The Population Ecology of an Invasive Social Insect, *Vespula germanica* (Hymenoptera: Vespidae) in South Australia.

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Since its introduction in the late 1970s, *Vespula germanica* has become one of the major urban pests in Australia. Absence of natural competitors and enemies, the ability for long-term sperm storage, plus human transportation have enabled the wasp to spread quickly. Despite costly eradication efforts, little is known of the species' biology under Australian conditions. In this study, the ecology of *V. germanica* was examined in South Australia. Results indicate that in comparison with its native range in England, milder seasons enable the wasp to be active for seven as opposed to four months. Additionally, three times as many workers and queens are produced per colony. However, at temperatures $\geq 35^{\circ}C$, foraging activity diminishes by 50%, while colony requirements for water increase. Hot summers also have a negative effect on nest densities in the following year.

The possible impact of *V. germanica* on the environment was assessed by determining its prey diet, and comparing it to the diet of a native paper wasp, *Polistes humilis*. Prey were identified using a combination of visual and molecular techniques. Results indicate that *V. germanica* feeds on at least nine arthropod orders, as well as vertebrate prey, while *P. humilis* is restricted to feeding on Lepidoptera. Phylogenetic analyses reveal that only a small overlap in prey exists. However, future studies need to determine if the numerical dominance of *V. germanica* gives it an advantage as a competitor.

Apart from hot temperatures, the rainfall in April and number of nests destroyed during the previous year were also significant predictors of future wasp densities in a population model. Thus, the spread and development of *V. germanica* may be inhibited by weather, and its distribution may be limited by water availability. The population model also indicates that current control measures are effective in suppressing wasp densities, and broad-area poison baiting should occur.

Although this study has provided a basic outline of the ecology of *V. germanica* in South Australia, future studies on the population genetics of the species and the importance of inter- and intra-specific competition are needed to determine the pathways responsible for its invasion success.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Marta Kasper

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CHAPTER 1:

INTRODUCTION

1.1 Population dynamics

A population is defined as the total number of individuals of the same species living in a given region of space (Nisbet and Gurney 1982). However, both the population size and the spatial distribution of the population can vary through time, and the factors causing such changes have long fascinated ecologists (e.g. Lele *et al.* 1998; Kendall *et al.* 1999). This section will concentrate on the dynamics of a population through time, while section 1.2 will examine small populations extending their ranges to new habitats, i.e. invasions.

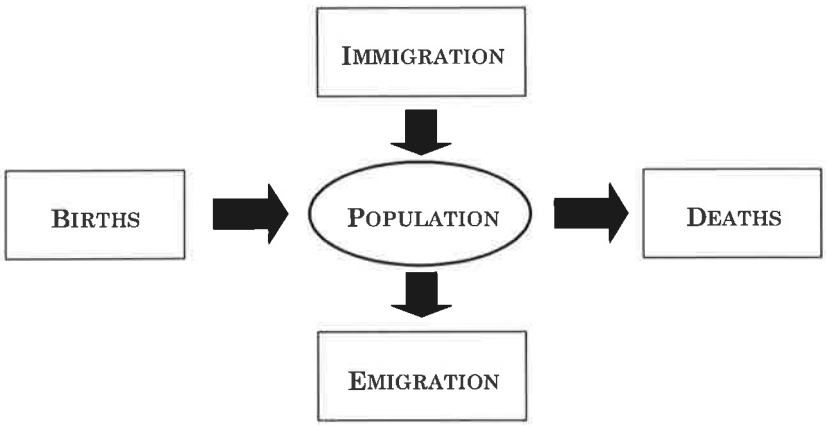

Figure 1.1: The four factors determining population size. Arrows pointing towards the population indicate an increase, while arrows pointing away indicate a decline in numbers.

The size of a population (N) can only change due to four factors: births (B), deaths (D), immigration (I) and emigration (E) (Fig. 1.1) (Nisbet and Gurney 1982). Therefore, in a time interval Δt, the change in a population (ΔN) can be summarised as:

$$\Delta N = (B - D + I - E)\,\Delta t$$

In a simple model of population growth, where no immigration or emigration occurs, the number of births usually corresponds to the number of offspring surviving to adulthood per female times the number of females, and the number of deaths corresponds with the number of individuals in the population who have died. Thus, in a simple theoretical population under ideal conditions, where there is no crowding, no competition or predation, no immigration or emigration, food is unlimited, and the climate is benign, the contribution of an individuals to the population is constant. The population grows in an exponential fashion:

$$N_t = N_0 e^{rt}$$

where N_t is the population size at time t, N_0 is the population size at time 0, and r is the intrinsic growth rate (Roughgarden 1979).

However, natural populations do not live under such conditions, and their rates of mortality and fecundity can be expected to be regulated by factors operating in a density-dependent manner. Both predator/prey interactions as well as interspecific and intraspecific interactions for resources (e.g. food, mates, space) occur within a population, and if the effect of these forces changes with population size, then they are termed 'density-dependent'. The effect of other factors, such as climatic conditions or the quality of food, are less likely to change with population size, and these are termed 'density-independent' (Fig. 1.2).

In a population regulated by density dependent factors, the contribution of an individuals to population growth is not constant, but changes with the size of the population, following a logistic growth model equation:

$$N_t = N_0 e^{r[1-\frac{N_t}{K}]t}$$

where N_t is the population size at time t, N_0 is the population size at time 0, r is the growth rate and K is the carrying capacity, or the number of individuals of a population that an environment can support (Roughgarden 1979). In this model, an individual's contribution to population growth decreases linearly as the population increases.

Often, there is a delay between an organism's reproductive output and population size. For example, most insects show seasonal reproductive activity. In such cases, a steady pattern of exponential growth to equilibrium is not observed, but various forms of cyclicity occur. If the discrepancy between *r* and time between generations is high, chaos may result (e.g. May 1974; Roughgarden 1979; Turchin and Taylor 1992).

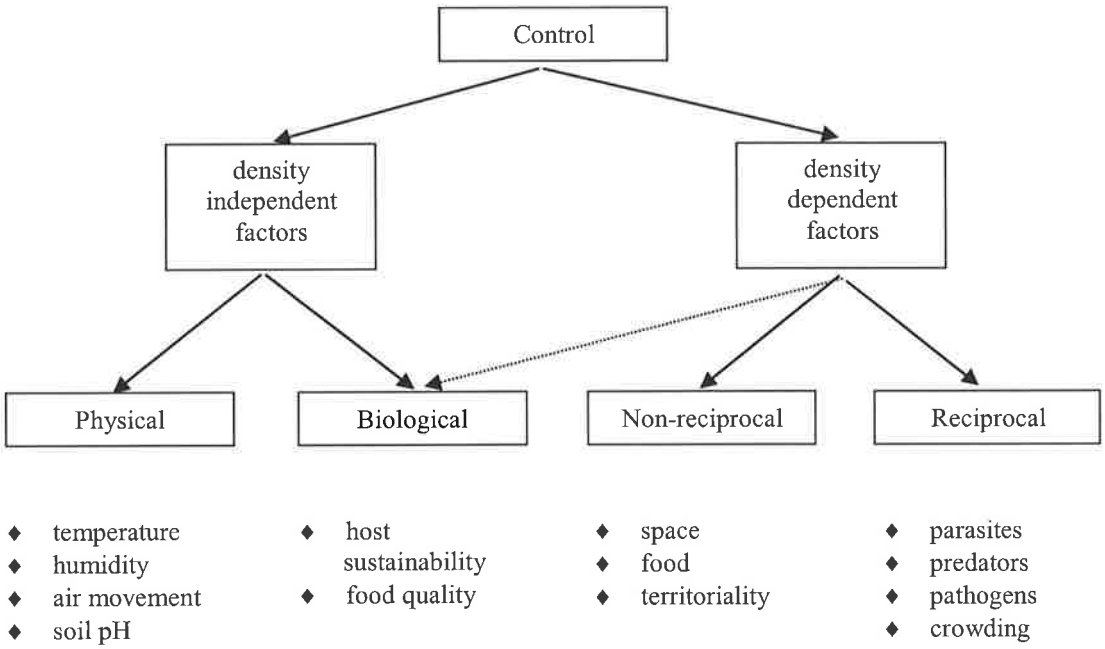

Figure 1.2: Density dependent and density independent factors influencing births and deaths in a population (modified from Gutierrez 1996).

Alternatively, in a population regulated by density-independent factors, the intrinsic rate of increase varies with each generation. In this model, a population may never reach its carrying capacity due to unfavourable physical or biological conditions (Fig. 1.2). Populations regulated by density-independent factors usually show periodic (e.g. seasonal) or non-cyclic oscillatory patterns (e.g. Jaksic *et al.* 1997; Hunter and Price 1998; Lima *et al.* 2002).

The action of density-dependent and density-independent factors on organisms differs with the length of their life cycle. Species with an annual cycle or long

generation times are thought to be regulated by density-independent factors, while species with short life cycles or producing several generations in one year tend to be regulated by density-dependent factors (Nisbet and Gurney, 1982).

As it is often difficult to prove that a field population is regulated by density dependence, much debate exists about the relative applicability of density-dependent and density-independent factors in natural populations (e.g. Nicholson 1933; Andrewartha and Birch 1954; Murray 1999; Turchin 1999; Sale and Tolimieri 2000). Recent studies acknowledge that often both density-dependent and density-independent factors drive population changes simultaneously (e.g. Leirs *et al.* 1997; Lewellen and Vessey 1998; Lima *et al.* 2002).

1.2 Biology of invasions

An invasion occurs when an organism (invader) arrives at a place beyond its previous geographical range (Elton 1958), i.e. it represents an immigration of a small part of a population into a new habitat. Invasions are often well-studied as many invasive species become pests, causing economic losses (especially in agriculture), or drastically affect biodiversity in natural habitats (Vitousek *et al.* 1996).

1.2.1 Stages of a biological invasion

The arrival of new species happens mostly due to commerce, agriculture, or travel of humans, and has been made easier in the past century due to the increased speed of travel. Not all new species that arrive outside their geographical range are successful. It has been suggested that, on average, only 10% of invaders become established, and only 10% of these become pests (Williamson and Fitter 1996). However, this success varies greatly depending on type of the invader (Williamson 1996).

Most successful invaders arrive in numbers much lower than the carrying capacity (*K*) of the new environment, but with time build towards it, expanding both in numbers and in range. The population dynamics of the species at this spread phase depend mostly on how fast the organism can reproduce. The

spread of invaders is often modelled to predict the speed and range of their dispersal.

The final stage of invasion is integration into the new environment, and equilibrium in terms of population numbers of the invading species. Evidence suggests that invaders quickly establish interactions with members of the recipient communities, and can have both ecological and evolutionary effects (Vermeij 1996). Many invaders affect the abundance of species, usually leading to a change in community structure, reduction in biodiversity, and sometimes extinctions (Vinson 1997; Holway 1998). However, introduced species can also be beneficial. They can lead to an increase in local biodiversity, successful biological control of other pests, or simply be aesthetic (Williamson 1996). As integration and equilibrium requires the invading population to have developed strategies to co-exist with native species, apart from the effects the invader has on the invaded community, aspects of the invader's biology may be altered as well (Holway and Suarez 1999).

1.2.2 Characteristics of successful invaders

Table 1.1: Some characteristics present in populations of successful invaders. Modified from Lodge (1993).

r selected traits: populations with a high rate of intrinsic increase. However, *K*-selected traits will be chosen in a stable environment.
high dispersal rate: populations with high reproductive rates often also have high dispersal rates. However, air or water mediated dispersal is often the fastest.
single parent reproduction: sperm storage in animals, and dioecious characteristics in plants
high genetic variability: ability to best adapt genetically and behaviourally to the new environment
vegetative reproduction: responsible for success of weed invaders
phenotypic plasticity: occupying vacant niches
large native range: often predictive of stable populations
eurytopy: ecological generalism
polyphagy: specialist feeders have lower chances of finding suitable food sources
human commensalism: humans often homogenise habitats, as well as providing numerous nesting sites in artificial structures and food sources

Invasion biologists have proposed many ecological, genetic, life-history and behavioural traits that characterise an invasive species (e.g. Lodge 1993;

Williamson and Fitter 1996; Holway and Suarez 1999). These, like the chances of success of establishment, vary depending on the species. However, most invasive species possess a number of characteristics in common (Table 1.1). These include a high rate of increase, polyphagy, and a number of other traits that enable the organism to become adaptable to new environments.

1.3 Social Insects

Although altruism and philanthropy are widespread throughout the animal kingdom, reproductive altruism, or eusociality, is rare, and is restricted to some groups of Hymenoptera, termites (Wilson 1971), and one species of mammal (the naked mole rat *Heterocephalus glaber* Rüppell; Honeycutt 1992). A species is only considered to be eusocial if its reproductive behaviour satisfies three criteria: there is an overlap of generations, reproduction is restricted to a few individuals, and there is cooperative brood care (Wilson 1971). However, many social insects only satisfy some of these criteria, and exhibit different levels of sociality (Table 1.2). As this thesis deals only with eusocial insects, hereafter they will be referred to as 'social'.

Apart from separation into reproductive and non-reproductive castes, social insect societies also exhibit division of labour among the worker castes (polyethism). The workers can usually be divided either on the basis of physical (size or shape) or temporal (age) castes. Ants (Formicidae) and termites (Isoptera) have the most developed physical castes; honey-bees (*Apis* spp.) and stingless bees (*Trigona* spp.) have well-developed temporal castes, while in halictine bees, bumblebees (*Bombus* spp.) and wasps (Vespoidea), worker polyethism does not seem to be as pronounced (Naug and Gadagkar 1998).

These differences make social insects quite unique both in terms of their population dynamics and potential as invasive species. Firstly, there is the question of defining population size – is it the number of colonies, or the number of individuals across all colonies? In terms of population biology only the number of reproductives is important, but it is the foraging workers that have the largest impact on the environment. Secondly, factors affecting the population dynamics of social insect colonies are very complex, as the number of

reproductives produced per colony depends not only on the fecundity of the queen but also on the ability of workers to gather resources and gradually expand the colony (Michener 1964). These, as well as weather, competition and predation all interact to influence population size of social insects.

Table 1.2: The various levels of sociality found among social insects (from Cowan 1991).

Solitary: Females nest alone and mass provision their nests. They do not interact with their developing young.
Presocial: Females exhibit social behaviour beyond sexual interactions, yet short of eusociality.
Subsocial: Females nest alone but interact with their developing larvae by progressive provisioning. Females that live sufficiently long may occur on the nest with their adult daughters.
Parasocial: Females of the same generation interact on the same nest.
Communal: Each female builds, oviposits in, and provisions her own cells.
Quasisocial: All females cooperate in building and provisioning brood cells, and all females oviposit.
Semisocial: Some females (reproductives) lay most or all of the eggs. Other females (workers) with limited egg-laying opportunities are relegated to foraging, nest building, and caring for the young.
Eusocial: Multiple females cooperate in nesting and exhibit reproductive division of labour (as in semisocial), but there is also and overlap of generations, so that adult offspring assist their parents.
Primitively eusocial: Colonies are relatively small and short-lived, and morphological differences between reproductive and non-reproductive females are minimal or non-existent.
Highly eusocial: Colonies are relatively large and complex and often are long-lived. Reproductive castes often are morphologically distinct from non-reproductive castes.

Similar problems are encountered when examining the ability of social insects to invade new habitats. Not surprisingly, they possess several of the identified characteristics of successful invaders (see Table 1.1). Their social organisation, and ability of new reproductives to fly, enables them to have a high dispersal rate. Unlike most non-social species, the reproductives of social insects are capable of long-term sperm storage, and thus only a single inseminated female is required to arrive in a new habitat to start a viable population. Studies of invasive ants, such as the red imported fire ant (*Solenopsis invicta* Buren), or Argentine ant (*Linepithema humile* Mayr) indicate that colonies are able to adapt both behaviourally and ecologically to new environments by lowering inter-colonial aggression, and modifying their life cycle and reproductive patterns (Vinson 1997; Holway 1999; Holway and

Suarez 1999). Most social insects also have large native ranges, and many are introduced into habitats where they can fill an empty niche (e.g. *Polistes* and *Vespula* spp. introduced into New Zealand, where no native social wasps exist; Clapperton *et al.* 1989c; Clapperton *et al.* 1994; Clapperton *et al.* 1996). In addition, social insects are well protected against predators and usually exhibit effective defensive mechanisms such as a sting, large mandibles or defensive chemicals (Spradbery 1973). Also, predation on reproductives only occurs before the establishment of the colony, as workers perform all out-of-nest activities once a nest is established, and therefore effective predation can only occur on the nest itself, which is usually well-defended (Moller 1996). Social insects are also extremely competent at removing any foreign organisms, such as parasitoids and fungi, or sick individuals from the nest (Moller 1991; Barlow *et al.* 1996).

Thus, sociality in insects not only distinguishes them from non-social species in terms of population biology, but also makes them potentially successful invaders. In the next section, a case study of an invasive social insect, *Vespula germanica* (Hymenoptera: Vespidae) is considered in terms of its ecology and population biology in a new habitat. Other *Vespula* species are also examined, where appropriate, and their biology compared.

1.4 Introduction to *Vespula* spp.

1.4.1 Classification

Vespula germanica (F.), or the European wasp, is a member of the Vespidae, a family that comprises both highly eusocial and solitary nesting wasps. Most species in the subfamilies Euparigiinae, Masarinae and Eumeninae are either solitary or presocial (see Table 1.2 for definition) (Cowan 1991), the Sterogastrinae are subsocial and parasocial (Turillazzi 1991), the Polistinae primitively social (Gadagkar 1991; Jeanne 1991; Reeve 1991), while the Vespinae are highly eusocial (Spradbery 1973; Edwards 1980). The genus *Vespa* comprises the hornets, while *Dolichovespula* and *Vespula* are commonly referred to as 'yellowjackets' (mostly in the U.S.A.). *Vespula germanica* belongs to the *V. vulgaris* group, which, apart from morphological differences, is

often considered to be the 'scavenger group' within the genus (Akre *et al.* 1981; Carpenter 1987). (See Figure 1.3 for relationships)

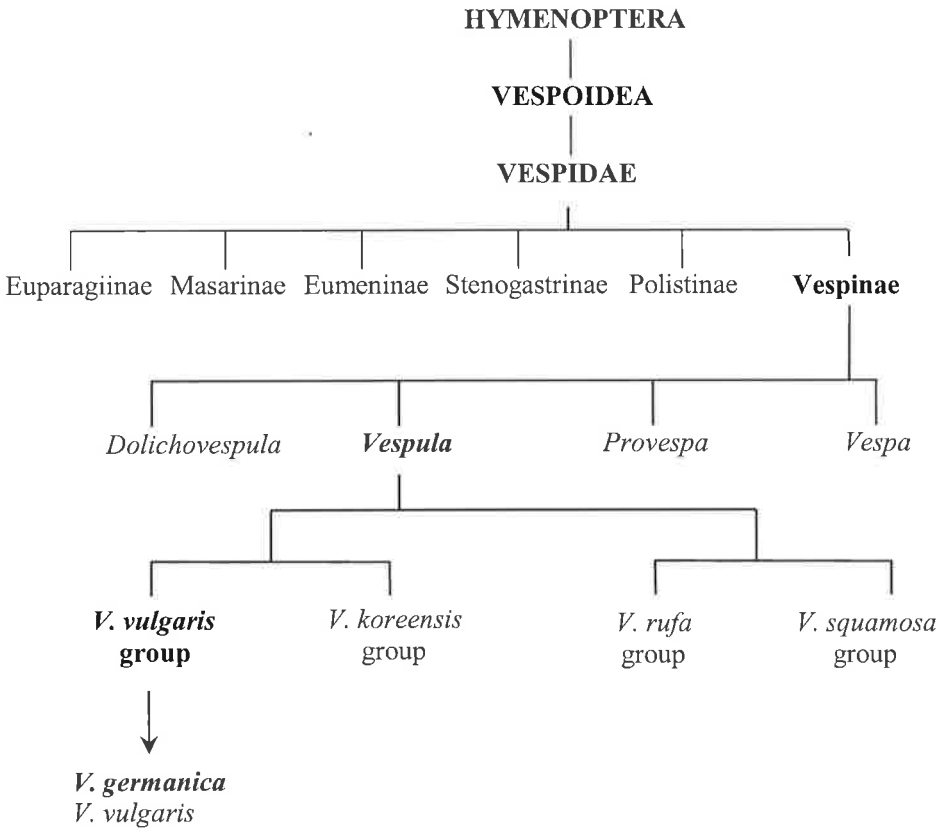

Figure 1.3: Phylogeny of the Vespoidea showing the position of *Vespula germanica* (modified from Carpenter 1982 and Carpenter 1991).

1.4.2 Morphology and social organisation of V. germanica

Vespula germanica has a highly eusocial organisation. There are three separate, easily distinguishable, castes present in the colony. These are the relatively rare reproductive males (drones) and reproductive females (gynes and queens), and the numerous sterile females (workers). All adult *Vespula*, regardless of caste, have a similar morphology. The metasoma is striped black and yellow, and this aposomatic colour pattern presumably advertises the presence of a painful sting to their predators. The taxonomy of vespid wasps relies heavily on the size and shape of their colour patterns (Spradbery 1973; Edwards 1980). Figure 1.4 shows the differences in abdominal markings of

V. germanica and *V. vulgaris*. However, Clapperton *et al.* (1989b) found a considerable overlap in markings between the two species in New Zealand, as well as some intermediate patterns, and suggested that other characteristics, such as markings on other parts of the body, nesting characteristics or behavioural patterns should also be considered when distinguishing between the species.

