Habitat use by bumble bees
(Hymenoptera: Apidae: Bombus spp.) in New Zealand.

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by
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Pollination is a valuable ecosystem service in which wild insect pollinators play a major role. Currently, managed pollination of crops relies almost entirely on one insect species, the honey bee *Apis mellifera* L. Interest in alternative pollinator species has increased in recent years with declines in honey bee and wild pollinator populations in Europe and North America. Bumble bee colonies are managed successfully for greenhouse pollination of tomatoes; however, field management of bumble bees has not been proven to be reliable and cost-effective. Habitat manipulation has been suggested as a low input means of increasing bumble bee populations but so far has not led to a reliable method of improving bumble bee numbers.

Improving the scientific basis of habitat manipulation and the provision of artificial nest sites on farmland may help increase bumble bee populations in the vicinity of a crop. Eighty, four-unit nest boxes have been situated on Kowhai Farm at Lincoln University for four years, and occupancy by bumble bee queens has been recorded during that time. The results collected during the four years are summarised and used along with appropriate literature to suggest how the bumble bee nest boxes on Kowhai Farm could be used to investigate the factors influencing bumble-bee queen nest site selection.

Twenty commercially-produced *Bombus terrestris* (L.) colonies were placed at 20 field sites. Sites were divided into two treatments, based on the predominant habitat within 10 m of the colony. The landscape surrounding the colonies out to a radius of 500 m was divided into one of four habitat categories: flowering crop, non-food crop, pasture, and ‘other’. The proportion of each habitat category was calculated for each colony. Colony performance was measured by a productivity index. The performance of
B. terrestris colonies was highly variable and no significant habitat effects were observed.

Highly variable microsatellite loci have been used to differentiate nest mates from non-nest mates in several social insect species. Such markers could be used to investigate bumble bee forager movement within a landscape. Twelve microsatellite loci isolated in the bumble-bee B. terrestris were tested for applicability to New Zealand populations of Bombus hortorum (L.). Three loci could repeatedly produce informative gels. Regression relatedness was calculated between B. hortorum individuals collected from three naturally founded colonies. The three useful loci provided sufficient information to distinguish between related and unrelated workers. A high proportion of the workers collected from within nests appeared to be unrelated to each other.

Keywords: Bumblee bees, Bombus terrestris, Bombus hortorum, habitat manipulation, pollinations, microsatellites, relatedness, marking insects.
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Completing this thesis has given me the opportunity to learn about and work with bumble bees, which have proven to be entertaining and interesting creatures, this is something that I don’t think I’d have ever done had I not been given the opportunity to study this topic.
Chapter 1 Introduction

1.1 Bumble-bee life history

Temperate bumble bee species, such as *Bombus subterraneus* (L.), *Bombus hortorum* (L.), *Bombus terrestris* (L.) and *Bombus ruderatus* (Fabricius), which were introduced into New Zealand, develop through an annual cycle including seasonal phases of solitary and social behaviour. It is during the social phase of the life cycle that bumble bees are most valuable as pollinators. During that time a colony can contain several hundred workers and foraging workers must visit many flowers to collect pollen for the developing brood. In addition, a bumble bee colony is safe from many of its natural enemies, is largely resistant to weather and has a large work-force that can search for patchy floral resources. However, most colonies do not reach this point as they are vulnerable during earlier stages of the colony cycle (Goulson 2003).

New queens and males emerge from colonies at the end of summer (Alford 1975). The only function of male bumble bees is to find and mate with a queen. There are three types of queen-finding behaviour performed by males of different species: territoriality, nest surveillance, and patrolling (Goulson 2003). Males of territorial species occupy a prominent landmark and defend the surrounding area from other males. The males attempt to mate with any queen that enters their territory. None of the four bumble bee species in New Zealand display this behaviour (table 1.1). Nest-surveillance males, such as *B. subterraneus*, congregate near the entrance of nests containing new queens. Large groups of males may be observed outside a colony during the breeding season. Patrolling males mark prominent landmarks along a route and patrol the route, ‘looking’ for new queens; the queens are attracted by the scent marks left by the males. Different species patrol different areas; for example, *B. hortorum* males patrol approximately 1 m above ground level, whereas *B. terrestris* males patrol much higher (Goulson 2003).

The queens of most bumble bee species mate with only one male (monoandry). Copulation in bumble bees lasts for 36-44 minutes (Goulson 2003), sperm is transferred in the first two minutes, during the remaining time the male transfers the mating plug. The mating plug remains in place for approximately three days and partially blocks sperm transfer by other males, which allows time for the sperm to reach the spermatheca (Goulson 2003). The effectiveness of the mating plug varies between species, for example, the mating plugs of the facultatively polyandrous (queens mate
with several males) bumble bee *Bombus hypnorum* (L.) do not remain in place as long as those of the monoandrous *B. terrestris* (Brown *et al.* 2002).

Mated bumble bee queens hibernate during winter (Alford 1975). Queens excavate a burrow and chamber several centimetres below the surface of the ground; the burrow is blocked with soil and the queen remains in hibernation until spring (Alford 1975). Hibernation is not an obligatory part of the bumble bee life cycle. Some tropical South American species do not hibernate (Cameron and Jost 1998) while some temperate species, such as *B. terrestris* and *B. hortorum*, show variation in this behaviour in different parts of their geographic range (Goulson 2003). For example, foraging workers of both species have been observed during winter in parts of New Zealand (Donovan and Wier 1978) where the winters are milder than in their native range of Britain where winter foraging workers are rarely observed (Goulson 2003).

In spring, queens emerge from hibernation to feed and search for suitable nest sites. Upon discovering a suitable site, the queen builds two wax structures: a honey cup for storing nectar, and an egg cup for the brood (Donovan and MacFarlane 1984). Several eggs are laid in the egg cup, which the queen incubates with her own body heat to speed up development (Donovan and MacFarlane 1984).

During this time, the queen must perform all of the tasks of a worker. This stage of the life cycle possibly places a selection pressure on bumble bee queen morphology, preventing many of the reproductive specialisations seen in advanced social Hymenoptera, such as honey bees (*Apis* spp.), stingless bees (Melliponinae) and ants (Formicidae), developing in bumble bees. A queen must leave the nest to collect pollen and nectar to feed the developing larvae (Donovan and MacFarlane 1984), while the queen is away, the nest becomes vulnerable to attack by predators such as mice (*Mus musculus* L.), or usurpation by another queen (Plowright and Laverty 1984). Bumble bee queens will often enter a developing nest and try to usurp the resident queen, because this will considerably reduce the time and effort required to get the first active workers in the colony and therefore shorten one of the most vulnerable periods in the colony cycle (Plowright and Laverty 1984). Queens will fight over a developing brood, which usually results in the death of one or both bees. Queens will even try to enter a colony that already has workers, although the probability of successful usurpation decreases after the first workers emerge (Goulson 2003).

There are two types of larval feeding behaviour exhibited by different bumble bee species. Species referred to as pocket-makers, such as *B. hortorum*, force pollen into
one or two wax pockets beneath or adjacent to the growing brood (Goulson 2003). The larvae feed on the pollen mass collectively and some larvae consume more food than others, which affects their adult size. In the later stages of development, pocket-maker larvae are fed regurgitated food directly from adults; developing queen larvae receive regurgitated food earlier than worker larvae. Pollen-storers, such as *B. terrestris*, keep a supply of pollen-stored in storage pots and disused pupal cells within the colony (Goulson 2003). In pollen-storers the brood clump breaks up and the larvae build individual cells from wax and silk in which they grow and feed individually. The larvae are fed a mixture of pollen and honey by the queen or workers, which regurgitate the mixture through a hole in the larval cell. The feeding rate of all larvae is directly controlled by the adult bees.

Larval feeding is correlated with adult size. Pocket-maker larvae compete with each other for food, with some individuals consuming more than others. As a result, there is a continuous size range from the smallest workers up to the largest workers and queens (Goulson 2003). Consequently distinguishing queens from workers in pocket-maker species is difficult because of the size overlap between large workers and small queens. In comparison, the adult size range of pollen-storer species is more bimodal, and although workers vary in size, they are smaller than queens and the two castes can be easily distinguished based on body size (Goulson 2003).

The first workers emerge 3-4 weeks after the queen selects a nest site (Donovan and MacFarlane 1984). The colony then enters a phase of growth during which the workers are produced. As the worker population increases, the queen stops foraging and spends all her time inside the nest. During the growth phase, a bumble bee colony displays a typical social hymenopteran division of labour. Foragers supply all the food, house bees manage hygiene, nest maintenance as well as thermoregulation of the brood, and large colonies generally have one or two bees guarding the entrance of the colony, inspecting the colony odour of returning foragers to prevent foreign queens and other intruders from entering the nest (Goulson 2003). The queen can now function solely as an egg layer.

As with honey bees, there is an age-based polyethism with most of the in-nest jobs being performed by younger workers, whereas foraging is performed by older workers (Goulson 2003). Task specialisation is also correlated with size; large workers begin foraging earlier than small workers and some small workers never leave the nest (Goulson 2003). There is also a degree of individual specialisation by workers to
specific tasks; different workers have been observed spending a disproportionate amount of time performing particular tasks such as foraging, nursing, and guarding. There is, however, a larger degree of plasticity in bumble bees than in honey bees and workers will switch tasks in response to colony requirements (Goulson 2003).

The duration of this period and the size to which the colony grows varies within and between species. Of the species in New Zealand, *B. terrestris* produces the largest colonies, which may comprise over 2000 individuals, including hundreds of new queens. *Bombus hortorum* and *B. ruderatus* colonies are usually smaller, but can also produce hundreds of individuals throughout the season. *Bombus subterraneus* colonies have a short growth phase, producing reproductives early and, as a result, do not produce a large worker population.

The growth phase ends when the colony begins rearing males, or when the development of female larvae produces queens rather than workers (Goulson 2003). The colony now enters the reproductive phase of the colony cycle. During the growth phase, a pheromone produced by the queen influences the caste of female larvae at a key stage of larval development (Goulson 2003). Presence of the pheromone, at this stage causes female larvae to develop into workers. However, in the absence of the pheromone female larvae can also develop into queens if provided with sufficient food during their final larval instar (Goulson 2003).

The determination of sex in Hymenoptera is genetic. In bumble bees there is a single sex-determining locus (Cook and Crozier 1995). If an individual is heterozygous at this locus, it becomes a female. If it is homozygous or hemizygous (contains only one copy of the gene) it becomes male. The majority of males are hemizygous because they develop from unfertilised haploid eggs and therefore only have one copy of every gene. However, it is also possible for some fertilised eggs to produces diploid individuals with two identical copies of the sex-determining locus; these individuals become diploid males rather than workers. Diploid males can mate, but the queens that mate with diploid males can not produce colonies (Duchateau and Marien 1995).

The timing of the switch from growth to reproduction varies greatly. Some colonies switch early and produce mostly males, whereas some colonies switch late and mostly produce queens (Goulson 2003). Once the colony begins producing males and queens no more workers are produced. As the workers age and die, the colony starts to deteriorate. Males generally leave the colony shortly after emergence; the young queens return to the colony to feed and build up energy stores before winter. In most cases, new
queens make no further contribution to the old colony, although *B. terrestris* queens have been observed collecting pollen for a declining pre-winter colony suggesting that at least some queens remain in the colony and function as workers for a time (B. Donovan pers. comm.).

### 1.2 Bumble bees in New Zealand

Bumble bees were successfully introduced to New Zealand in 1885 and 1906 for the purpose of red clover (*Trifolium pratense* L.) pollination. Four species have established in New Zealand: *B. terrestris*, *B. hortorum*, *B. ruderatus*, and *B. subterraneus*, each with different characteristics relating to their ecology and pollination (table 1.1).

*Bombus terrestris* is a generalist pollinator, and forages on a wide range of introduced plants as well as some native species (MacFarlane and Gurr 1995). Of the four introduced species, it is the least efficient in pollinating red clover as its tongue is too short to reach the nectar in a red clover flower. Instead workers “bite” into the side of red clover flowers to gain access to the nectar and, in doing so, avoid pollinating the plant. *Bombus terrestris* is, however, a valuable pollinator in New Zealand and is commercially reared for greenhouse pollination of tomatoes (*Solanum lycopersicum* L.) and is a valuable pollinator of other outdoor crops, such as Lucerne (*Medicago sativa* L.) and kiwifruit (*Actinidia deliciosa* C. S. Liang. & A. R. Fergusson.).

*Bombus ruderatus* is a long-tongued bumble bee species, and an effective pollinator of flowers with long corolla tubes such as red clover. *Bombus ruderatus* is also widespread in New Zealand though generally less common than *B. terrestris* (MacFarlane and Gurr 1995). It is found throughout most of the South Island and in many parts of the North Island, especially areas with the warmest and driest climates (MacFarlane and Gurr 1995).

*Bombus hortorum* is similar to *B. ruderatus* in appearance and flower preference (Alford 1975). However, in New Zealand it has a more restricted distribution than *B. ruderatus*. It is found mostly in Canterbury, concentrated around Christchurch, where it is more common than *B. ruderatus*. It is also found in parts of Otago and in the North Island. *Bombus hortorum* is generally found at highest densities in suburban habitats within its range and is rarer in rural areas. The distribution of *B. hortorum* in New Zealand would suggest that it is more sensitive to early season drought and limited flower sequence than the other bumble bee species (MacFarlane and Gurr 1995). *Bombus hortorum* has the longest tongue of the four New Zealand species and, like
*B. ruderatus*, it is an effective pollinator of red clover. Compared with *B. ruderatus*, *B. hortorum* workers are smaller, but *B. hortorum* colonies grow larger and are active for longer. *Bombus hortorum* is thought to be able to produce as many as three generations in a single season in the New Zealand climate and can remain active all year round (B. Donovan, pers. comm.).

*Bombus subterraneus* has the most restricted distribution of the four species. It is the only bumble bee species no longer found at the original release site near Christchurch (MacFarlane and Gurr 1995); *B. subterraneus* can now only be found in inland Otago and Canterbury. These regions have harsh winters by New Zealand standards and over wintering colonies of *B. terrestris* and *B. hortorum*, which occur in other parts of the country, do not persist here (B. Donovan, pers. comm.). *Bombus subterraneus* is a long-tongued species, and like *B. ruderatus* and *B. hortorum*, it is an effective pollinator of red clover.

*Bombus terrestris* is the only pollen-storer species in New Zealand. There is a clear difference in size between queens and workers. Of the four species, it produces the largest colonies and has the widest flower preference, which probably contributes to it being the most common species throughout New Zealand. The three long-tongued species are all pocket-makers, so distinguishing queens from workers can be difficult. As long-tongued bumble bees, they have a more restricted flower preference range than *B. terrestris* but are useful for pollinating different crops.
1.3 Bumble bees for crop pollination

Pollination is a valuable ecosystem service provided in part by wild insect pollinators. Costanza et al. (1997) estimated the value of pollination as US$112 billion to the world’s economy. Currently, managed pollination of crops relies almost entirely on one insect species, the honey bee *Apis mellifera* L. This critical service is now compromised by declines in beekeeping and wild pollinators (Kremen et al. 2002).

In many crops, insect pollination is essential for seed production and seed set can be enhanced by insect visitation for several reasons. Self-pollinating plant species, such as tomatoes (*L. esculentum*), may require insects to move pollen from the anthers to the stigma of the same flower (Corbet et al. 1991). Insects may move pollen between flowers on different plants; for some plants such as red clover such cross-pollination is a prerequisite for seed set. Insect pollination improves the quality of the seed and fruit produced by some plants such as kiwifruit. Pollination by insects may also help uniformity of a crop such as in oilseed rape (*Brassica napus* L.) (Corbet et al. 1991).

