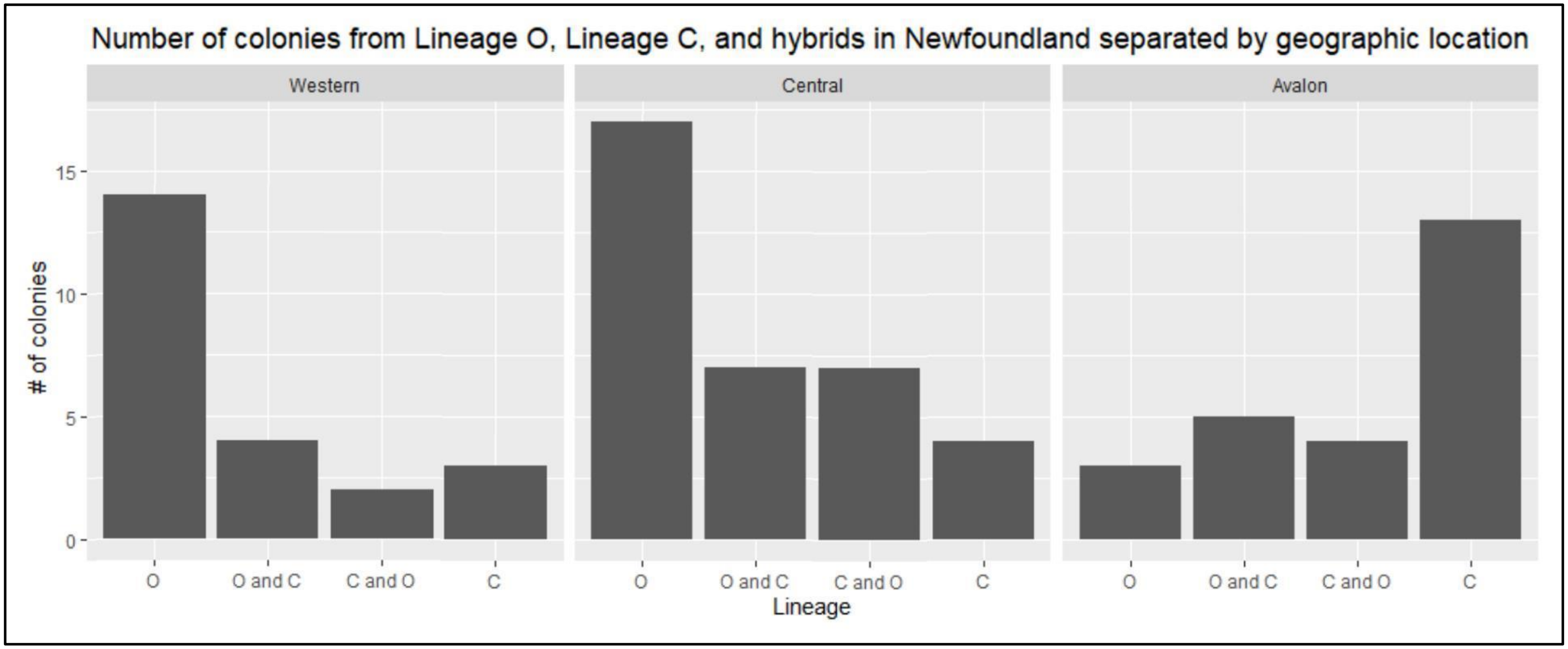


Figure 3.3. The number of colonies from Lineage O, Lineage C, and hybrids of the two from each geographic location in Newfoundland. "O and C" represents a colony that was classified as most likely coming from Lineage O but also shows characteristics of Lineage C. "C and O" represents a colony that was classified as most likely coming from Lineage C but also shows characteristics of Lineage O. These colonies most likely came from a mixing of lineages.

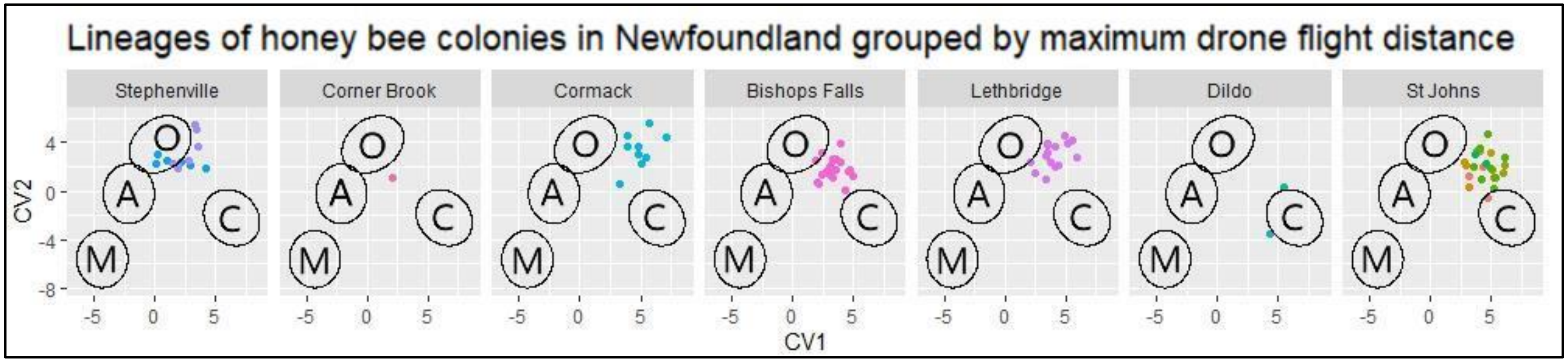


Figure 3.4. A graphical representation of lineages identified using IdentiFly and multivariant statistical analysis. CV scores calculated through the program create points representing individual colonies. Each graph represents apiaries within the maximum drone flight distance of each other (seven kilometers) and shows a more refined image of interbreeding on the Island. Each lineage is represented as an ellipse; the closer a point is to the center of the ellipse, the higher similarity the colony has to the lineage.

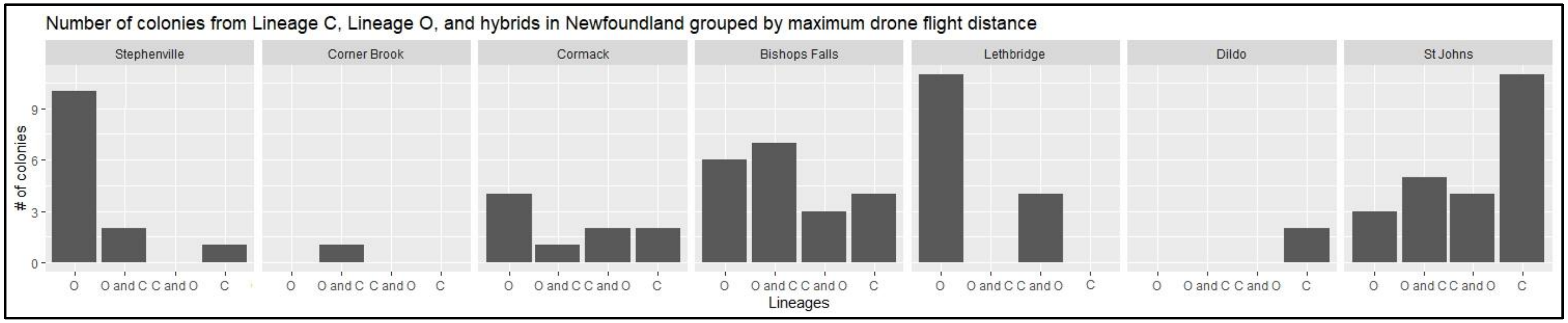


Figure 3.5. The number of colonies from Lineage O, Lineage C and hybrids of the two. Each graph represents apiaries within the maximum drone flight distance of each other (seven kilometers) and shows a more refined image of interbreeding on the Island. Apiary mixing is unlikely with locations further than seven kilometers.