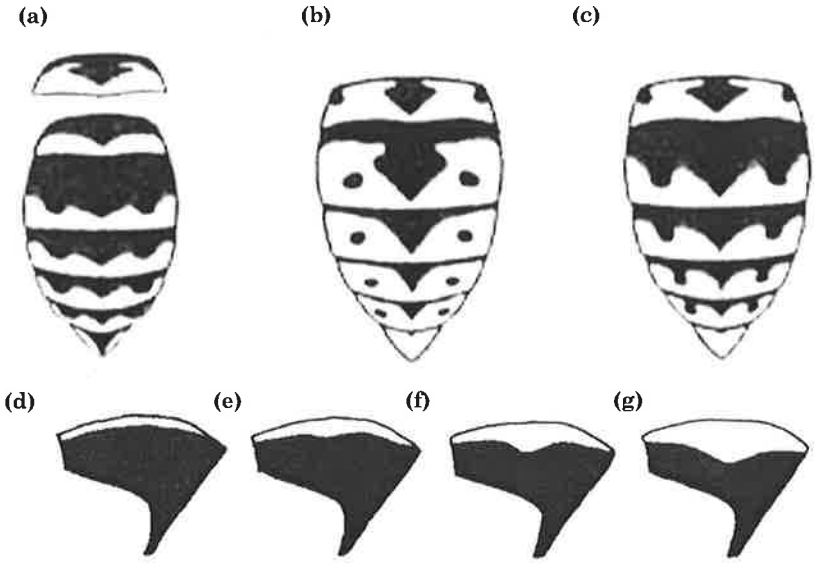

Figure 1.4: The distinguishing dorsal metasomal marks of (a) *Vespula vulgaris* and (b and c) *Vespula germanica* (from Akre *et al.* 1981), and lateral pronotal markings of (d) *Vespula vulgaris* and (e,f,g) *Vespula germanica* (from Clapperton *et al.* 1989b).

Males and females can be easily separated as males have eight metasomal and 13 antennal segments, while females have seven metasomal and 12 antennal segments (Spradbery 1973). The queen of *V. germanica* is the largest caste, measuring 15 to 20 mm in length. The males and workers are smaller, 12 to 13 mm (Edwards 1980), although there is variation in size throughout the season, with the trend being towards larger workers (Potter 1964; Spradbery 1973).

Both reproductive and worker females of *Vespula* spp. possess a sting, connected to the sting gland, which is usually retracted between the seventh tergite and sternite, and is used for defence (Spradbery 1973). Another gland in the sixth metasomal sternite, the Van der Vecht gland, is thought to produce

pheromones, probably for the control of the colony (queen pheromones). In males, the most posterior metasomal segments enclose the genital structure instead of the sting.

1.4.3 Determination of sex and caste

One of the characteristics of Hymenoptera, and thus of *Vespula*, is their haplodiploid method of sex determination. Males develop from unfertilised eggs and are haploid, while females are diploid, carrying maternal as well as paternal genes. Haplodiploidy is thought to be one of the pre-adaptations that enabled the rise of sociality in Hymenoptera (Trivers and Hare 1976). Under this system of sex determination, sisters are ¾ related to each other, but only ½ related to their offspring. However, if queens multiply-mate, sister-sister relationships lessen.

Differentiation between worker and queen castes occurs during the fourth larval instar, with queen larvae being fed more than workers (Potter 1964; Spradbery 1973). Male eggs are normally laid by the queen, although worker laid eggs have been detected in some colonies (e.g. Akre and Reed 1983; Ross 1985; Goodisman *et al.* 2002).

Males and queens are only produced in the nests towards the end of the season. For this purpose, large cells are built. It is not certain what cues are responsible for the onset of large cell production, but queen age is thought to be involved (Potter 1964).

1.4.4 World distribution of *V. germanica* and *V. vulgaris*

Vespula germanica is native to Europe, northern Africa, the Middle East, northern India, China and Korea, between the latitudes 23°N and 62°N (Edwards 1976; Spradbery and Maywald 1992; Fig. 1.5). Several species of *Vespula*, including *V. vulgaris*, are also present in parts of North America. Both *V. germanica* and *V. vulgaris* are normally associated with a Mediterranean climate, i.e. hot, dry summers, and cool, wet winters (Edwards 1980). Both are predominantly lowland species (Spradbery 1973), however *V. vulgaris* has been shown to exist in sub-alpine environments in its introduced range (Beggs 1991).

Since 1945, both *V. germanica* and *V. vulgaris* have also been introduced to many other parts of the world. They are now established in parts of New Zealand, Ascension Island, South Africa, Chile, Argentina, Canada, U.S.A. and Australia (Edwards 1976; Akre *et al.* 1989; Spradbery and Maywald 1992; Tribe and Richardson 1994; Vetter *et al.* 1995; D'Adamo *et al.* 2002; Fig. 1.5).

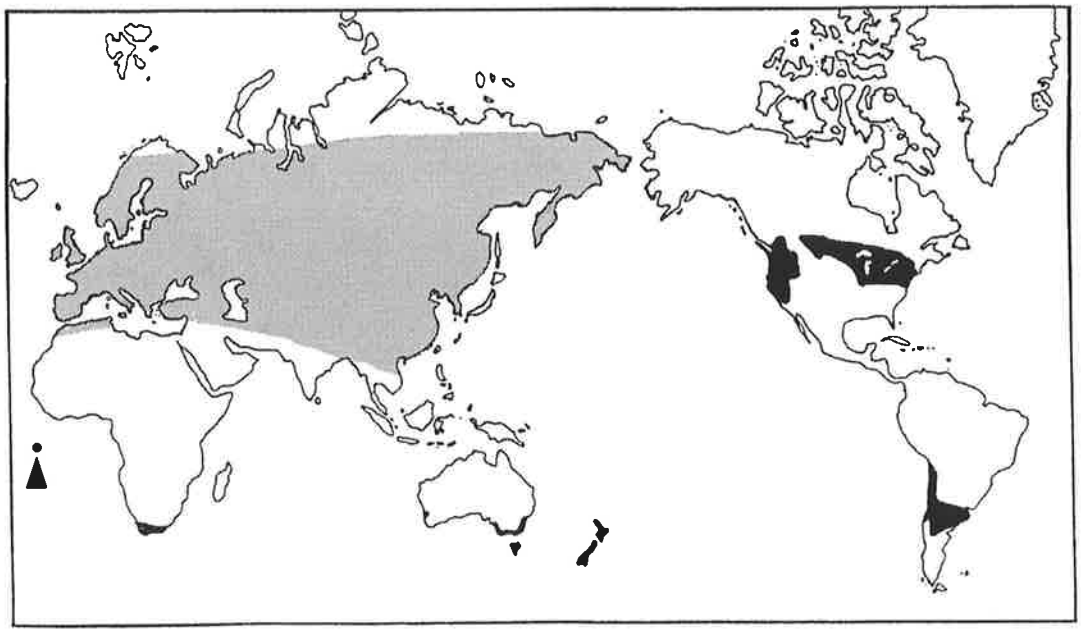

Figure 1.5: The native (shaded) and introduced (black) world distribution of *Vespula germanica* (modified from Greene 1991). The Ascension Island population is marked with an arrow.

1.4.5 Introduction and spread of V. germanica and V. vulgaris

Apart from being studied in their native range in England, *V. germanica* and *V. vulgaris* have also been examined in New Zealand and Australia.

Vespula germanica was introduced into New Zealand in 1945 (Thomas 1960), and despite extensive attempts to eradicate it, the species is now well established throughout both main slands (Thomas 1960; Clapperton *et al.* 1989c; Clapperton *et al.* 1994). *Vespula vulgaris* was first recorded in New Zealand in 1921, but did not established there until the early 1980s (Donovan 1984). Since then, it has spread rapidly and the two species now coexist, except in beech (*Nothofagus* spp.) forests, where *V. vulgaris* has outcompeted *V. germanica* (Clapperton *et al.* 1994). *Nothofagus* forests present a unique

environment, as they are naturally infested with honeydew producing scale insects *Ultracoelostoma assimile* (Maskell) and *U. brittani* (Morales), providing a rich carbohydrate food source (Thomas *et al.* 1990). No native vespid wasps are present in New Zealand; in fact, there are no native social wasps (Clapperton *et al.* 1989c; Clapperton *et al.* 1996). The introduction of the two species therefore has the potential to have an enormous impact on both the biodiversity and composition of native communities (Beggs and Wilson 1991; Fordham 1991; Moller *et al.* 1991b; Barr *et al.* 1996).

Several species of the primitively eusocial Polistinae are native to Australia, but no members of the Vespinae were present until *V. germanica* was first discovered in Hobart in 1959. It was subsequently recorded on the mainland in 1975 when it was found in Sydney (Spradbery and Maywald 1992). It arrived in Melbourne in 1977; by 1992 it was widespread in the metropolitan area, and by 1989 it could be found in most major towns throughout Victoria (Crosland 1991). A rapid eradication program in Perth led to the destruction of first nests in 1977, and the wasp did not reappear until 1983. Between 1983 and 1989 small numbers of nests (5-20 per season) were detected and destroyed after presumably being initiated by queens transported from the eastern states (Crosland 1991). In 1984 and 1985 some nests were also destroyed in Albany, Western Australia.

In South Australia, the wasp was first detected in 1978, near Port Adelaide. The nest was destroyed, and the next sighting was not until 1984 at Stirling. Since then the wasp has become permanently established, and it is now found throughout most of metropolitan Adelaide, from Gawler south to McLaren Vale, and eastwards into the Adelaide Hills. Small outbreaks also occur in regional towns, and have been recorded from Port Lincoln, Whyalla, Port Augusta, the Riverland, Bordertown and Mt. Gambier. However, populations in these towns are prevented from permanently establishing by rapid nest destruction (Fig. 1.6) (Local Government Association, pers. comm.).

Vespula vulgaris arrived in Australia in 1958, but compared with *V. germanica*, its range has been restricted to a few suburbs in metropolitan Melbourne (Crosland 1991). However, it has recently been recorded throughout Tasmania,

and is now thought to have established there permanently (Matthews *et al.* 2000).

The potential spread and range extension of *V. germanica* in Australia has been modelled using the ecoclimatic matching program, CLIMEX (Spradbery and Maywald 1992). This model predicts that wasps could colonise all areas on the south and east coast, up to, and including, Brisbane. Although nests have been destroyed in Brisbane in the past, the species has not established there.

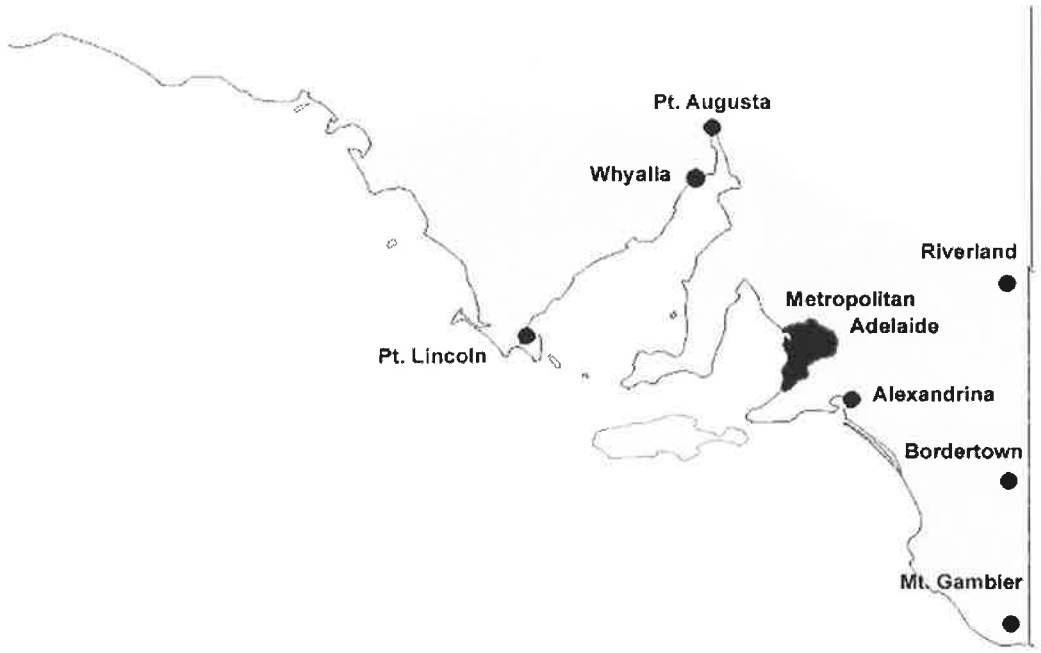

Figure 1.6: The current (black) and potential (shaded) distribution of *V. germanica* in South Australia. The potential distribution is as predicted by ecoclimatic matching by Spradbery and Maywald (1992).

The summary of *V. germanica* invasions presented here illustrates the different conditions in both invaded environments (other social wasps in Australia, and no social wasps in New Zealand), giving rise to potentially different selection pressures on each invading population. It also highlights the need for caution when extrapolating from ecological and behavioural studies undertaken in one climatic zone (e.g. England) to another (e.g. Australia).

1.5 Basic biology and ecology of *Vespula* spp.

1.5.1 Nest site selection and preferences

In their native range in Europe, nests of both *V. germanica* and *V. vulgaris* are found in rural rather than urban areas, and both species prefer nesting in subterranean locations (Spradbery 1973; Pawlikowski 1990). In contrast, in New Zealand, 77% of *V. germanica* nests are found below ground, 20% in aerial sites, and 3% in artificial structures (Donovan *et al.* 1992). Moller and co-workers (1991a) found that nest site preferences of *V. germanica* and *V. vulgaris* varied between different locations. In beech forests and in horticultural areas 100% of nests were located underground, in urban areas only 60% were subterranean, while 30% were found in artificial structures.

In Australia, Crosland (1991) noted that 42-72% of *V. germanica* nests were found underground, 24-33% were in buildings, and 1-3% were aerial (see Ch. 2). These figures are not separated into urban and rural areas, and therefore comparisons are difficult. However, it appears that *V. germanica* is less likely to nest underground in Australia than in its native range.

1.5.2 Life cycle

The colony life cycle of *Vespula* spp. is usually annual, with nests being founded by a single queen in spring (Spradbery 1973; see Fig. 1.7). After emergence from hibernation, the queen searches for a suitable nest site, usually in a dry, dark cavity, and starts building an embryonic nest. The nest is constructed from wood scrapings mixed with salivary excretions and water to produce a brownish or greyish paper. The colour of the paper varies between species of *Vespula* (Edwards 1980; Akre *et al.* 1981).

The nest consists of a series of hexagonal cells used for rearing young, arranged in a roughly circular pattern. Each layer of cells forms a comb, and a mature nest in England may comprise up to 15,000 cells and 11 combs (Spradbery 1973; Archer 2001b). Nest construction begins with the attachment of the first layer of 4-5 cells to a buttress (such as a twig) by a spindle. Next, an envelope is built around the nest, presumably for protection from predators and

for thermoregulation (Potter 1964). More cells are added to the first comb, and the envelope is also expanded to enclose the whole nest.

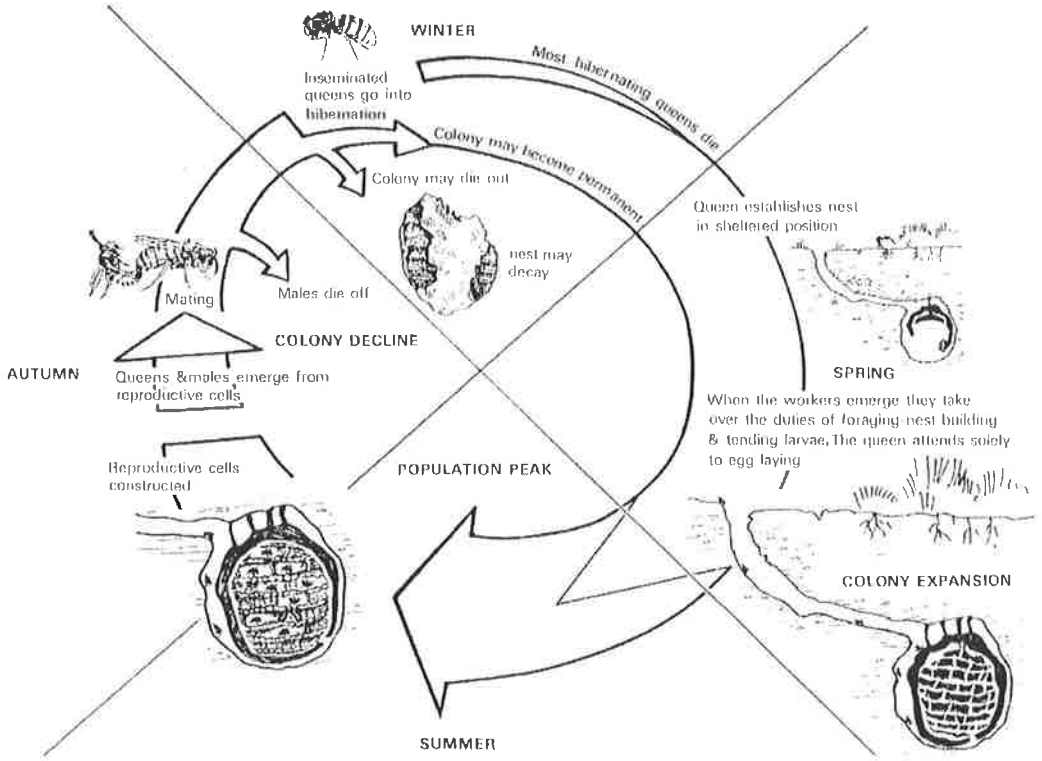

Figure 1.7: The life cycle of *Vespula* spp.

The time from establishing the nest until the first workers emerge can be referred to as the 'critical stage' at it is a very vulnerable time (Akre and Reed 1981). During this period energy demands on the queen are extremely high, as she has to continue nest building, forage for herself and the developing larvae, as well as protect the nest by herself. Loss of the queen when foraging, or usurpation by other queens is common (Ross *et al.* 1981; Donovan 1991; Spradbery 1991b; Archer 2001b). Success or failure at this stage is possibly correlated with the fitness of the queen and her ability to protect the nest from usurpations (Leathwick 1997). Queens that have the largest fat reserves, typically found in successful nests (Harris and Beggs 1995), may be energetically more able to survive the 'critical' period and successfully establish a colony.

After approximately 40 days (for *V. germanica*), the first generation of workers reach adulthood and take over all nest expansion, building, foraging, larva nursing and nest protection activities. The envelope is thickened, and more cells are added. As the nest expands soil is excavated from the bottom of the nest and new layers (combs) are built underneath the old ones (Spradbery 1973). The queen is confined to the nest to continue laying eggs for the rest of the season and her lifetime (Edwards 1980). The workers exhibit a mild form of temporal polyethism, where they start off as nurses, but in time shift to foraging for pulp, prey and liquids, and performing nest guarding duties before dying.

At the end of summer, cells that are 30-40% larger are constructed (Archer 2001b). Most of the males and new queens (gynes) are produced in these large cells, although males can also be produced in small cells. The reproductives disperse and mate, while the drones die off and the nest usually breaks down (variations to this pattern are explained in section 1.5.4). The inseminated queens find a dark, dry place and enter reproductive diapause until next spring, when they emerge and start the life cycle again (Spradbery 1973; Edwards 1980; Greene 1991).

Life cycle duration from nest construction to hibernation varies between species of *Vespula*. In their native ranges it is longest (4 to 5 months) for species in the *Vespula vulgaris* group (see Fig. 1.3 for classification), and shorter (3 to 4 months) for members of *Vespula rufa* group (Akre *et al.* 1981).

1.5.3 Colony development

The development of each colony may be determined by examination of natural colonies in the field as well as ones reared in the laboratory. Development during the various stages of the life cycle may be described by several characteristics, such as number of cells, brood, adults and meconia, rate of cell building, production of each brood stage, mortality of each brood stage, and length of life characteristics of the brood, adults and colony (Archer 1997). Further, there are a number of phases the colony can go through, depending on the success of the colony. These are listed in Table 1.3.

There are also some critical times in the development of the colony. These include queen emergence from hibernation, the emergence of first workers,

foundation of the nest, the commencement of large cell production, emergence of all queens and males, and end of the colony life (Archer 1997).

Typically, a mature *V. germanica* colony in England, averages 6,540 small and 1,563 large cells, with the largest colonies producing up to 7,991 workers, 3,215 males and 1,326 queens (Archer 2001b). In comparison, a *V. vulgaris* colony, which averages 7,400 small and 2,300 large cells produces 10,293 workers, 1,011 males but only 962 queens (Archer 1980a). This is due to *V. vulgaris* utilising more of its large cells for male rather than queen production. Comparable data on nest sizes does not exist for New Zealand or Australia, apart from the large overwintered nests. This is urgently needed as it forms the basis of any population ecology studies.

Table 1.3: Nest phases of a *Vespula* colony. At the end of the season, the success of the colony is proportional to the position of the phase in the table (from Archer 1980a).