Honey bees are the most easily managed and readily available pollinators for commercial crop pollination (Corbet et al. 1991). Honey bees collect nectar and pollen from a broad range of plant species and so can be used to pollinate most crops. A honey
bee hive contains thousands of individuals; the hive can be moved into a crop during flowering and then moved away when flowering finishes providing a large number of pollinators precisely when they are needed. The worker population of a hive can be manipulated by the beekeeper to match the phenology of important crops, so that when the crop comes into flower there are hives ready to be used (Crane 1990). However, some crops, such as tomatoes and Lucerne, are poorly pollinated by honey bees because the morphology and behaviour of honey bee workers are not suitable for the flowers (Corbet et al. 1991). Wild pollinators such as bumble bees (*Bombus* spp.) and solitary bees may contribute significantly to pollination of these crops, but their populations are unpredictable and fluctuate widely from year to year (Donovan and Wier 1978). The use of other insect pollinators for certain crops has been explored, leading to the development of management strategies for alternative pollinators such as the alkali bee (*Nomia melanderi* Cockerell) and the lucerne leafcutting bee (*Megachile rotundata* (Fabricius)) for the pollination of lucerne (Donovan and MacFarlane 1984; Kevan and Phillips 2001) and bumble bees for pollination of tomatoes and red clover (Donovan 2001).

Of the wild bees, bumble bees are among the most important pollinators, at least at higher latitudes, because of the features associated with sociality, abundance, an extended flying season, and broad flower preference (Corbet et al. 1991). Whilst bumble bee colonies do not provide the large manageable populations of honey bee hives, they possess several morphological and behavioural attributes that make them superior pollinators to honey bees in certain situations (Goulson 2003). Due to their large size and thermoregulatory abilities, bumble bees are able to forage in the windy and wet conditions that deter honey bees from foraging (Corbet et al. 1991). Therefore in regions with unpredictable climate or where there is a high chance of poor weather at the time a crop is in flower, bumble bees can be valuable (Goulson 2003). In addition, the flowers of some plants, such as lucerne, must be tripped to release pollen and require a large heavy insect to do this (Corbet et al. 1991). Large pollinators like bumble bees are also able to pollinate crops such as kiwifruit where smaller insects like honey bees do not make contact with the styles and stamens when entering the flower (Corbet et al. 1991).

Bumble bees have longer tongues than honey bees. As a result, when foraging for nectar, bumble bees often, whereas honey bees visit smaller, open flowers. Tongue length also varies among bumble bee species and influences their flower preferences.
Long-tongued bumble bees foraging in crops having large deep flowers with long corollae such as red clover visit flowers faster and more efficiently than short-tongued bumble bees and honey bees, which in turn more efficient pollinators of plants with small open flowers. Bumble bees are also capable of buzz-pollination where, by contracting their flight muscles they shake the anthers sufficiently to dislodge the pollen. Therefore they collect pollen from the tomato flowers and pollinate the plants; tomato flowers produce pollen but no nectar and are therefore of little interest to honey bees (Corbet et al. 1991). However, if provided with an artificial source of sucrose, bumble bees will forage for pollen on the tomato plants.

Large bumble bee colonies can be purchased at any time of year for crop pollination. Due to the absence of wild pollinators in a green house commercial bumble bee colonies are cost effective for indoor crops. Bumble bee colonies can also be bought for pollinating field crops such as Lucerne, however, colonies are expensive and with the availability of other non-target floral resources plus the presence of wild pollinators, the contribution of a single colony to the pollination of a particular field crop is difficult to know, although, it has been calculated that a *B. hortorum* colony can potentially pollinate sufficient flowers to produce seed worth NZ$999 (Donovan 2001). In New Zealand, only *B. terrestris* colonies can be produced commercially. Provision of crops such as red clover with the long-tongued bumble bees *B. hortorum* or *B. ruderatus* relies on trap-nested colonies (Donovan 2001) but the availability of such colonies can be unpredictable.

Other bumble bee management options have been tested for red clover crops, such as release of spring-collected queens near the crop to boost the local bumble bee population (MacFarlane et al. 1983), placement of artificial nest sites for wild queens to occupy near the crop (Barron et al. 2000), conservation of habitat containing suitable natural nest sites, and floral resources to maintain bumble bee populations when the crop is not in flower (Kells and Goulson 2001; Goulson et al. 2002). These methods have shown some effect on pollinator numbers but the direct benefits of such measures are hard to quantify and so far have not been proven to be financially worthwhile.

### 1.3.1 Tracking the movement of foraging bees
Understanding the movement of foraging bumble bees in relation to the location of a colony is important because it helps define the appropriate level of investment that should go into increasing bumble bee numbers. For example, if a *B. hortorum* colony is placed next to a paddock of red clover, it is not known what proportion of the foragers
from that colony forage in the red clover or what proportion of the total number of pollinators visiting the crop are from the colony, and therefore whether or not a purchasing bumble bee colony is value for money.

In order to track forager movements, individual insects need to be recognisable, but very few bumble bee researchers claim to be able to recognise individual bees by sight (B. McCarthy, personal observation), therefore a marker of some kind must be attached to a bee to identify it individually, or to its colony. The ideal marker should persist without inhibiting the insect's normal biology, be environmentally safe, cost-effective, and easy to use (Hagler and Jackson 2001). Individual marks, usually in the form of a painted label or a physical tag, permit the identification of a specific individual in a population. Mass-marking, in the form of an application of dust, paint or dye, permits the identification of a group of insects within a larger population e.g., bees from a single colony foraging in a field. The major advantages of using paint or tags are that they are inexpensive and can be used to identify individual insects (Hagler and Jackson 2001). The disadvantage is that application is tedious and time consuming and is impractical for mass marking insects. Dusts are excellent markers for most insects because they are inexpensive, readily available, environmentally safe, and are easily applied and detected. Dusts are especially useful for marking large insects with hairy surfaces, such as bumble bees. Self-marking techniques have been developed where dust containers are attached to the hive entrance so that foraging bees are automatically covered with coloured as that enter and exit the hive. Different colours can be used to mark different groups (Hagler and Jackson 2001) so that several hive can be used together. Self marking can also be achieved with pollen but identification of pollen can be difficult and requires a level of expertise.

Mark-reobservation studies of bumble bee foraging using coloured tags, dusts and paints to identify bees from different colonies have been done with commercial and natural colonies of several bumble bee species in Europe and New Zealand (Barron 1998; Walther-Hellwig and Frankl 2000; Dramstad et al. 2003). Reobservation rates in these studies are generally low, even though the marking method has been shown to reliably mark the majority of foraging bees from the nest (Barron 1998). Results from these studies have shown that there are interspecific differences in the mean distance that bumble bees forage from the nest (Walther-Hellwig and Frankl 2000), and the traditional idea that bumble bees forage in the immediate vicinity of the colony is
incorrect (Walther-Hellwig and Frankl 2000; Dramstad et al. 2003). However, the foraging sites of most of the foragers from a colony remain unknown (Barron 1998).

Osborne et al. (1999) used harmonic radar to track the movements of foraging B. terrestris workers in relation to their nest. This data provided information about the areas of forage and how B. terrestris workers moved in the landscape. However, the method was claimed to have several limitations. The range of the radar was 700 m, which is less than the maximum foraging range of B. terrestris workers reported in other studies. The signal from the radar was also blocked by objects such as buildings and hedges, so there was often incomplete coverage of the outward and return flights of foragers. The data, however, indicated that often bees flew further than expected, given the distribution of forage patches, and that most bees flew more than 200 m from the nest, even when a similar resource was closer to the colony.

An alternative means of tracking insects is by genetic analysis range of such techniques can be used to identify individual members of family groups such as social insect colonies and there is the potential that some of these methods may also be used to investigate aspects of bumble bee foraging.

1.4 Genetic markers

1.4.1 Introduction
Molecular genetic data are becoming increasingly easy to collect and, molecular methods are becoming more commonly used in applied research. DNA markers are especially useful for distinguishing morphologically similar individuals or groups. Analysing genetic markers offers a means of studying the ecology and behaviour of animal species by allowing a researcher to trace gene flow between populations and see details of mating systems and dispersal that would be otherwise difficult to observe.

A wide range of molecular techniques producing different types of data are available for analysing genetic variability e.g., DNA sequencing, restriction site analysis. In addition, there are several different regions of the genome that can be analysed to reveal different levels of genetic information depending on the taxa being studied ranging from highly conserved ribosomal DNA to variable non-coding regions and microsatellites for closely related species and populations (Caterino et al. 2000; Navajas and Fenton 2000; Cruickshank 2002). Genetic markers that exhibit intraspecific variability, i.e. below the species level, can be used to study genetic structuring within
and between populations of a species. This information can assist in the understanding of how biology, behaviour and habitat affect gene flow within a species.

Such markers can be used to identify which population an individual belongs to (Bogdanowicz et al. 1997; Eldridge et al. 2001; Jenkins et al. 2001) or the geographic source of an introduced population (Tsutsui et al. 2001). The intra-specific genetic structure of a species can be used to study the micro-evolutionary history of a species, such as, the expansion of *A. mellifera* from Asia into Europe and Africa (Franck et al. 2000). Genetic markers can also be used to identify members of social groups such as eusocial insect colonies (Ross 2001).

### 1.4.2 Microsatellite DNA

Microsatellites are short DNA fragments containing two to six base-pair sequence arranged in tandem repeats (Navajas and Fenton 2000). PCR primers matching sequence in the conserved flanking regions either side of the tandem repeats allow specific loci to be selected for PCR amplification. Microsatellites have a high rate of mutation and often reveal high levels of polymorphism. They have therefore been particularly useful for examining relationships among individuals and breeding groups within a population (Caterino et al. 2000). Polymorphisms result from replication slippage altering the number of tandem repeat units within the microsatellite (Navajas and Fenton 2000; Zhu et al. 2000), effectively changing its length. PCR amplified microsatellite fragments are run through an electrophoresis gel and separated according to size (Navajas and Fenton 2000).

In the case of diploid species, one copy of each locus is inherited from each parent. Microsatellites are co-dominant, therefore both copies are amplified during PCR, and both produce bands in the electrophoresis gel (Navajas and Fenton 2000). Hence, a diploid organism, if it is a heterozygote, will reveal two separate bands on the gel. In this way, heterozygotes can be recognised from homozygotes, which produce one band. The co-dominant nature of microsatellites is a useful feature for population level analysis, especially if information relating to breeding or parentage is wanted (Caterino et al. 2000; Navajas and Fenton 2000).

Highly polymorphic markers such as microsatellites can be used to study aspects of behaviour by tracking the movement of individuals and observing gene flow. For example, Carmichael et al. (2001) found the genetic structure of grey wolf populations (*Canis lupus* L.) in Canada matched the migration patterns of local caribou (*Rangifer*...
tarandus (L.)) herds more so than distance and topological features in the area. This information showed that wolves followed the caribou herds (Carmichael et al. 2001). In another study, gene flow in populations of two species of ground beetle (Carabidae) revealed the effects of un-forested areas on movement of individuals through the landscape; gene flow was limited between populations separated by open habitat, showing that the movement of individual beetles was largely restricted to forested areas (Brouat et al. 2003).

Co-dominant markers such as microsatellites have been used to provide detailed information about mating systems such as the identity of parents, dominant individuals, breeding territories, mate choice and fidelity in various organisms e.g., alligators (Alligator mississippiensis Daubin) (Davis et al. 2001). The ability to distinguish between family groups make microsatellites particularly useful for studying aspects of social insect behaviour because the genotypes of both parents and offspring can usually be collected from one place i.e. a colony generally contains at least one queen plus a large number of worker offspring, and often the paternal genotype(s) can be determined directly by analysis of the king (in Isoptera) or of sperm from the spermatheca of the queen (in Hymenoptera) (Ross 2001).

If microsatellite loci have already been located for the species being studied, this is a simple and rapid means of genotyping individual organisms. To locate and isolate new loci for a particular species can be difficult and expensive (Caterino et al. 2000, Navajas and Fenton 2000). In some cases, some loci been used in closely related species although often they are less polymorphic in the non-target species (Estoup et al. 1995).

1.4.3 Social Insects

Using microsatellites markers to identify individuals from different patrilines (offspring with the same mother but different fathers) and colonies has revealed details about the behaviour of social insects especially honey bees and ants (Kryger et al. 2000; Gadau et al. 2003). The social structure of ant colonies is diverse, with variation between species in the number of reproductive females per colony and the mating frequency of queens (Strassmann 2001). The coexistence of several queens in a single colony is called polygyny. Genetic analysis of queens in colonies of different polygynous species reveals different ways in which a polygynous colony can function. Genetic analysis of Leptothorax rugatulus (Emery) revealed that polygynous colonies
contain many closely related queens (Ruppell et al. 2001). In contrast, genetic analysis of polygynous *Camponotus ligniperdus* (Latreille) colonies showed that coexisting queens were unrelated to each other (Gadau et al. 1998). These two species represent very different polygynous systems. Polygyny in *L. rugatulus* colonies is a result of newly mated queens returning to their natal colony. This is a behaviour observed in many polygynous ant species and results in the queens are all related to each other. In such colonies the reproductive contribution of queens may not be equal and some polygynous species are, in effect, functionally monogynous; only the dominant queen laying eggs and the other queens act as replacement reproductives. Polygyny in *C. ligniperdus* colonies is different. Queens do not return to their natal colonies after mating and the colony gains additional queens by adopting newly-mated queens that are unrelated to the resident queen (Gadau et al. 1998). Worker offspring of all queens interact with each other and with all queens as if they were normal nestmates. However, the queens are highly intolerant of each other and contact between *C. ligniperdus* queens from the same colony can lead to fatal fighting (Gadau et al. 1998). In *C. ligniperdus* there are no dominance hierarchies; queens are spatially segregated within the colony and produce both worker and sexual offspring. The two forms of polygyny produce very different genetic relationships within a colony, which are easily recognised with molecular analysis.

Dulosis is the term used to describe a parasitic behaviour among ants where colonies of one species raid colonies of another species, taking the brood back to their own nest. Workers that emerge from the stolen brood in the parasitic nest behave as they would in their natal colony. *Protomagnathus americanus* colonies that raid colonies of *Leptothorax* spp. (Foitzik and Herbers 2001). Microsatellite analysis of *Leptothorax* workers in a *P. americanus* colony showed that the *Leptothorax* workers were not highly related indicating that the workers were from several *Leptothorax* colonies (Foitzik and Herbers 2001). The genotypes of the slave workers did not match that of any free living *Leptothorax* colonies indicating that host colonies are either destroyed during the raid or that they migrate after the raid (Foitzik and Herbers 2001). A different type of parasitic behaviour occurs when ants raid conspecific colonies. Brood are often collected from the defeated colony and used for food, as slaves, or both. Genetic analysis of *Pogonomyrmex* colonies showed that colonies contained workers that could not have been offspring of the queen. This suggests that conspecific raids in this species result in slavery not predation of the collected brood (Gadau et al. 2003).
Honey bees, and some species of ants, live in large monogynous, highly polyandrous colonies. Within these colonies, workers are all offspring of the queen, but there are several patrilines with in the colony that are offspring of different males. It has been observed in *A. mellifera* and *Apis florea* Fabricius that workers of different subfamilies are more likely to perform certain tasks within the colony (Kryger et al. 2000). Experiments using allozyme analysis have shown patriline differences in nectar and pollen foraging, guarding, and nest site scouting. However, with allozyme analysis, only three patrilines could be distinguished per colony, whereas a colony may contain as many as 50. Microsatellite markers provide greater resolution in distinguishing subfamilies within a colony and have shown patriline differences in responses to waggle dancing and in water collection (Kryger et al. 2000). It is thought that genetic differences between different patrilines create different threshold levels that initiate these behaviours; once a worker starts performing a task, it becomes specialised (Kryger et al. 2000). A recent study has shown similar task specialisation among patrilines in the leaf cutter ant *Acromyrmex echinatior* (Forel) (Hughes et al. 2003).