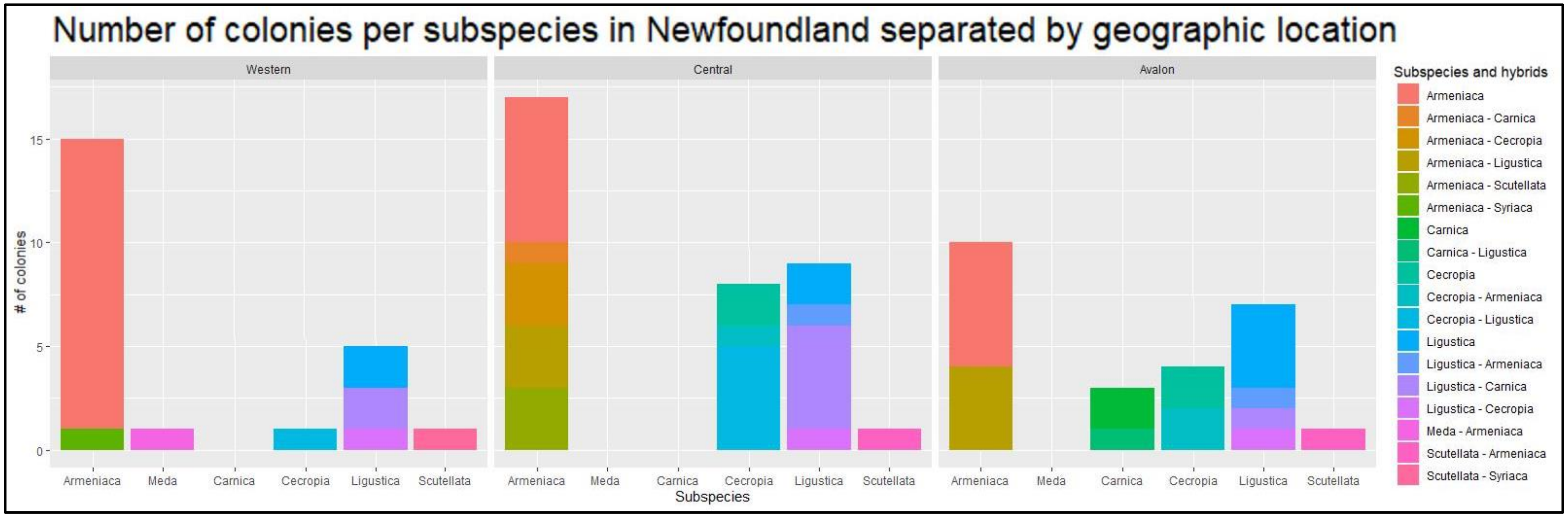


Figure 3.6. The number of colonies identified as the corresponding subspecies grouped by geographic region of Newfoundland. Colonies with two high subspecies probabilities relative to all others indicates a hybrid of those subspecies. The subspecies with the higher probability is considered the dominant subspecies of the colony. Subspecies are ordered based on lineages with Lineage O comprised of *A. m. armeniaca* and *A. m. meda*, Lineage C comprised of *A. m. carnica, A. m. cecropia, and A. m. ligustica*, and Lineage A comprised of *A. m. scutellata*.

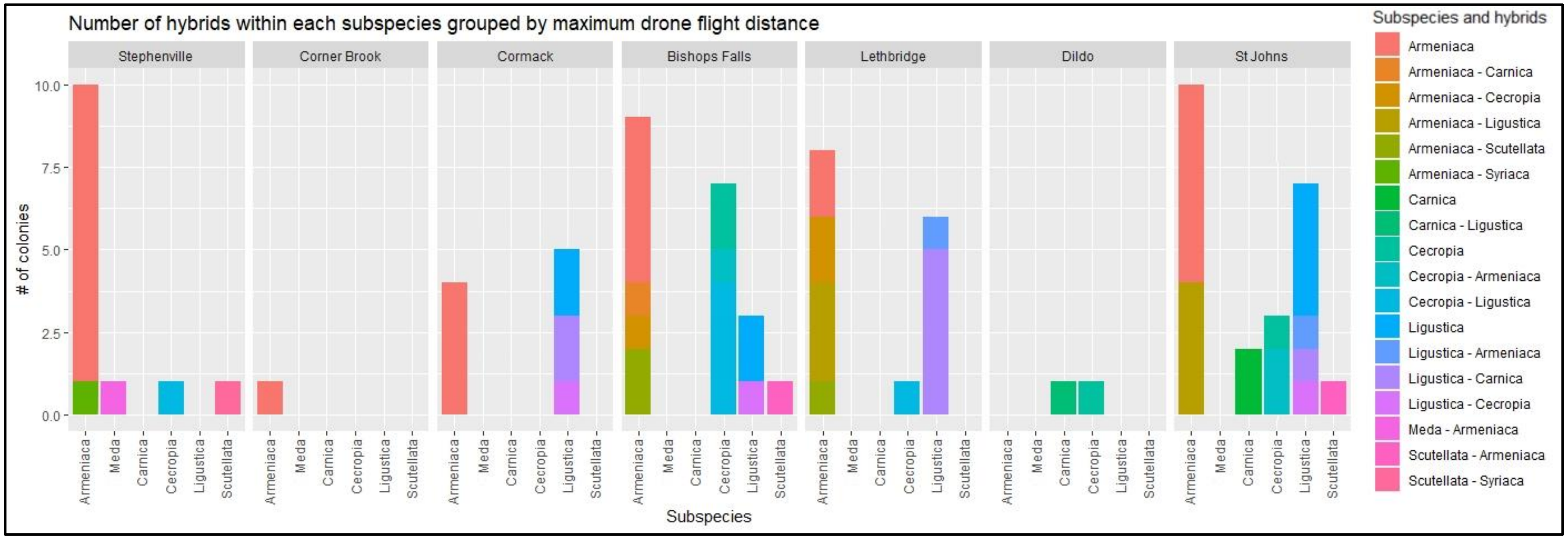


Figure 3.7. The number of colonies identified as the corresponding subspecies grouped by maximum drone flight distance. This shows a more refined image of interbreeding between apiaries. Colonies with two high subspecies probabilities relative to all others indicates a hybrid of those subspecies. The subspecies with the higher probability is considered the dominant subspecies of the colony. Subspecies are ordered based on lineages with Lineage O comprised of *A. m. armeniaca* and *A. m. meda*, Lineage C comprised of *A. m. carnica*, *A. m. cecropia*, *and A. m. ligustica*, and Lineage A comprised of *A. m. scutellata*.

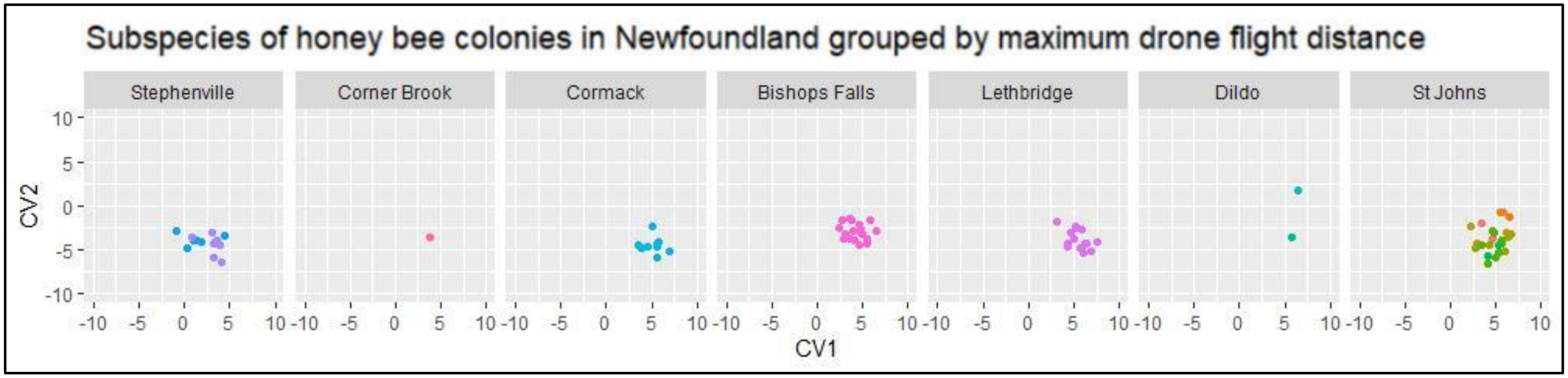


Figure 3.8. A graphical representation of subspecies identified using IdentiFly and multivariant statistical analysis. CV scores calculated through the program create a point representing a single colony. Each graph represents apiaries within the maximum drone flight distance of each other (seven kilometers). In order to better view apiary diversity, subspecies ellipses have been omitted. Refer to Figure 2.5 for subspecies grouping.