Queen colony: only the queen present before first workers hatch
Small cell colony: queen and workers present but no large cells built
Large cell colony: large cells are present in the colony
large cell colony with eggs
large cell colony with sealed brood
adult have emerged
<100 adults emerged
>100 adults emerged
all adults left

1.5.4 Overwintering and polygyny

Overwintering is a modification to the usual colony life cycle where a colony becomes biennial or perennial (Ross and Matthews 1982; Ross and Visscher 1983; Plunkett *et al.* 1989; Greene 1991; Pickett *et al.* 2001). Overwintered polygynous (containing multiple queens) colonies only occur in species that produce reproductives later in the year, i.e. species with a long colony cycle. These include the *V. vulgaris* and *V. squamosa* groups, while the short cycle species, such as the *Vespula rufa* group, have not been reported to overwinter (Ross and Matthews 1982). Polygyne colonies are also only found in places

experiencing mild winters, including North Africa, Florida, California, Australia and New Zealand (Edwards 1980).

Overwintering colonies of *V. germanica* usually begin as annual colonies, produce reproductives at the end of the season, but continue on instead of dying off during winter (Donovan *et al.* 1992). Most overwintered nests are formed when gynes produced late in the year are thought to return to and reproduce in the natal nest, as offspring produced in such nests are more related than would be expected if non-nestmate recruitment occurred (Spradbery 1991a; Goodisman *et al.* 2001b).

Overwintered nests can produce many workers and reproductives and grow extremely large in size. In Australia and New Zealand *V. germanica* nests have been found to have up to 3-4 million cells and 180 combs (Spradbery 1973). The smallest overwintered nests have at least 25 egg-laying queens, and the largest up to 400 (Spradbery 1991a).

It is difficult to determine the importance of overwintering on the population dynamics of *Vespula* as the frequency of overwintering nests is largely unknown. Three out of 36 colonies (8.3%) of *V. vulgaris* collected during one season in Palmerston, New Zealand overwintered (Leathwick and Godfrey 1996). Ratnieks and Miller (1993) report an even smaller percentage for *V. vulgaris* in its native habitat in California (about 3%), while Edwards (1980) gives a figure of 10% for *V. germanica* in New Zealand. It is unknown why overwintered polygynous nests have not become more common in *Vespula* spp., considering they appear to be viable and are capable of having a greater reproductive potential than annual nests. Moreover, polygynous colonies are typical among members of the Polistinae (Jeanne 1991; Spradbery 1991a), and in social insects in general, particularly among ants (Brian 1989; Vinson 1997). Future research is needed to clarify why polygyny is not more widespread in *Vespula*.

1.5.5 Foraging

Vespula forage for four main resources. These are pulp for nest building, water for thermoregulation, and carbohydrate fluids and arthropod prey to feed on (Edwards 1980; Raveret Richter 2000). Because of their morphology, adult

wasps are unable to process solid food items. After capturing an item, foragers may either leave it intact, chew off certain parts such as wings and appendages, or extensively masticate it (Archer 1977). Foragers then take the item back to the nest, where worker-larvae trophallaxis takes place. During this process, workers feed larvae portions of prey items, while the larvae regurgitate a solution containing sugars, protein and free amino acids (Hunt 1991).

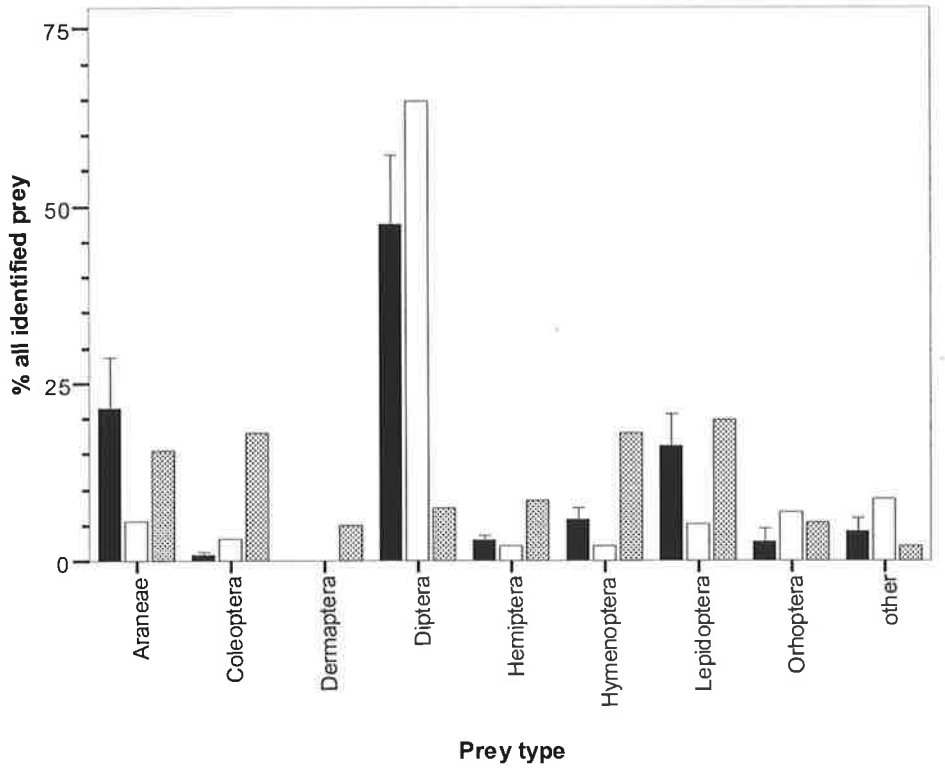

Figure 1.8: Summary of prey items captured by *Vespula germanica* in three introduced habitats: New Zealand (black), Australia (white) and Argentina (grey). Data was obtained from three studies in New Zealand (Harris 1991; Harris and Oliver 1993; Harris 1996), one study in Australia (Madden 1981), and one study in Argentina (Sackmann *et al.* 2000). Data from the three New Zealand studies was summarised by site and by study. Error bars represent standard error of mean.

The returning foragers can be intercepted when returning to the nest, and any load brought back can be removed and identified. Detailed lists of prey items have been compiled for *V. germanica* and other *Vespula* spp. (e.g. Gambino 1986; Gambino *et al.* 1990; Harris 1991; Gambino 1992; Harris and Oliver 1993; Sackmann *et al.* 2000). Spradbery (1973) summarised some of the

insect prey collected by *V. germanica* in Britain; however, the relative abundance of these items in the wasps' diet is unknown. Studies of wasp diet in New Zealand, Australia and Argentina indicate that *V. germanica* is an opportunistic feeder, foraging on prey from many different arthropod orders (Fig. 1.8). In Australia and New Zealand, the predominant prey type is Diptera, while in Argentina the species feeds on Araneae, Coleoptera, Hymenoptera, and Lepidoptera equally (Madden 1981; Harris 1991; Harris and Oliver 1993; Harris 1996; Sackmann *et al.* 2000).

Apart from internal needs for food, water and pulp, foraging in social insects is also governed by weather (Porter and Tschinkel 1987). *Vespula* spp. are visually navigating insects, and will not forage below a certain light threshold. Similarly, a minimum temperature threshold has been suggested (Gaul 1952a; Blackith 1958; Potter 1964). Rainfall has also been described to reduce wasp activity, however, this has not been studied quantitatively (Gaul 1952b; Potter 1964). Moreover, specific daily patterns have been described, where activity peaks in the morning, and declines to a steady level for the remainder of the day (Gaul 1952a; Potter 1964; Edwards 1980). What and how environmental factors influence foraging activity in *Vespula* spp. needs to be determined.

1.6 Annual fluctuations in *Vespula* populations

As explained in section 1.1, the size of a population can only depend on the number of births, deaths, immigrations and emigrations. Thus, in an established population, the seasonal abundance of wasps is dependent on birth and mortality rates of queens and of colonies. Birth rates, or rate of production of new queens can be derived from examination of late season field colonies. Mortality rates are more difficult to assess. Archer (1980a) suggested that the greatest period of mortality in England occurs during winter, when up to 98% of queens die. Other mortality factors thought to be driving wasp population cycles, both in England and elsewhere are: the abundance of predators and parasites, availability and selection of nest sites, the quality of the queen, and weather effects. These factors have been studied both in the wasps' native habitat, and in their introduced ranges (Table 1.4).

Table 1.4: Factors found responsible for yearly fluctuations in *Vespula* species.

Vespula species	Correlation	Country	Source
Vespula spp.	-ve with spring rainfall	England	Beirne 1944; Fox-Wilson 1946
V. vulgaris	-ve with autumn weather (but only in small colonies)	England	Archer 1980b
V. germanica	+ve with autumn and spring rain	Australia	Madden 1981
V. vulgaris *V. pensylvanica*	+ve with spring temperature and –ve with spring rainfall	USA	Akre and Reed 1981
Vespula spp.	no correlation with rainfall (re-evaluation of Beirne 1944 and Fox-Wilson 1946 data)	England	Archer 1985
V. germanica	+ve with annual rainfall	Australia	Horwood *et al.* 1993
V. vulgaris	queen quality	New Zealand	Harris and Beggs 1995
V. germanica and *V. vulgaris*	2-yearly cycles caused by density-dependence	England	Archer 2001a
V. vulgaris	density-dependence and spring rainfall	New Zealand	Barlow *et al.* 2002

Although similar factors have been found to regulate *Vespula* populations across their geographical distribution, the factors vary among populations and also the direction of the correlation. Thus, for example, in England and USA a negative relationship has been noted between abundance and spring rainfall (Beirne 1944; Fox-Wilson 1946; Akre and Reed 1981), whereas in Australia this relationship is a positive one (Madden 1981). Archer (1985) proposed that some or all of these factors were acting together with an endogenous regulating mechanism. He later showed wasp populations exhibited two-year cycles which could be explained by density-dependence (Archer 2001a). A recent study of population dynamics of *V. vulgaris* in New Zealand's honeydew beech forests modelled rates of increase as a function of both density-dependent and density-independent factors (Barlow *et al.* 2002). They found that a Ricker model, employing wasp densities as a function of previous densities and spring rainfall, could explain 66% of variation in abundance. Further research needs to

confirm if factors affecting population dynamics of *Vespula* in such a unique environment are the same as in other locations (see Ch. 6).

1.7 Impact and control of *Vespula* spp.

1.7.1 Impact on native communities

There are few quantitative data regarding the ecological impacts of *V. germanica* on the native fauna and flora of Australia. In New Zealand's native honeydew beech forests, *V. vulgaris* has been shown to remove large amounts of honeydew, also utilised by many native insects and birds (Beggs and Wilson 1991; Moller *et al.* 1991b). It has been estimated that 80-340 litres/ha/season of honeydew is removed by *V. vulgaris*, reducing the standing crop of honeydew by up to 99% (Moller *et al.* 1991b). Predation by wasps may also reduce invertebrate abundance, with 1-8 kg of native invertebrates/ha/year being removed from beech forests (Harris 1991). Although wasp densities in Australia do not reach the levels experienced in New Zealand (see Ch. 6), they may still have a great effect on invertebrate biodiversity, and studies need to be carried out to examine this.

1.7.2 Impact on humans

Vespula spp. are perceived as dangerous to humans because of their painful sting, and potential to attack in large numbers near nest sites (New 1994). Due to their scavenging nature, wasps are often present in recreational areas, and can be a nuisance when they are foraging on human food and drinks (New 1994). *Vespula* stings can be a potential health risk to humans. Wasps will only sting in self-defence, however they are able to sting several times. For most people, the sting is a painful experience and some swelling may occur. However, a few people can develop anaphylaxis, which, if not treated immediately, could be potentially life-threatening (Levick *et al.* 2000; McGain *et al.* 2000).

The extent of impact that *Vespula* has on the agricultural, horticultural or viticultural industries has not been examined in detail. However, as wasps forage in orchards and near ripening fruit, they can be a problem to fruit pickers.

Further anecdotal evidence indicates that foraging wasps may damage large numbers of pears, strawberries (New 1994) and thin-skinned grapes (D. Hopkins, pers. comm.).

In New Zealand, losses of 5-35% of honeybee hives due to invasion by wasps are reported (Clapperton *et al.* 1989a), while in Israel, up to 65% of cattle are reported to be injured by wasps (Braverman *et al.* 1998).

1.7.3 Chemical and biological control

Due to their status as a pest in invaded regions, especially in New Zealand and Australia, various control methods have been used in an attempt to reduce *V. vulgaris* and *V. germanica* populations. The traditional way of killing *Vespula* wasps is to treat the nest with a pesticide powder (Edwards 1980). This approach has frequently been used in Australia, where many local council authorities employ nest destruction schemes. However, the disadvantage of this method is that nests have to be located prior to being killed this way, and in areas with high nest densities such as in New Zealand, remote regions, or densely populated urban areas, this is virtually impossible. A more effective alternative is using poison baits, which can be taken back to the nest by foraging wasps. Problems arise in what bait type to use as an attractant, as even the same species of wasps show preferences for different baits in different locations (e.g. Ross *et al.* 1984; Spurr 1995). Ideally, a specific, non-perishable bait should be used, but once again, these work in some places while not in others (Reierson and Wagner 1975; Spurr 1996; Landolt 1998; Landolt *et al.* 2000). Additionally, a suitable poison needs to be used, and fipronil has recently surfaced as a potential candidate (Harris and Etheridge 2001; Sackmann *et al.* 2001).

An alternative approach to controlling *Vespula* populations is using biological control agents. Possible candidates include fungi, nematodes, bacteria, viruses, parasites and microsporidia (Moller 1991; Gambino *et al.* 1992; Barlow *et al.* 1996; Glare *et al.* 1996; Rose *et al.* 1999). Research to date has been mostly unsuccessful with this approach, mostly due to the prompt and effective removal of any foreign organisms from their nests (Moller 1991; Barlow *et al.* 1996).

1.8 Research objectives and thesis outline

1.8.1 Overall objectives

This review has shown that when an invasive species establishes in a new environment, it has the potential to restructure the invaded community through predation or competition. In addition, the invader may also alter its own biology to better adapt to the new conditions (sections 1.2.1, 1.5.1, 1.5.4, 1.7.1). Social insects especially make very efficient invaders, but their social structure also makes them unique as each nest supports numerous workers apart from the reproductive individuals (section 1.3).

Although a considerable amount of information exists for *Vespula* spp., the majority of research on *V. germanica* and *V. vulgaris* has been conducted in New Zealand and in England. Both of these locations experience different climates, and very different community structures. In England, where *Vespula* spp. are native, they live sympatrically with other vespids (seven species in total; Edwards 1980), whereas New Zealand has no native social wasps. Indeed, differences in nest sites (section 1.5.1) and colony development (1.5.3 and 1.5.4) suggest that *V. germanica* behaves quite differently in these two locations. Little comparable information exists for Australia, but is necessary in order to assist with the development of control methods, and to understand the invasion biology of this social insect species. Thus, the broad aim of this thesis is to describe the biology and ecology of *V. germanica* under South Australian conditions. Furthermore, using available time series data from the ongoing nest destruction program, the aim is to develop a population dynamics model that can predict wasp densities from year to year. Thirdly, this study will add to the broader knowledge of *Vespula* biology, and the ecology of social insects as invaders.

1.8.2 Specific aims

These three broad objectives are addressed within five separate but related studies, presented as the main research chapters of this thesis. Each chapter introduces the specific aims of these studies, and discusses their relevance and implications separately.

Chapter 2 investigates the basic biology of *V. germanica* in South Australia. The questions that it answers include:

1. What nest sites are utilised by *V. germanica*?
2. How many months of the year are wasps active?
3. How fast and how large do colonies grow?
4. How many workers, males and queens are produced by each colony?
5. Is there a relationship between observed traffic rates and colony size?

Additionally, data on seasonal changes in the number of eggs laid per cell, thought to be representative of queen control and be crucial to colony cohesion in social insects, are also presented.

Having ascertained that traffic rates represent the size of the colony and are representative of colonial needs (Ch. 2), Chapter 3 examines how these needs change through time. This is assessed by documenting the inflow of resources into the nest. In particular, this chapter analyses changes in:

1. Prey/pulp/carbohydrate/water/nothing foragers;
2. Prey types;
3. Crop carbohydrate concentration.

To complement findings from Chapter 3, Chapter 4 examines the environmental factors restricting foraging activity in *V. germanica*. The specific questions asked are:

1. What environmental factors (light, temperature, humidity, rainfall) influence foraging activity?
2. What is a 'typical' daily activity pattern for the species?

The next chapter, Chapter 5, concentrates on what the predominantly arthropod diet of *V. germanica* is and how the wasp's predation may be affecting native insect communities. The use of molecular techniques and phylogenetic analyses of prey items also enables comparisons of prey consumed by *V. germanica* and a native paper wasp, *Polistes humilis*. Questions answered by this chapter are:

1. What is the diet of *V. germanica* in South Australia?
2. Is there evidence of an overlap in prey, and thus possibly competition between, *V. germanica* and *P. humilis*?

In the final experimental chapter, Chapter 6, the focus is on annual variation in *V. germanica* population densities in South Australia. Data collected by local council authorities as part of the ongoing nest destruction program are used to build a predictive model of wasp populations. The specific aim of this chapter is to determine how previous nest densities and weather variables affect population densities.

Each chapter has been written to stand alone in a format suitable for submission as a journal article, and thus some overlap between individual chapters exists. Chapter 5 has recently been accepted for publication in Molecular Ecology (Kasper *et al.* 2004).

CHAPTER 2:

NEST SITE PREFERENCES AND SEASONAL COLONY GROWTH OF *V. GERMANICA* IN SOUTH AUSTRALIA

2.0 Chapter summary

Vespula germanica has been accidentally introduced into Australia, where it has now established as a horticultural, agricultural, environmental and nuisance pest. Despite comprehensive and often expensive eradication programs, remarkably little basic information exists for the species in Australia. Most previous studies were conducted in its native range in England and in New Zealand, where *V. germanica* has also been introduced. Data on nesting sites in Adelaide were collected over two seasons, with a total of 2,640 nests surveyed. The majority of nests (67%) were found below ground, mostly in soil. Twenty-nine percent of nests were found in artificial structures, while the remainder of nests was located above ground. Variation between different habitat types suggests that the species can utilise whatever nest sites are available.

Colony duration, timing and seasonal development were also examined by collecting wasp nests over three seasons. Nests were found between November and May. Average mature colonies contained over 9,500 small and 3,600 large cells, with some large nests consisting of over 27,500 cells. By the end of May, such colonies could produce 21,000 workers, 7,500 males, and 4,000 queens. This is two to three times more than the numbers of adults produced in *V. germanica* colonies in England. Other differences included the presence of overwintered nests and multiple eggs per cell. A significant linear relationship was found between wasp traffic and various measures of nest size. These results indicate that in the absence of an adverse climate, *V. germanica* nests can attain substantial sizes. Greater numbers of queens produced in nests may also facilitate a faster rate of population increase in Australia.

2.1 Introduction

Vespula germanica is a highly invasive social wasp. Its native range includes Europe, south-east Asia and northern Africa (Edwards 1976; Spradbery and Maywald 1992). Due to human transport, it has also become accidentally established in New Zealand, Ascension Island, South Africa, Chile, Argentina, Canada, U.S.A. and Australia (Edwards 1976; Akre *et al.* 1989; Spradbery and Maywald 1992; Vetter *et al.* 1995). Along with *V. vulgaris*, a closely related species belonging to the same group within the genus, *V. germanica* has been the most widely studied social wasp in the past decade (Akre 1991). In their introduced range, these two species are considered substantial agricultural, horticultural, viticultural, environmental and human pests. Workers of both species can attack bee hives, kill foraging bees and damage horticultural crops (Clapperton *et al.* 1989a; New 1994). They have also been known to injure cattle (Braverman *et al.* 1998). These wasps may also restructure ecosystems by removing large amounts of invertebrate prey, and through competition for resources with other species (e.g. Beggs and Wilson 1991; Harris 1991; Bashford 2001; Beggs 2001). Additionally, they can be a nuisance to humans by scavenging on food and drinks (New 1994). *Vespula* stings are painful and in a small proportion of cases may be lethal due to anaphylactic shock or mass envenomation (Vetter *et al.* 1999; Levick *et al.* 2000; McGain *et al.* 2000). Where wasps are present in high densities, they can present a problem for the tourism industry (Thomas 1960).

In Australia, the first *V. germanica* nest was discovered in Tasmania in 1959, but the species was not recorded on the mainland until 1975 (Crosland 1991; Spradbery and Maywald 1992). In South Australia, the first nest was discovered in 1978. Following its destruction, no more nests were found until 1984, however, since then numbers have increased (see Ch. 6). At present, the species is widespread throughout Tasmania, Victoria, A.C.T. and New South Wales, and is also permanently established in metropolitan Perth and Adelaide (Crosland 1991). In South Australia, outbreaks have also occurred in regional towns, but these are thought to originate from queens transported from Adelaide and interstate rather than as a result of permanent *V. germanica*

populations in these towns. The second wasp, *V. vulgaris*, has been recorded from parts of Melbourne, and recently its presence has been confirmed in Tasmanian forests (Matthews *et al.* 2000).

In New Zealand, *V. germanica* has been established since 1945 (Thomas 1960). *Vespula vulgaris* was first detected in 1921, but is thought to have only established there in the 1980s (Donovan 1984; Clapperton *et al.* 1989c). Now, both species exist sympatrically throughout New Zealand, except for honeydew producing beech forests in the South Island, where *V. vulgaris* has displaced *V. germanica* (Harris *et al.* 1991b; Clapperton *et al.* 1994).