*Acromyrmex echinatior* has a dimorphic worker caste; the large workers, called majors, specialise in foraging and nest maintenance, whereas the small workers, called minors, specialise in rearing brood and tending the fungus gardens. Microsatellite analysis of *A. echinatior* colonies has shown that the proportion of major and minor workers differs between subfamilies (Hughes et al. 2003). The mechanism is thought to be a genetic difference in the threshold of developing larvae to the nutritional and pheromonal controls of caste development within the colony, so that workers of different patrilines differ in their response to environmental conditions.

The above examples demonstrate ways in which genetic markers can be used to infer behaviour of social insects. Though the development of microsatellites for bumble bees is not as advanced as for honey bees and ants, use of the markers currently available could enable questions about the biology and foraging behaviour of bumble bee species in New Zealand to be addressed. For example: recognising foragers from a known nest site could provide information about foraging distances; distinguishing between bumble bees from different nests could be used to investigate the number of colonies that contribute to the pollination of a crop; investigating whether queens inhabiting nest boxes are offspring of the occupant of the previous season; and whether occupants of artificial nest boxes are related to each other.
1.4.4 Genetic relatedness of nest mates in social insects

Determining the relatedness of nest mates can be complex, and depends on the breeding system involved. The number of patrilines in a colony can have a significant effect on the level of relatedness within a colony (Schmid-Hempel and Schmid-Hempel 2000). In a monoandrous system, the genetic relationship between the queen and her male and female offspring is 0.5, the relationship between workers and their sisters (both worker or queen) is 0.75 given the haploid nature of drones, whereas the relationship of workers to their brothers is only 0.25. Therefore, a female ant, bee or wasp has a greater genetic ‘interest’ in raising sisters (0.75) than she would in raising daughters (0.5). However, a female also has a greater genetic ‘interest’ in her own and her sister’s sons than she does in raising her brothers. The majority of hymenopteran species appear to be monoandrous (Strassmann 2001). However, there are several examples of polyandry in Hymenoptera, and the mating systems for most hymenopteran species have not been assessed (Payne et al. 2003). In a polyandrous colony with workers of several patrilines a worker is not equally related to all her sisters. If they have the same father, their relatedness is 0.75; if they have different unrelated fathers the relatedness is 0.25.

The average relatedness between individuals within a colony decreases as the number of males the queen mates with increases and, as a result, genetic conflicts arise (Schmid-Hempel and Schmid-Hempel 2000). Species that have high levels of polyandry, such as honey bees (Apis spp.) and leaf-cutter ants (Atta spp.), live in colonies with a single queen and large populations of morphologically specialised workers (Strassmann 2001). It is thought that the high level of polyandry evolved after the formation of a highly specialised worker caste that can no longer reproduce. In most other cases of polyandry, the number of males is very low (Strassmann 2001).

Bumble bees do not live in large, highly organised colonies, and the morphological differences between workers and queens are small. In fact, worker reproduction is not uncommon in bumble bees; unmated workers can lay haploid male eggs (Paxton et al. 2001; Brown et al. 2003). Based on observation of mating behaviour and sperm counts, most bumble bee species were traditionally thought to mate with only one male; however, both these methods are considered unreliable (Schmid-Hempel and Schmid-Hempel 2000; Strassmann 2001). With the availability of microsatellite markers, several studies have readdressed this question and have found that bumble bees are, for the most part, monoandrous. In the few cases of polyandry, mate number is low.
Estoup et al. (1995) used microsatellites to look for queen mate number in five European bumble bee species. Young queens were collected in spring and used to produce colonies in the laboratory. The number of patrilines was inferred from the genotypes of a sample of workers, and then confirmed by genotyping the founding queen or by genotyping several males, which can inherit only maternal alleles. Estoup et al. (1995) were the first to use the loci isolated in *B. terrestris* in other bumble bee species; they found that some loci were highly variable in some species but monomorphic in others. Of the five species sampled, *B. terrestris* (2 colonies), *Bombus pratorum* (L.) (2 colonies), *Bombus lucorum* (L.) (1 colony), *Bombus lapidarius* (L.) (1 colony), and *B. hypnorum* (3 colonies), only two *B. hypnorum* colonies were found to contain multiple patrilines. Genotypes of the remaining colonies were consistent with monoandry.

Schmid-Hempel and Schmid-Hempel (2000) repeated this experiment using greater colony sample sizes with eight European bumble bee species, *B. terrestris* (17 colonies), *B. hypnorum* (17 colonies), *B. lucorum* (12 colonies), *B. pratorum* (5 colonies), *B. lapidarius* (11 colonies), *Bombus siccheli* (Radoszkowski) (2 colonies), *B. hortorum* (5 colonies), and *B. pascuorum* (6 colonies). Spring queens were captured and reared in the laboratory as by Estoup et al. (1995), except for two colonies of *B. hypnorum* that were collected from the wild. Pedersen and Boomsma (1999) described difficulties with estimating effective queen mating number from worker genotypes where additional patrilines can be missed by sampling too few individuals (non-sampling error) or by two patrilines appearing to be the same due to lack of variation (non-detection error). Schmid-Hempel and Schmid-Hempel (2000) calculated the chances of non-sampling and non-detection error that may affect their results and found both to be negligible.

The polyandrous colonies of *B. hypnorum* studied by both Estoup et al. (1995) and Schmid-Hempel and Schmid-Hempel (2000) were collected from the same areas of western Europe, whereas the single-mated colonies in both studies came from north and south of the Swiss Alps (Schmid-Hempel and Schmid-Hempel 2000). These authors commented on the possibility of geographic variation in queen mating frequency for *B. hypnorum*, but suggested that further sampling would be required before conclusive statements could be made.

Payne et al. (2003) investigated polyandry in North American bumble bees using a single microsatellite locus (B10). There were eleven species: *Bombus auricomus*
(Robertson) (1 colony), Bombus affinis Cresson (1 colony), Bombus fervidus (Fabricius) (1 colony), Bombus griseocollis (DeGeer) (1 colony), Bombus bimaculatus Cresson (4 colonies), Bombus impatiens Cresson (11 colonies), Bombus mixtus Cresson (1 colony), Bombus ternarius Say (1 colony), Bombus vagans Smith (4 colonies), Bombus citrinus (1 colony), Bombus bimaculatus (4 colonies), Bombus impatiens (11 colonies), Bombus mixtus (1 colony), Bombus ternarius (1 colony), Bombus vagans (4 colonies), Bombus citrinus (2 colonies), and Bombus insularis (Smith) (3 colonies). B. citrinus and B. insularis are members of the obligatory social parasitic subgenus Psithyrus (Lepeletier), or cuckoo bumble bees, which had not previously been investigated in America or Europe for polyandry, using microsatellites. Five of the 11 species contained polyandrous colonies, including B. citrinus, one of the cuckoo bumble bees. The four other polyandrous species, B. impatiens, B. bimaculatus, B. ternarius, and B. mixtus, are all members of the subgenus Pyrobombus (von Dalla Torre), which also includes the only polyandrous European species, B. hypnorum. It was concluded that the queen must have mated with more than one male if more than three alleles were observed in any colony. With information from a single locus the possibility of non-detection error is high. Therefore, the effective mate numbers per queen are the minimum number of males with which the queen could have mated with. Consequently, polyandry may be more common in North American bumble bees than was indicated by the results in Payne et al. (2003). Subsequent studies investigating queen-worker conflict over male production in B. hypnorum have found it to be both polyandrous and monoandrous (Paxton et al. 2001, Brown et al. 2003). For polyandrous colonies, one male dominates fathering of the worker offspring, so the effective mating frequency was considerably lower than the observed mating frequency. This is common in Hymenoptera, and would be anticipated in other polyandrous bumble bee species. Both these studies showed that workers contributed to the production of males in B. hypnorum colonies (Paxton et al. 2001, Brown et al. 2003).

Brown et al. (2003) used laboratory-reared colonies from wild queens. Paxton et al. (2001) and Brown et al. (2003) used a combination of wild colonies (i.e. colonies that established and developed with no human interference until sampling) and colonies reared from wild queens (i.e. colonies reared in the lab from wild queens that have mated without human interference). In several of the wild colonies, foreign workers were discovered. These were workers with a microsatellite genotype that could not have been inherited from the queen. Paxton et al. (2001) attributed the presence of these workers to colony usurpation, the foreign workers having developed from the brood of the first queen. Paxton et al. (2001) reasoned that because the foreign genotypes did not match with genotype of other sampled colonies they were unlikely to
be drifted workers. The foreign workers were closely related to each other and were small in body size, suggesting that they were from the first brood of a queen. Both factors indicate usurpation as the probable source of foreign workers.

In these studies, the distinction between worker-laid or queen-laid males, monoandrous or polyandrous queens, genetic offspring and foreign workers, is made by assessing the presence and absence of different alleles. The haplodiploid pattern of inheritance allows the identification of maternal and paternal alleles; the distribution of these alleles within a colony reveals the social structure and breeding behaviour of the species.

1.5 Thesis aims and objectives

This thesis aimed to evaluate aspects of habitat manipulation that can be used to enhance local bumble bee populations. Because the scale at which habitat may effect a colony is dependent on the foraging behaviour of individual bees, the potential of using microsatellite DNA as markers for bumble bee colonies was also investigated. The main objectives were:

- To collate records of bumble bee occupancy of nest boxes on Kowhai Farm over the last four years and make recommendations for their future management.
- To evaluate the effect of near-by habitat on the performance of commercial *B. terrestris* colonies.
- To assess the feasibility of using microsatellite markers to study the foraging behaviour of New Zealand populations of *B. hortorum*.
Chapter 2 The management of artificial nest-sites for wild bumble bees (*Bombus* spp.)

2.1 Introduction

Barron et al. (2000) constructed 80 four-unit bumble bee nest boxes for an experiment investigating bumble bee nest site selection. At the conclusion of the Barron et al. (2000) experiment the nest boxes were placed on Kowhai Farm, an organic research farm at Lincoln University. The intention was that the occupancy of the nest boxes on Kowhai farm would be monitored and provide data comparing nest box occupancy between different habitats on Kowhai farm, and look for possible trends that may be associated with conversion from conventional to organic farming practices. The availability of ongoing nest occupancy data has the potential to provide valuable data on factors that may affect bumble bee nest site selection such as local habitat and previous occupation.

The Kowhai farm experiment was not initially part of this thesis; however, during the course of this thesis, I was given responsibility of managing of the Kowhai farm nest boxes for the 2002/2003 season. It was clear that from the instructions available and from looking at previous data that there was are inconsistencies in the way nest box occupancy had been recorded. Cleaning and preparation of the boxes for subsequent seasons also varied. In addition to this, the boxes were set out without any clear objectives; as a result the design of the experiment was not balanced between potential treatments (e.g. habitat types on Kowhai farm) restricting statistical analysis.

This chapter describes the management of artificial nest boxes on Kowhai Farm at Lincoln University and summarises the occupancy trends as they have occurred to date. Recommendations are made for the future management of these nest boxes and suggestions for future research are given.

2.2 Bumble bee nesting sites

Bumble bees are widely regarded as valuable pollinators of many crops important to humans, but their numbers can fluctuate widely from one year to the next (Donovan and Wier 1978). The pollination value of bumble bees would be enhanced if their numbers could be managed (Donovan and Wier 1978). In order to manipulate bumble bee numbers, it is necessary to identify the factors influencing their populations.
The availability of nest sites and continuous forage resources are two factors that are thought to limit bumble bee populations in farmland (Goulson et al. 2002).

One approach to addressing the availability of nest sites, is to supply artificial nest boxes to augment or replace natural nesting habitat that may be limiting bumble bee populations in New Zealand. Because of the relative freedom from enemies, it is therefore likely that measures to increase populations of bumble bees by provision of field nest boxes could be more successful in New Zealand than elsewhere (Donovan and Wier 1978, Barron et al. 2000). The ideal nest site must give adequate protection against the weather and provide a supply of nest material such as grass, hair or moss (Alford 1975). The burrows of small mammals and areas of undisturbed, tussocky grass are common natural places for bumble bee nests in Europe (Alford 1975). Bumble bee nest-site preference is species-specific (Alford 1975) with some species nesting below the ground and others on, or just below, the soil surface (Kells and Goulson 2001).

According to the descriptions in Alford (1975), three of the four bumble bee species introduced to New Zealand, *Bombus terrestris* and *Bombus ruderatus* (Fabricius), and *Bombus subterraneus* (L.), generally occupy subterranean nests, whereas *Bombus hortorum* (L.) usually nests near the soil surface.

Locating and studying wild bumble bee colonies is difficult. However, a method of inferring nest site preference is to observe queen nest-searching behaviour. Nest-searching queens display a typical behaviour pattern very different from that of foraging individuals (Kells and Goulson 2001). Nest-searching queens are common in spring and observation of them can provide useful data on preferred nesting sites (Svensson et al. 2000; Kells and Goulson 2001). Two studies, Svensson et al. (2000) and Kells and Goulson (2001), used this method to infer habitat preferences of several European bumble bees including three of those now present in New Zealand. *Bombus terrestris* queens preferred to search along banks (Kells and Goulson 2001) and in areas of open ground (Svensson et al. 2000). Nest searching *B. subterraneus* queens were common in open habitats, similar to that favoured by *B. terrestris* (Svensson et al. 2000). *Bombus hortorum* queens were most commonly observed searching for nests in habitat containing tussocks (Kells and Goulson 2001). Most species showed searching behaviour in patches of withered grass (Svensson et al. 2000). Interestingly, no nest-seeking bumble bees were observed within annual crop fields (Svensson et al. 2000), suggesting that disturbance makes the areas unsuitable for nesting. *Bombus ruderatus* is a rare species in much of Europe and no observations of nest-searching behaviour by its
queens were made in either study. In European farm landscapes, the abandoned burrows of small mammals and tussocky areas, where different bumble bee species commonly build their nests, are generally found along uncultivated field boundaries (Svensson et al. 2000, Kells and Goulson 2001). Intensification of farming practices, such as increasing paddock size, can reduce these areas. Therefore protection of undisturbed areas has been suggested as a means of increasing bumble bee populations.

In addition to protecting nesting habitat, artificial nest boxes can be provided additional nest sites for bumble bees. Occupancy rates of artificial nest boxes have been higher in New Zealand than in the Northern Hemisphere, indicating that a lack of nest sites may be a major limiting factor in New Zealand (Donovan and Wier 1978). This is possibly because New Zealand has a limited fauna of small burrowing mammals (Alford 1975) which provide a large proportion of bumble bee nest sites in Europe (Donovan and Wier 1978, Pomeroy 1981, Barron et al. 2000). Various designs of artificial nest boxes have been tested in New Zealand (Donovan and Wier 1978, Pomeroy 1981, Barron et al. 2000). Acceptance rates have ranged from <1-93%, depending on surrounding habitat, domicile design and placement (Barron et al. 2000).