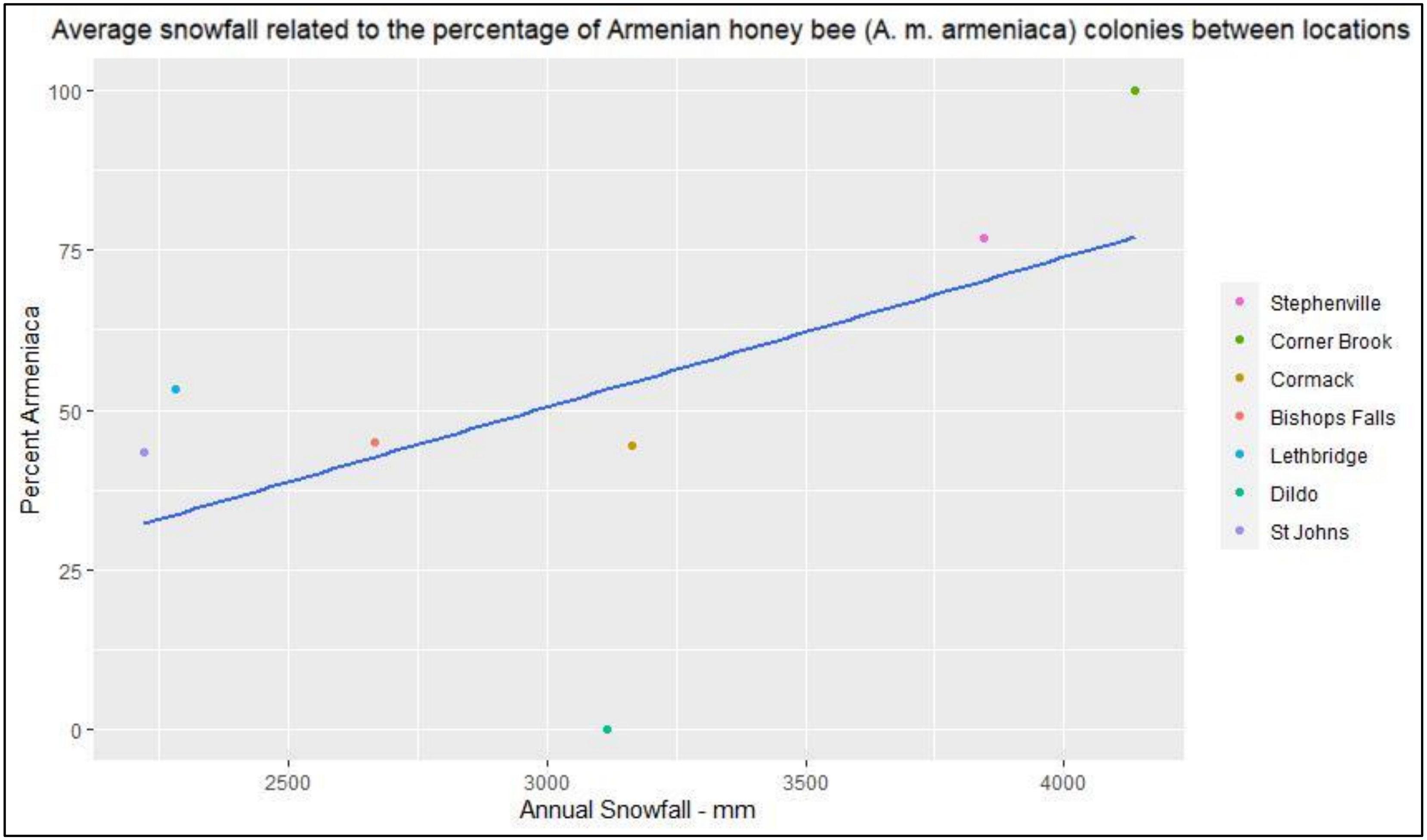


Figure 3.9. Correlation between the average annual snowfall (mm) in each location (based on maximum drone flight distance) and the percentage of colonies identified *A. m. armeniaca*. Blue line represents the line of best fit. Snowfall data obtained through Environment Canada (2010

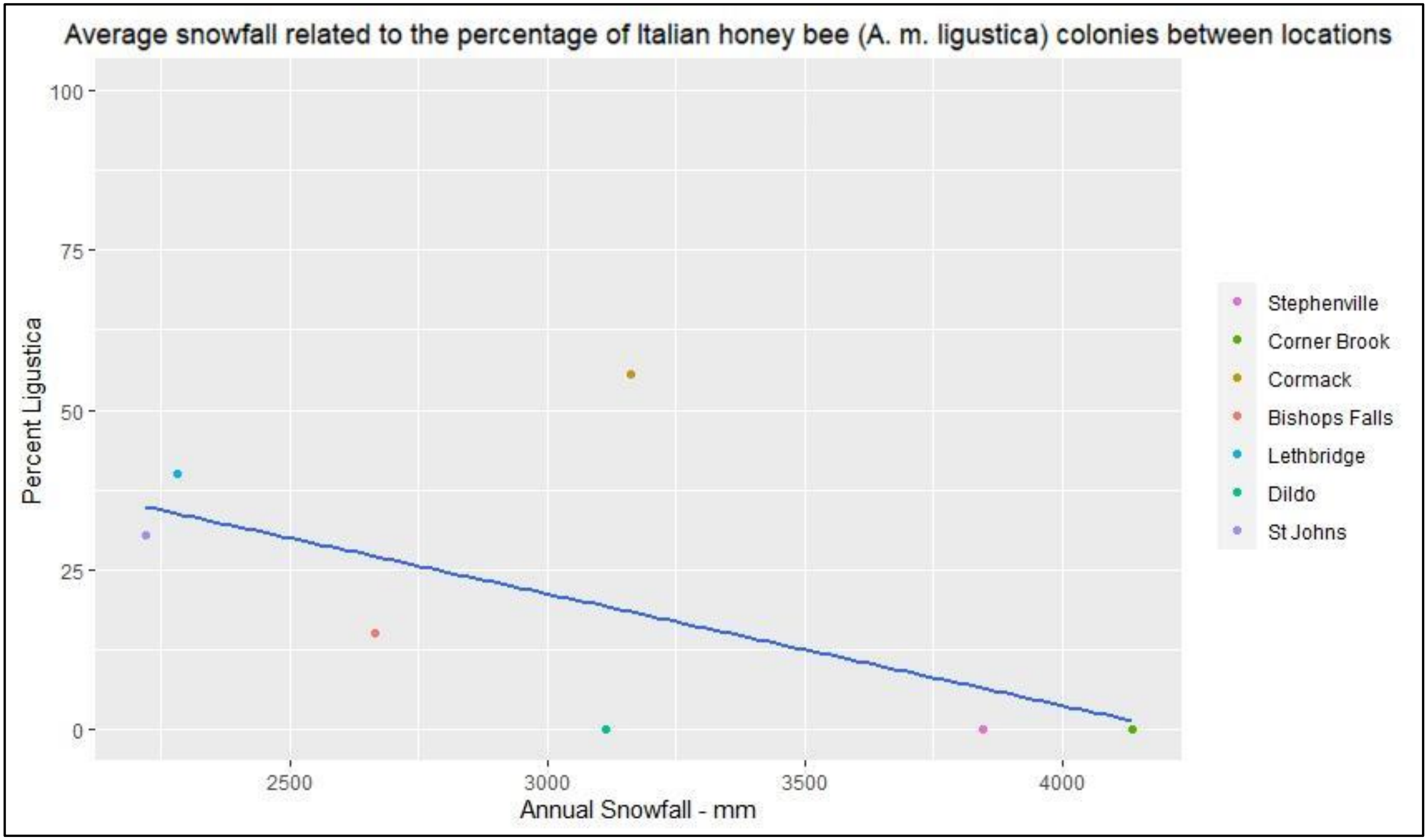


Figure 3.10. Correlation between the average annual snowfall (mm) in each location (based on maximum drone flight distance) and the percentage of colonies identified as *A. m. ligustica*. Blue line represents the line of best fit. Snowfall data obtained through Environment Canada (2010).

Chapter 4 – Discussion

4.1. Morphometric analysis using IdentiFly

Morphological differences between honey bee subspecies have been categorized for a century (Ruttner, 1988; Cochlov, 1916) and programs such as IdentiFly (Tofilski, 2008) give researchers the ability to classify colonies to evolutionary lineage and subspecies using morphometric data. Unlike genetic testing, morphometric analysis can be done using free software and requires little training, allowing both researchers and beekeepers the opportunity to better understand the origins of their colonies.