Vespula spp. usually have an annual life cycle (Potter 1964; Spradbery 1973; Edwards 1980). A single inseminated queen initiates nests in spring. Each queen constructs an embryonic nest, consisting of a series of hexagonal cells, each housing the immature stages of one wasp. These stages include eggs, five larval instars, and pupae. During summer, rapid colony growth occurs, both in numbers of workers and nest size. Colony expansion slows towards the end of summer, and increasingly more males than workers are produced. The addition of small cells is replaced by building cells that are 30-40% larger (Archer 2001b). These large cells house the new reproductive queens, as well as more males. In early autumn, new queens disperse, the old queen dies, and the colony disintegrates. In some instances, *V. germanica* and *V. vulgaris* nests do not die but continue through the winter and into the next season, becoming polygynous and reaching very large sizes (Spradbery 1973; Plunkett *et al.* 1989; Goodisman *et al.* 2001b).

Despite comprehensive and often costly eradication programs being in place in several states (e.g. South Australia, Australian Capital Territory, Western Australia), there is a lack of basic information on the biology and ecology of *V. germanica* in Australia (Crosland 1991; Ward *et al.* 2002). Previous studies have modelled the potential spread of the wasp (Spradbery and Maywald 1992), and examined changes in annual abundance (Madden 1981; Horwood *et al.* 1993; Goodall and Smith 2001). Crosland (1991) examined nesting sites of *V. germanica* in parts of Adelaide and Melbourne, while Ward *et al.* (2002) inspected seasonal changes in nest size. Some recent studies have examined the genetic patterns within and between *V. germanica* populations (Goodisman

et al. 2001a; Goodisman *et al.* 2001b; Goodisman *et al.* 2002). Other demographic information on the species exists only from studies conducted in its native range in England, and the introduced populations in New Zealand.

The aim of this chapter is to document the basic biology of *V. germanica* under South Australian conditions, and compare and contrast the data with information available for the species in England and in New Zealand. In particular, nest sites are surveyed, length of season is examined, and seasonal changes in colony development are followed.

2.2 Materials and Methods

2.2.1 Nest destruction scheme

Since the discovery of the first *V. germanica* nest in South Australia, local council authorities have undertaken a nest destruction scheme to reduce wasp populations in urban areas. Under this scheme, *V. germanica* nests reported by property owners and members of the public are destroyed free of charge by local councils. Records of numbers, dates and addresses of nests destroyed have been kept by councils participating in the scheme. The data on frequency of nests destroyed throughout the year presented in this chapter were collated from 11 of the 20 metropolitan Adelaide council records for six wasp seasons, 1994/95 - 1995/96, and 1997/98 - 2000/01.

2.2.2 Local councils supporting research

In 1999, seven of the 20 metropolitan Adelaide councils (Adelaide Hills, Prospect, West Torrens, Mitcham, Tea Tree Gully, Unley and Playford City Councils, Fig. 2.1) volunteered to participate in the current study. Although the level of involvement varied among councils, most nests used to determine wasp nest sites and colony development characteristics were located within these 'research council' regions. Thus, the study site from which nests were sampled covered most of the permanent distribution of *V. germanica* in Adelaide, and included a range of habitats, from warmer and drier suburbs on the Adelaide plains, to wetter, cooler and sparsely populated areas in the hills (see Appendix 1 for nest locations).

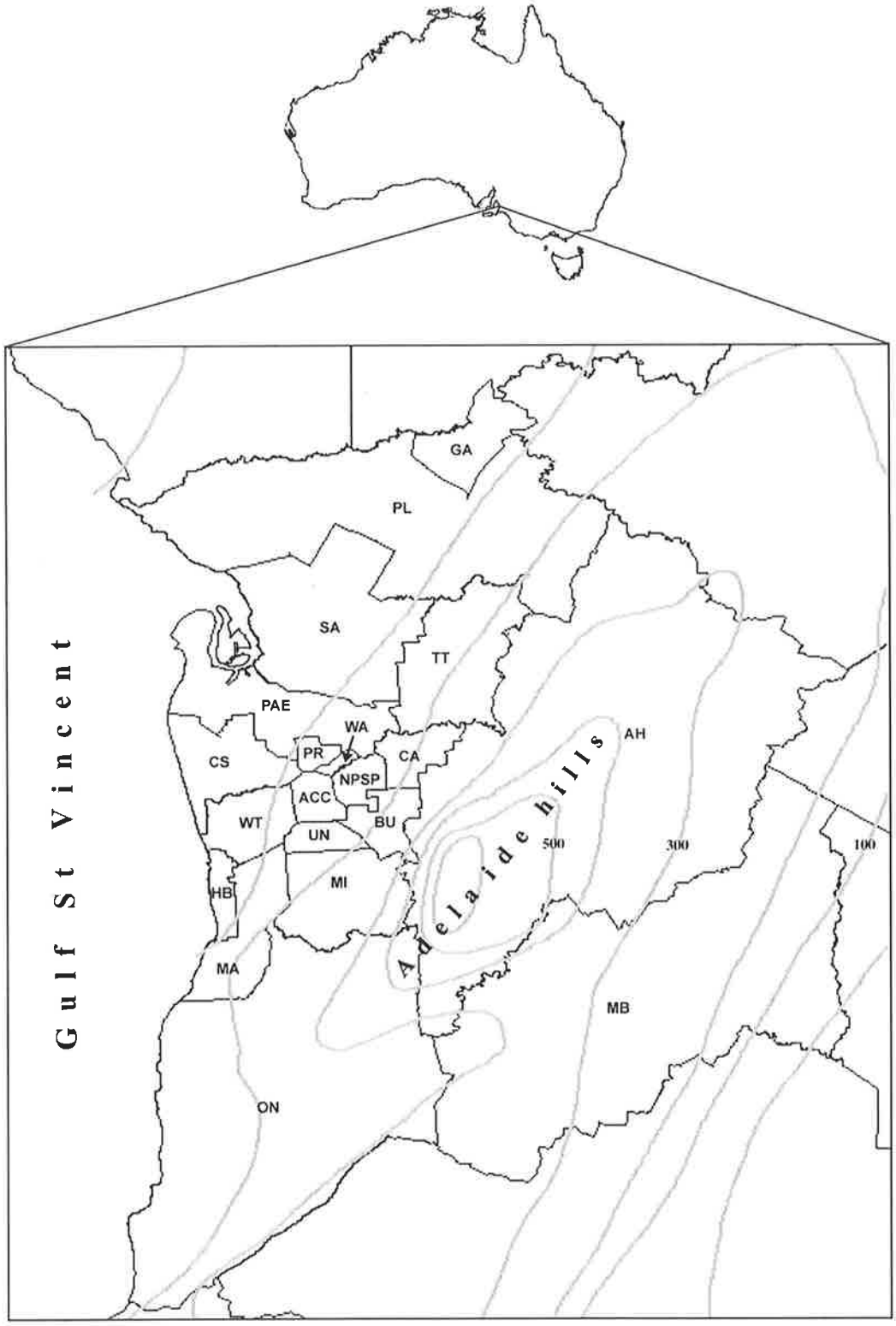

Fig. 2.1: Map showing the relative sizes and positions of the twenty Adelaide metropolitan councils: Adelaide City Council (ACC), Adelaide Hills (AH), Burnside (BU), Campbelltown (CA), Charles Sturt (CH), Gawler (GA), Holdfast Bay (HB), Marion (MA), Mitcham (MI), Mt Barker (MB), Norwood, Payneham and St Peters (NPSP), Onkaparinga (ON), Prospect (PR), Port Adelaide Enfield (PAE), Playford (PL), Salisbury (SA), Tea Tree Gully (TT), Unley (UN), Walkerville (WA), and West Torrens (WT).

2.2.3 Recording of wasp nest sites

During the 1999/00 and 2000/01 wasp seasons, data on the location of each nest destroyed by the 'research councils' was recorded on a standardised form. The date, address of property, and specific location of each nest were recorded. These were then categorised as being 'above ground', 'below ground' or in an 'artificial structure'. The distinction between 'below ground' and 'above ground' is based on whether nests were enclosed in soil and excavation occurred. Thus, 'below ground' included nests in compost heaps and in soil behind garden walls, such as retaining walls and railway sleepers. Nests located in or on any human structure were placed in the 'artificial structure' category. For details of all specific location categories, see Table 2.1.

Table 2.1: Location categories of nest sites included on questionnaire sheets

Location categories
Above ground:
Inside a tree trunk (*e.g. eucalypt*)
Between tree/shrub branches (*e.g. staghorn*)
Other above ground
Below ground:
In a compost heap
In roots of trees/shrubs (*e.g. bamboo*)
Behind a retaining wall (*e.g. railway sleepers*)
In a hole in the ground (*e.g. front lawn*)
Other below ground
In an artificial structure:
In a roof cavity (*e.g. under eaves*)
Inside a wall cavity (*e.g. between bricks*)
Inside an air vent
Under a dwelling floor (*e.g. wooden house floor*)
Inside furniture (*e.g. in a couch arm*)
Other artificial

Nest site proportions were compared with human population densities in individual council regions. Nest site proportions were arcsine square root transformed prior to the analysis to satisfy regression assumptions. Demographic data was obtained from the 2001 Census (Australian Bureau of Statistics 2001).

2.2.4 Nest collection

While the majority of nests used for seasonal nest analysis were obtained from the seven 'research councils', some were also reported by people who have visited the University's wasp web site (developed as a part of this project but no longer live), from personal communication, and also by intensive searching of likely wasp sites. Prior to collection, nests were anaesthetised with ~200 ml of diethyl ether poured directly into the nest entrance through a funnel with a 30 cm plastic pipe attached at the end, or over the nest envelope in cases of aerial nests. Nests were then left for 15-60 min to allow the majority of foraging workers to return to the nest, and then removed. As nest collection occurred during daylight hours, rarely were all foragers present in the nest at the time of collection. Aerial nests were simply collected, or cut out if necessary, while subterranean nests were excavated using a shovel, a pick and hand trowel. Care was taken to keep the nest structure intact.

Upon collection, nests were placed in plastic bags, labelled, and subsequently frozen at -20°C for at least 24 hours to ensure all workers within were dead before being analysed.

2.2.4 Nest analysis

Collected nests were pulled apart comb by comb and visually analysed. Each cell was classified as being large or small, and scored as containing one of the three developmental stages of wasps (egg, larva, sealed brood), or being empty. The number of meconia at the bottom of each cell was also recorded, as this reflects the number of generations that have been reared in each cell.

Each comb was sampled for sex ratios of sealed brood. This was done by randomly selecting a 30° wedge from an approximate centre of the comb and scoring identifiable sealed brood within as either worker, male, or queen.

The same wedge sub-sampling technique was used to determine the number of eggs laid in each egg-containing cell.

Numbers of emerged wasps were calculated based on generations (as per Archer 1980a). Briefly, all cells with one meconia (first generation empty and second generation egg, larva and pupa cells) were considered to have

produced one adult each; all cells with two meconia, two adults each, etc. Ultimately, data collected for each nest included the date of collection, and numbers of combs, small and large cells, emerged wasps, cells never used, meconia, and eggs, larvae, sealed brood and empty cells in each generation of wasps.

2.2.5 Traffic as an indicator of nest size

Prior to nest collection, an average wasp traffic rate was obtained. This was the mean of five one-minute counts of wasps entering and five one-minute counts of wasps leaving the nest. Traffic counts were made visually.

2.3 Results

2.3.1 Nest sites

Examination of 1,872 nest sites in 1999/00 and 768 sites in 2000/01 indicates that nesting patterns were similar for both seasons (Fig. 2.2). Overall, most nests were located 'below ground' (67 and 63%), with the majority found 'in a hole in the ground'. In 2000/01, 'below ground' nests were also frequently found amongst tree and shrub roots and behind garden walls. Nests located in 'artificial structures' were the second most abundant (29 and 32%), comprising mostly of nests inside wall cavities. Nests located above ground - inside tree trunks and hanging among tree/shrub branches - formed only a small proportion of nests (4 and 5%). While there was little variation in nest sites across Adelaide between seasons, this was not the case when the data were examined on a council by council basis (Fig. 2.3). In some council regions, the pattern of nest sites was similar to that across the whole of Adelaide (e.g. Adelaide Hills, Playford, Tea Tree Gully), but in other councils nests were found in artificial structures in preference to below ground (e.g. Prospect, Unley). In fact, proportion of nests below ground significantly reduced with human population density (Fig. 2.3; R^2 = 0.60, $F_{1,5}$ = 7.557, p = 0.04). An opposite trend was observed for nests in artificial locations, but this was statistically non-significant (Fig. 2.4; R^2 = 0.25, $F_{1,5}$ = 1.658, p = 0.25). On a seasonal level, patterns remained similar between councils (Fig. 2.3b).

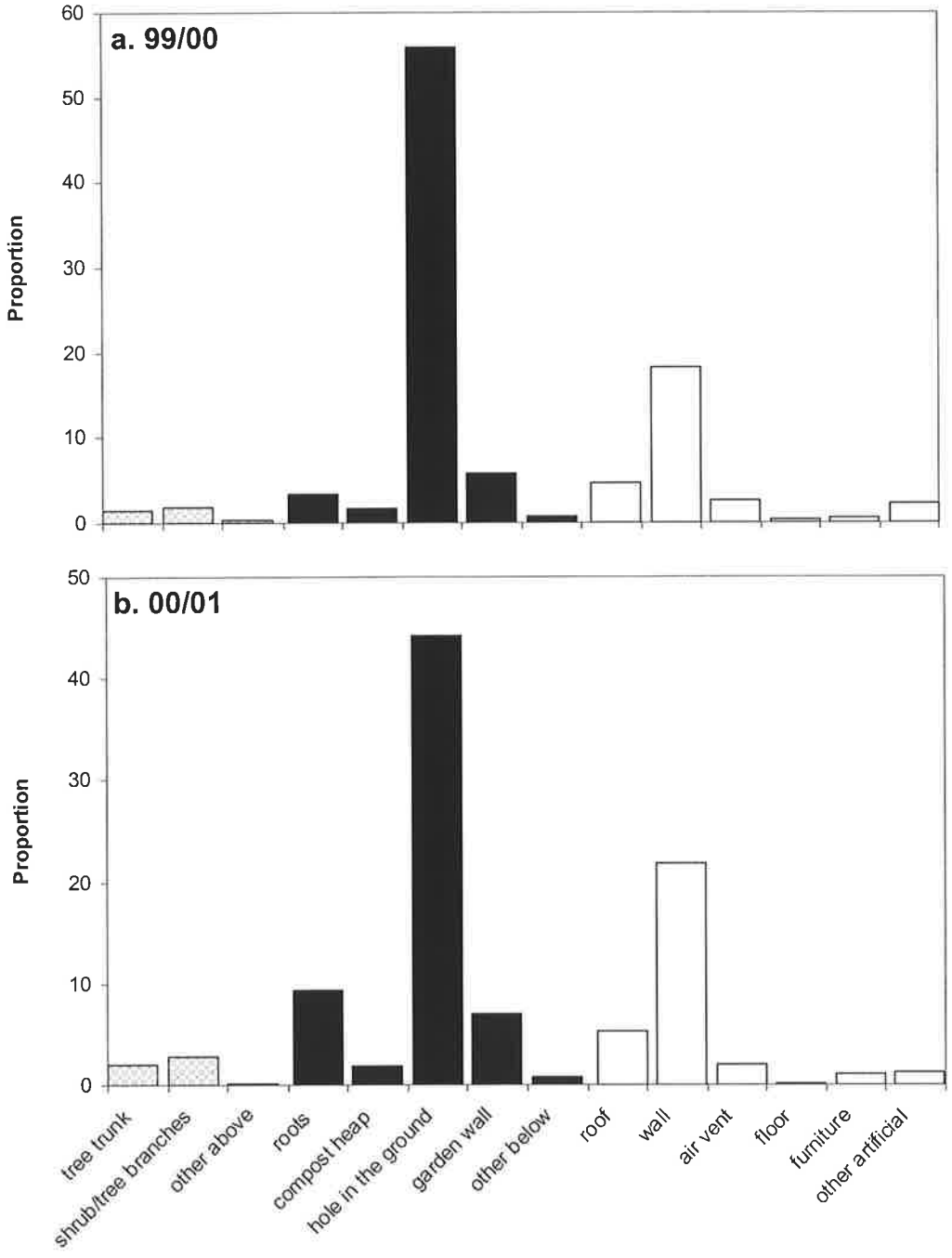

Fig. 2.2: Proportions of *V. germanica* nests found above ground (grey), below ground (black) and inside artificial structures (white) during (a) 1999/2000, and (b) 2000/2001 wasp season. Data is compiled from locations of nests destroyed by councils (see text).

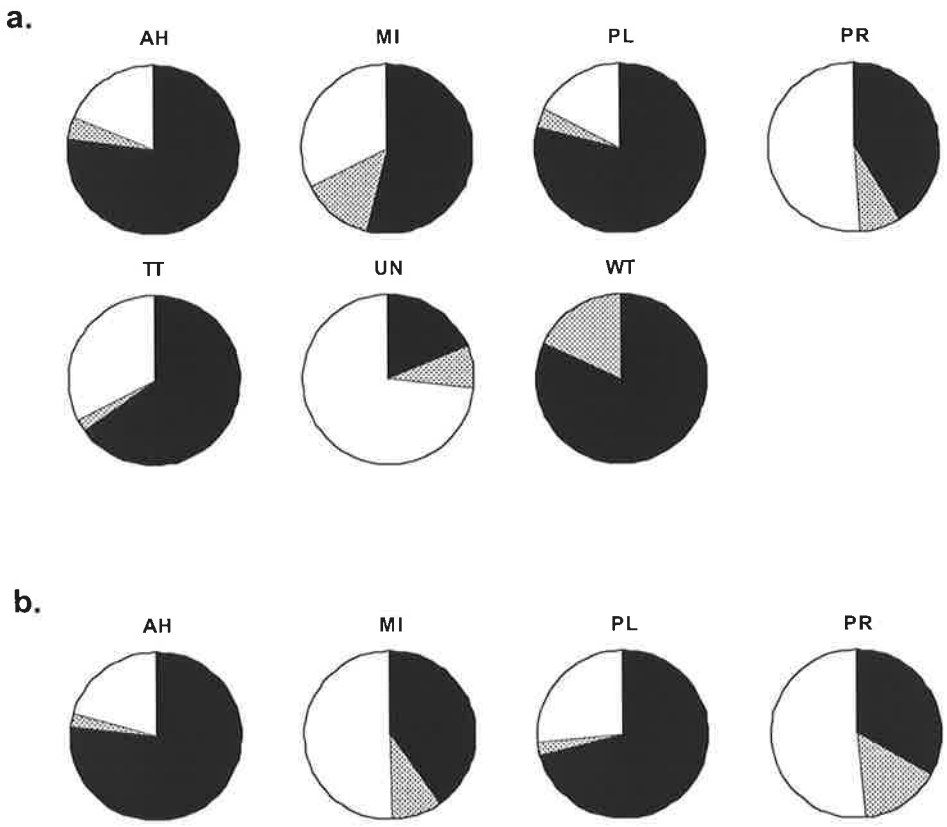

Fig. 2.3: Proportions of *V. germanica* nests found above ground (grey), below ground (black) and inside artificial structures (white) during (a) 1999/2000, and (b) 2000/2001 wasp season. Each pie represents a different council region. Council codes are as follows: AH = Adelaide Hills, MI = Mitcham, PL = Playford, PR = Prospect, TT = Tea Tree Gully, UN = Unley, and WT = West Torrens.

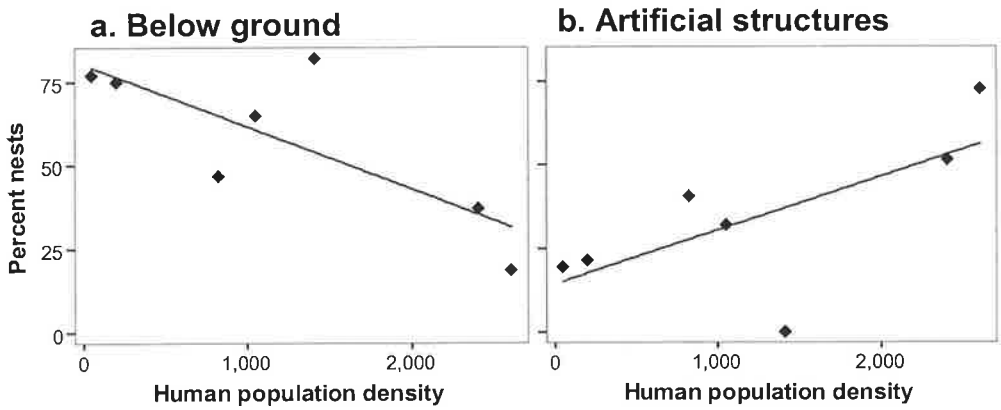

Fig. 2.4: Regression of percent of nests found (a) below ground and (b) in artificial structures on human population density. Mean nest data were used for each council region.