Due to nesting differences between the species described by Alford (1975) it could be expected that artificial nests designed with or without entrance tunnels to simulate subterranean nests could be used to selectively encourage the long-tongued *B. hortorum* or the short-tongued *B. terrestris*, depending on the pollination requirements of a crop. However, studies have produced conflicting results. Donovan and Wier (1978) found that all four species in New Zealand readily accepted nest boxes without tunnels but, comparing the number of initiated nests with the number of pollen gathering queens, indicated that nest boxes were much more acceptable to *B. hortorum* than to *B. terrestris* (Donovan and Wier 1978). Pomeroy (1981) tested several nest box designs for *B. ruderatus* and observed an above-ground occupancy rate of 32%, whereas the underground hives were occupied at near capacity 93%. Alford (1975) described *B. ruderatus* as a subterranean nester; it is possible that the tunnel entrances used by Donovan and Wier (1978) were not as close an imitation of a subterranean cavity as to satisfy *B. ruderatus* and *B. terrestris* queens. Barron et al. (2000) also used above-ground nest boxes without entrance tunnels and found *B. hortorum* to be more a common occupant (61%) than either *B. terrestris* (25%) or *B. ruderatus* (a single colony), although it should be noted that *B. ruderatus* is uncommon in the vicinity of the Barron et al. (2000) study.
Donovan and Wier (1978) and Pomeroy (1981) reported evidence that over-wintered queens returned to the site of their natal nest. Barron et al. (2000) observed that colonies were more likely to be established in units that had been occupied the previous year. The preference for previously occupied sites may be due to over-wintered queens returning to their natal nest in spring when searching for nest sites. Alternatively nest odour may attract over-wintered queens, possibly acting as an indication of an incipient colony to usurp or the presence of a successful colony the previous year. Occupancy rates increase over time (Barron et al. 2000). This has been attributed to improved design and placement of nest boxes (Barron et al. 2000), or the weathering of nest boxes which may remove treatment chemicals from the wood that could deter queens from nesting. The results of these studies show that the occupancy rate of artificial nest boxes can be high if the design and location of the nest box is suitable.

2.3 Materials and methods

In summer 1999/2000, 80 bumble bee nest boxes were placed on field margins at Kowhai Farm, Lincoln University. The nest boxes were those used by Barron et al. (2000). Each box contained four separate compartments permitting four bumble bee colonies to use the nest box at once (Figure 1); the 80 nest boxes therefore provided nest sites for 320 bumble bee colonies.

![Figure 2.1. A four-unit bumble bee nest box used by Barron et al. (2000) set out at Kowhai farm.](image)

The field margins of Kowhai Farm included four different microhabitats, defined by cover vegetation, which functioned as treatments. The microhabitats were: oak trees (*Quercus* sp.), alders (*Alnus* sp.), macrocarpa hedges (*Cupressus macrocarpa* Hartw. ex...
Gordon.), and open grass. Nest boxes had been distributed haphazardly between these habitats on the farm, the treatments did not contain equal numbers of motels.

A single layer of upholsterers' underlay was placed in each of the 320 nest box units. In subsequent years, the nesting material was replaced if it was damaged after occupation by bumble bees or mice (*Mus musculus* L.).

The number and species of bumble bee colonies using the nest boxes was recorded 1-5 times a year from January 2000 to March 2003, the presence of mice in the domiciles was recorded during some inspections. However it is not clear whether these records referred to actual mouse nests, or the presence of middens, faeces, or the mice themselves. Four different observers recorded occupancy during the four year sampling period; the definition of mouse occupancy may have differed between observers.

The original placement of some nest boxes made them prone to wind disturbance, these nest boxes were moved, some to different treatments. In 2002, two sections of macrocarpa hedge were removed, the boxes beneath these sections were moved to new locations on the farm beneath oak trees and alders. The change of treatments was not recorded, therefore the habitat treatment of nest boxes before 2002 is unknown.

### 2.4 Results

Table 2.1 illustrates that occupancy of the nest boxes during the four years they were on Kowhai farm ranged from 3.1-11.3%. This is lower than that recorded by Donovan and Wier (1978) and Pomeroy (1981) but higher than that of Barron *et al.* (2000). The occupancy rate appeared to increase annually for the first three years, but declined in year four. The majority of the colonies inhabiting the nest boxes were *B. hortorum* (Table 2.1). Most of the remaining colonies were *B. terestris*; there were no recorded colonies of *B. ruderatus* or *B. subterraneus*. Nest boxes were also occupied by colonies of wasps (*Vespula* spp.) and honey bees (*A. mellifera*) one honey bee swarm was removed, while the wasps and other honey bee colonies either abandoned the nest box or died naturally within a year of establishment.

The highest number of colonies initiated in any habitat was 22 beneath oak trees in 2001-2002. It appears that the decrease in occupancy from 2001-02 to 2002-03 was habitat specific, with large declines in occupancy beneath oak trees and *Macrocarpa* spp., whereas occupancy in the open habitats of grass increased.
Table 2.1. Bumble bee (*Bombus* spp.) occupancy of 320 nest box units on Kowhai Farm, Lincoln University, describing the proportions of occupation by different bumble bee species, and the number of colonies occupying nest-boxes in different habitats. (Note: Bh = *Bombus hortorum*; Bt = *B. terrestris*; indet = unidentified colonies; n = the total number of nest-box units in each habitat; Po = occupied units that were also occupied during the previous year; No = occupied units that were not occupied during the previous year; * = no data)

<table>
<thead>
<tr>
<th>Season</th>
<th>Number of nests founded</th>
<th>% occupancy</th>
<th>Po</th>
<th>No</th>
<th>Bt</th>
<th>indet</th>
<th>grass (n=80)</th>
<th>alder (n=56)</th>
<th>oak (n=120)</th>
<th>macracarpa (n=64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999/2000</td>
<td>12</td>
<td>3.8</td>
<td>*</td>
<td>41.7</td>
<td>41.7</td>
<td>16.7</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2000/2001</td>
<td>26</td>
<td>8.1</td>
<td>1</td>
<td>88.5</td>
<td>3.8</td>
<td>7.7</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>2001/2002</td>
<td>36</td>
<td>11.3</td>
<td>13</td>
<td>80.6</td>
<td>11.1</td>
<td>2.8</td>
<td>2</td>
<td>5</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>2002/2003</td>
<td>10</td>
<td>3.1</td>
<td>2</td>
<td>70.0</td>
<td>20.0</td>
<td>10.0</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

2.5 Discussion

In summary, overall occupancy rate of bumble bee nest boxes was low compared to several previous studies in New Zealand, but was higher than is observed in the Northern Hemisphere (Delaplane and Mayer 2000). Movement of nest boxes between vegetation types meant that the effect of habitat on bumble bee occupancy could not be analysed. As in previous studies, *B. hortorum* was the most common species to use the nest boxes (Donovan and Wier 1978, Barron *et al.* 2000). Units were more likely to be occupied if they had been occupied the previous year, but with such low occupancy rates detailed analysis of this trend was not possible.

2.5.1 Recommendations for the management of nest boxes

First, the field placement of nest boxes on Kowhai Farm can be improved to increase occupancy rates and produce useful scientific data about bumble bee nesting preferences. Maintenance and inspection of the nest boxes needs to be standardised between years. Currently, the number of layers of nesting material in the nest boxes varies between different nest boxes; each box should contain two layers of upholsterers’ underlay as nesting material, giving the queen an insulated space between layers to begin a colony. Currently, the bumble bee nest boxes are also more likely to contain earwigs, spiders, and mice than bumble bees; the presence of dead bees indicates that spiders and mice prey on bees inside nest boxes (B. McCarthy, personal observation), which could be an important cause of queen mortality. Some form of mouse control or exclusion from nest boxes, and the removal of spiders and webs during nest box
inspections are recommended in future years. Standardising the frequency and timing of inspections would improve the comparability of data between years. For example, an inspection in early spring, mid summer and late summer scheduled in early November, early January, and March respectively, would cover the early, middle, and late stages of the colony cycle.

An unavoidable problem with an ongoing experiment such as this is that different observers conduct inspections in different years. Discrepancies between recording mouse occupancy and bumble bee species identification have occurred over the four years of data collection. It is recommended that 'type' specimens from each bumble bee colony be taken, so that species identification can be checked. It may also be useful to store collected sample bees in 95% ethanol, so that specimens are available for genetic analysis. It is important to note that a type specimen should be taken only from colonies that contain several workers, and that small individuals should be selected to ensure that the queen is not sampled.

Mouse occupancy is difficult to define because there are numerous ways a mouse can affect a nest box; some units contain nests where a mouse has raised a litter, others contain piles of faeces indicating that a mouse has either resided there for some time or is a regular visitor. Mice can also leave middens of grass seed and acorns. Barron et al. (2000) found that previous occupation by mice had no effect on the probability of bumble bee occupancy and, since almost every nest box on Kowhai Farm contains some indication of mouse activity, there seems to be little value in recording mouse data.

2.5.2 Future research
The long term collection of data from the occupancy of nest boxes on Kowhai Farm by naturally initiated bumble bee colonies provides several opportunities for future research.

First, in relation to habitat, the nest boxes need to be divided equally between the different vegetation types along boundaries on Kowhai Farm. Boundaries containing oak and grass provide the most space for placement of nest boxes and could easily be incorporated into a balanced experimental design. Both vegetation types contain relatively open terrain and undisturbed grass that nest searching queens are reported to favour (Svensson et al. 2000), the effect of the different type of shelter that oak trees and thick grass provide would be interesting.
Measurement of microclimate variables inside the nest boxes located in different vegetation types, and whether any of these variables can be associated with occupancy rate, could provide valuable information relating to the requirements of nest box design and construction. The significantly lower occupancy rates observed in this study and that of Barron *et al.* (2000), which used the same nest boxes, compared with other studies in New Zealand (Donovan and Wier 1978; Pomeroy 1981) suggest that the current design of the nest boxes on Kowhai Farm may be flawed. Therefore, an improvement could increase occupancy dramatically. The differences observed by Pomeroy (1981) between above ground and below ground nest boxes warrants investigation. If modification of the entrance of the nest boxes could duplicate the qualities of an underground nest, then the current nest boxes could be improved without altering the entire structure.

The association of previous occupancy of units with subsequent use by bumble bees should be explored by placing old nest material in half of the motel units, which should produce an odour that may attract over-wintered queens. This method would be preferable to the methodology used here that simply observed nest box occupancy from year to year without manipulation. The four unit design of the motels is ideal for paired sampling by treating two units of each nest box throughout all vegetation treatments. The results of such a trial would be easier to interpret than those observed in this study.

This study, and that of Barron *et al.* (2000) treated data from the four units of each nest box as being independent. Consequently the sample size in both studies was 320 as opposed to 80. This assumption seems reasonable because there is no record of robbing or other interference behaviour between mature bumble bee colonies and colonies do, from time to time, occupy adjacent units (Barron *et al.* 2000). However, occupancy rates in this study and that of Barron *et al.* (2000) were too low for this behaviour to be analysed. If bumble bee occupancy rates can be increased, then the effect of neighbouring colonies should be investigated.
Chapter 3 The effects of habitat on colony performance of the bumble bee *Bombus terrestris* (L.)

3.1 Introduction

Bumble bees (*Bombus* spp.) live in annual colonies that go through periods of development with only a small number of workers. Even colonies with large worker populations do not store large amounts of food in the colony. This means that bumble bees are sensitive to food shortages and require a continual supply of food throughout the entire colony cycle. Loss of habitat due to agricultural intensification has been linked to declining populations of wild bees, including bumble bees, in Europe and North America. (Osborne et al. 1991)

Recent studies have indicated that the foraging distance of *Bombus terrestris* (L.) is 1-3 km (Walther-Hellwig and Frankl 2000; Chapman et al. 2003), which is considerably further than previously thought. (Dramstad 1996) Within this 1-3 km range, workers can usually find sources of pollen and nectar, however, food shortages can still occur and can weaken a colony. The likely result of such food shortages is that the number of workers a colony produces and its reproductive success are likely to be reduced. In the interests of increasing pollination rates as an ecosystem service (Costanza et al. 1997) and for the conservation of rare bumble bee species, it is important to understand what features of a landscape can affect the quantity and quality of bumble bee colonies.

Habitat manipulation by the provision of nesting sites and floral resources has been suggested as a method to increase the number of bumble bees for pollination, and for the conservation of rare bumble bee species (Kells et al. 2001). Observed occupancy rate in artificial nest boxes has been positively linked to floral diversity (Donovan and Wier 1978, Pomeroy 1981, Barron et al. 2000), although this hypothesis has not been fully tested. Increasing the number of bumble bee colonies is part of the solution, but in order to increase the number of bumble bees pollinating a crop, the size of the colonies is as important as the of colonies.

3.1.1 Foraging habitat

It has traditionally been accepted that semi-natural areas, with a high floral diversity and suitable nesting sites are important for increasing wild populations of bumble bees (Barron et al. 2000; Goulson et al. 2002). However, some studies have also reported that
the presence of mass flowering crops, which provide a large short term food source, can also increase pollinator densities in a landscape (Westphal et al. 2003).

Westphal et al. (2003) recorded bumble bee densities in 4.5 m² experimental plots of *Phacelia tanacetifolia* (Bentham) and compared them with the proportion of semi-natural habitat and flowering crops at various scales out to a radius of 3000 m the *Phacelia* plot. The strongest correlation was between bee densities and the proportion of flowering crop at the largest scale; there was no beneficial effect from semi-natural habitat. In agricultural habitats with a minimum of 2% semi-natural habitat, the numbers of bumble bees were apparently not limited by the availability of nesting sites or other factors associated with this type of habitat. The most common bumble bee species observed were: *B. terrestris*/*B. lucorum* (L.) (these species are difficult to distinguish in the field), *B. lapidarius* (L.), and *B. pascuorum* (L.). These species are important pollinators of crops, possibly due to their ability to forage on mass flowering crops, which are so common in farmland landscapes. However, this factor is less important for conserving rare bumble bee species, such as *B. subterraneus* (L.), which appear to get little benefit from large short term food sources.

Goulson et al. (2002) investigated the effect of farmland conservation measures on colony performance (see section 3.1.2). They compared the performance of *B. terrestris* colonies on conventional farmland, with colonies on farmland with flower-rich conservation measures, and colonies in suburban gardens. There was no difference between colonies for either of the farmland treatments; however, colonies in suburban gardens grew significantly larger than colonies in both farmland treatments. Goulson et al. (2002) concluded that habitat differences between the two farmland treatments were restricted to individual farms, whereas the foraging area of each colony was spread over the wider landscape, which included more than one farm. Therefore, the actual habitat differences between the two treatments may have been negligible, with habitat modification at the farm level having little effect on local bumble bee colonies.

The higher growth rate among colonies in suburban gardens suggests that floral diversity and or the quantity of floral resources available is an important contributor to colony success. Goulson et al. (2002) showed that suburban gardens provide a more suitable habitat for the growth of *B. terrestris* colonies than farmland. However, the differences in colony growth do not identify the causal factors, or the scale at which these factors are effective, so no recommendations about habitat improvements can be made.
Another method to assess the effect of habitat quality is to directly observe foraging bumble bees. Bee walk transects have been used to assess the use of different farmland habitats by foraging bees (Walther-Hellwig and Frankl 2000, Kells et al. 2001). Kells et al. (2001) examined the role of naturally regenerated field margins as forage sites for bees. The number and species of bees and the chosen forage plant were recorded along with the number of individual flowers of each flower species. With this information, Kells et al. (2001) constructed an index of flower preference for each species of bumble bee. Significantly more bumble bees foraged in naturally regenerated field margins than cropped field margins. The flower species preferences between honey bees and bumble bee species indicated that field margins containing open herbaceous vegetation free from disturbance for 5-10 years provided the greatest benefit to long-tongued bumble bee species. (Kells et al. 2001)

Walther-Hellwig and Frankl (2000) used transect counts of bees foraging in habitat associated with semi-natural vegetation, and arable crops to compare how bees use these two types of habitat. Walther-Hellwig and Frankl (2000) recorded the species and sex of each bee along with the species of plant visited and the coverage of all flowering, insect pollinated, plant species. They reported that the abundance of bumble bees was greatest in flowering crops and that short-tongued bumble bee species \textit{B. terrestris} and \textit{B. lapidarius} dominated this habitat. Abundance was lower in semi-natural vegetation, but the diversity of bumble bees, especially long-tongued bumble bees, was higher. These results suggest that the generalist short-tongued bumble bee species are better able to take advantage of the large short term resources provided by flowering crops, whereas rarer long-tongued species rely more on semi-natural areas.