Analyses of forewing vein patterns of worker bees on the Island of Newfoundland found two major lineages most associated with these colonies: Lineage O and Lineage C. Colonies were further classified into six different subspecies and hybrids (Figure 3.1): Italian honey bee (*A. m. ligustica*), Carniolan honey bee (*A. m. carnica*), Greek honey bee (*A. m. cecropia*), Armenian honey bee (*A. m. armeniaca*), Iranian honey bee (*A. m. meda*), and African honey bee (*A. m. scutellata*).

Subspecies diversity and numbers varied between locations on the Island. Colonies identified as subspecies from Lineage O (specifically *A. m. armeniaca*) were more commonly observed in Western Newfoundland (Stephenville and Corner Brook) while colonies identified as subspecies from Lineage C (*A. m. ligustica, A. m. cecropia,* and *A. m. carnica*) were found in the Avalon Peninsula (Dildo and St. John's). In most Central Newfoundland locations (Cormack and Bishop's Falls), colonies were identified as a hybrid between subspecies of Lineage O and Lineage C. The one exception in

Central Newfoundland was Lethbridge where the majority of colonies were identified as either Lineage O or a hybrid of Lineage O and Lineage C.

The morphological analysis program, IdentiFly, was developed using reference images of honey bee wings from the Morphometric Bee Data Bank in Oberursel, Germany (Tofilski, 2008). Similar programs, such as ApiClass, have used databases of reference images of genetically identified subspecies. The Morphometric Bee Data Bank operates differently; great care has been taken to collect samples from regions where no known importation or hybridization of colonies has occurred (M. Meixner, personal communication, August 4, 2021). Instead of collecting samples and then identifying the subspecies based on genetics, references are only taken from isolated colonies in their area of origin which are then used to identify unknown colonies. Currently, work is being done to confirm reference samples in the Oberursel database by performing genetic analysis on the bees that have contributed to the current morphometric catalogue.

During the development of IdentiFly, Nawrocka *et al*. (2018) noted that "the available reference data set varied in the number of colonies per subspecies". Of the subspecies potentially in Newfoundland, the number of colonies analysed using IdentiFly are as follows: *A. m. ligustica* (11), *A. m. carnica* (15), *A. m. cecropia* (9), *A. m. armeniaca* (6), *A. m. caucasica* (12), *A. m. meda* (8), *A. m. scutellata* (14). The subspecies with fewest reference colonies is *A. m. armeniaca* with six reference colonies. Though no colonies were identified as *A. m. caucasica* by the IdentiFly program, this subspecies will be discussed because of the similar characteristics between it and *A. m. armeniaca*, their similar area of origin, and the popularity of *A. m. caucasica* among beekeepers.

Nawrocka *et al*. (2018) explained that classification probabilities need to be reviewed in order to correctly identify a colony's most probably subspecies origin and influences. If the identified subspecies probability is much higher than all other probabilities then it is safe to conclude that the identified subspecies is correct (Nawrocka *et al*., 2018). However, if there are multiple moderately high probabilities then you can conclude that the colony is similar to the identified most likely subspecies but markedly different from the reference samples (Nawrocka *et al*., 2018). This could indicate either (1) the colony is a hybrid of the highest probable subspecies or (2) the colony belongs to a subspecies not covered by the model (Nawrocka *et al*., 2018).

When Węgrzynowicz *et al*. (2019) compared worker wings of hybrid and backcross colonies to their paternal and maternal colonies, they found that wings of hybrid workers were more similar to those of workers from the maternal than the paternal colony. Wegrzynowicz *et al*. (2019) speculated that the effects can be related to the mitochondrial DNA that is only inherited from the mother. Even with a small number of genes that do not directly affect morphology, Wegrzynowicz *et al*. (2019) hypothesized that these genes within mitochondrial DNA can still alter the wing shape indirectly. This indicates that wing vein patterns may not represent the true hybridization of a colony and might be skewed towards the maternal colony's morphology. Of the 83 colonies sampled in Newfoundland, 42 colonies had a relatively high probability in two subspecies. This is not surprising as hybridization is expected to happen in an isolated area, especially when importations are restricted and uncommon, with the last recorded importation in 2016. In order to be completely accurate in the identification process, genetic analysis of worker samples would need to be completed. All samples taken during this research have been labelled and stored in a -80° freezer to preserve worker bee DNA in preparation for future research. Ideally, regular samples taken of colonies should be taken in order to track genetic diversity and hybridization of honey bees across the Island. These samples can be used to compared pre-*varroa* infection DNA in case of any future spread of the parasitic mite.

4.2 Causes of lineage and subspecies variation

As stated earlier, local selection, the combination of natural and artificial pressures on colony survival and reproduction, influences the lineages and subspecies varieties seen across Newfoundland. Figures 3.2 and 3.3 show how colonies gradually drift from Lineage O to Lineage C as we move from west to east. This gradient is most likely caused by the numerous different influences associated with the different apiaries.

As shown in figure 3.8, areas with large amounts of annual snowfall had a larger percentage of colonies classified as *A. m. armeniaca* than areas with less snowfall, whereas the percentage of colonies classified as *A. m. ligustica* was negatively correlated with the annual snowfall. To better understand the importance of this correlation, we need to refer to the Köppen-Gieger maps (Beck *et al*., 2018) of the origin countries of the various subspecies in of honey bees identified in my analyses and relate them to the climate of Newfoundland. Figure 4.1 displays the climate maps of Newfoundland and the country of origin of the most commonly identified subspecies in Newfoundland: *A.m. ligustica* (Italy), *A. m. cecropia* (Greece), *A. m. armeniaca* (Armenia), and *A. m. caucasia* (Georgia). Visually, it is easy to see the climate and biome similarities between

Newfoundland and Armenia & Georgia. Even though Italy does have some similar regions, they are extremely small in relation to the areas in Armenia and Georgia. As Meixner and colleagues (2015) have confirmed, locations with similar climates to the area of origin of honey bee subspecies will perform better due to their specific genotype-environment interactions. Figure 4.2 shows the climate map of Canada and the similarities between Newfoundland and Guelph, Ontario, a known location of colony importation into Newfoundland (Hicks, 2014; Armitage, 2018). Based on the climate map, we can predict that subspecies raised and bred in Ontario would be well adapted for Newfoundland ecosystems with similar weather.

The similarities in climate between areas of origin for Lineage O subspecies and Newfoundland help explain the larger proportion of colonies identified as *A. m. armeniaca*. Much of Newfoundland is divided into two climate zones: Subarctic (Dfc) and Warm-summer humid continental (Dfb). These two climate zones make up a majority of the landmass of Armenia and Georgia, making any subspecies originating from those areas a prime candidates for Newfoundland beekeepers. Comparing the Newfoundland climate maps with those of Italy and Greece, the countries where *A. m. ligustica* and *A. m. cecropia* are endemic, we can see that the climates of those countries are much warmer and not similar to Newfoundland. Even though these subspecies tend to be the most popular among beekeepers around the world, as pure subspecies they would not be best adapted to Newfoundland's specific environment.

Figure 3.9 shows the positive correlation between lower annual snowfall and the percentage of colonies identified as *A. m. ligustica*. This is expected since *A. m. ligustica* is accustomed to Mediterranean climates and would subsequently be expected to do poorly in areas of high snowfall. In fact, there were no colonies identified as *A. m. ligustica* in the two most western locations, Stephenville and Corner Brook (Figure 3.6). Strong, cold winds that blow off the Gulf of St. Lawrence and Labrador cause a climate that is almost arctic-like on the western coast of Newfoundland where both Stephenville and Corner Brook are located (Boland, 2017). Lineage C subspecies would not be able to survive such harsh temperatures that Lineage O subspecies might be more adapted for. Further collecting of samples along the Great Northern Peninsula would help to clarify the potential impact of snowfall on subspecies designations.

Honey bees require a minimum temperature of 12.2°C in order to achieve flight (Vicens & Bosch, 2000). There is a difference in average temperatures throughout the Island which can affect the length of the foraging season (Figure 1.3). Bishop's Falls and Lethbridge can reach minimum flight temperature by late April while St. John's won't reach this temperature until early May. Cormack experiences temperatures below the foraging threshold earlier than other areas in the study, with an average high below 12.2°C by mid-September. Lethbridge and Dildo usually maintain an average high above this temperature until October. Based on the average high temperatures, Lethbridge has the longest season with opportunities for bees to forage from late April to October.