2.3.2 Seasonal abundance

Ninety-five percent of *V. germanica* nest reports in Adelaide were between November and May (n = 3,720; Fig. 2.5). The small percentage of nests found outside of this period (June-October) indicates the presence of some perennial nests. While the changes in numbers of nests destroyed between most months were gradual, there was a sharp increase between December (8%) and January (28%). To test whether this corresponded to an increase in wasp activity rather than being due to a delay in nests being reported and destroyed over the Christmas/New Year period, numbers of nests destroyed in the first half of January were compared with those destroyed in the second half. There were no differences between the two (sign-test, p = 0.481).

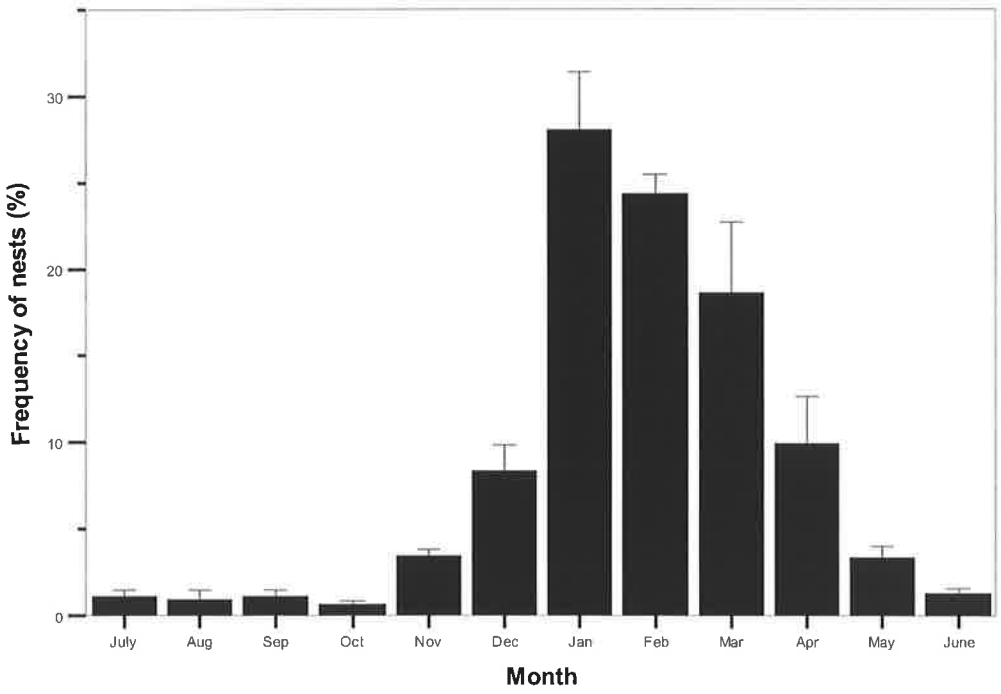

Fig. 2.5: Monthly frequencies of nests destroyed by 11 of the 20 metropolitan Adelaide councils pooled for the 1994/95-1995-96 and 1997/98-2000/01 wasp seasons. Error bars represent SE.

2.3.3 Nest size

Seventy two *V. germanica* nests were collected and analysed between November and May (1999/00 to 2001/02 wasp seasons; Table 2.2). This corresponds to the same time period when most nests in Adelaide are reported (2.3.2 above). Figure 2.6 shows the changes in nest size in terms of numbers of cells through time for each of the three study seasons. There appears to be an overall increase in nest size, with some nests reaching 20,000 small cells by January, however, a large variation between nests is also evident. For example, in the second half of January 1999/00, numbers of small cells varied between 204 and 21,902. Seasonal variation may also exist. It appears that nests in 1999/00 grew faster and reached larger size earlier on, however this may be due to relatively smaller sample sizes in the following two seasons. Unlike small cells, the variation in numbers of large cells among nests and seasons is small, with nests containing 3,000 - 5,000 large cells by May.

Table 2.2: Monthly frequencies of nests used in the current study.

Month	No. nests
NOVEMBER	3
DECEMBER	10
JANUARY	15
FEBRUARY	10
MARCH	14
APRIL	14
MAY	6

An exception to these patterns is evident in November 1999, when a nest containing worker and large cells was found (marked AH-002 on Fig. 2.6a). This was probably a nest surviving from the previous season. During the three seasons of the study, three other nests collected in November and December contained large cells, but these were not analysed due to time constraints. The perennial nest (AH-002) is excluded from all subsequent analyses.

Figure 2.6 summarises monthly cell totals during the study period. Numbers of small cells increased rapidly between November and February, and level off at an average of 9,593 cells for the remainder of the season. Large cells also increased in number between March and May, averaging 3,622 cells (Fig. 2.7).

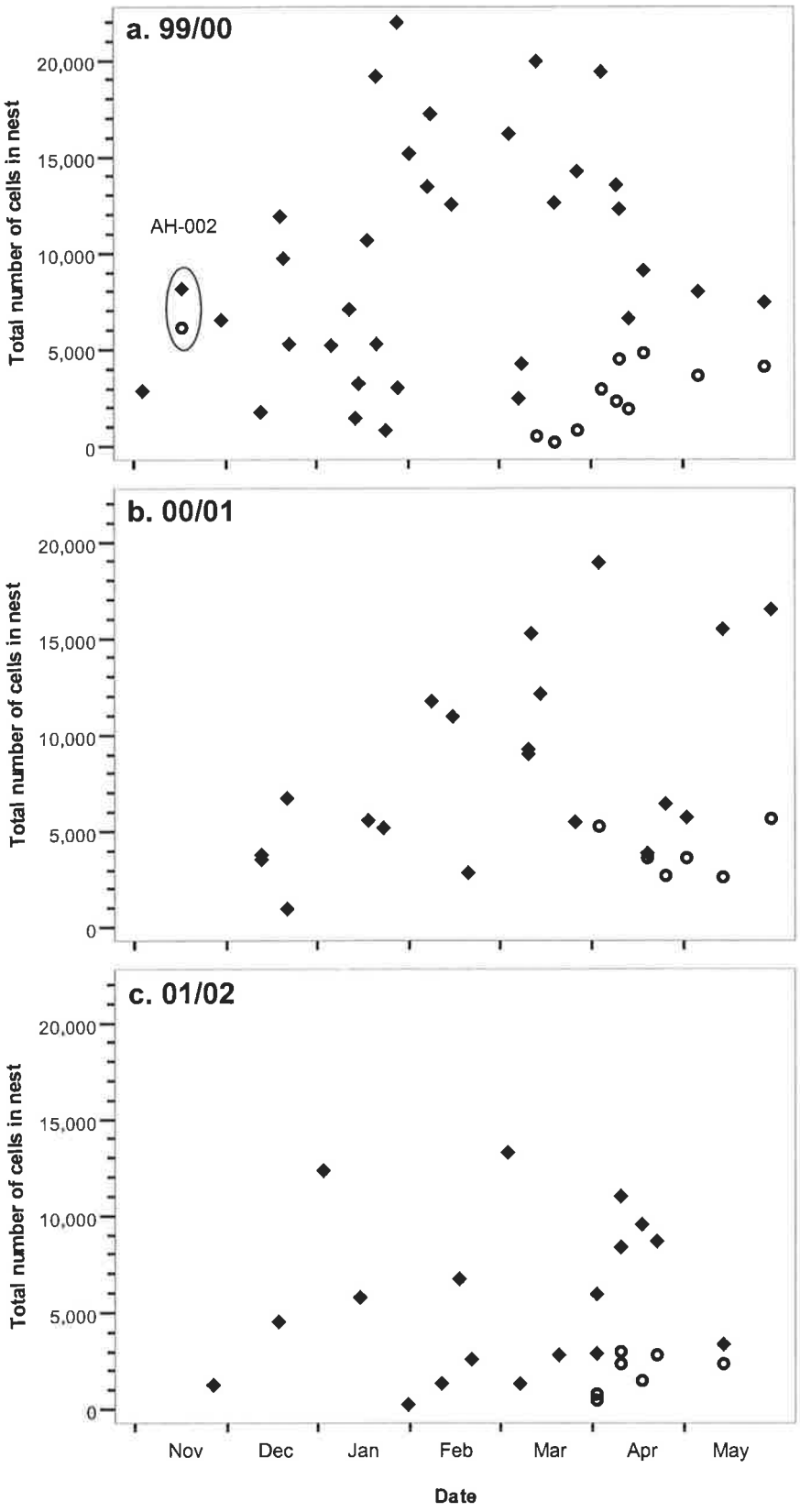

Fig. 2.6: Numbers of small (diamonds) and large (circles) cells inside *V. germanica* nests during (a) 1999/00, (b) 2000/01, and (c) 2001/02 wasp season. Nest marked AH-002 was an overwintered nest.

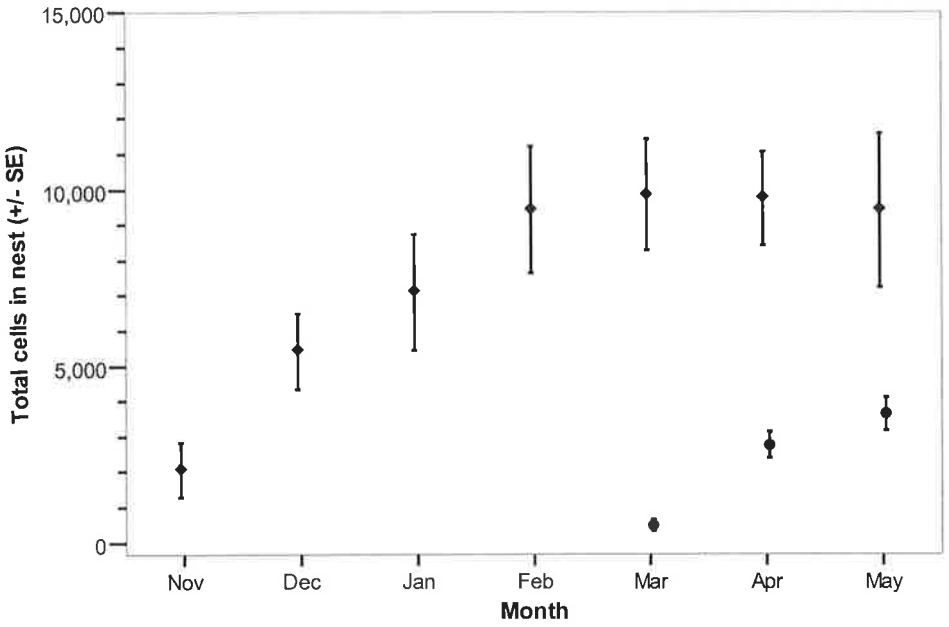

Fig. 2.7: Total numbers of small (diamond) and large (circle) cells each month inside annual *V. germanica* colonies with data pooled over three seasons (1999/00-2001/02). Error bars indicate SE.

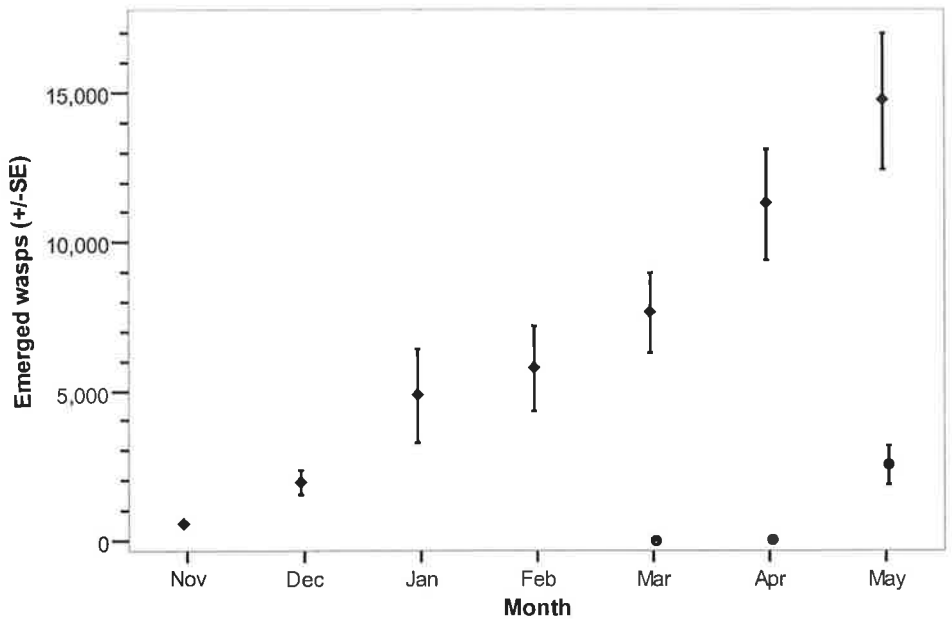

Fig. 2.8: Numbers of wasps emerged from small (diamond) and large (circle) cells inside annual *V. germanica* nests collected over three wasp seasons (1999/00-2001/02). Error bars show SE.

In contrast, when taking into account not only cell numbers but also numbers of times cells have been re-used, the increase does not reach a plateau (Fig. 2.8). In the large cells, emerged wasps are only evident in May, with numbers in March and April close to zero. This suggests that although building of new small cells stops in February, the rate of production of small-cell adults does not, and old cells are re-used for this purpose. Proportions of second and third-generation cells increased through the season, and by April, only 30% of small cells held first-generation wasps, and in May fourth generation cells were not uncommon. Large cells were generally only used once, but in May 50% had been re-used for the second time (data not shown).

Another measure of nest size that has previously been used to describe *Vespula* spp. nests is number of comb layers. From Fig. 2.9, it is evident that although the mean number of combs in the nest shows a seasonal increase, substantial variation exists among nests.

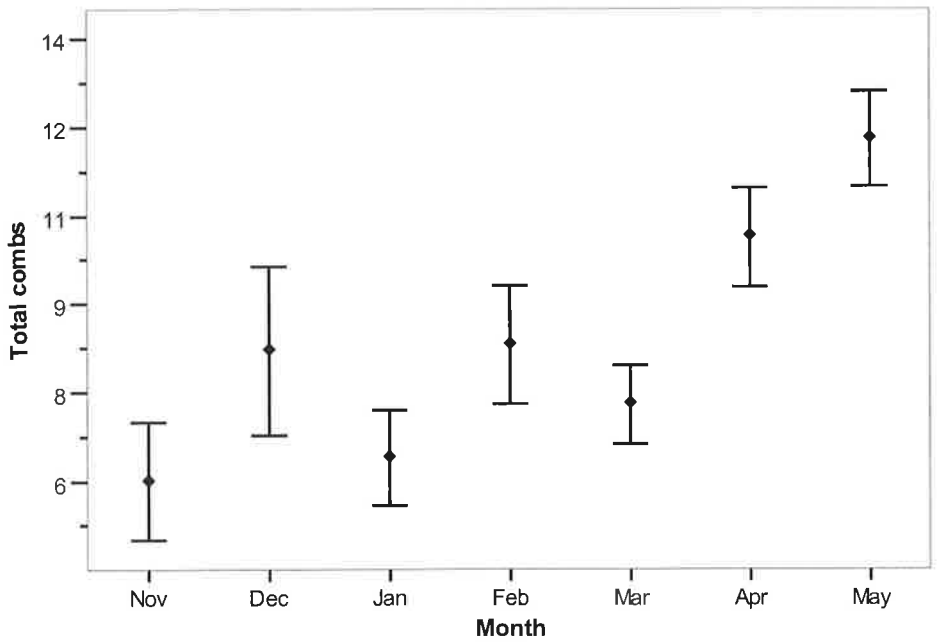

Fig. 2.9: Average numbers of combs (±SE) inside annual *V. germanica* nests each month for nests collected over three wasp seasons (1999/00-2001/02). Small, large and mixed combs are not distinguished.

2.3.4 Proportions of small and large cells

Large cells were not produced in nests until the end of March, but from then on their number increased sharply to constitute approximately 50-60% of all cells by May (Fig. 2.10). Again, the perennial nest, AH-002, can be seen as an exception, containing over 40% large cells in November.

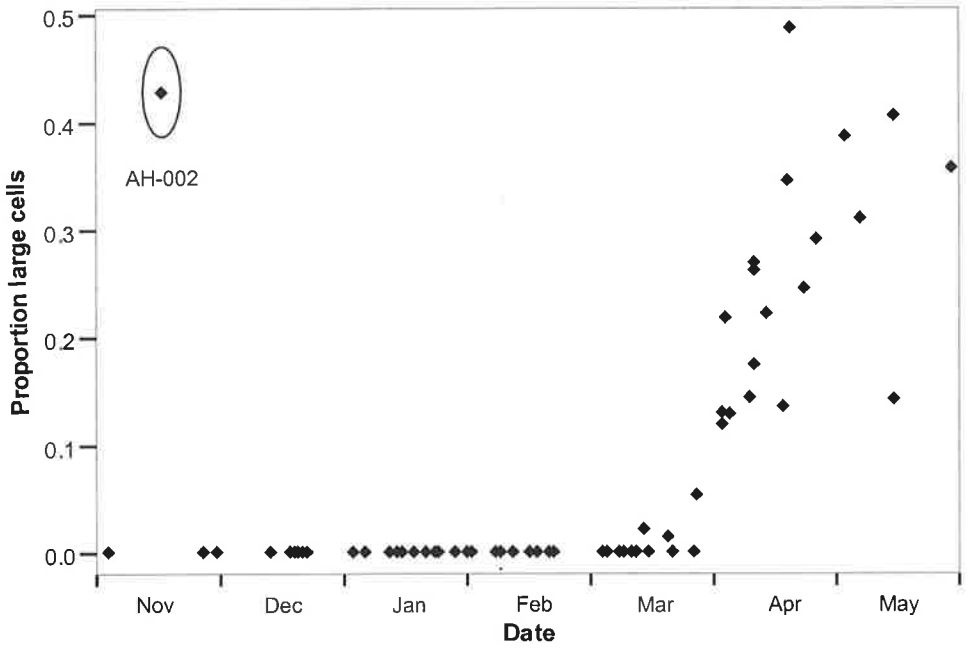

Fig. 2.10: Changes in proportion of large cells from November to May in *V. germanica* colonies. Data were collected over three wasp seasons, 1999/00-2001/02. Nest marked AH-002 is an overwintered nest.

2.3.5 Colony development

Proportions of cells taken up by different developmental stages were not constant over time (Fig. 2.11). Proportions of small cells containing eggs and larvae showed a gradual decrease from November to May, while the opposite trend was observed for empty cells. The number of cells with pupae remained proportionally constant from December to April, but were lower in November and May. In large cells, all three developmental stages showed an increase over time, while the proportion of empty cells decreased. Variation in the

relative proportions of the four stages was small among nests, except for proportions of eggs and empty cells in November and March for small and large cells respectively.

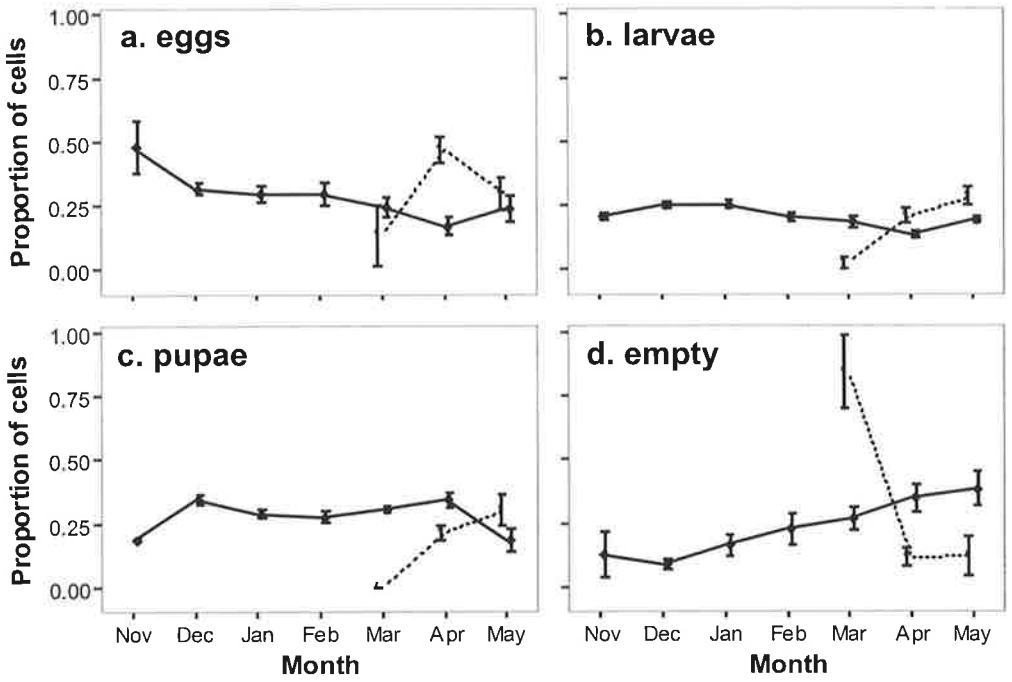

Fig. 2.11: Monthly averages of proportions of small (solid line) and large (dotted line) cells containing (a) eggs, (b) larvae, (c) pupae, and (d) no brood in *V. germanica* colonies. Data is pooled for three seasons (1999/00-2001/02). Error bars show SE.