Transect walks identify what habitat bumble bees are foraging in whereas bumble bee densities on attractive flower plots reflect the density of bumble bees in the surrounding landscape; the scale of the area depends on the foraging range of bumble bees, which is not well known. The performance of experimental colonies should reflect the ability of the surrounding habitat to support bumble bee colonies and give the researcher the ability to control factors such as nest sites, parasitism, and predation.

3.1.2 Colony performance

Colony performance can be measured in a variety of ways. Simply using the size or weight of a colony can indicate growth and the number of pollinating bees that it has produced. Alternatively, the number of reproductive individuals produced by a colony
may be a more significant assessment of success, as production of these individuals is the focus of colony development.

Studies of the performance of an endangered bumble bee species in its natural habitat should focus on reproduction. In contrast, studies of common species that are important pollinators should incorporate details about worker numbers, because it is the workers that do the majority of the pollinating. In addition, any measure of colony success should take into account both the reproductive output and ergonomic growth of a colony, and allow for the differential investment of different castes.

Pomeroy (1981) investigated the use of artificial nest sites by wild B. ruderatus (F.) colonies. To measure the productivity of colonies in different artificial nest designs, a productivity index based on the number and size of cocoons in the colony was calculated. Pollen intake by larvae bears a linear relationship to cocoon diameter (Pomeroy 1979). For B. ruderatus, the mean external diameter for worker–male cocoons was 8.7 mm and of queen cocoons was 13 mm. Pomeroy (1981) calculated from the mean cocoon diameter that the weight of pollen consumed by queen larvae is 3.3 times greater than that of worker and male larvae. The productivity index calculated by Pomeroy (1981) for B. ruderatus colonies was the number of worker/male cocoons plus 3.3 times the number of queen cocoons. The productivity index represents the total amount of food consumed by the developing brood in the colony, which reflects the amount of 'work done' by the colony throughout the entire colony cycle.

Goulson et al. (2002) used the growth rate of B. terrestris colonies to compare habitats. Goulson et al. (2002) placed commercial B. terrestris colonies in the field, weighing each colony before the bees were released and then at weekly intervals for four weeks, by which time some colonies started to produce males and the experiment was terminated. In addition to colony weight, Goulson et al. (2002) also recorded the number of adult workers, queens, males, eggs, larvae, and pupae, noting whether pupae were healthy, hatched or damaged/dead, the number of nectar and pollen pots and the number of wax moth larvae. The only variables that differed significantly between treatments were weight, number of dead and damaged pupae, and the number of wax moth larvae. This methodology provided a comprehensive view of the stage of development of each colony. The major limitation was that colonies were monitored for only 4 weeks, which included only the growth phase, so the colonies did not reach their full size or reproductive development. The reason for the short experimental period was that commercial B. terrestris colonies in England are imported from Europe, and the
commercial *B. terrestris terrestris* colonies are a different subspecies from the native English populations of *B. t. audax*. So the experiment was ceased to prevent reproductive *B. t. terrestris* individuals escaping and potentially interbreeding with local populations of *B. t. audax*

Baer and Schmid-Hempel (2003b) compared the performance and immune response of laboratory-reared colonies exposed to field conditions at different stages of the colony cycle. Colonies were reared in the laboratory from wild *B. terrestris* queens until 15 workers had emerged. Each colony was then allocated into one of four treatments according to the age at which they were exposed to field conditions: control, early, middle, and late. Control colonies were put into the field once the first 15 workers had emerged, and left to develop through the entire colony cycle. Each of the other three treatments was kept in the laboratory except for a 14 day period in the field timed according to their treatment. Once the 14 day field period was over the colonies were returned to the laboratory for the remainder of the colony cycle. The number of workers in each colony was recorded each week for the duration of the colony cycle. Queens and males were removed from the colony every second day, so that newly emerged individuals would be collected before leaving the colony. Colony fitness was calculated by the number of males plus two times the number of queens. This method focuses on the reproductive output of colonies rather than the number of pollinators produced. The immune response of workers from the early brood of each colony correlated with both colony size and colony fitness at the end of the cycle (Baer and Schmid-Hempel 2003b). Baer and Schmid-Hempel (2003b) found that exposure to short selection episodes during different stages resulted in only marginal variation in the size and fitness of colonies whereas continuous exposure reduced fitness considerably. The method used by Baer and Schmid-Hempel (2003b) measured both the reproductive and ergonomic growth of each colony and monitored the colonies throughout the colony cycle. However, it involved regular inspection of the colonies, which is labour intensive and potentially disruptive to the colonies.

Pelletier and McNeil (2003) investigated the effect of food supplementation on the reproductive success of field colonies of *B. impatiens* Cresson and *B. ternarius* (Say). Wild queens were collected and reared in the laboratory. Colonies were placed into the field once the first brood (5-10) workers had emerged. Colonies receiving additional resources were given a sucrose solution in a gravity feeder and 5 g of fresh pollen a day. The number of workers, reproductives and pupal cells of different
sizes were recorded every 12-15 days. Reproductive success was calculated by the number of males plus three times the number of queens. The total number of sexual pupal cells and adults was used. Pelletier and McNeil (2003) assumed that, once the first males appeared, worker production had stopped and that all subsequent small cocoons were males. Pelletier and McNeil (2003) multiplied the number of queens by three to compensate for the different investment in males and queens. Reproductive success in this case is only an index; because both adults and pupae were included some individuals will have been counted twice. As with Baer and Schmid-Hempel's (2003b) method, the method of Pelletier and McNeil (2003) records both the ergonomic and reproductive success of each colony, allowing further comparisons of how colonies are investing in reproduction, and records the colonies' development throughout the whole colony cycle.

3.1.3 Objectives

Habitat manipulation is a recommended method of increasing bumble bee populations on farmland. One objective of this experiment was to use the performance, as indicated by an index of colony productivity, of experimental *B. terrestris* colonies to assess the effects of habitat immediately adjacent to the colony, and in the wider landscape i.e. test the hypothesis that the floral habitat of the immediate or wider landscape has an effect on colony performance.

Habitat modification adjacent to artificial nests can encourage wild queens to initiate colonies. A second objective of this experiment was to investigate whether the provision of floral resources adjacent to the nest also assists colony development at later stages in the colony cycle i.e. test the hypothesis that provision of floral resources colony size. In addition, an assessment of the effects of distant floral resources on colony performance was made.

3.2 Materials and Methods

3.2.1 Preparation of colonies

Twenty starter-cup stage *B. terrestris* colonies were purchased from Zonda Resources Ltd. Commercial bumble bee colonies were used to reduce inter-colony variation during the initial stages of colony development and differences in queen productivity. Starter-cup stage colonies consist of a single queen with developing brood, all colonies contained pupae, and some colonies had one or two active workers that had already
emerged. The colonies were kept in the laboratory at Lincoln University for a further three weeks to develop a worker population that would reduce the chance of queen mortality. In the laboratory, the colonies were fed on white clover, *Trifolium repens* L. pollen and sucrose solution in a controlled temperature of 26°C and 24 hour darkness. After three weeks in these conditions all 20 colonies had at least eight active workers; the largest colonies had over 20 active workers. At the time of placement in the field, each colony was classified as having a large (>20), or small (<20) worker population so that the variation in starting size could be considered during analysis.

3.2.2 Domiciles
In the field the colonies were housed in wooden domiciles with a metal covered roof (see Donovan and MacFarlane (1984) for design details). The internal measurements of the domicile were 30 cm × 30 cm, which allowed room for the colonies to grow. Upholsterers’ underlay was provided as nesting material.

The entrance of each colony was covered with a queen excluder to protect the queen from usurpation and risks associated with foraging. The excluder consisted of a piece of wood with two 6 mm holes that allowed access by *B. terrestris* workers but not queens. Each domicile was placed under a hedge or shrub or an artificial shelter was provided if neither of these were available. The queen excluders were removed after three weeks, when it was judged that the worker population was sufficient for the queen to remain within the nest. The colonies were then left to develop until brood production ceased i.e. there were no developing larvae in the colony.

3.2.3 Habitat
Colonies were placed in 20 separate locations around Lincoln, Ellesmere, and Darfield. The land area around each colony was classified into four categories:

Category 1: crops that *B. terrestris* could to use as a source of pollen or nectar (Table 3.1).

Category 2: crops that provide no nectar or pollen resources (Table 3.1).

Category 3: pasture.

Category 4: other areas that do not fit into any of the previous three categories.

These categories were used to assess the habitat at two levels: the immediate habitat within 10 m of the colony; and the wider habitat within 500 m of the colony. The
proportion of land area in each category at each location was calculated with Arcview GIS 3.2 using aerial photographs from Topomap NZ and information from landowners.

The 20 locations were divided into two treatments based on the predominant habitat within 10 m of the colony. The treatments were based on the habitat categories above, combining categories 1 and 4 into treatment 1 and combining categories 2 and 3 into treatment 2. Categories 1 and 4 through volume and diversity of food sources near the colony were thought likely to be beneficial to a growing colony i.e. floristically rich, whereas the sparse floral resources of categories 2 and 3 would provide none of these advantages i.e. floristically poor.

3.2.4 Colony performance
Colonies were collected once brood development ceased. The number and caste of pupae in each colony were counted. Colony performance was measured by the productivity index of Pomeroy (1981), the total population i.e. the number of cocoons, and by the dry weight of the colony at the end of the season.

This productivity index assigns a value of 1 to each worker or male pupae, which are indistinguishable, and 3.5 to each queen cocoon in a colony; the productivity index represents the amount of food consumed by the colony. The queen value used in our study differs from that used by Pomeroy (1981) because the relative size of queens and workers differs from B. ruderatus studied by Pomeroy (1981).

No distinction was made between opened and unopened pupae because larval food intake is not affected by whether or not a viable adult emerges (N. Pomeroy, pers. comm.), so both are included in the performance count.

3.2.5 Data analysis
Generalised Linear Model analysis was used to assess the effect the immediate habitat, wider habitat, and initial worker population had on the performance of the colonies. Correlations were calculated in R 1.7.0; all other statistics were calculated using Systat 9 1999.
Table 3.1: Crop species within a 500 m radius of experimental *Bombus terrestris* colonies, divided according to value as food source for colony: category 1 (food crops), and category 2 (non-food crops).

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Common name</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1 crops</td>
<td></td>
<td>Category 2 crops</td>
<td></td>
</tr>
<tr>
<td><strong>Rape</strong></td>
<td><em>Brassica napus</em> L.</td>
<td><strong>Barley</strong></td>
<td><em>Hordeum vulgare</em> L.</td>
</tr>
<tr>
<td><strong>Onion (seed crop)</strong></td>
<td><em>Allium cepa</em> L.</td>
<td><strong>Hazelnut</strong></td>
<td><em>Corylus</em> spp.</td>
</tr>
<tr>
<td><strong>Peas</strong></td>
<td><em>Pisum sativum</em> L.</td>
<td><strong>Walnut</strong></td>
<td><em>Juglans</em> spp.</td>
</tr>
<tr>
<td><strong>White clover</strong></td>
<td><em>Trifolium repens</em> L.</td>
<td><strong>Grape</strong></td>
<td><em>Vitis</em> spp.</td>
</tr>
<tr>
<td><strong>Apple</strong></td>
<td><em>Malus domestica</em> Borkh</td>
<td><strong>Wheat</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Carrot (seed crop)</strong></td>
<td><em>Daucus carota sativus</em> (Hoffm)Arcang</td>
<td><strong>Maize</strong></td>
<td><em>Triticum aestivum</em> L.</td>
</tr>
<tr>
<td><strong>Lupin</strong></td>
<td><em>Lupinus</em> spp.</td>
<td><strong>Rye</strong></td>
<td><em>Secale cereale</em> L.</td>
</tr>
<tr>
<td><strong>Pear</strong></td>
<td><em>Pyrus communis sativa</em> DC.</td>
<td><strong>Italian ryegrass</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Stonefruit</strong></td>
<td><em>Prunus</em> spp.</td>
<td><strong>Grass seed</strong></td>
<td><em>Lolium multiflorum</em> Lam.</td>
</tr>
<tr>
<td><strong>Pumpkin</strong></td>
<td><em>Cucurbita</em> spp.</td>
<td><strong>Pine</strong></td>
<td><em>Pinus</em> spp.</td>
</tr>
<tr>
<td><strong>Broadbeans</strong></td>
<td><em>Vicia faba</em> L.</td>
<td><strong>Broccoli (vegetable crop)</strong></td>
<td><em>Brassica oleracea italica</em> Plenck.</td>
</tr>
<tr>
<td><strong>Blackcurrant</strong></td>
<td><em>Ribes nigrum</em> L.</td>
<td><strong>Onion (vegetable crop)</strong></td>
<td><em>Allium cepa</em> L.</td>
</tr>
<tr>
<td><strong>Gum tree</strong></td>
<td><em>Eucalyptus</em> spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Red clover</strong></td>
<td><em>Trifolium pratense</em> L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lucerne</strong></td>
<td><em>Medicago sativa</em> L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Beans</strong></td>
<td><em>Phaseolus vulgaris</em> L.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Radish (seed crop)</strong></td>
<td><em>Raphanus sativus</em> L.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.3 Results

There was substantial variation in the productivity index \( (872 \pm 161.9, \text{mean} \pm \text{S.E.}) \), total population \( (677 \pm 123.9) \), and dry weight \( (100.4 \pm 21.3 \text{ g}) \) of the *B. terrestris* colonies in this trial. The productivity index correlated with total population \( (r = 0.937, P < 0.01) \) (Figure 3.1), and dry weight \( (r = 0.893, P < 0.01) \) (Figure 3.2). The proportion of queen cocoons in each colony also varied greatly, but did not correlate strongly or significantly \( (r = 0.412, P = 0.07) \) (Figure 3.3) with the productivity index, indicating that the allocation of resources towards reproduction differed between colonies.

![Figure 3.1](image1.png)  
**Figure 3.1** The relationship between total population (the total number of cocoons in the colony), and colony productivity.

![Figure 3.2](image2.png)  
**Figure 3.2** The relationship between dry weight and productivity of bumble bee colonies.
Figure 3.3. The relationship between colony productivity and the proportion of queen pupae within colony.

Separate analyses by the generalised linear model were carried for the variants productivity index and proportion of queen cocoons against the factors: 10 m habitat, initial population, and 500 m habitat category proportions (Table 3.3.); none of these analyses indicated that any factor was significant ($P > 0.05$). Interactions between factors were also investigated with no significant interactions.