Outside of the Avalon Peninsula, Lethbridge has the most colonies identified as *A. m. ligustica* (Figure 3.6). A longer foraging season provides these Mediterranean colonies with ample foraging opportunities and supports their survival. As excellent producers of surplus honey, colonies with *A. m. ligustica* foraging characteristics might be selected by beekeepers in the Lethbridge area. This would explain the higher proportion of colonies identified as "Italian" in the region compared to the rest of central and western Newfoundland. Lethbridge has a lower average annual snowfall compared to much of the Island which would benefit *A.m. ligustica* and other eastern European honey bee lineages' survivability (Figure 3.10).

Fog and rain can limit the foraging capabilities of honey bees and will influence the survivability of specific subspecies. Table 4.1 shows the annual average days of precipitation and the annual average precipitation in millimeters (Environment Canada, 2010). Stephenville has the most days of precipitation with an average of 221 days per year while Bishop's Falls records the least number of days of precipitation with an average of 159 days per year. St. John's has the most annual precipitation with an average of 1534 millimeters while Cormack has the least amount of annual precipitation with an average of 1099 millimeters. A higher average number of days of precipitation per year can limit the foraging of honey bee colonies in these areas. The combination of limited opportunities to forage due to precipitation or low temperature could affect honey bee subspecies differently. Colonies derived from Lineage O subspecies that evolved under conditions of limited resources and poor weather conditions may be better adapted to thrive in these harsh conditions.

Plant diversity is also influenced by the climate differences around the Island and can have a drastic affect on nutrition in the colony. Table 1.1 lists some common flowering plants in Newfoundland that are often visited by honey bees. Western Newfoundland has the largest flowering plant diversity compared to Central

Newfoundland and the Avalon Peninsula. Pollen and nectar availability could be more widely available and more diverse in this region. Because pollen and nectar quality and diversity can alter honey bee physiology, the survivability of specific subspecies can vary (Pasquale *et al*., 2013). Higher quality pollen has been shown to improve resistance to pesticides (Schmehl *et al*., 2014) while high quality nectar can increase pathogen resilience of bees (Mao *et al*., 2013). This can help explain the differences in diversity of subspecies between Western, Central, and the Avalon Peninsula.

Differences in elevation can also affect plant diversity and abundance which in turn can influence honey bee diversity. Corner Brook, whose elevation ranges from 0 to 0.304 kilometers, offers the most drastic change in elevation of the sampled locations. The only honey bee sample taken from the area was identified as *A. m. armeniaca* which supports the idea that this subspecies is best adapted for the specific environment of Western Newfoundland. Further sampling of colonies in Corner Brook would be needed to confirm this idea. The higher diversity and quality of pollen/nectar sources in Western Newfoundland, ecosystem similarities between Newfoundland and Middle Eastern countries of origin, and the ability of *A. m. armeniaca* to maximize their energy efficiency while foraging (Cakmak & Wells, 2001) can help explain their success in the area. Naturally occurring flora account for a portion of the pollen and nectar sources available to honey bees. Agricultural land, specifically vegetable and fruit cultivation, can provide even more nutritional sources for colonies.

It has been anecdotally noted by many beekeepers that colonies located in the St. John’s region were stronger, developed more honey, and survived the winter better than colonies located outside of the city. This may be due to the greater diversity and abundance of floral resources in urban areas. No scientific evidence has been collected to support this idea, yet future studies may focus on understanding the differences in colony strength between urban and rural communities. In these areas there is also a presumed increase in pesticide use for lawn care, both on personal property and public property. Lineage O subspecies may be more tolerant to pesticides and take advantage in the variety of pollen and nectar sources which could explain their larger numbers in urban areas. In contrast, Lineage C subspecies may be more sensitive to pesticides which would reduce their survival in these areas.

As discussed previously, *A. m. armeniaca* evolved in a very similar climate and biome as Newfoundland which has given them an adaptive advantage. These colonies may be better producers of colony products, tend to create large amounts of brood, survive harsher winters, or exhibit any other beneficial traits that beekeepers value. Those colonies that demonstrate these characteristics will be split into new colonies and carry on the *A. m. armeniaca* lineage.

In the Avalon Peninsula, the majority of apiaries consist only of a few colonies that beekeepers tend to and maintain. These smaller apiaries include bees of greater morphological diversity compared to apiaries in central and western Newfoundland (Figure 3.5). In contrast to apiaries on the Avalon, beekeepers elsewhere on the Island raise and split colonies in order to create new colonies. These apiaries regularly consist of a few dozen colonies at any given time. Unsurprisingly, colonies in these apiaries are more closely related to one another as seen by the CV scores (Figure 3.7). The major nuc suppliers on the Island create their bee stock using this method and then sell their colonies to smaller operations or to hobbyist beekeepers (many of which are located in larger cities such as Corner Brook and St. John's). This commercial dispersal of colonies would explain the wider range in subspecies diversity in the St. John's area (Figure 3.7). As beekeeping expands in popularity in Newfoundland, we can expect urban areas will continue to have the most subspecies diversity. Sampling more colonies in Corner Brook would provide a better understanding of urban versus rural apiaries.

An interesting pattern is seen in Bishop's Falls: the majority of colonies at the one apiary at this location are identified as either Armenian honey bees (*A. m. armeniaca*) or Greek honey bees (*A. m. cecropia*). In fact, Bishop's Falls contains the highest number of colonies identified as Greek honey bees (Figure 3.7). The beekeeper who manages this specific beekeeping operation has been expanding their colony numbers in the last two to three years without bringing in new stock. We can see this trend in Figure 3.8 with the high precision in identification between colonies housed in this apiary. The presence of bees most similar morphologically to Greek honey bee is unusual when comparing the climates of Greece and Newfoundland (Figure 4.1). Beekeeper influence, such as wrapping hives during winter, could be artificially supporting this subspecies on the Island. Bishop's Falls also experiences the least number of days of precipitation annually (Table 4.1) and has a relatively low snowfall compared to the other locations, which could support the survival of the Greek honey bees. Their gentleness, low tendency to swarm, and quick spring build up may have made beekeepers unknowingly favor them compared to other colonies. Further analysis of the Greek honey bee colonies' characteristics such as aggression, honey production, tendency to swarm, and colony growth would help clarify the reason for their propagation in this specific area.

Though over 50% of sampled colonies in Newfoundland were identified as *A. m. armeniaca*, it is possible that these colonies are not actually Armenian honey bees. Discussions with beekeepers in Armenia and Georgia suggested that the export of Armenian honey bees is non-existent. Because of their high performance in sub-arctic climates, care was taken in order to isolate the *A. m. armeniaca* stock during the Soviet Era in order to preserve their unique genetics (G. Tabakyan, personal communication, August 4, 2021). Imports and exports of honey bee colonies was strictly enforced. Even after the fall of the Soviet Union, colonies were kept under tight regulations making exportation unlikely. Historically, there is no evidence the bees have been imported to North America from Armenia (Carpenter & Harpur, 2021).

When we look at the CV graph identifying subspecies (Figure 2.5) we can see how much subspecies overlap with each other, especially with those coming from the same lineage. Combined with the history of honey bee imports into the Island, the more likely subspecies of these colonies are *Apis mellifera caucasia* (Caucasian honey bee). Both *A. m. armeniaca* and *A. m. caucasia* belong to the same lineage (Lineage O) and have very similar characteristics. A map of the distribution of honey bees (Figure 1.2) shows how close the region of origins are for *A. m. armeniaca* and *A. m. caucasia*. In the Caucasus Mountains, *A. m. caucasia* more often occurs at higher elevations compared to *A. m. armeniaca* which are often seen in the lowlands. It should be noted that the “lowlands” sit at a higher elevation (average 1,800 metres) than Newfoundland (highest elevation is 814 metres) [Howe, 2021; Hiller & Harris, 2021]. These subspecies are known to interbreed in areas of sympatry along the elevation gradient (G. Tabakyan, personal communication, August 4, 2021). Unlike the Armenian honey bee, *A. m. caucasia* has a long history of exportation out of the Caucasus region (Cobey, Sheppard, & Tarpy, 2011; Carpenter & Harpur, 2021). Therefore, it is more likely that colonies identified as *A. m. armeniaca* are descendents of the related and more regularly exported *A. m. caucasia*.