2.3.6 Sex ratios of brood

In November, only the worker caste was recorded from small cells (Fig. 2.12a). Males were first produced in small numbers in December, and their relative proportion in small cells increased gradually to an average of 73% by the end of May. Occasionally, small cells also held queens (3%). Large cells only contained pupae in April and May. During both of these months, queens occupied 70-72% of all cells, with the remainder being used to rear males (Fig. 2.12b). Approximately 1% of large cells contained worker pupae.

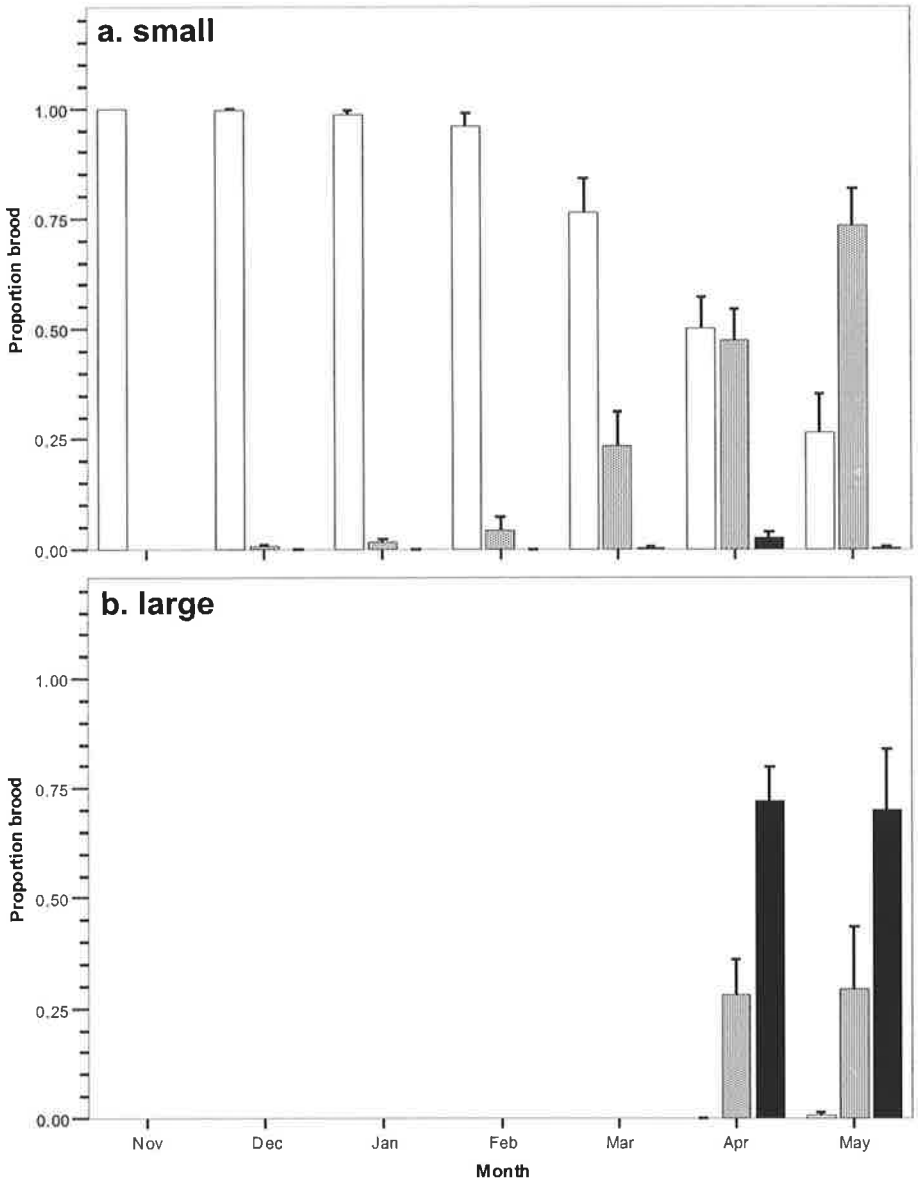

Fig. 2.12: Monthly average proportions of worker (white), male (grey) and queen (black) brood inside annual *V. germanica* nests with data pooled over three seasons (1999/00-2001/02). (a) brood in small cells, (b) brood in large cells. Error bars show SE.

2.3.7 Eggs per cell

From November to February, the mean number of eggs per cell was one, increasing to 1.5 in March and April (Fig. 2.13). This higher value of 1.7 eggs per cell in January was mostly due to one nest. By May, the average number of eggs per cell was above three, for both small and large cells.

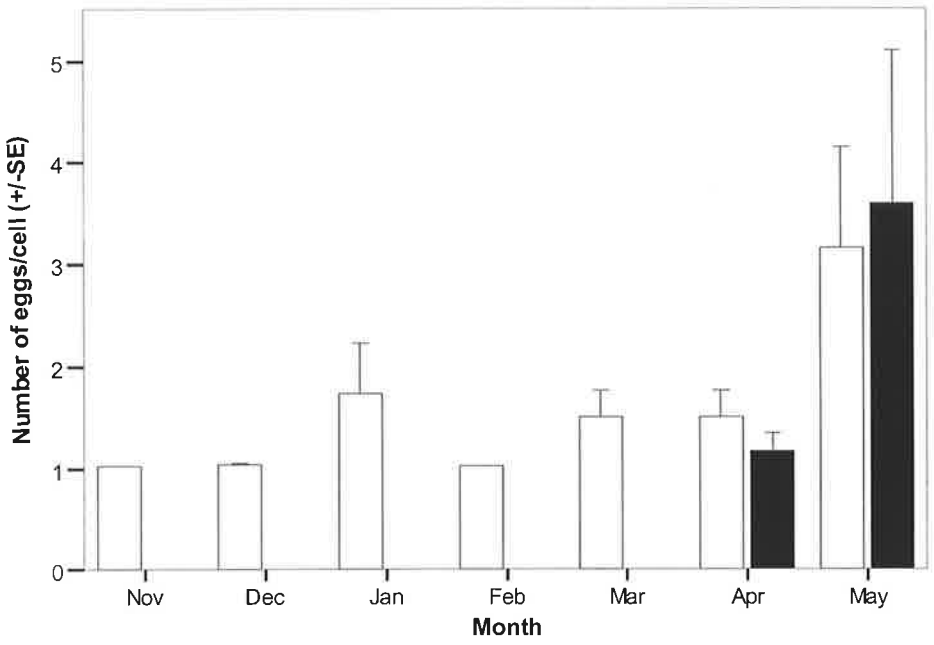

Fig. 2.13: Monthly means of numbers of eggs per cell inside annual *V. germanica* nests with data pooled over three seasons (1999/00-2001/02). Small cells are represented in white, and large cells appear in black. Error bars show SE.

For small cells, the seasonal increase in multiple eggs is caused by a decreasing proportion of cells containing one egg in the lower combs, as well as an increasingly larger variation among nests (i.e. in newer parts of the nest, Fig. 2.14a). In large cells, there is no pattern of change in the proportion of multiple egg cells with comb number, but large variation among nests is evident once again (Fig. 2.14b). In some instances, up to 16 eggs were recorded in one cell, while other cells on the same comb contained only one egg.

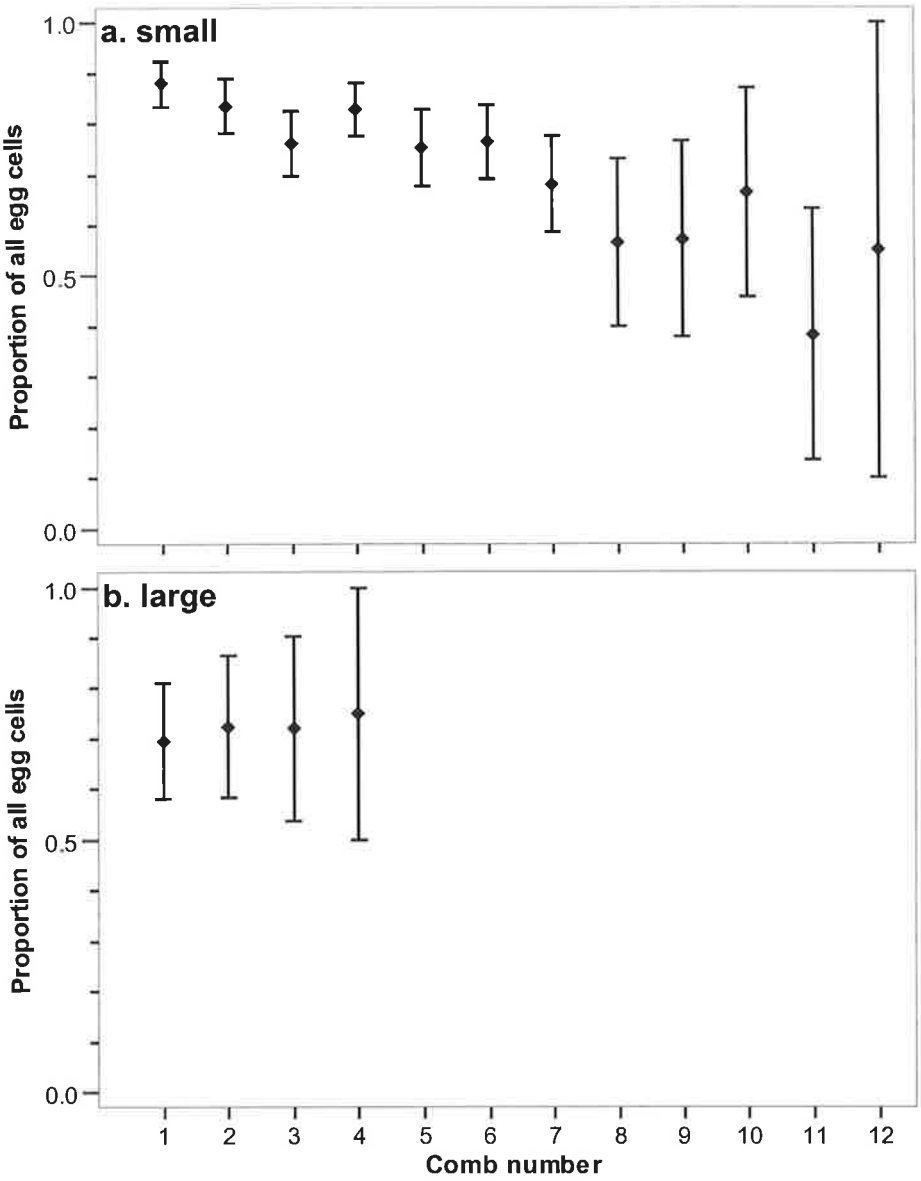

Fig. 2.14: Mean proportion (±SE) of egg-cells containing only one egg at various levels in the nest in (a) small and (b) large cells of annual *V. germanica* colonies. Combs were numbered from the top of the nest. Data are for the 1999/00-2001/02 wasp seasons.

2.3.8 Traffic as an indicator of nest size

Seasonal wasp traffic followed a pattern similar to that observed for the total number of cells; reaching a plateau after an initial increase (Fig. 2.15). Linear regressions of traffic rates with number of cells, emerged wasps, eggs, larvae, pupae and empty cells were all significant for small cells and total cells but not for large cells (Table 2.3). The best fit was for total number of cells (R^2 = 0.37).

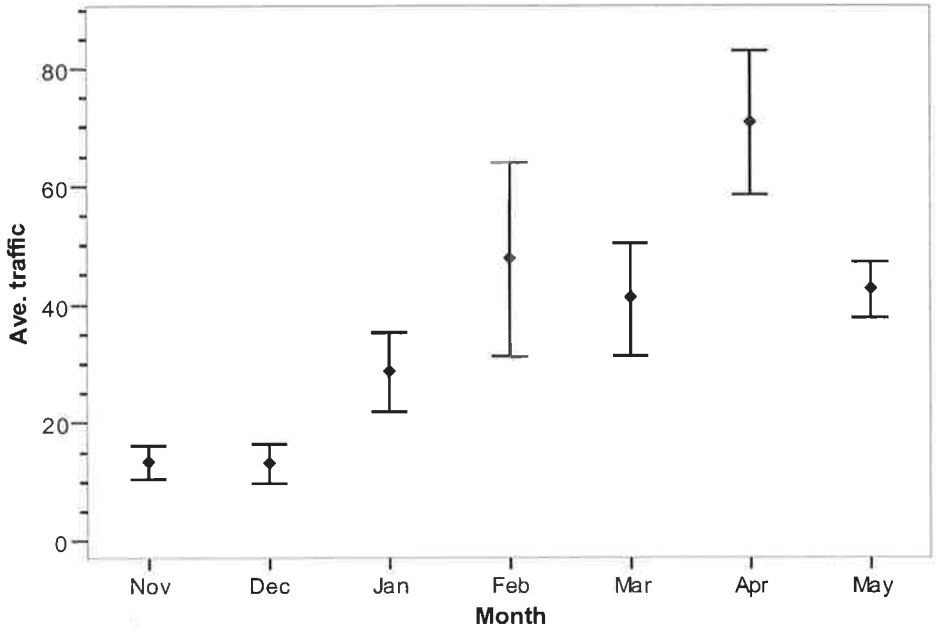

Fig. 2.15: Monthly means of traffic rates observed at the entrance of annual *V. germanica* nests (1999/00-2001/02). Error bars show SE.

Table 2.3: Linear regressions of traffic rate as a function of several measures of nest size. Significance of α is indicated by asterisks, where * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.

Nest size	Size	R^2	d.f.	F	α	β	p
TOTAL CELLS	SMALL	0.291	62	25.466	11.11	3.63×10^{-3}	<0.001
	LARGE	0.136	20	3.154	34.30	9.96×10^{-3}	0.091
	TOTAL	0.369	62	36.270	7.16	3.68×10^{-3}	<0.001
EMERGED WASPS	SMALL	0.239	62	19.472	20.89***	2.82×10^{-3}	<0.001
	LARGE	0.040	20	0.844	66.32***	-6.00×10^{-3}	0.369
	TOTAL	0.219	62	17.337	22.24***	2.54×10^{-3}	<0.001
EGGS	SMALL	0.117	62	8.230	24.21**	8.74×10^{-3}	0.006
	LARGE	0.097	20	2.045	46.30**	1.53×10^{-2}	0.169
	TOTAL	0.176	62	13.248	22.16**	8.35×10^{-3}	0.001
LARVAE	SMALL	0.154	62	11.323	21.33**	1.13×10^{-2}	0.001
	LARGE	0.081	20	1.774	47.04**	2.05×10^{-2}	0.198
	TOTAL	0.250	62	20.652	14.89*	1.31×10^{-2}	<0.001
PUPAE	SMALL	0.282	62	24.330	15.03*	9.26×10^{-3}	<0.001
	LARGE	0.117	20	2.647	42.49**	2.79×10^{-2}	0.119
	TOTAL	0.366	62	35.787	9.83	1.02×10^{-2}	<0.001
EMPTY CELLS	SMALL	0.195	62	15.043	26.06***	8.60×10^{-3}	<0.001
	LARGE	0.006	20	0.121	64.21***	-7.32×10^{-3}	0.732
	TOTAL	0.197	62	15.173	25.91***	8.20×10^{-3}	<0.001

2.4 Discussion

2.4.1 Nest sites

Previous studies of nesting preferences of *V. germanica* show great variation in the predominant nest site for this species. Studies in Melbourne, England, and New Zealand have shown that 45-90% nests are found below ground, 3-20% are in trees, and 3-52% are in artificial locations (Spradbery 1971; Archer 1989; Crosland 1991; Donovan *et al.* 1992). Moller *et al.* (1991a) observed marked variation in nesting patterns between urban and rural/forest locations in New Zealand. They showed that in beech forests and in horticultural areas, 100% of nests were located underground, but in urban areas only 60% were subterranean, while 30% were found in artificial structures such as roofs, attics or walls (Moller *et al.* 1991a). The current study found similar variation between individual locations, with wasps building fewer nests below ground in highly populated council regions (Fig. 2.4). In these heavily built-up locations, subterranean nest sites are replaced by buildings, concrete and asphalt, and thus their availability is diminished. Where subterranean nests are constructed, they are more susceptible to flooding from run-off water, and may therefore die before being reported. Some densely populated council regions in Adelaide also have high water tables (e.g. Unley, Prospect; G. Tassel pers. comm.), preventing the establishment of subterranean nests.

These studies suggest that *V. germanica* does not have a preference for nesting sites as such, but rather will utilise available sites. Furthermore, as nest sites reported in the current study were collated from records of already established nests, they do not necessarily reflect the wasps' preference for nesting locations as such, but rather a survey of sites of successful nests. Indeed, Spradbery (1973) proposed that many queens select sites that are not suitable for the establishment or the eventual growth and expansion of colonies from the beginning.

2.4.2 Seasonal abundance

The duration and timing of wasp nests found in the current study is comparable to that reported from nest destruction programs in other parts of Australia

(Crosland 1991). However, maximum nest destruction in Adelaide occurs in January compared to February in Hobart and Melbourne, while no peak is observed in Sydney. As the pattern of mean monthly temperatures and rainfall for Adelaide is closer to those for Hobart and Melbourne rather than Sydney (Spradbery and Maywald 1992), the finding that the wasp season in Adelaide is less similar to the one in Sydney is not surprising. In New Zealand, studies of *V. germanica* seasonality in Christchurch showed large annual differences (Donovan *et al.* 1992). In some years, seasonal abundance was remarkably similar to the one described for Sydney, with wasp nests being found from November till June, while in other years the numbers of nests destroyed remained relatively constant throughout the year, indicating a large proportion of overwintered nests. Comparable nest destruction data does not exist for England, however nests were found by active searching between July and October (corresponding to January - April in the Southern Hemisphere; Spradbery 1971; Archer 2001b).

The disadvantage of relying on nest destruction data is that queen and early small-cell nests, sustaining only small wasp traffic and thus not being immediately obvious, are unlikely to be recorded. During the three study seasons in Adelaide, flying *V. germanica* queens were observed between August and January (pers. obs.). In a survey of artificial hives set up for bumble bees in Christchurch, New Zealand, Donovan (1991) reported *V. germanica* to initiate nests between mid-September and November. In another study in the Manawatu region, also in New Zealand, Leathwick *et al.* (1999) indicated that wasp colonies were present from September till May. In England, queens were described searching for nest sites as early as May, with queen nests being found by mid-June (Archer 2001b). Thus, it appears that queen nests are started six weeks to two months before the emergence of workers. In Adelaide, this occurs in about September.

Therefore, the length of *V. germanica* season in Adelaide is mostly similar to other places in Australia and New Zealand, lasting at least seven months. In its native range in England, the season is much shorter, lasting only four months. These differences are undoubtedly due to longer summers and milder conditions experienced in the wasps' introduced range.

2.4.3 Nest size

Patterns of seasonal nest growth obtained for *V. germanica* in Adelaide are comparable with other studies that measured nest size in a similar fashion. For example, data from England show numbers of cells increasing to a plateau, while numbers of emerged adults increase linearly with season (Spradbery 1971; Archer 2001b). In New Zealand, the plateau reached March and May was not as pronounced, with a slight increase in cell numbers continuing (Leathwick *et al.* 1999).

Other studies have used different methods of determining nest size. These include numbers of comb layers (e.g. Fordham *et al.* 1991; Donovan *et al.* 1992; Ward *et al.* 2002) and total area of combs (e.g. Donovan *et al.* 1992; Ward *et al.* 2002). While these methods are quicker, they are less accurate and not as informative as detailed cell-by-cell analysis, for example they do not distinguish between small and large cells, or various stages of brood development. When results obtained in this study are compared with studies using other measures of nest size, the patterns differ. Donovan *et al.* (1992) noted a general seasonal increase in numbers of combs in Christchurch, New Zealand, also observed in this study. However, Fordham *et al.* (1991) in Manawatu, New Zealand, and Ward *et al.* (2002) in eastern Australia found a linear relationship between sampling date and number of combs present. When two of these studies examined nest size in terms of nest area, the results show the same pattern of linear increase with season (Donovan *et al.* 1992; Ward *et al.* 2002).

Although the patterns of seasonal changes in nest size are similar between Australia, New Zealand and England, differences arise in the length of the wasp season, as already described in 2.4.2, and maximum nest sizes reached by the end of the season, measured as numbers of cells in the current study. In Adelaide, mature colonies contained an average of 9,593 small cells and 3,622 large cells. In England, colonies contained an average of 6,540 small and 1,563 large cells (Archer 2001b). When considering the maximum rather than average sizes, the differences are even greater, with nests in Adelaide producing up to 21,902 small and 5,599 large cells, while nests in England contained up to 11,961 small and 2,886 large cells (Archer 2001b). Similarly, more wasps were produced in nests in Australia, with almost 15,000 small-cell

brood and over 2,500 large-cell brood having emerged by May. In England, the estimated mean number of adults produced from nests was 11,190 in small cells, and 1,342 in large cells (Archer 2001b). Unfortunately, no comparable data exists for nests in New Zealand as these were not measured in terms of cell numbers. However, areas of combs reported by Fordham *et al.* (1991) in Manawatu, New Zealand, and Ward *et al.* (2002) in eastern Australia fall within the same range, indicating that nests in New Zealand and Australia are of approximately similar sizes. These figures indicate that nests in Adelaide are larger, and produce more wasps than those in England.

2.4.4 Large and small cell proportions

Large cells were present in wasp nests for the last three months of the wasp season, corresponding to previous studies of the species in Australia (Ward *et al.* 2002) and England (Archer 2001b). However, while in Australia nests contained 45% large cells by the end of this period, in England this was only 20%. Therefore, in Australia, *V. germanica* not only build larger nests with more cells (2.4.3), but also produce proportionally more large (reproductive) cells than in England. This is possibly due to nests containing greater numbers of workers during the large-cell colony phase.