At the end of this experiment, 25% of colonies contained dead queens; this is likely to be the result of attempted usurpation by wild queens. Colony 4 was infested with a parasite, which was possibly Melittobia acasta (Walker); this appeared to have killed most of the pupae in the colony. This colony was also inhabited by a mouse. There appeared to be no damage to the bumble bee nest and it is not known whether the colony was active at the time the mouse was present. Domestic pigs ($\textit{Sus scrofa}$ L.) damaged colony 9; the nest had been exposed to the weather but not physically damaged.

Table 3.2. Summary of performance data of experimental \textit{B. terrestris} colonies placed in different field locations. All numbers are mean $\pm$ S.E.

<table>
<thead>
<tr>
<th>10 m habitat treatment</th>
<th>productivity index</th>
<th>dry weight of colony (g)</th>
<th>proportion of queen cocoons</th>
<th>Total population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floristically rich (category 1+4)</td>
<td>1019.3 $\pm$ 268.3</td>
<td>122.7 $\pm$ 38.8</td>
<td>0.11 $\pm$ 0.04</td>
<td>797.8 $\pm$ 220.0</td>
</tr>
<tr>
<td>Floristically poor (category 3+4)</td>
<td>724.7 $\pm$ 183.9</td>
<td>78.1 $\pm$ 17.3</td>
<td>0.10 $\pm$ 0.05</td>
<td>556.2 $\pm$ 114.9</td>
</tr>
</tbody>
</table>
Table 3.3. Performance and habitat data for all 20 experimental *B. terrestris* colonies.

<table>
<thead>
<tr>
<th>Colony</th>
<th>initial worker population</th>
<th>Number of initial worker pupae</th>
<th>Number of pupae</th>
<th>Number of pupae</th>
<th>Number of pupae</th>
<th>Productivity index</th>
<th>Dry mass of colony (g)</th>
<th>10 m habitat treatment</th>
<th>Dead queens</th>
<th>Proportion of habitat within 500m radius of colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L</td>
<td>403</td>
<td>103</td>
<td>0.20</td>
<td>763.5</td>
<td>47.63</td>
<td>rich</td>
<td>no</td>
<td>0.06</td>
<td>0.25, 0.58, 0.11</td>
</tr>
<tr>
<td>2</td>
<td>S</td>
<td>674</td>
<td>388</td>
<td>0.37</td>
<td>2032</td>
<td>173.15</td>
<td>poor</td>
<td>yes</td>
<td>0.19, 0.9, 0.36, 0.36</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>L</td>
<td>583</td>
<td>25</td>
<td>0.04</td>
<td>670.5</td>
<td>81.86</td>
<td>poor</td>
<td>no</td>
<td>0.0, 0.02, 0.94, 0.03</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>L</td>
<td>205</td>
<td>0</td>
<td>0.00</td>
<td>205</td>
<td>22.88</td>
<td>poor</td>
<td>no</td>
<td>0.34, 0.25, 0.37, 0.04</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>L</td>
<td>71</td>
<td>0</td>
<td>0.00</td>
<td>71</td>
<td>12.33</td>
<td>rich</td>
<td>no</td>
<td>0.18, 0.46, 0.25, 0.10</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>L</td>
<td>216</td>
<td>50</td>
<td>0.19</td>
<td>391</td>
<td>50.62</td>
<td>rich</td>
<td>no</td>
<td>0.0, 0.00, 0.48, 0.52</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>S</td>
<td>1902</td>
<td>124</td>
<td>0.06</td>
<td>2336</td>
<td>233.95</td>
<td>rich</td>
<td>no</td>
<td>0.08, 0.2, 0.52, 0.21</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>S</td>
<td>147</td>
<td>86</td>
<td>0.37</td>
<td>448</td>
<td>38.85</td>
<td>poor</td>
<td>no</td>
<td>0.22, 0.21, 0.16, 0.40</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>S</td>
<td>761</td>
<td>108</td>
<td>0.12</td>
<td>1139</td>
<td>158.7</td>
<td>rich</td>
<td>no</td>
<td>0.31, 0.22, 0.32, 0.16</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>L</td>
<td>215</td>
<td>0</td>
<td>0.00</td>
<td>215</td>
<td>18.98</td>
<td>poor</td>
<td>yes</td>
<td>0.3, 0.63, 0.00, 0.00</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>S</td>
<td>637</td>
<td>29</td>
<td>0.04</td>
<td>738.5</td>
<td>99.89</td>
<td>poor</td>
<td>no</td>
<td>0.04, 0.65, 0.29, 0.02</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>L</td>
<td>563</td>
<td>377</td>
<td>0.40</td>
<td>1882.5</td>
<td>149.9</td>
<td>rich</td>
<td>no</td>
<td>0.0, 0.02, 0.63, 0.34</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>S</td>
<td>843</td>
<td>19</td>
<td>0.02</td>
<td>909.5</td>
<td>96.83</td>
<td>poor</td>
<td>no</td>
<td>0.11, 0.02, 0.77, 0.10</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>L</td>
<td>983</td>
<td>76</td>
<td>0.07</td>
<td>1249</td>
<td>148.65</td>
<td>poor</td>
<td>yes</td>
<td>0.08, 0.00, 0.81, 0.11</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>S</td>
<td>461</td>
<td>1</td>
<td>0.00</td>
<td>464.5</td>
<td>43.7</td>
<td>rich</td>
<td>no</td>
<td>0.0, 0.00, 0.06, 0.94</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>S</td>
<td>113</td>
<td>0</td>
<td>0.00</td>
<td>113</td>
<td>14.62</td>
<td>rich</td>
<td>yes</td>
<td>0.0, 0.3, 0.21, 0.76</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>L</td>
<td>1848</td>
<td>108</td>
<td>0.06</td>
<td>2226</td>
<td>405.88</td>
<td>rich</td>
<td>no</td>
<td>0.35, 0.13, 0.33, 0.19</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>S</td>
<td>521</td>
<td>51</td>
<td>0.09</td>
<td>699.5</td>
<td>85.93</td>
<td>poor</td>
<td>no</td>
<td>0.19, 0.01, 0.72, 0.08</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>S</td>
<td>754</td>
<td>15</td>
<td>0.02</td>
<td>806.5</td>
<td>109.76</td>
<td>rich</td>
<td>yes</td>
<td>0.2, 0.21, 0.49, 0.10</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>S</td>
<td>80</td>
<td>0</td>
<td>0.00</td>
<td>80</td>
<td>14.78</td>
<td>poor</td>
<td>no</td>
<td>0.0, 0.00, 0.85, 0.15</td>
<td></td>
</tr>
</tbody>
</table>
3.4 Discussion

There was a large amount of variation in the performance of *B. terrestris* colonies in our study as revealed by the large S.E. values (Table 3.2); none of the factors measured explained this variation. The 10 m habitat treatment, floristically rich vs. poor, appeared to have no effect, suggesting that any benefits of habitat manipulation in the immediate vicinity of artificial nest boxes are due to factors other than food availability or that the benefits of food availability are restricted to the very early stages of colony development, which was not part of this experiment.

Other studies measuring performance of bumble bee colonies have also found high variation between colonies (Pomeroy 1981; Pelletier and McNeil 2003). Intrinsic factors are thought to have a large influence on the development of a colony (Baer and Schmid-Hempel 2003b). For example, *B. terrestris* colonies reared under the same laboratory conditions with no parasites and plenty of food still varied in performance, (Muller and Schmid-Hempel 1992) cited in (Pelletier and McNeil 2003). The queens in our study had already shown differences in productivity before they were placed in the field, shown by the variation in worker and brood populations produced before placement into the field although the number of active workers did not correlate with colony productivity.

Colonies are exposed to more than just floral resources of the surrounding environment. Other factors, such as competition, parasites, and abiotic factors, can affect the development of the colony. Measures were taken during this experiment to eliminate or standardise the effect of as many of these factors as possible. The colonies were purchased at an early stage of development so that as much of the colony cycle could be exposed to the habitat treatments as possible. If the colonies were placed outdoors with such a small population of workers the queen would begin foraging and there would be a high risk that wild *B. terrestris* queens could attempt to usurp the colony. The worker populations reared in the laboratory, and the queen excluders, were provided to protect the queens from the risks of foraging and usurpation. In spite of these measures, wild queens invaded at least a quarter of our experimental colonies and one colony was killed due to parasitism.

The success of a bumble bee colony depends on a number of different factors, and controlling most of them is difficult. However, our experiment could have been improved in several ways. Workers that have just emerged from pupae are known as
callow workers. Callow workers can be introduced into different colonies without triggering an aggressive response; they will then join the new colony and exhibit normal worker behaviour. Callow workers could have been added to the colonies to give them an equal initial worker population that would have been sufficient to protect the queen, while keeping the colonies in the laboratory for as short a time as possible. This would also minimise the laboratory conditions which can trigger bumble bee colonies to produce reproductives earlier than normal (Ptacek 2001), which would lower the cumulative population of the colony and therefore its productivity index. This may have been the case with some of the colonies used in this experiment.

Marking the queens with honey bee queen tags would have enabled us to identify whether the original queen of each colony had survived through to the end of the colony cycle. This relatively simple modification was not thought to be necessary because the workers and queen excluders were expected to ensure the queens' safety. This would have been especially useful in colonies that contained dead queens, because the original queen would have been instantly recognisable.

Categorising habitat that was not a crop or pasture was difficult. Habitat category 4 included a wide range of different habitats from gardens to car parks, and designating these diverse habitats as a single category may not provide an accurate representation of landscape foraging quality. Defining every habitat within a landscape is unrealistic for this kind of experiment, but category 4 could have been divided into more biologically relevant categories. Similarly for category 1, which included all crops that *B. terrestris* was known to visit. Division of this category according to the value of each crop to *B. terrestris*, or time of flowering may have been more informative.

The habitat was assessed only within 500 m of each colony. Bumble bee workers are known to forage further than 500 m from the nest (Walther-Hellwig and Frankl 2000). The reason for using a 500 m radius was that obtaining information about the landscape beyond this was difficult. Westphal *et al.* (2003) reported the most significant correlation between habitat and bee numbers was for an analysis using a radius of 3000 m, which was the largest distance used by Westphal *et al.* (2003). It is possible that using a larger radius could provide useful results. Walther-Hellwig and Frankl (2000) noted differences in the foraging range of different bumble bee species, it is possible that the habitat treatments used in this experiment may have had some impact on *B. hortorum* colonies in which workers are known to forage closer to the nest.
Only a small proportion of the queens that emerge in spring successfully initiate new colonies, and only a small proportion of these new colonies develop to the stage of producing males and new queens (Donovan and Wier 1978). The success of a colony is affected by many factors, and a high failure rate of bumble bee colonies is commonly recognised. The large number of factors affecting the survival of wild bumble bee colonies may mean that colony performance is not the best way of measuring habitat suitability. Instead observations of wild bumble bees will more likely deliver clear results, especially if a standard food source such as *Phacelia* is used as in Westphal *et al.* (2003). Nevertheless, Understanding what factors affect colony performance is valuable in its own right. Colony performance measurements will be more useful for analysing aspects of artificial nest design and placement.
Chapter 4 Genetic analysis of *Bombus hortorum* colonies using microsatellite DNA

4.1 Introduction

In order to make the most of bumble bees as pollinators it is necessary to understand where workers forage in relation to the colony. The distance an individual worker travels in search of food will influence the contribution that a colony is likely to make to the pollination of a crop, and therefore the economic benefit of a bumble bee colony placed near a crop. Distinguishing bumble bees from different colonies is necessary in order to study the movement of bees from a nest.

Microsatellite DNA has been used as a means of gathering genetic data. Loci have been developed for use in *Bombus terrestris* (L.) (Estoup *et al.* 1995, 1996). These loci have also been used for other bumble bee species in Europe (Estoup *et al.* 1995, Estoup *et al.* 1996, Wildmer *et al.* 1998, Wildmer and Schmid-Hempel 1999, Schmid-Hempel and Schmid-Hempel 2000, Paxton *et al.* 2001, Brown *et al.* 2003, Chapman *et al.* 2003) and North America (Payne *et al.* 2003). They have also been used in the giant honey bee, *Apis dorsata* (Fabricius) (Oldroyd *et al.* 2000). Not all loci reveal variation in every species and it is possible that the information from these loci may not adequately represent polymorphism in species other than *B. terrestris*, although this point has never been systematically investigated (Schmid-Hempel and Schmid-Hempel 2000).


The social behaviour of hymenopteran insects is diverse, and colony structure varies between species. Whether a colony has a simple or complex caste system, is perennial or annual, monogynous or polygynous (contains one or several queens)
(Gadau et al. 2003), monodomous or polydomous (occupies one or several nests) (Debout et al. 2003), monoandrous or polyandrous (queens mate with one or several males) (Schmid-Hempel and Schmid-Hempel 2000) is important to the understanding the genetic structure of the colonies, which is necessary for interpretation of relatedness within a colony.

4.1.1 Population structure and foraging behavior
Microsatellites can be used to identify members of different populations. Allele frequencies can be used to calculate a coefficient of relatedness between individuals within a population or for assignment tests to estimate the likelihood that a multilocus genotype is derived from the allele distribution of a population from which it was sampled (Bogdanowicz et al. 1997). The population can be a geographic location or a colony. These kind of data can be used to investigate gene flow and population structuring (Estoup et al. 1996, Wildmer and Schmid-Hempel 1999), geographic origins (Bogdanowicz et al. 1997), foraging behaviour (Chapman et al. 2003), worker drift, (Pfeiffer and Crailsheim 1998)dulosis (social parasitism/slavery), (Foitzik and Herbers 2001)dispersal, (Liautard and Keller 2002)and colony founding behaviour. (Pirk et al. 2001; Fournier et al. 2002)

Microsatellites have been used in conjunction with DNA sequence information from the microsatellite DNA gene regions cytochrome B and cytochrome oxidase II to investigate intraspecific geographic structuring in European bumble bee species (Estoup et al. 1996; Wildmer et al. 1998; Wildmer and Schmid-Hempel 1999). Allele frequencies are compared between different locations. Most of the variation at these loci is within rather than among populations. Bombus pascuorum was shown to have two separate populations in Europe separated by the Swiss Alps (Wildmer and Schmid-Hempel 1999). Allele frequencies with B. terrestris showed differentiation between continental and island populations, but not between populations within continental Europe (Estoup et al. 1996). Both these studies found that population differentiation was associated with geographic barriers rather than with distance.

Chapman et al. (2003) used microsatellites to investigate foraging behaviour in two bumble bee species, B. terrestris and B. pascuorum. They sampled workers of both species foraging on patches and used information from several loci to calculate (i) relatedness values to determine whether a patch was dominated by one or a few colonies, or whether workers foraging in a patch were from many different colonies, (ii) the average number of colonies foraging per hectare for each species, and (iii) the
average foraging range of workers for each species from an estimated value of natural colony density.

The objective of the present investigation was to assess the application of microsatellites developed for European *B. terrestris* in New Zealand populations of *B. hortorum*. The future aim is to be able to use microsatellite data to study foraging behaviour of New Zealand bumble bees. The first step in this process is to identify which loci can be used for studying New Zealand bumble bee populations, and whether the information from these loci can be used to distinguish between bumble-bee colonies.

### 4.2 Materials and methods

#### 4.2.1 Collection
A sample of 19-34 bees were collected from each of five colonies located in artificial nest boxes on Kowhai farm at Lincoln University (see Chapter two); all bees were collected from within the nest. Carbon dioxide gas was used to anaesthetise the bees, which were then placed into a freezer at −80°C to prevent degradation of the DNA.