But how did these bees get to Newfoundland?

Newfoundland is known for being a porous island; tales have been told of illegal importation of a wide range of items, ranging from primates to drugs. Even today, stories of illegal importation of honey bees make their way into the news ("Sting operation: N.L. beekeeper sounds alarm after queen bee illegally imported by mail", 2021). Though honey bee import restrictions have been in place for many years, little government support has been put into ensuring these laws are followed. Even if every citizen followed the restrictions, swarms are known to survive on cargo ships and shipping containers (Barry *et al*., 2010). This has made Newfoundland's ports of entry hot spots for mainland honey bee colonies entering the Island (Newfoundland and Labrador Beekeeping Association, 2020). Australia has been able to adopt strategies in order to reduce the accidental importation of colonies such as using bait hives to collect swarms, strict record keeping, and monitoring of ports (Barry *et al*., 2010). Newfoundland does not currently have any such protections in place and the Island could have already had a rogue colony enter unknowingly. Feral colonies which have survived at least one winter have been detected in Newfoundland which begs the question, has a swarm entered the Island and survive to reproduce? It is unlikely but should still be considered.

We can speculate on what genetic diversity exists on the Island by examining the subspecies diversity of the most recent locations colonies were exported from, including Nova Scotia, Ontario, and Australia. In Nova Scotia, the most kept colonies are *A. m. carnica* but there are also colonies mixed with *A. m. ligustica* and Buckfast (NSBA, personal communication, August 16, 2021). In Ontario, selective breeding has been going on since 1997 in order to breed a bee for low *Varroa* populations, large colonies, high honey production, gentleness, and hygienic behaviour (Szabo, n.d.). Attempts were made to reach out to this apiary in order to determine what subspecies make up their stock, but there was no response. Australia's earliest known honey bee imports were from Western Europe around the early 1860's (Chapman *et al*., 2016). In the early 1880's, *A. m. caucasia* was imported, making it the first colonies originating from Lineage O in Australia. Today, the favored subspecies cared for by beekeepers in Australia are *A. m. ligustica*, *A. m. carnica*, and *A. m. caucasia* [Chapman *et al*., 2016; Chapman *et al*., 2008].

There are two likely points of origin for the introduction of Lineage O subspecies into Newfoundland: Szabo stocks in Guelph, Ontario and Western Australian stocks. Between 2000 to 2014, four separate importations of eggs and queens occurred from the Szabo stock in Ontario (Newfoundland and Labrador Beekeeping Association, 2020). No records were found on where exactly these colonies were imported in Newfoundland. *A. m. caucasia* colonies might have also been brought into Newfoundland when 130 colonies were imported from Australia in 2016; 100 of these colonies were placed in Bonne Bay, Western Newfoundland and the rest were located in St. John’s. Unfortunately, no samples were taken from colonies in either location. We might see the genetic influence of the Australian colonies imported into St. John’s due to their proximity to the sampled apiaries, but we cannot be certain.

4.3. Potential for future research and general discussion

Care was taken to insure proper long-term storage of worker samples for future genetic testing to take place. Genetic testing would be able to identify subspecies of honey bee worker samples more precisely than the wing vein pattern method. The insect bodies from each colony are being held at -80° and include hive number, colony location, and the date sampling took place. If genetic testing provides a specific lineage or subspecies then that information in turn can help to support the IdentiFly program in learning vein pattern variations between colonies.

With this foundation, future researchers can build on the accuracy of identification software using wing vein morphology. As more samples of wing veins from bees representing pure subspecies and various crosses between are taken around the world and with genetic testing to confirm lineage and subspecies, programs such as IdentiFly become more reliable and offer a wider range in subspecies identification. Wing images taken during this research will be shared with the IdentiFly development team in hopes to contribute to the further development of this free program.

Regular sampling of worker bees and analysis of wing vein patterns over the years would also provide researchers the ability to track introgression on the Island. These “snap-shots” of colony diversity would help to identify areas of increased interbreeding between colonies and would allow the further development of a *Varroa* action plan through the provincial government. Knowing where colonies are more likely to socialize with each other would help in defining natural breaks between colonies; if *Varroa* is ever introduced onto the Island, then beekeepers can take action in isolating colonies to stop the spread to the rest of Newfoundland.

Because IdentiFly is a free, easy to use software, beekeepers are able to take advantage of this program and are able to track their own apiaries without the need for assistance from researchers. The extensive access to this program can offer an increase in citizen collected data that researchers can use to track diversity over time. These data can then be shared with researchers in order to focus on other questions that have arisen.

There is also ample opportunity to focus more on the behavioural aspect of honey bee subspecies. Characteristics important to beekeepers such as aggressiveness and hygienic behaviour can also be analyzed to identify the best subspecies of honey bees for agricultural and commercial apiaries. Newfoundland’s stock has been initially tested for their hygienic behaviour and a variety of degrees of hygienic behaviour had been observed. Based on 14 colonies assessed in August 2019, five colonies met the strict requirement to be considered “hygienic” with at least 95% of dead brood removed within 24 hours (Newfoundland and Labrador Beekeeping Association, 2020). In other studies, colonies with hygienic traits also demonstrate resistance to *Varroa*, American foulbrood, and chalkbrood, making this trait highly desirable (Spivak & Gilliam, 1998). Further analysis of the hygienic behaviour of specific subspecies variety could benefit the selective breeding of colonies in Newfoundland.

Potential sources of error can be found in any research project. Due to COVID-19 restraints, sampling of colonies was done over two separate summers. The summer of 2020 sampling was done only in the Avalon Peninsula while the summer of 2021 sampling was done in Central and Western Newfoundland. Instead of offering a single "snap-shot" of the diversity seen on the Island, instead we are looking at two separate points in time. Over this period, the diversity on the Avalon Peninsula could have changed as queens lay eggs using sperm from different drones, beekeepers split and create new colonies, and the natural die-off of colonies over winter. Preferably, sampling of colonies across Newfoundland would be done in a relatively short time span during the same time each year. Future researchers should take note and plan accordingly.

Though the IdentiFly program takes advantage of the slight differences in wing vein patterns between subspecies to identify colonies, errors can still occur. Figure 2.5 shows how subspecies overlap one another, especially between subspecies of the same lineage. Due to the isolated nature of Newfoundland, hybridization is bound to occur, which creates even more difficulty in defining subspecies of colonies. Genetic testing of worker bodies would eliminate this error and in turn could help to improve the accuracy of the software, though this would require financing and specialized training/equipment. Wing images taken during this project can be used in the future with updated versions of IdentiFly to receive a more accurate classification of colonies.

IdentiFly requires the user to place points on wing vein intersects and placement can easily vary between one researcher and another. Care was taken to double check that points were placed appropriately on each intersect but variation can still occur which can slightly alter the final result. Some wing images were too out of focus to be used and new photos could not be taken because wings were disposed of in order to re-use slides. Some images were slightly out of focus but could still be used to pin-point intersects. Preferably, clear images would be used in future projects and wing slides would be held until an image's clarity is verified. There were also some colonies sampled where we did not collect at least 20 workers. Whether due to poor image quality or low number of worker bees collected, four colonies in the Avalon Peninsula were re-sampled during the summer of 2021 in order to obtain a minimum of 20 wing images per colony. Colonies B.3, C.1, E.2, and E.5 all had more workers collected and wing images taken to use in the IdentiFly program. The use of different generations of worker bees may have influenced the identification due to the change in sperm used by the queen between seasons. Two other colonies (C.3 and G.1) also had a low number of worker bees collected yet unfortunately these colonies did not survive the winter of 2020 and thus could not be re-sampled. These two colonies were still processed but both had less than 20 wing images uploaded to IdentiFly. These low numbers could have influenced the identifications.

The creators of IdentiFly are continuing to improve the program and as more reference images are collected, the program becomes more accurate in identifying both lineages and subspecies. Images taken during this study can be re-used in future studies and potentially provide a more accurate snap-shot of the genetic diversity on the Island.

Worker bodies that have been preserved can be used to determine the exact genetic make up of colonies in Newfoundland. Not only would this provide beekeepers with an accurate analysis of their colonies' genetics, it would also support the development of the IdentiFly program.