2.4.5 Colony development

Seasonal trends in the relative proportions of immature life stages in this study corresponded well with previous research in Australia, New Zealand and England. Ward *et al.* (2002), Fordham *et al.* (1991), and Archer (2001b) agree that the proportion of empty cells increases with time while the proportion of larval cells decreases. However, where Ward *et al.* (2002) found these increases to be linear, the current study identified non-linear trends. For example, the proportion of pupae in November and May was much lower than in other months (Fig. 2.11b). A similar non-linear trend was described by Fordham *et al.* (1991) and Archer (2001b). However, Ward *et al.* (2002) only examined the second half of the wasp season, February to May, and thus any differences in the earlier part of the wasp lifecycle were not identified.

It is remarkable that the variation in proportions of eggs, larvae and pupae observed in Adelaide is relatively small between nests, despite the fact that samples were taken from various locations and over three seasons. The exceptions are empty and egg-containing small cells in November, and empty and egg-containing large cells in March. The variation most likely reflects differences between habitats, and thus optimal conditions for cell building/egg laying being reached at slightly different times.

2.4.6 Sex ratios

2.4.6.1 Workers, males and queens produced in nests

The results obtained in this study suggest that by the end of the wasp season, males form 73% of all small cell brood, and 29% of large cell brood (Fig. 2.12). These ratios are more male biased than previously reported for mature *V. germanica* colonies in England. Archer (2001b) found only 42% of small and 1% of large cells being occupied by males. Using these sex ratios and nest sizes, the largest colony in England produced 7,991 workers, 3,215 males and 1,326 queens (Archer 2001b). In comparison, an average colony of *V. germanica* in South Australia produced 11,537 workers, 3,832 males and 1,848 queens, while the largest colony produced 21,283 workers, 7,443 males and 4,045 queens. Thus, an average colony in Adelaide produced more workers, males and queens than the largest colony in England, while the largest colony in Adelaide produced nearly three times as many workers and queens, and twice as many males than in England.

Larger nests produce more queens (this study), and queens from large nests are also bigger and heavier than ones produced in smaller nests (Harris and Beggs 1995). Preliminary findings suggested that heavier queens can hibernate for longer (see Appendix 2). Additionally, smaller sizes are under represented in founding queens, possibly due to winter mortality (Harris and Beggs 1995). Winter mortality may be reduced in Australia due to the milder climate (compared with 98% winter queen mortality suggested in England, Archer 1980a). Thus, producing a larger number and better quality queens per nest may facilitate a faster rate of annual population increase in Australia (but see Ch. 6).

2.4.6.2 Queen production in small cells

Queen production in small cells was previously described by Spradbery (1993) in *V. germanica* nests collected in New South Wales, ACT and Victoria, Australia. In *Vespula* spp., queen/worker caste is determined during the fourth larval instar, and depends on feeding regimes (Potter 1964). Normally, the amount of food administered to a larva is determined by cell size, with larvae in large cells being fed more. Spradbery (1993) suggested that queen brood produced in small cells were simply due to 'overfeeding'. The current study found up to 3% of worker cells to be occupied by queen brood, however, this only occurred in April. In contrast, Spradbery (1993) found that only 0.5% of small cells held queens, but they were present at all times from January to early March. Fordham (1991) reported that multiple queens were occasionally found in immature nests in New Zealand. These may have been new queens produced in small cells.

Production of workers from large cells has not been reported previously. Workers in large cells were clearly distinguishable from queens by their slimmer bodies, and cell caps that were sunk below the edge of the cell, so chances of misidentification were small. Workers in large cells could be produced by 'underfeeding'. Clearly more research needs to be conducted into what factors determine queen/worker caste and, indeed, whether small-cell produced queens, or large-cell produced workers only occur in Australia, or are also found elsewhere.

2.4.7 Eggs per cell

Excess numbers of eggs per cell has previously been noted as being one of the distinguishing features of overwintered *Vespula* colonies, as it suggests loss of control by the foundress queen (Spradbery 1973). One such colony from Tasmania, containing almost 34,000 small cells, averaged 2.6 eggs per cell. Another overwintered colony had up to 20 eggs per cell (Spradbery 1973). For annual colonies of *Vespula* spp., data on numbers of eggs per cell have not generally been recorded. An average of 13 cells containing more than one egg was reported per *V. germanica* colony in England (Spradbery 1973). In an average mature colony, where the total number of cells is 8,103 (Archer 2001b),

this equates to 0.1% of all cells containing multiple eggs. In the U.S.A., the proportion of cells containing more than one egg in colonies of *V. vulgaris* did not drop below 92% at any one time (Akre and Myhre 1991).

In polygynous overwintered colonies, multiple eggs are thought to result from a combination of pressure on new queens to lay eggs, and a lack of empty cells (Spradbery 1973). In the current study, it is unclear whether multiple eggs were laid by the foundress queen, by new queens, or by workers. Multiple eggs generally occurred towards the end of the season, and it is possible that the original queen had died in some colonies. However, large numbers of worker and queen brood were present in all colonies, suggesting the presence of at least one queen. Alternatively, excess eggs could have been laid by workers. Normally, development of worker ovaries is suppressed by a queen secreted pheromone (Akre and Reed 1983). But workers with developed ovaries have previously been recorded from larger nests and late-season colonies (Ross 1985). Furthermore, a recent study of genetic relatedness of workers within annual *V. germanica* colonies suggested that although most egg laying is done by the queen, workers produced males in some nests (Goodisman *et al.* 2002). However, multiply-mating species such as *V. germanica* (Goodisman *et al.* 2002) should experience worker policing of worker laid eggs (Ross 1986) and thus worker reproduction should be minimal. Once again, this area requires further study.

2.4.8 Traffic as indicator of colony size

The present study found that traffic rates followed a seasonal pattern similar to that for the total number of cells in a colony. Furthermore, a significant linear relationship existed between traffic rate and various measures of colony size, with total number of cells providing the best fit. These findings support previous research on *V. germanica* and *V. vulgaris* wasps in England and in New Zealand. Working with mature colonies, Malham *et al.* (1991) found a correlation between traffic and number of workers present in the nest. In contrast, Potter (1964) found a significant correlation between traffic and number of workers present in a nest during the initial rapid nest expansion, while in mature colonies there was a significant relationship between traffic and

number of larvae. Adult wasps present in the nest were not counted in the current study so it is impossible to determine whether a more significant relationship could be obtained between traffic and numbers of workers. As the worker to larvae ratio does not stay the same during the season (Spradbery 1971; Fordham *et al.* 1991), differences similar to those observed by Potter may be expected. However, despite being based on data obtained over seven months and from three seasons, the current study found significant relationships between traffic and colony size. Thus, it is likely that not one but a combination of factors determine traffic rate. Changes in colony needs, such as larval and worker food requirements, pulp for nest building and expansion, and water for nest thermoregulation (see Ch. 3), but also environmental factors constraining foraging (e.g. temperature, see Ch. 4) should be considered. Also, some traffic measurements obtained in this study were made at temperatures that are sub-optimal for wasp foraging (Ch. 4), and these would have influenced the data.

CHAPTER 3:

CHANGES IN DAILY AND SEASONAL FORAGING CHARACTERISTICS

3.0 Chapter summary

Vespula wasps are known to forage for protein prey, carbohydrates, pulp, and water. The first two resources provide food for the colony and the wasps themselves. Pulp, when mixed with water, is used for nest expansion. Water is also used for thermoregulation inside the nest. Changes in daily and seasonal proportions of these resources were followed by sampling returning foragers over the most active part of the season – February to May. Over 3,000 foragers were sampled returning to 16 nests. Overall, 54% of all wasps returned with carbohydrate in their crops. Approximately 13% of foragers brought prey, while pulp and water foragers made up 3% each. About 27% of wasps returned to the nest with nothing. Changes in these resource proportions were not significant on a daily basis. When these changes were examined on a seasonal basis, proportions of foragers returning with prey increased, proportions of foragers returning with nothing decreased, while foragers returning with pulp, water or carbohydrate did not change. However, the proportion of wasps foraging for water increased with rising ambient temperature, while the proportion of carbohydrate foragers decreased. Further examination of crop carbohydrate revealed that sucrose concentration did not change during the season. These results contradict previous hypotheses that *Vespula* foragers switch from protein prey to carbohydrate food sources with the onset of autumn, as the results obtained did not show a seasonal increase in the proportion of carbohydrate over protein foragers, nor did they show an increase in sugar concentration with time. This suggests that the increasingly higher energy requirements of nests are satisfied by an increase in the number of loads rather than quality of each load.

3.1 Introduction

In social insect colonies, foraging is governed by an interaction between internal colony needs, food resources and the physical environment (Porter and Tschinkel 1987). Internal needs are controlled by hunger and rates of brood production (Wallis 1962; Harris 1995). These needs depend on the availability and the nutritional value of food resources brought into the nest by foraging workers (e.g. Howard and Tschinkel 1981; Traniello *et al.* 1984). However, even with abundant resources, foraging may often be restricted by the physical environment – daylight controls navigation, while temperature and humidity may limit foraging trip frequency and duration (e.g. Talbot 1943; Wehner 1976; Marsh 1985). Environmental factors may also determine the distribution and availability of resources.

Changes in internal needs are especially evident in social insect colonies with an annual life cycle, such as the eusocial Vespidae. In such species, nests are typically initiated by a single inseminated queen in spring; increase in numbers of workers, larvae and physical size during summer; and produce large numbers of reproductive males and queens before finally disintegrating in early autumn (Potter 1964). As nests grow and expand, their needs for food and building materials can also be expected to change.

The resources utilised by social wasps include wood fibres (pulp), water, and food (Edwards 1980; Raveret Richter 2000). Pulp and water are mixed to construct and expand nests. In the Vespinae, which build protected nests and keep them at constant temperatures, water is also used for thermoregulation (Wilson 1971). Food resources collected by these wasps include carbohydrates in the form of nectar or honeydew, and proteins, predominantly in the form of other invertebrates, but also small portions of vertebrate remains (Edwards 1980; Kasper *et al.* 2004, see Ch. 5). Prey and pulp are carried back to the nest in the wasps' legs and mandibles, while water and carbohydrates are carried in the crop and can be later regurgitated (Hunt 1991). Both carbohydrates and protein prey are used to satisfy energy requirements of brood and adults, egg maturation in the queen, and to build up fat reserves in new reproductives (Archer 1998b). It has been suggested that protein foods

are predominantly fed to the larvae, while carbohydrate foods are mostly used by adult wasps (Archer 1977; Hunt 1991). Therefore, an increase in the relative proportion of wasps foraging for carbohydrate might be expected with increased worker to larvae ratios within the nest, as occurs towards the end of a colony season (Edwards 1980). Such changes have been suggested to exist, especially as wasps are frequently seen visiting carbohydrate rich sources towards autumn (Thomas 1960). Changes in other resources should also occur on a seasonal basis depending on internal needs and environmental factors.

Few previous studies have examined how the composition of *Vespula* resource use changes due to nest requirements and environmental conditions. The relative amounts of prey, pulp and fluids collected by various vespids have been studied to some extent (see Edwards 1980 for summary). These studies of laboratory-kept colonies were built upon by Archer (2000), who sampled colonies of *V. vulgaris* in England over a three month period, and compared the results with colony growth patterns. Other studies have examined the changes in composition of prey orders with season, and concluded that a shift in prey types occurs (Gambino 1986; Harris 1991; Sackmann *et al.* 2000). No previous studies have examined variation in seasonal crop carbohydrate concentrations.

Although *V. germanica* is native to Europe and Asia (Edwards 1976; Spradbery and Maywald 1992), it has recently been widely studied in its introduced range in New Zealand and Argentina, where it has become a human and an environmental pest (e.g. Clapperton *et al.* 1989a; Beggs and Wilson 1991; Harris *et al.* 1994; Sackmann *et al.* 2001). The species has also become established in Australia, but despite its potential impact on the native flora and fauna (Bashford 2001), relatively little research has been conducted on the species to date. This chapter aims to provide the first comprehensive picture of changes in daily and seasonal foraging characteristics of *Vespula* wasps. To achieve this, the combined effects of colony needs and environmental factors on daily and seasonal changes in resources collected by foraging workers were examined. Furthermore, to determine if seasonal changes in dietary use occur, variation in prey types collected and carbohydrate concentration in the crop were also measured.

3.2 Materials and Methods

3.2.1 Study area

The study was carried out in metropolitan Adelaide, South Australia and surroundings (34°55'S, 138°36'E). Adelaide has a Mediterranean climate with average maximum daily temperatures of 30°C in January to 12°C in July, average minimum daily temperatures of 16°C in February to 7°C in July, and an average annual rainfall of 400 mm (Commonwealth Bureau of Meteorology 2004). Samples were collected from nests in a variety of habitats, ranging from heavily built up urban areas to patches of native vegetation. Locations of nests were reported by local councils, as well as members of the public (Table 3.1).

Table 3.1: Details of locations of nests used to determine changes in daily and seasonal foraging characteristics of *V. germanica*.

Month	Nest ID	Location	Foragers captured
February	Sad	34° 56.298'S, 138° 34.676'E	39.8 ± 2.267
	Ar1	34° 58.228'S, 138° 37.762'E	44.6 ± 4.020
	K8	35° 01.034'S, 138° 43.600'E	51.0 ± 4.219
	K2	35° 00.980'S, 138° 43.685'E	33.0 ± 3.507
March	K4	35° 00.950'S, 138° 43.540'E	50.4 ± 4.665
	Ver	35° 00.335'S, 138° 48.350'E	45.8 ± 0.860
	KW	34° 55.350'S, 138° 50.279'E	31.0 ± 0.707
	Wl1	34° 58.032'S, 138° 37.012'E	27.8 ± 0.860
April	Wi2	35° 01.525'S, 138° 36.600'E	36.2 ± 1.655
	Vd3	35° 00.341'S, 138° 48.358'E	34.0 ± 1.049
	SC	35° 00.156'S, 138° 43.956'E	48.8 ± 1.715
	LRd	34° 55.380'S, 138° 80.200'E	34.4 ± 0.678
May	Vd1	35° 00.341'S, 138° 48.359'E	33.4 ± 1.288
	Wl4	34° 58.021'S, 138° 37.138'E	33.8 ± 1.393
	Ar3	34° 58.242'S, 138° 37.794'E	34.0 ± 0.837
	WCp	34° 58.185'S, 138° 38.095'E	34.8 ± 0.583

3.2.2 Forager sampling

All nests sampled in this study were subterranean. Foragers returning to the nest were intercepted at the entrance and collected with a hand net. To ensure that only incoming workers were sampled, wasps inside the nest were anaesthetised by pouring a small amount of diethyl ether (~10 ml) into the

entrance tunnel. This prevented workers from leaving the nest for up to 30 mins, after which activity resumed. Organic solvents are not known to harm the brood inside the nest, and the amount used was much smaller than the amount recommended to anaesthetise nests prior to collection (200ml; Spradbery 1973; Edwards 1980). All collected wasps were killed by crushing the thorax, and subsequently stored at -20°C. Prey and pulp items were removed and stored in 70% ethanol until analysed.

Immediately prior to each sampling period, traffic rate counts of numbers of wasps entering or leaving the nest in any one minute period (see Ch. 2) were measured. As traffic rates are correlated with the number of larvae and workers present in the nest (Potter 1964; Malham *et al.* 1991, Ch. 2), these were used as an indicator of the internal needs of the colony.

At the same time, ambient air temperature, light intensity and humidity were measured. Temperature was recorded to the nearest 0.5°C using a hand held thermometer shielded from direct sun and positioned at least 1 m above ground level. Light intensity was determined using a LI-COR Inc. quantum meter (Model LI 185B). Relative humidity was measured with a Dick Smith Electronics™ hygrometer (Cat. No. Y5189) to the nearest percentage. These three measures served as an evaluation of environmental factors.

3.2.3 Experimental design

A total of 16 nests were sampled from February to May 2001, four each month, to determine seasonal changes of resource proportions as well as resource quality (Table A1). Each nest was sampled for one day only. Each sampling day was divided into 5 sampling periods, corresponding to proportions of the wasps' foraging day (see Ch. 4). Depending on day length, each sampling period was 2-3 hours, and during that time, 30-60 wasps were captured. This was done once, usually in the middle of the sampling period, rather than throughout the whole period, to enable wasps to recover from the ether and resume normal activity. Thus, for example, for a 15 hour wasp foraging day, sample one was taken at 1½ hours after the first activity, sample two was taken three hours later, and so on.

3.2.4 Changes in resource proportions

During each sampling period, the number of wasps carrying prey (PREY foragers), pulp (PULP) and no external load was recorded. Crops of foragers with no external loads were further examined by firstly cutting off the thorax from the rest of the body, and consequently, applying pressure to the bottom of the wasp's abdomen with a pair of forceps (method modified from Harris 1991; Harris *et al.* 1991a). Liquid expunged from crops was examined for presence of carbohydrate (see 3.2.6 below). Foragers were classified as foraging on carbohydrates (CARBOHYDRATE) when the sucrose concentration in their crop was greater than zero, otherwise they were considered to forage for water (WATER). Wasps whose crops were empty or contained minute amounts of liquid were classified as not having brought anything back (NOTHING).

Daily changes, as well as overall differences between proportions of these five resource types, were analysed with a univariate ANOVA. To satisfy conditions of normality, all resource proportions were arcsine square root transformed prior to analysis. Only resource proportions and changes on a daily scale were examined; differences due to season or environmental variables were not considered in this analysis.

Seasonal changes in proportions of each of the resources (arcsine square root transformed) were examined using a repeated measures ANOVA. This was necessary as the results obtained for each sampling period were not independent as they were taken from the same nest. Mean temperature, humidity, light intensity and traffic rates were modelled as covariates. Further analysis, concentrating only on the changes of the relative proportions of food (prey and carbohydrate), were also subsequently performed.

3.2.5 Changes in relative proportions of prey orders

Prey items were visually identified using distinguishing morphological characters to at least order level (see Ch. 5). The relative proportions of five most abundant prey orders, as well as the unidentified items, were determined based on the total number of PREY foragers captured. Due to the small numbers of prey of each type collected, daily changes could not be examined.

Seasonal variation in each prey order was analysed using a repeated measures ANOVA on arcsine square root transformed data in a design similar to the one described above.

3.2.6 Changes in sucrose concentration

Liquid expunged from crops of foragers was analysed for sugar content using a hand held refractometer (ATAGO Type 500). Although the quantity of each sample was not determined, the refractometer was able to take accurate readings of liquid drops larger than 2 µL. As the mean volume of fluid in the crop is normally much larger than that (14.7-16.7 µL; Harris *et al.* 1991a), wasp crops containing amounts smaller than 2 µL were not considered to contain fluid. The refractometer reading was corrected for temperature using the inbuilt compensating thermometer. This was then converted from Brix to sucrose concentration, in g/L, using conversion tables for concentrative properties of aqueous solutions (Weast and Astle 1980). Because of uneven numbers of replicates within each time period, a linear mixed effects model was applied to test the effects of month and temperature on sucrose concentration, allowing for random variation among nests.

3.3 Results

The types of resources collected by foragers were recorded for 842, 775, 767, and 680 wasps in February, March, April and May, respectively (Table 3.2).

Table 3.2: Numbers of wasps used to determine changes in daily and seasonal foraging characteristics of *V. germanica* captured each month and at each sampling period.

Month	Sample period	No. foragers
February	1	195
	2	151
	3	148
	4	192
	5	156
March	1	166
	2	152
	3	145
	4	163
	5	149
April	1	156
	2	160
	3	145
	4	155
	5	151
May	1	142
	2	134
	3	134
	4	133
	5	137

3.3.1 Changes in resource proportions

Wasps were not seen returning with more than one resource type. Across all samples, most wasps foraged for CARBOHYDRATES (53.7% ±1.60 SE). This was followed by wasps returning with NOTHING (26.5% ±1.91 SE), and PREY (13.2% ±1.10 SE). WATER and PULP foragers were most infrequent (3.4% ±0.80 SE and 3.2% ±0.4 SE, respectively).