#### 4.2.2 DNA Extraction
Ten female bees from each colony were taken at random and used for DNA extraction. Wing muscles were removed and digested in 500 μl digestion buffer for 1.5-2.5 hours at 55°C on a rocking platform. The digestion buffer contained 100 μl 10× SET, 100 μl 10% SDS, 20 μl 10 mg/ml proteinase-K and 760 μl of water. DNA was extracted using a silica based purification matrix (Prep-A-Gene® BIO-RAD) following the methods of Armstrong *et al.* (1997). The genomic DNA extracted was resuspended in 100 μl of TE pH 8 and stored at 4°C.

#### 4.2.3 PCR amplification
In the first instance 12 loci from Estoup *et al.* (1995, 1996) were assessed for amplification in *B. hortorum*. The PCR was carried out in 15 μl volumes containing 0.17 μl of Expand™ High Fidelity *Taq* polymerase, 1.5 μl 10× Expand™ High Fidelity buffer, 2.25 μl 1 mM dNTPs, 2 μl 2 mM each primer, 0.6-1.0 μl genomic DNA and 6.88 μl dH₂O. A GeneAmp® PCR System 2400 (Perkin-Elmer) and A GeneAmp® PCR System 9700 (Applied Biosystems) thermal cyclers were used with the following temperature profiles of 94°C for 2min denaturation followed by *n* cycles of 94°C for 15 sec, *x*°C for 30 sec, 68°C for 30 sec and a final extension of 72°C for 10 min (see table
4.1 for annealing temperatures and PCR cycles. The presence and quality of the PCR products were assessed using submerged gel electrophoresis on a 2% agarose gel. Approximate molecular weight was determined against a 100 bp ladder (XIV ROCHE), then stained with 0.08 μg/ml ethidium bromide and visualized with UV light.

Table 4.1. Annealing temperatures and number of PCR cycles for loci used in *Bombus hortorum*. Details of primer sequences in Estoup et al. (1995, 1996).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Optimal annealing temperature (°C)</th>
<th>Optimum number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10</td>
<td>52</td>
<td>35</td>
</tr>
<tr>
<td>B11</td>
<td>58</td>
<td>35</td>
</tr>
<tr>
<td>B96</td>
<td>58</td>
<td>35</td>
</tr>
<tr>
<td>B100</td>
<td>58</td>
<td>40</td>
</tr>
<tr>
<td>B101</td>
<td>58</td>
<td>35</td>
</tr>
<tr>
<td>B116</td>
<td>58</td>
<td>35</td>
</tr>
<tr>
<td>B118</td>
<td>58</td>
<td>40</td>
</tr>
<tr>
<td>B121</td>
<td>52</td>
<td>35</td>
</tr>
<tr>
<td>B124</td>
<td>57</td>
<td>35</td>
</tr>
<tr>
<td>B126</td>
<td>57</td>
<td>30</td>
</tr>
<tr>
<td>B131</td>
<td>54</td>
<td>35</td>
</tr>
<tr>
<td>B132</td>
<td>58</td>
<td>35</td>
</tr>
</tbody>
</table>

4.2.4 Microsatellite separation

PCR products were resolved on non-denaturing Spreadex® EL400 gels (Elchrom Scientific) and electrophoresed according to the manufacturer’s recommendations of 120 V at a constant temperature of 55°C using an Elchrom SEA2000 submerged electrophoresis apparatus. The volume of PCR product used in the Spreadex® gel was determined by the intensity of the bands in the 2% agarose. 1-2 μl of PCR product was mixed with 1-2 μl of H2O and 3μl of Elchrom loading buffer to a total volume of 6 μl per sample. The 100 bp ladder XIV (ROCHE) and 10 bp ladder (Invitrogen) were used to determine the size of DNA fragments. Bands were made visible by staining gels with in 0.3 μg/ml ethidium bromide and a permanent digital photographic record made. 5-10 lanes were used for control samples so that samples run on separate gels could be compared.
4.2.5 Data analysis
Alleles were distinguished visually, the length (base pairs) of alleles was determined using Quantity One® (BIO-RAD) to assist comparisons between gels. Heteroduplex bands were not included in analysis.

Regression relatedness (Queller and Goodnight 1989) was calculated using the program RELATEDNESS 5.0.8 (http://gsoft.smu.edu/Gsoft.html) which compared the pair-wise relatedness values of individuals within and between colonies. Individuals were confirmed as nest mates by visual assessment of their genotype at each locus; if an individual had an allele at any locus that not could have been inherited from the same mother and father as majority members collected from the same nest, they were classified as foreign workers.

4.3 Results

4.3.1 Usefulness of loci
The potential of 12 microsatellite loci for use on wild populations of *B. hortorum* in New Zealand was assessed. Of the 12 loci only B11, B100, and B126 could be repeatedly amplified and produced clear banding patterns. These were then used to further to evaluate relatedness within and between *B. hortorum* colonies. Locus B126 exhibited the highest number of alleles with seven, B11 exhibited four alleles and B100 exhibited three. B126 and B11 were the most useful for visually discriminating between colonies due to the number of alleles and the presence of alleles unique to different colonies.

The clarity of the gel images varied between loci and was improved by reducing the cycle number and annealing temperature. Banding patterns of B11 were very clear and easy to score (Figure 4.1A). Bands of B100 were also clear and scoring was straightforward (Figure 4.1B). B126 showed some smearing (Figure 4.1C), which made scoring the bands difficult, however, with proper planning in regard to which samples needed to be run on the same gel, alleles could assigned for all 30 individuals.

Most of the remaining loci showed little or no potential for use on *B. hortorum*. Repeated attempts to produce PCR product from B118 failed for most individuals, and no samples from this locus were run on Spreadex gels. PCR product could be acquired for B10, B131 and B132 but these loci produced unreadable smears on the Spreadex gels. B116, B124, B101, and B96 exhibited no variation when viewed on Spreadex gels. B121 initially produced ample PCR product that separated into clearly defined bands on
the Spreadex gel (Figure 4.2), however, samples for some individuals did not show up and repeated attempts to produce further PCR product failed. Consequently, a complete data set for this locus could not be produced.

Figure 4.2. Example of PCR products resolved on Spreadex gel at locus B121. Samples from different colonies separated by the molecular weight marker in lane 13.
Figure 4.1. Examples of PCR products resolved on Spreadex EL400 gels at locus B11 35 cycles (A), B100 (B), and B126 (C). Products from different colonies separated by the molecular weight marker in lane 13. $\alpha =$ bee 18, $\beta =$ bee 19.
4.3.2 Heteroduplex bands

Heteroduplexes are double stranded DNA molecules formed in vitro between two different alleles and, therefore, contain mismatches (Perez et al. 1999). Heteroduplexes can be detected because they move slower than the corresponding homoduplexes in an electrophoresis gel (Perez et al. 1999). Heteroduplex bands were produced with the PCR product of some samples (Figure 4.3). In some cases one or two heteroduplex bands could be eliminated by reducing the number of cycles during the PCR reaction. The reduction in PCR cycles eliminated the largest band(s) in all but one sample (Figure 4.2A and Figure 4.3, compare \( \alpha \) and \( \beta \) where the smallest band was removed.

Figure 4.3. Example of multiple heteroduplex bands on Sreadex EL400 gel at locus B11 40 PCR cycles. \( \alpha = \) bee 18, \( \beta = \) bee 19 appear to be the same (compare with Figure 2A). The banding patterns on this gel were not included in analysis.

4.3.3 Relatedness within and between colonies

Using combined data for the three loci the mean relatedness within each colony was calculated (Table 4.2). These values were affected by the number of foreign workers (see section 4.3.4) found in each colony. The mean relatedness within colonies 1 and 3 were calculated excluding the foreign workers (Table 4.2). Colony 2 contained a higher proportion of foreign workers than either colony 1 or colony 2, and is described further in section 1.3.3.

The mean relatedness between workers collected from different colonies was 0.0112 ± 0.028 (range 0.83 – 1.0). However, 11 pair-wise relatedness values between
individuals collected from different colonies were higher than 0.50 (Table 4.3). In most cases the genotypes of these pairs were consistent with that expected of monoandrous haplodiploid nest mates if limited to a pairwise comparison but not when placed in the context of the colony. Therefore these pairwise relatedness values could be recognised as being misleading.

Table 4.2. Mean relatedness within each colony using combined data for the three loci.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Foreign workers included</th>
<th>Foreign workers excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SE</td>
<td>max</td>
</tr>
<tr>
<td>1</td>
<td>0.702 ± 0.1311</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.1779 ± 0.0028</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0.3968 ± 0.0928</td>
<td>1</td>
</tr>
</tbody>
</table>

4.3.4 Foreign workers
All three colonies sampled contained foreign workers. Colony 1 contained two foreign workers and colony 3 contained four foreign workers; the foreign workers did not appear to be related to each other in either colony. Colony 2, contained such a variety of genotypes that it was difficult to decide which individuals should be classed as foreign although there were groups of workers with high pair-wise relatedness values and genotypes consistent with full sisters (Table 4.4). The largest group consisted of three individuals, bees 13, 17, and 19. Bees 22 and 15 also had high pair-wise relatedness values and compatible genotypes, as did bees 18 and 20. Bees 14 and 17 could have been nest mates, but 14 could not be related to bees 13 and 19. Bees 16 and 21 appeared to have no close relatives in any nest.
Table 4.3. Genotypes of each bumble bee at all three loci. B11 alleles (bp): A = 138, B = 142, C = 150, D = 166. B100 alleles (bp): A = 144, B = 146, C = 156. B126 alleles (bp): A = 179, B = 184, C = 188, D = 190, E = 196, F = 201, G = 207.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Bee ID</th>
<th>B11</th>
<th>B100</th>
<th>B126</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>AA</td>
<td>BB</td>
<td>AF</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>AC</td>
<td>BC</td>
<td>FF</td>
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<tr>
<td></td>
<td>3</td>
<td>AA</td>
<td>AB</td>
<td>FF</td>
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<tr>
<td></td>
<td>4</td>
<td>AA</td>
<td>AB</td>
<td>AF</td>
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<tr>
<td></td>
<td>5</td>
<td>AA</td>
<td>BB</td>
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<td></td>
<td>6</td>
<td>AA</td>
<td>BB</td>
<td>AF</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>AA</td>
<td>BB</td>
<td>AF</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>AC</td>
<td>AC</td>
<td>DF</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>AA</td>
<td>AB</td>
<td>AF</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>AA</td>
<td>BB</td>
<td>AF</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>CD</td>
<td>AB</td>
<td>FF</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>AA</td>
<td>AA</td>
<td>FF</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>CC</td>
<td>AB</td>
<td>DD</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>AD</td>
<td>AB</td>
<td>DF</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>AC</td>
<td>AC</td>
<td>FF</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>CC</td>
<td>CC</td>
<td>FG</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>CD</td>
<td>AB</td>
<td>FF</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>CD</td>
<td>BC</td>
<td>FG</td>
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<td>19</td>
<td>BC</td>
<td>AC</td>
<td>CE</td>
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<td></td>
<td>20</td>
<td>CC</td>
<td>AA</td>
<td>DD</td>
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<tr>
<td>3</td>
<td>21</td>
<td>CC</td>
<td>AC</td>
<td>FG</td>
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<td></td>
<td>22</td>
<td>AC</td>
<td>CC</td>
<td>BC</td>
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<tr>
<td></td>
<td>23</td>
<td>AD</td>
<td>BC</td>
<td>BD</td>
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<td></td>
<td>24</td>
<td>AC</td>
<td>BC</td>
<td>FF</td>
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<td></td>
<td>25</td>
<td>BC</td>
<td>BC</td>
<td>BB</td>
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<td></td>
<td>26</td>
<td>BC</td>
<td>CC</td>
<td>BG</td>
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<td></td>
<td>27</td>
<td>BC</td>
<td>CC</td>
<td>BB</td>
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<td>28</td>
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<td>BC</td>
<td>BG</td>
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<tr>
<td></td>
<td>29</td>
<td>BC</td>
<td>BC</td>
<td>BB</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>AB</td>
<td>BC</td>
<td>BG</td>
</tr>
</tbody>
</table>
Table 4.4. Pair-wise relatedness values between all *Bombus hortorum* individuals, bold values indicate relatedness of 0.5 or greater, lines indicate comparison within and between colonies.

|       | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  | 25  | 26  | 27  | 28  | 29  | 30  |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 2     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 3     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 4     | 0.46 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 5     | 0.72 | 0.62 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 6     |     | 0.84 | 0.83 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 7     |     |     | 1.00 | 0.72 | 0.84 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 8     |     |     |     | 1.00 | 0.72 | 0.84 | 1.00 | 0.84 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 9     |     |     |     |     | 0.53 | 0.05 | 0.32 | 0.32 | 0.21 | -0.05 | -0.05 | -0.05 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 10    |     |     |     |     |     | 0.32 | 0.32 | 0.21 | -0.05 | -0.05 | -0.05 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 11    |     |     |     |     |     |     | 0.17 | 0.18 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 12    |     |     |     |     |     |     |     | 0.17 | 0.18 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 13    |     |     |     |     |     |     |     |     | 0.07 | 0.07 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 14    |     |     |     |     |     |     |     |     |     | 0.07 | 0.07 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 15    |     |     |     |     |     |     |     |     |     |     | 0.07 | 0.07 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 16    |     |     |     |     |     |     |     |     |     |     |     | 0.07 | 0.07 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 17    |     |     |     |     |     |     |     |     |     |     |     |     | 0.07 | 0.07 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 18    |     |     |     |     |     |     |     |     |     |     |     |     |     | 0.07 | 0.07 |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 19    |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 0.07 | 0.07 |     |     |     |     |     |     |     |     |     |     |     |     |
| 20    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 0.07 | 0.07 |     |     |     |     |     |     |     |     |     |     |     |
4.4 Discussion

4.4.1 Bombus hortorum

*Bombus hortorum* was used in this study instead of *B. terrestris* because of the availability of naturally established colonies in bumble bee nest domiciles (Barron *et al.* 2000). These colonies were established by wild queens so should exhibit all the natural behaviours and genetic variation of wild *B. hortorum*.

All four species of bumble bees in New Zealand have been through the same genetic bottleneck when introduced from England and may contain substantially less genetic variation than European populations of the same species. As the selected loci were developed in *B. terrestris*, it would be expected that they should be more informative in this species than in any of the other three. It seems reasonable to assume that if the loci from Estoup *et al.* (1995) and Estoup *et al.* (1996) can be used in New Zealand *B. hortorum* then they should also be useful for New Zealand *B. terrestris*, which is the most common species bumble bee in New Zealand and a valuable pollinator.

4.4.2 Usefulness of loci

The variation at some loci appears to be different in New Zealand and European populations of *B. hortorum*. Schmid-Hempel and Schmid-Hempel (2000) sampled five colonies of *B. hortorum* from Europe using loci B10, B11, B124 and B126. They found variation at loci B10 and B124, which in contrast showed no variation in the New Zealand population sampled in this study. They did not publish results for locus B100, which did reveal variation for *B. hortorum* in New Zealand. They do not state whether B100 was excluded because it revealed little or no variation, or was not tested in *B. hortorum* at all.

The different choice in loci between this study and that of Schmid-Hempel and Schmid-Hempel (2000) may also result from intra-specific variation within the natural range of *B. hortorum*. The bees used by Schmid-Hempel and Schmid-Hempel (2000) were from continental Europe, whereas the bees introduced into New Zealand were from Britain. Intra-specific variation in microsatellites has been observed across geographic barriers such as mountain ranges and oceans in *B. terrestris* (Estoup *et al.* 1996) and *B. pascuorum* (Wildmer and Schmid-Hempel 1999), so it is possible that there are differences between *B. hortorum* populations in England with those in
continental Europe that could explain the difference at B100. It is also possible that B100 was used by Schmid-Hempel and Schmid-Hempel (2000) and produced similar results in both studies, but with only three alleles, B100 was not included in the analysis because with the availability of alternative loci B100 did not add any useful information.