There are many possibilities for the future of apiculture in Newfoundland. With renewed public interest, continued apiculture research, and the increase in beekeeping across the Island, Newfoundland has the unique opportunity to be a larger producer of apiculture products in Canada.

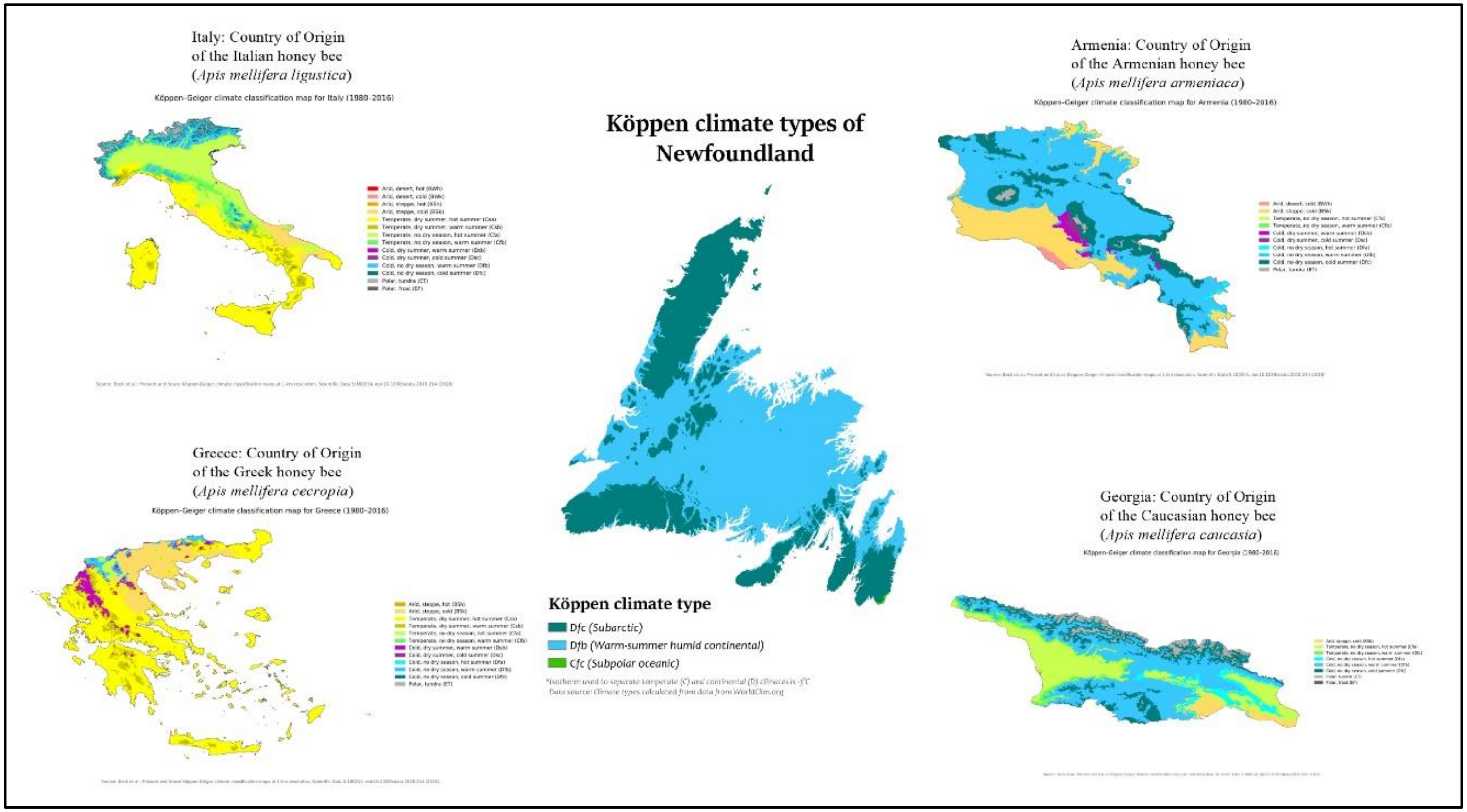


Figure 4.1. Köppen-Gieger climate maps of Newfoundland and the four areas of origin of some of the subspecies found on the Island: Italy (*A. m. ligustica*), Armenia (*A. m. armeniaca*), Greece (*A. m. cecropia*), and Georgia (*A. m. caucasia*). Köppen climate classification is widely used in the scientific community and identifies not only climates but also ecosystem conditions and types of vegetation within these areas. Areas sharing the same color also share similarities in climate and vegetation. Map of Newfoundland retrieved from Peterson, 2016. All other maps retrieved from Beck *et al*. 2018.

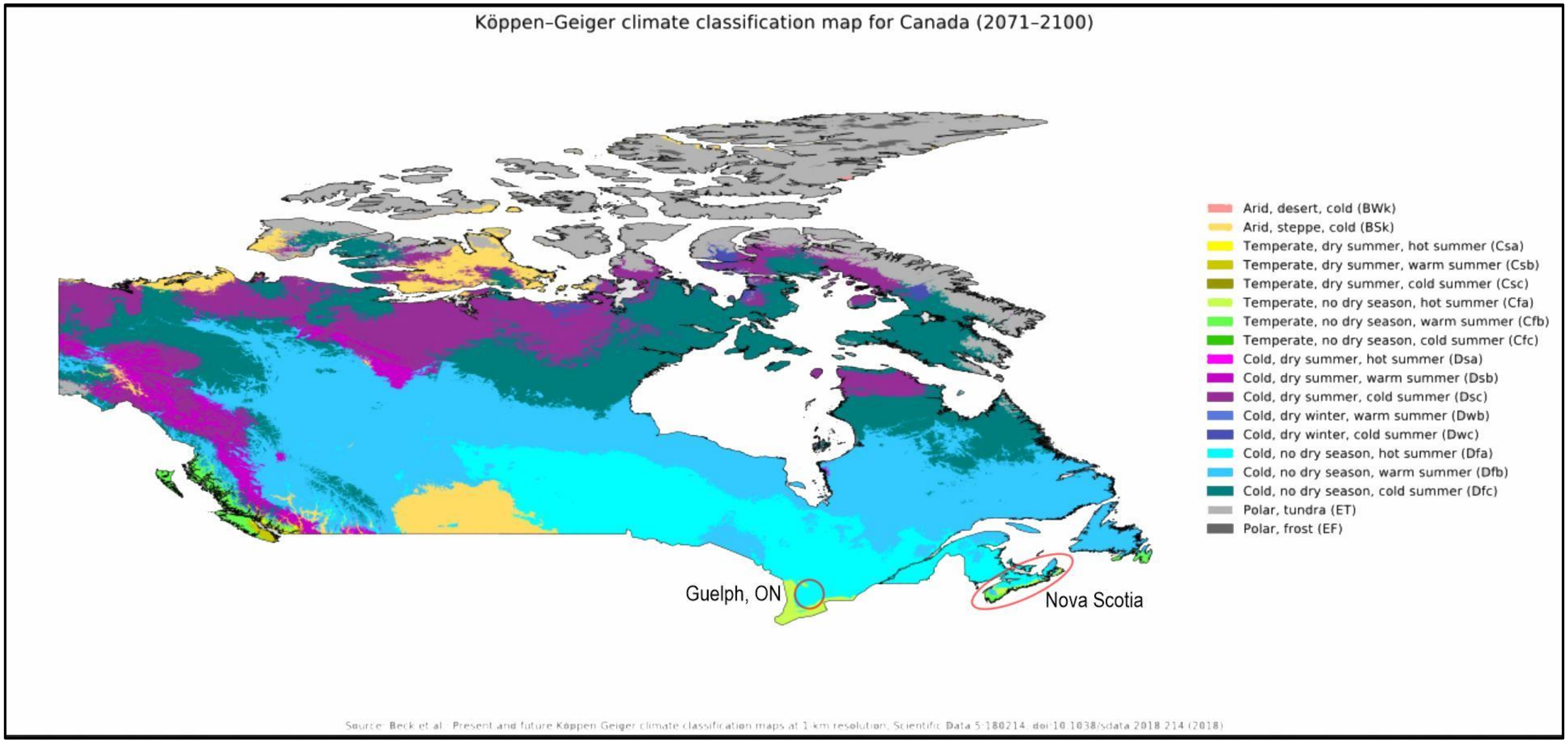


Figure 4.2. Köppen-Gieger climate map of Canada. Areas of interest for this project include Guelph, Ontario and Nova Scotia. Both of these locations have a history of exporting colonies to Newfoundland. Areas sharing the same color also share similarities in climate and vegetation. Map retrieved from Beck *et al*., 2018.