In addition to the three loci that produced data for this study variation was also observed at locus B121 which contained at least six alleles. Further optimisation of the PCR was required, but this locus should be included in future genetic studies of B. hortorum. The addition of information from more loci would be beneficial.

The number of loci used in studies of social structure and relatedness within and between colonies varies between studies and taxa Payne et al. (2003) used a single microsatellite locus to investigate polyandry amongst North American bumble bees. Gadau et al. (2003) used two loci to investigate intra-colonial relatedness of ants, although they had a large sample size (474 workers from 20 colonies). Takahashi et al. (2003) used four microsatellite loci to investigate the social structure of Vespa analis Fabricius. Giraud et al. (2000) used eight microsatellite loci to investigate the population structure and mating biology of the ant Gnamptogenys striatula Mayr.

The number of loci and loci needed to be able to assign an individual to a colony or population is difficult to define. Increasing the number of loci will increase the precision of the data (Bernatchez and Duchesne 2000; Neff et al. 2000) and reduce the chances of non-detection error in parentage analysis (Tarpy and Nielsen 2002). Loci with a moderate allelic diversity, for example 6-10 alleles, are ideal (Bernatchez and Duchesne 2000).

The three useful loci found during this study should be sufficient for investigations of social structure and relatedness of New Zealand B. hortorum; however, to be able to assign field collected workers to a particular colony, further information about the allele frequencies in the wider population will be needed. For the population sampled in this study, individuals could be included or excluded from a given colony by comparing their genotypes with a number of other workers from that colony, but the population used in this case included bees collected only from three nests.

4.4.3 Heteroduplexes
While some heteroduplex bands were eliminated by reducing the number of PCR cycle, and remaining heteroduplexes were not included in analysis, it was interesting to note
that in one sample the smallest band was eliminated. This band may not have been a heteroduplex, but no other explanation could be found for additional bands observed in a non-denaturing gel. The analysis of this individual was based on the banding patterns in Figure 4.2A. Heteroduplex bands were not included in this analysis, but they have been found informative in other studies (Perez et al. 1999).

4.4.4 Relatedness within and between colonies
The mean relatedness of $0.436 \pm 0.0407$ within the colonies sampled during this study was low. However, with foreign workers removed the relatedness values of colony 1 and colony 3 were closer to the expected 0.75. As expected, the average relatedness between individuals collected from different nests was low, $0.0112 \pm 0.028$ (range - 0.83 – 1.0).

This study shows that individuals from the same colony can be recognised from within a population containing several colonies with the loci available. However, from inspection of the pair-wise relatedness values, pairs of individuals from different colonies may have high relatedness values; it is only by comparison with other members of the colony that some individuals could be excluded as nest mates.

4.4.5 Foreign workers
There were a high number of unrelated workers in individual nests. Foreign workers were not found in colonies in most of the previous microsatellite-studies of bumble bees (Estoup et al. 1995, Schmid-Hempel and Schmid-Hempel 2000, Brown et al. 2003, Payne et al. 2003). Most of these studies collected wild queens and raised colonies in the laboratory; these colonies would not have been exposed to individuals from other colonies. Paxton et al. (2001) used a combination of laboratory-reared and outdoor colonies; foreign workers were found in some of the outdoor colonies. The colonies used in this study developed with no human interference other than the provision of nest boxes. These colonies were exposed to the same environment as were wild colonies. There are five biological possibilities that may explain the presence of unrelated workers in a colony: polyandry, polygyny, queen replacement, worker drift, and null alleles (Paxton 2000). A sixth possibility is experimental error such as accidental mixing of bees from different nests.

Polygyny
Most bumble bees live in colonies headed by a single queen and queens will not tolerate other queens in the same nest. There are two situations when two or more queens may
occupy a single nest: several bumble bee queens may coexist in a single colony for a period of time in some perennial-nesting neotropical bumble bee species such as *Bombus atratus* Franklin (Cameron and Jost 1998). Also some cuckoo bumble bee species are able to coexist with the host species queen by use of appeasement chemicals (Alford 1975, Payne *et al.* 2003).

*Bombus hortorum* does not have a polygynous stage in the colony cycle, and there have been no records of cuckoo bumble bees in New Zealand; therefore, polygyny can be ruled out as an explanation for our results.

**Queen replacement**

Queen replacement is common in social Hymenoptera and happens in a variety of ways in different taxa. For bumble bees, queen replacement takes the form of colony usurpation that may occur during the early stages of colony development before and shortly after the first workers emerge (Alford 1975); once a number of workers have emerged they will prevent foreign queens from entering the nest. In most cases, if the invading queen is successful, some of the previous queen's brood will continue to develop and as adults these individuals will assist the new queen in raising her own brood.

As a result of colony usurpation, there will be a period when there are worker offspring of both queens present in the colony. Genetic analysis of such a colony would reveal two unrelated groups of individuals: the new queen and her offspring, and the remaining offspring of the previous queen. Over time, the offspring of the original queen will die and the colony will come to consist solely of the new queen and her daughters. Therefore, an old colony should contain no genetic trace of an earlier queen replaced by colony usurpation.

It is unlikely that the foreign workers observed in nests in this study were there as a result of colony usurpation. The colonies in this study were sampled late in the colony cycle, so any genetic trace of queen usurpation in the worker population should have disappeared; the foreign workers did not appear to be related to each other, which indicates that they were not offspring of the same queen.

**Polyandry**

Queen mating frequencies have not been reported for most social Hymenoptera (Payne *et al.* 2003). However, current information suggests that the majority of species of social Hymenoptera mate only once (Strassmann 2001). Even those species where some females mate multiple times they typically have mate numbers close to one (Strassmann
The unusual cases of multiple mating are most likely to be selected for because they increase genetic diversity in the brood (Strassmann 2001), which could potentially increase disease and parasite resistance (Baer and Schmid-Hempel 2003a). The rare exceptions to high mate numbers all come from highly social species such as honey bees (Apis spp.) and leaf cutter ants (Atta spp.) with single queens, morphological castes, and many workers (Strassmann 2001).

The mating frequency of bumble bee queens has been assessed through microsatellite genotyping of several species including B. hortorum (Estoup et al. 1995, Schmid-Hempel and Schmid-Hempel 2000, Payne et al. 2003). There is no indication that B. hortorum queens ever mate with more than one male. However, intraspecific variation in queen mating frequency from monoandry to low degrees of polyandry is not uncommon in eusocial Hymenoptera (Paxton 2000). Schmid-Hempel and Schmid-Hempel (2000) raised the possibility of intraspecific variation in queen mating frequency in B. hypnorum. So, the possibility of polyandry occurring in New Zealand populations of B. hortorum can not be totally excluded.

If polyandry was responsible for the foreign workers observed in B. hortorum colonies in this study, then the relatedness within each colony should be higher than the figures observed because all workers would share the same mother. It would also be expected that the foreign workers would be related to each other as full sisters. Most of the individuals from colony 2 and the foreign workers from colony 1 and colony 3 have low pair-wise relatedness across the whole population. It is unlikely that polyandry would explain this level of variation.

Worker drift
Worker drift occurs in honey bees and stingless bees (Meliponinae) (Pfeiffer and Crailsheim 1998, Oldroyd et al. 2000, Paxton 2000, Tarpy and Neilsen 2002), especially when colonies are close together or are in artificial hives (Pfeiffer and Crailsheim 1998, Paxton 2000). This happens in spite of workers guarding the colony entrance. Worker drift is known to occur among B. impatiens and B. occidentalis colonies in commercial greenhouses (Birmingham and Winston 2004) and between B. terrestris colonies located outdoors (Lopez-Vaamonde et al. 2004). Drifting workers have also been observed among outdoor B. hortorum colonies in artificial nests up to 10 m apart (M. Barron pers. comm.) in New Zealand. The colonies sampled for this study were naturally founded by wild queens in artificial nests. The colonies were not close together like honey bee hives in an apiary or commercial bumble bee colonies in greenhouses, but the
domiciles may have confused foraging workers or been easier to locate for robbing. The natural density of bumble bee colonies is not known (Chapman et al. 2003), so it is possibly that the artificial domiciles placed the colonies at a higher density than would naturally occur.

When drifting occurs, a portion of the worker population should be recognisable with genotypes completely different from the rest of the colony. Generally drifting bees may not be closely related to neighbouring colonies, suggesting that it is not confined to colonies that are closer together (Oldroyd et al. 2000), however, cases of worker drift in bumble bees have noted that most drifting workers are from nearby colonies (Birmingham and Winston 2004).

The foreign workers collected from colony 1 and colony 2 appeared to be unrelated to each other, which could suggest worker drift as a more likely explanation than usurpation or polyandry, but the high number of foreign workers found in colony 2 would require an extremely high rate of drifting in bumble bees.

Null alleles
Null alleles occur when a mutation in the primer site prevents an allele from amplifying during PCR. A null allele will make an individual appear homozygous when it is heterozygous (Hillis et al. 1996), which will mean that some genotypes within a population or colony will go unnoticed. Their existence has not been mentioned as a problem in previous studies using these loci on bumble bees. However, future sampling of haploid males may identify whether null alleles exist for any of these loci in New Zealand B. hortorum.

Experimental error
A total of 19-34 bees from each of a total of five nests were collected; but because of technical difficulties and time constraints only 10 workers from each of the three colonies were genotyped. Therefore, it is possible that the workers that do not fit into any of the three colonies used may have been mistakenly taken from one of the two other colonies. Each colony was sampled independently in order to minimise the possibility of such an error occurring, but with results as unusual as those found here the possibility of experimental error must be investigated. Without genotyping workers from the two remaining colonies it is impossible to be sure that a mix-up has not occurred, exaggerating the number of foreign workers in each colony. However, the relatedness values between individuals are very low, suggesting that the foreign workers observed in the sampled colonies originated from more than two colonies. It seems
unlikely that experimental error, if it had occurred, would explain the level of variation observed in this study.

4.4.6 Summary
Three loci could be PCR amplified and showed variation on Spreadex gels. A fourth locus appeared to contain a high number of alleles, but did not produce a complete data set for this study. Examining a population of *B. hortorum* collected from three nests showed that individuals from different colonies could be identified. However, the results of this study are clouded with respect to the use of microsatellites as unique colony markers by the extremely high number of unrelated workers collected from the same nests. Of the possible explanations for this observation, worker drift appears to be the one most likely to produce the observed variation. However, this would imply that the rate of worker drift in bumble bees is very high. The presence of unrelated workers was not expected and this study was not designed to distinguish between the possible reasons why unrelated workers would be in the same nest. Future genetic analysis of wild *B. hortorum* colonies should attempt to account for some or all of the possibilities discussed in section 4.4.5.
Chapter 5 General discussion and conclusions

Pollination as a critical ecosystem service is now compromised by declines in beekeeping and wild pollinators due to habitat degradation and the spread of pests and diseases (Kremen et al. 2002). Current management of pollination relies almost entirely on the use of honey bee (A. mellifera) hives. Little is known about the pollination services provided by wild bees, about their economic contribution or their susceptibility to environmental changes such as habitat loss.

Due to their social behaviour, bumble bees have greater potential to supplement honey bees as managed pollinators than native New Zealand bees and introduced solitary bees. However, research into the biology and behaviour of bumble bees introduced to New Zealand, especially in relation to nesting requirements and foraging behaviour in relation to habitat, is needed to produce successful management techniques for these species here. This thesis has aimed to address the lack of knowledge by investigating the effects of habitat on the initiation and performance of bumble bee colonies in farmland. Understanding this, for the purpose of increasing the number and size of bumble bee colonies near a crop, should be beneficial to the subsequent development of management techniques. Results from the experiments conducted here are discussed and conclusions made.

Habitat manipulation and the provision of nest boxes have been associated with increased bumble bee populations in some cases but in others these measures have been ineffective (Barron et al. 2000). The criteria of nest site selection used by bumble bee queens may not have been met in these instances and reasons for the differences need to be identified to make bumble bee management productive. In chapter 2, using data from an existing and ongoing field experiment, the occupancy rates of nest boxes on Kowhai Farm recorded from a number of years were considered as a means of identifying factors that influence queen nest-site selection. To date the experiment has not been managed to its potential; however, with some minor modifications to the distribution, preparation and inspection of bumble bee nest boxes on Kowhai Farm, factors affecting nest site selection can be investigated. Assessment of that experiment suggests that ongoing use of these nest boxes should consider aspects of nest box design and placement and investigate methods of specifically attracting both short-tongued and long-tongued species (Pomeroy 1981).
Successful establishment of colonies may be influenced by the nearby habitat for growth of colonies (Donovan and Wier 1978; Pomeroy 1981; Barron et al. 2000). Analysis here of habitat quality, in terms of floral resources, in the immediate vicinity of a colony (Chapter 3) suggests that this as a single factor has little or no effect on the size and development of a growing bumble bee colony once the first workers are produced. Nearby habitat may be important during the early stages of development, before a worker population has emerged. However, designing an experiment to test this will be difficult since the growth rate of colonies varied greatly even when they were maintained under standardised laboratory conditions before placement in the field. This suggests the involvement of intrinsic factors relating to the genetics or physiology of the queen (Baer and Schmid-Hempel 2003b), for which a much more complex experimental design is required. The effects of wider habitat and presence of mass flowering crops at different stages of the bumble bee nest cycle may be more relevant (Westphal et al. 2003), since the maximum foraging range of a bumble bee is several kilometres and extends well beyond the range of an individual farm.

In addition to using colony characteristics as a measure of habitat suitability, observation of foraging bumble bees may be a useful method to assess the importance of habitat types (Walther-Hellwig and Frankl 2000; Kells and Goulson 2001; Kells et al. 2001). To develop best practice for managed hives and understand their contribution within the overall pollinator community it is important to understand which and how many colonies are foraging in a particular area. Identification of individual bees is necessary and a simple mark-recapture method using coloured dust has been found elsewhere to be useful. This is appropriate in a situation where the foragers from one or a few colonies are to be located in the field irrespective of the presence of wild bees. However, more complex questions cannot be asked, such as the number of colonies (wild and managed) that use a resource (Chapman et al. 2003), or to find out whether queens inhabiting previously occupied nest sites are daughters of the previous occupant or are attracted by the qualities of the location. For this genetic markers have been proposed to be useful as a means of recognising parental-offspring relationships. They can distinguish between foragers from wild colonies that cannot be located and marked manually, and in some cases may enable identification of a foraging bee’s nest of origin. Such information could then be used to infer how far a bumble bee is willing to travel to reach such a resource, and therefore define the area affected by managed bumble bee colonies. However, development of the microsatellite DNA markers necessary for this level of resolution is expensive. Also if the suite of markers is not comprehensive and
sensitive enough, ambiguous data can be produced. Investigation through this thesis on the use of microsatellite markers developed for other species indicates that this is a viable method of studying bumble bee behaviour in New Zealand, but as yet is inadequate for field studies of *B. hortorum*. Interestingly, however, the genetic analysis revealed that there were a high number of unrelated individuals collected from within the nests. This suggests that neighbouring bumble bee colonies interact with each other and may mean that the location of colonies relative to each other also affects colony development. This is an additional point which may need to be considered in the design of studies and nest boxes.

In conclusion, continued long-term monitoring and manipulation of nest box use on Kowhai Farm combined with refinement of the microsatellite DNA technology provides a unique opportunity to study the behaviour and movement of *Bombus* spp. foraging workers and nest searching queens.
References