Table 4.1. Average number of days of precipitation and the average amount of precipitation in millimetres annually per location. Lethbridge, Bishop's Falls, and Cormack show the data for nearby communities. There were no data available for Dildo or nearby towns so this site was omitted. Retrieved from Environment Canada (2010).

Location	**Days of Precipitation Annually (Average)**	**Average Annual Precipitation (mm)**
St John's	212	1534
Dildo	NA	NA
Lethbridge (Terra Nova National Park)	192	1233
Bishop's Falls (Grand Falls)	159	1099
Cormack (Deer Lake)	204	1096
Corner Brook	212	1286
Stephenville	221	1340

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Appendix A

RStudio code to produce graphs listed in Chapter 1 and Chapter 3.

Figure 1.3 Average high and low temperatures of locations grouped by maximum drone flight distance

```
> high <- ggplot(Temps, aes(x = month, y = high, color = location, group = location)) + geom_point() + geom_line() + scale_x_discrete(limits = month.abb)

> high + geom_point(data = Temps, aes(x = month, y = low, color = location, group = location)) + geom_line(mapping = aes(x = month, y = low)) + scale_color_discrete(breaks = c('Stephenville', 'Corner Brook', 'Cormack', 'Bishops Falls', 'Lethbridge', 'Dildo', 'St Johns')) + labs(x = "Month") + labs(y = "Average Temperature - C") + labs(title = "Average high and low temperatures of locations grouped by maximum drone flight distance") + theme(legend.title = element_blank())
```

Figure 3.2. Lineages of honey bee colonies in Newfoundland separated by geographic location

```
> ggplot(data = Lineages) + geom_point(mapping = aes(x = cv_1, y = cv_2, color = location_id), position = "jitter") + facet_wrap(~ location_id, nrow = 1) + geom_ellipse(aes(x0=-2.151751602, y0=-0.21265441, a=2.45, b=2.1, angle=-pi / 2)) + geom_ellipse(aes(x0= 6.306834262, y0= -2.178250354, a=2.5, b=2.1, angle=-pi / 4)) + geom_ellipse(aes(x0= -4.339140175, y0= -5.585672678, a=2.35, b=2.1, angle=-pi / 2)) + geom_ellipse(aes(x0= 0.431102305, y0= 3.841924596,
```

a=2.75, b=2.1, angle= pi / 5)) + coord_fixed() + theme(legend.position = "none") + labs(x = "CV1") + labs(y = "CV2") + labs(title = "Lineages of honey bee colonies in Newfoundland separated by geographic location")

Figure 3.3 Number of colonies from Lineage O, Lineage C, and hybrids in Newfoundland separated by geographic location

> ggplot(Lineages) + geom_bar(mapping = aes(x = lineage_id_mix)) + facet_wrap(~factor(location_id, levels=c('Western','Central','Avalon')), nrow = 1)+ labs(x = "Lineage") + labs(y = "# of colonies") + labs(title = "Number of colonies from Lineage O, Lineage C, and hybrids in Newfoundland separated by geographic location")

Figure 3.4 Lineages of honey bee colonies in Newfoundland grouped by maximum drone flight distance

> ggplot(data = Lineages) + geom_point(mapping = aes(x = cv_1, y = cv_2, color = apiary_id), position = "jitter") + facet_wrap(~factor(group_id, levels=c('Stephenville','Corner Brook','Cormack','Bishops Falls', 'Lethbridge', 'Dildo', 'St Johns')), nrow = 1) + geom_ellipse(aes(x0=-2.151751602, y0=-0.21265441, a=2.45, b=2.1, angle=-pi / 2)) + geom_ellipse(aes(x0= 6.306834262, y0= -2.178250354, a=2.5, b=2.1, angle=-pi / 4)) + geom_ellipse(aes(x0= -4.339140175, y0= -5.585672678, a=2.35, b=2.1, angle=-pi / 2)) + geom_ellipse(aes(x0= 0.431102305, y0= 3.841924596, a=2.75, b=2.1, angle= pi / 5)) + coord_fixed() + theme(legend.position = "none") + labs(x = "CV1") + labs(y

= "CV2") + labs(title = "Lineages of honey bee colonies in Newfoundland grouped by maximum drone flight distance”)

Figure 3.5 Number of colonies from Lineage C, Lineage O, and hybrids in Newfoundland grouped by maximum drone flight distance

> ggplot(Lineages) + geom_bar(mapping = aes(x = lineage_id_mix)) + facet_wrap(~factor(group_id, levels=c('Stephenville','Corner Brook','Cormack','Bishops Falls', 'Lethbridge', 'Dildo', 'St Johns')), nrow = 1)+ labs(x = "Lineages") + labs(y = "# of colonies") + labs(title = "Number of colonies from Lineage C, Lineage O, and hybrids in Newfoundland grouped by maximum drone flight distance")

Figure 3.6 Number of colonies per subspecies in Newfoundland separated by geographic location

> ggplot(Subspecies) + geom_bar(mapping = aes(x = subspecies, fill = subspecies_mixes)) + facet_wrap(~factor(location_id, levels=c('Western','Central','Avalon')), nrow = 1)+ labs(x = "Subspecies") + labs(y = "# of colonies") + labs(title = "Number of colonies per subspecies in Newfoundland separated by geographic location")

Figure 3.7 Number of hybrids within each subspecies grouped by maximum drone flight distance

> ggplot(Subspecies) + geom_bar(mapping = aes(x = subspecies, fill = subspecies_mixes)) + facet_wrap(~factor(group_id,

levels=c('Stephenville','Corner Brook','Cormack','Bishops Falls', 'Lethbridge', 'Dildo', 'St Johns')), nrow = 1)+ labs(x = "Subspecies") + labs(y = "# of colonies") + labs(title = "Number of hybrids within each subspecies grouped by maximum drone flight distance") + theme(axis.text.x = element_text(angle = 90, hjust=1))

Figure 3.8 Subspecies of honey bee colonies in Newfoundland grouped by maximum drone flight distance

> ggplot(data = Subspecies) + geom_point(mapping = aes(x = cv_1, y = cv_2, color = apiary_id), position = "jitter") + facet_wrap(~factor(group_id, levels=c('Stephenville','Corner Brook','Cormack','Bishops Falls', 'Lethbridge', 'Dildo', 'St Johns')), nrow = 1) + coord_fixed() + theme(legend.position = "none") + labs(x = "CV1") + labs(y = "CV2") + labs(title = "Subspecies of honey bee colonies in Newfoundland grouped by maximum drone flight distance”)

Figure 3.9 Average snowfall related to the percentage of Armenian honey bee colonies between locations

> ggplot(Snowfall_Perc) + geom_point(mapping = aes(x = annual_snowfall_mm, y = perc_arm_hives, color = location)) + ylim(0,100) + geom_smooth(mapping = aes(x = annual_snowfall_mm, y = perc_arm_hives), method = "lm", se = FALSE) + scale_color_discrete(breaks = c('Stephenville', 'Corner Brook', 'Cormack', 'Bishops Falls', 'Lethbridge', 'Dildo', 'St Johns')) + labs(x = "Annual Snowfall - mm") + labs(y = "Percent Armenian") + labs(title = "Average snowfall related to

the percentage of Armenian honey bee (A. m. armeniaca) colonies between locations") + theme(legend.title = element_blank())

Figure 3.10 Average snowfall related to the percentage of Italian honey bee colonies between locations

```
> ggplot(Snowfall_Perc) + geom_point(mapping = aes(x = annual_snowfall_mm,
y = perc_lig_hives, color = location)) + geom_smooth(mapping = aes(x =
annual_snowfall_mm, y = perc_lig_hives), method = "lm", se = FALSE) +
ylim(0, 100) + scale_color_discrete(breaks = c('Stephenville', 'Corner Brook',
'Cormack', 'Bishops Falls', 'Lethbridge', 'Dildo', 'St Johns')) + labs(x = "Annual
Snowfall - mm") + labs(y = "Percent Italian") + labs(title = "Average snowfall
related to the percentage of Italian honey bee (A. m. ligustica) colonies between
locations") + theme(legend.title = element_blank())
```