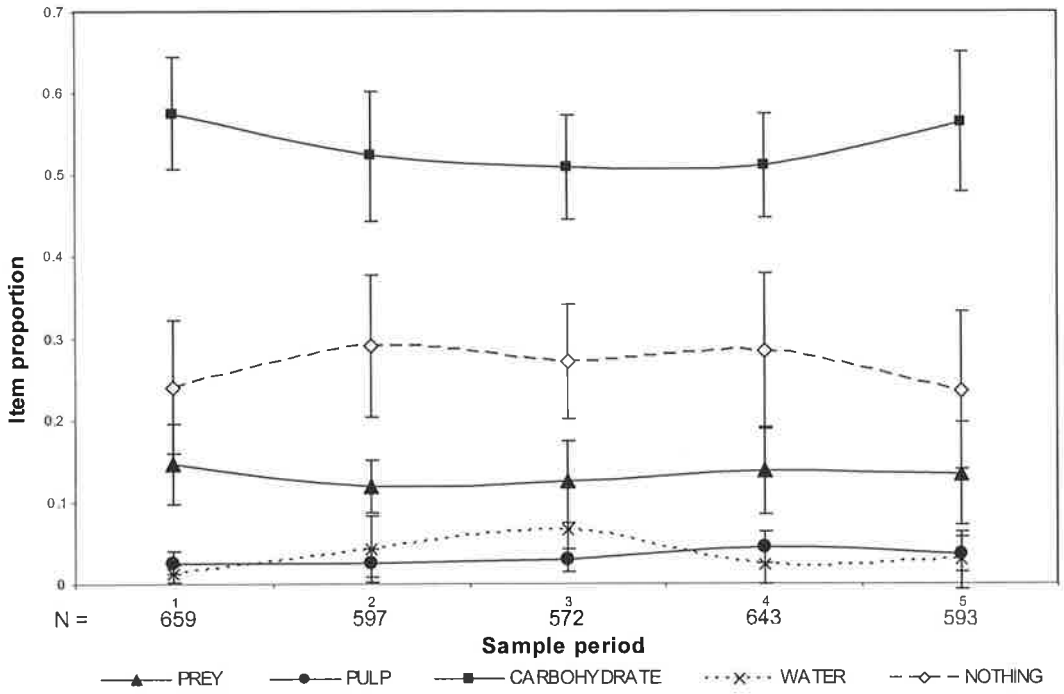

Fig. 3.1: Daily changes in proportions of workers foraging for PREY, PULP, CARBOHYDRATE, WATER and NOTHING. Error bars show 95% confidence limits.

Daily trends between proportions of workers foraging for these five resources are summarised in Figure 3.1. Statistical analysis shows that while there was no significant variation within a resource type during the day ($F_{4,375} = 0.272$, $p = 0.896$), the proportions of PREY, PULP, WATER, CARBOHYDRATE and NOTHING foragers were significantly different from each other ($F_{4,375} = 221.67$, $p < 0.001$). Multiple comparisons based on Tamhane's T2 test indicate that all resource proportions except for WATER and PULP differ significantly (Table 3.3).

Table 3.3: Results of Tamhane's T2 multiple comparison tests for daily changes in the five resource types foraged for. Values represent the observed arithmetic means for each resource type. Each subset is significantly different from the others.

Resource type	Subset			
	1	2	3	4
WATER	0.034			
PULP	0.032			
PREY		0.132		
NOTHING			0.245	
CARBOHYDRATE				0.537
Sig.	0.947	1.000	1.000	1.000

The seasonal trends for each resource type are presented in Figures 3.2 – 3.6. The best fit ANOVA model included month, temperature and traffic, with humidity and light intensity being non-significant (Table 3.4). Repeated measures analysis of variance on the transformed proportions shows that numbers of PREY foragers increase significantly with season (p = 0.039), while numbers of NOTHING foragers decrease (p = 0.042). CARBOHYDRATE proportions significantly decreased with increasing temperature (p = 0.046; Fig. 3.7), while proportion of WATER foragers significantly increased at higher ambient temperatures (p = 0.004; Fig. 3.8). All other resource types and factor/covariate interactions were non-significant.

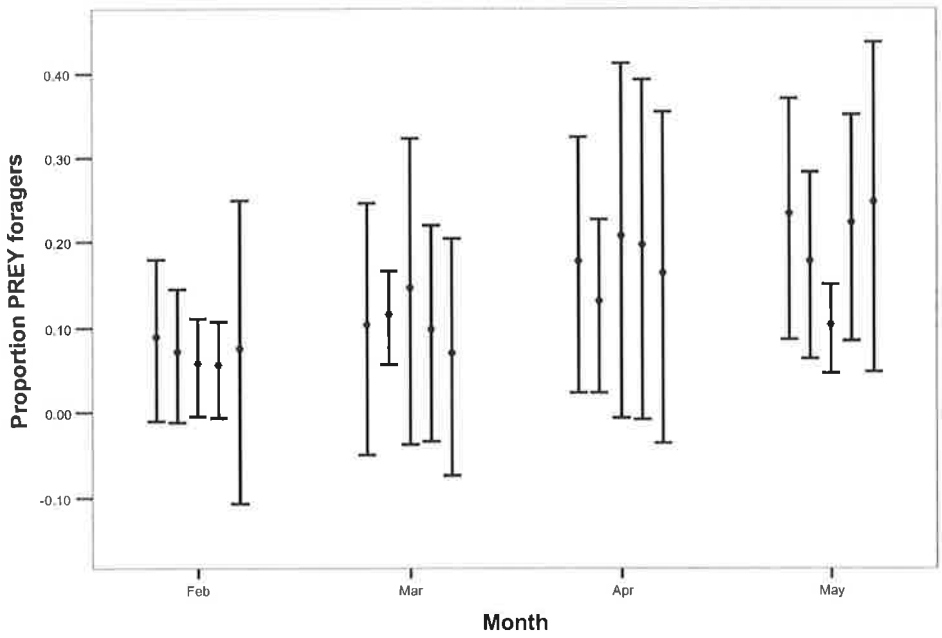

Fig. 3.2: Seasonal changes in proportion of prey collected by *V. germanica* foragers. Each point represents an average for each sampling period; sampling periods are represented sequentially. Error bars show 95% CI.

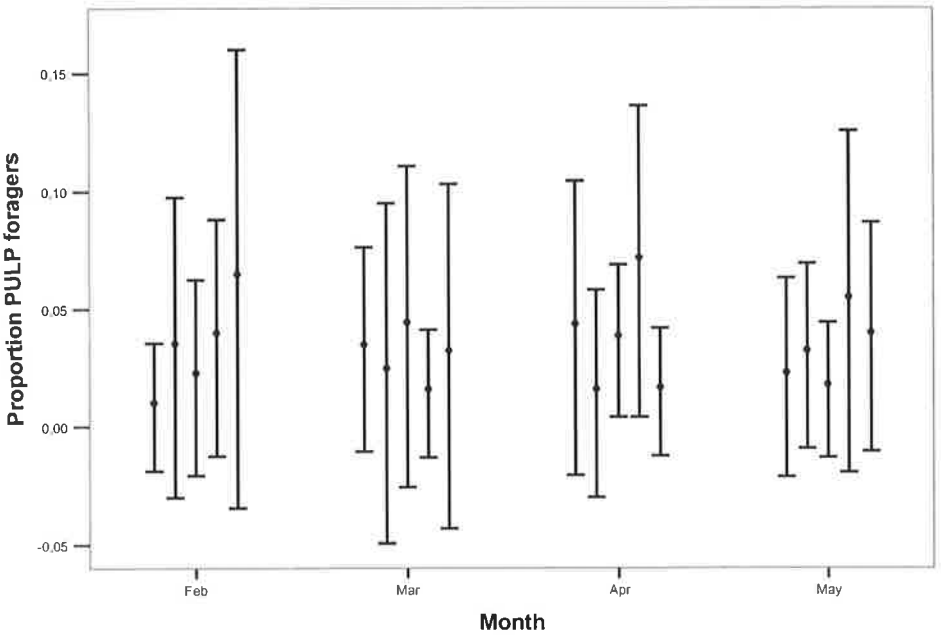

Fig. 3.3: Seasonal changes in proportion of pulp collected by *V. germanica* foragers. Each point represents an average for each sampling period; sampling periods are represented sequentially. Error bars show 95% CI.

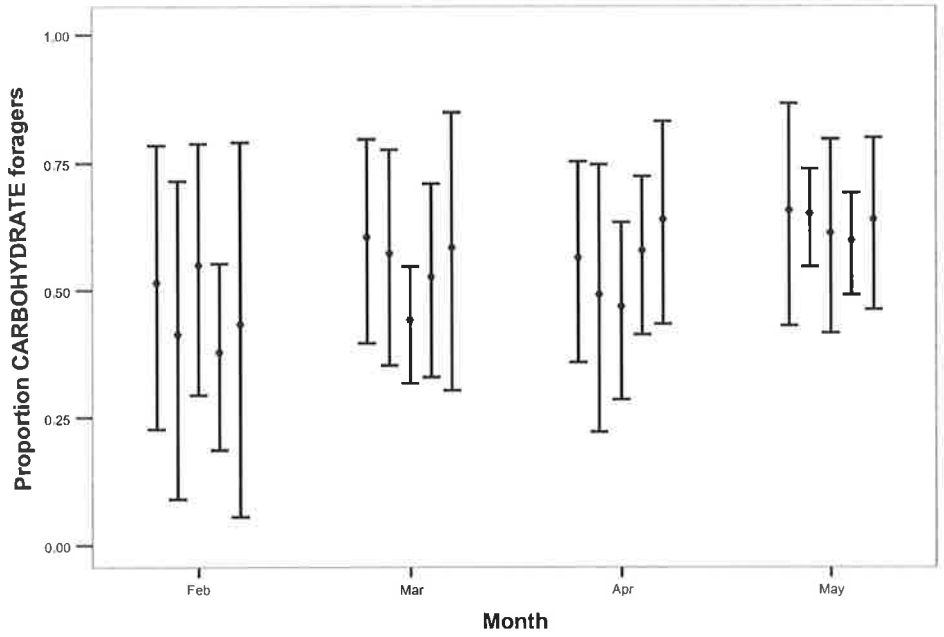

Fig. 3.4: Seasonal changes in proportion of carbohydrate collected by *V. germanica* foragers. Each point represents an average for each sampling period; sampling periods are represented sequentially. Error bars show 95% CI.

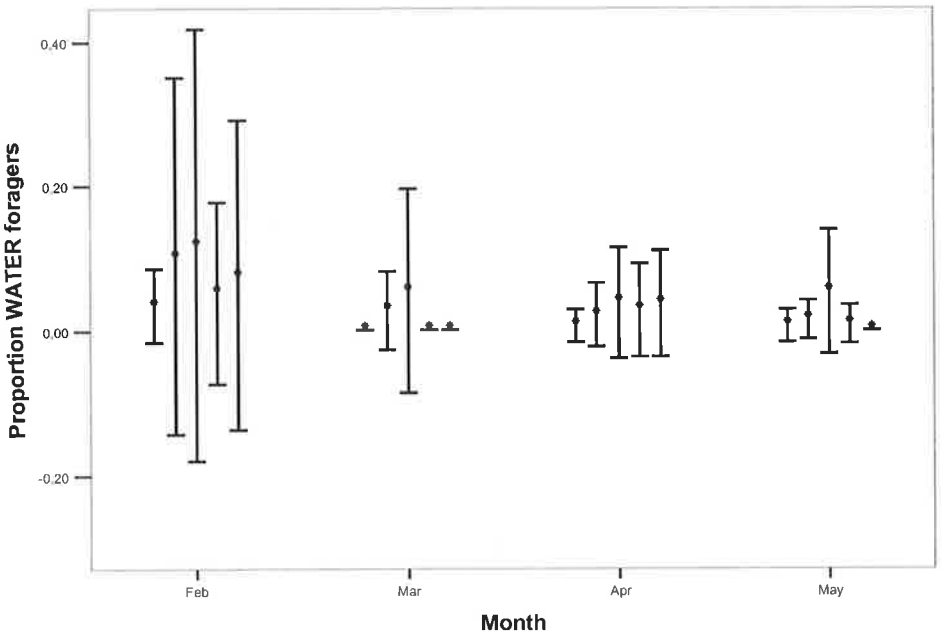

Fig. 3.5: Seasonal changes in proportion of water collected by *V. germanica* foragers. Each point represents an average for each sampling period; sampling periods are represented sequentially. Error bars show 95% CI.

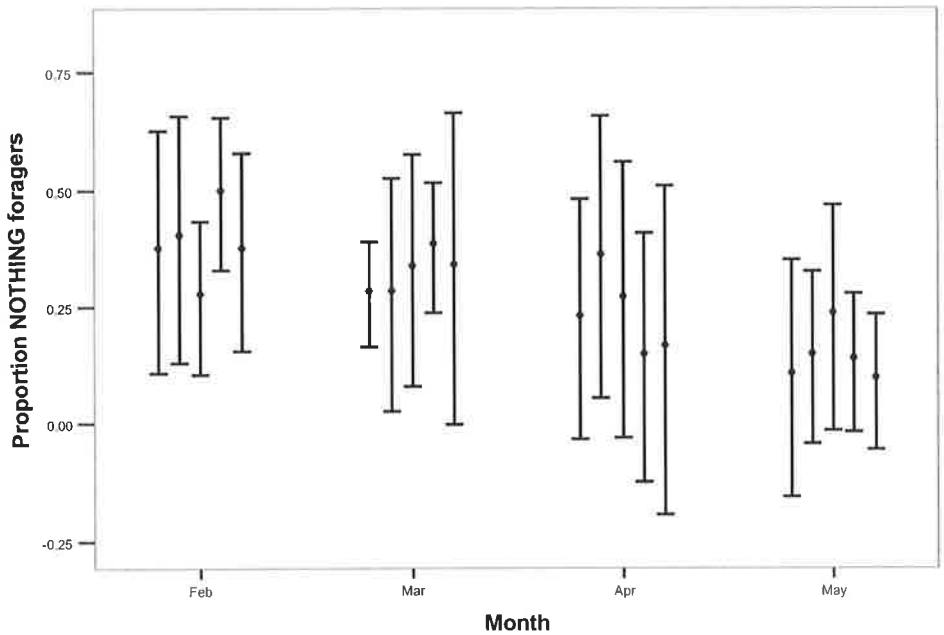

Fig. 3.6: Seasonal changes in proportion of *V. germanica* foragers returning with nothing. Each point represents an average for each sampling period; sampling periods are represented sequentially. Error bars show 95% CI.

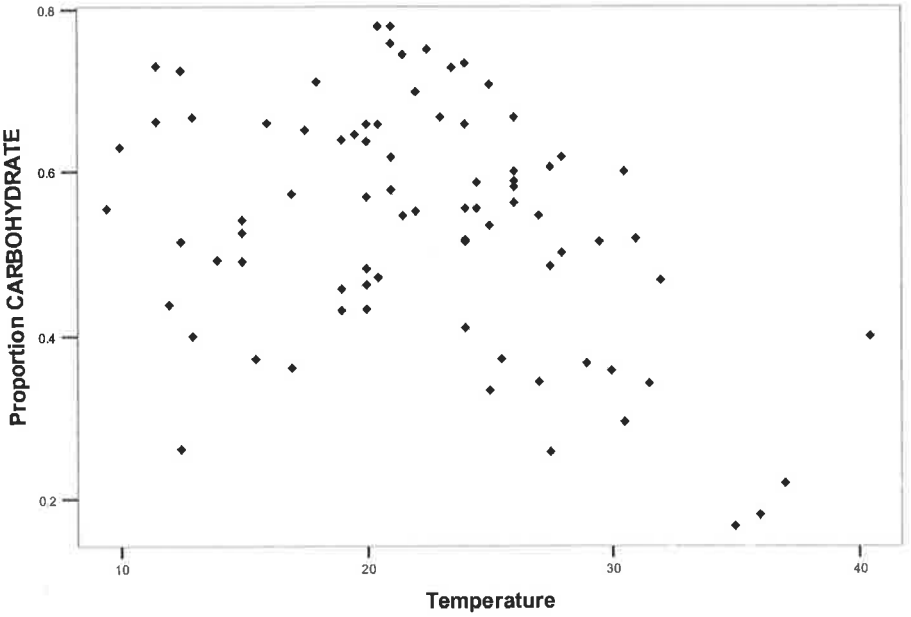

Fig. 3.7: Scatter plot of the influence of temperature (°C) on proportion of carbohydrate foragers of *V. germanica*.

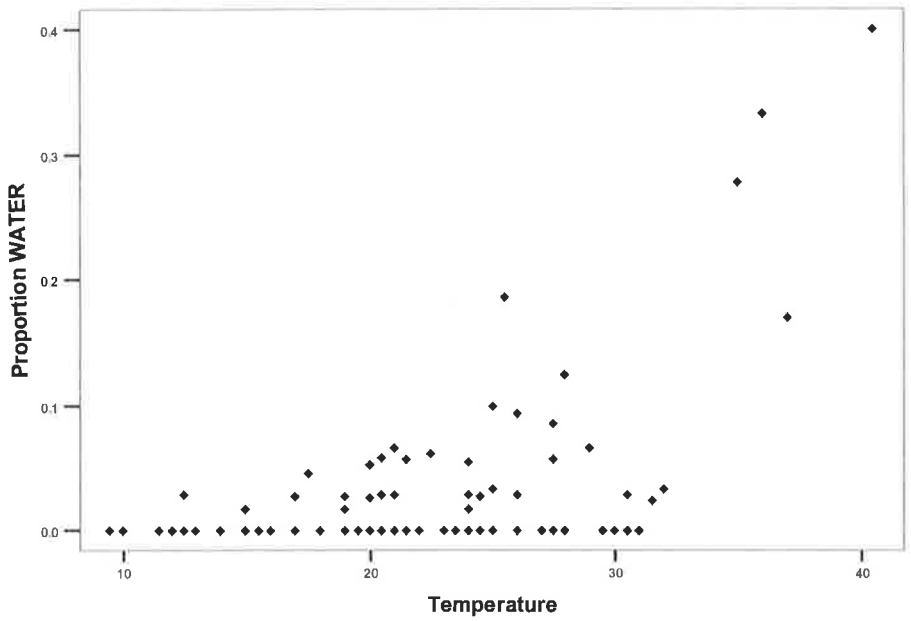

Fig. 3.8: Scatter plot of the influence of temperature (°C) on proportion of water foragers of *V. germanica*.

When the composition of food (in terms of proportion of prey to carbohydrate) was examined, the analysis shows that proportion of prey increases with increasing traffic rates (p = 0.046; Fig. 3.9). Although overall monthly changes are non significant, pair-wise comparisons show a significant increase in PREY proportion between February and May (p = 0.026).

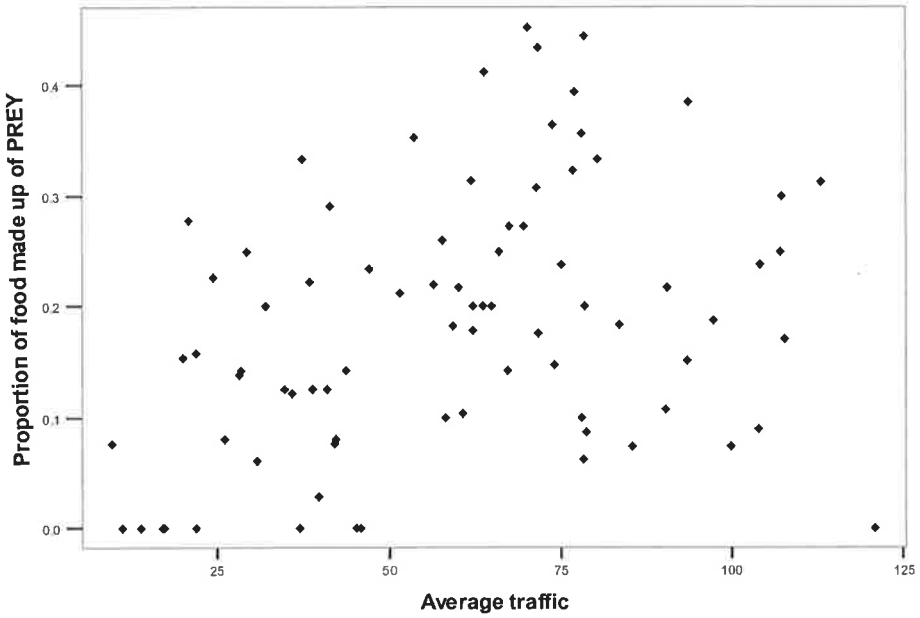

Fig. 3.9: Scatter plot of the influence of average traffic (wasps min^{-1}) on proportion of food made up of prey brought back to nest by *V. germanica*.

Table 3.4: Significance of factors and covariates on changes associated with each of the five resource types collected by *V. germanica*. Statistical significance is highlighted by asterisks (intercept excepted).

Resource	Source	d.f.	Mean square	*F*	*Sig.*	
PREY	Intercept	1	0.122	1.938	0.194	
	Traffic	1	0.258	4.092	0.071	
	Temperature	1	0.002	0.028	0.871	
	Month	3	0.258	4.106	0.039	*
	Error	10	0.063			
PULP	Intercept	1	0.057	2.842	0.123	
	Traffic	1	0.075	3.706	0.083	
	Temperature	1	0.033	1.611	0.233	
	Month	3	0.006	0.315	0.814	
	Error	10	0.020			
CARBOHYDRATE	Intercept	1	3.322	101.399	0.000	
	Traffic	1	0.019	0.593	0.459	
	Temperature	1	0.169	5.164	0.046	*
	Month	3	0.092	2.798	0.095	
	Error	10	0.033			
WATER	Intercept	1	0.267	8.528	0.015	
	Traffic	1	0.107	3.423	0.094	
	Temperature	1	0.443	14.147	0.004	**
	Month	3	0.062	1.970	0.182	
	Error	10	0.032			
NOTHING	Intercept	1	1.273	9.783	0.011	
	Traffic	1	0.169	1.298	0.281	
	Temperature	1	0.005	0.035	0.855	
	Month	3	0.516	3.964	0.042	*
	Error	10	0.130			

3.3.2 Changes in prey orders

During the survey, a total of 339 prey items were collected from returning foragers. The five most abundant types, apart from the unidentified items, were Araneae, Diptera, Hymenoptera, Lepidoptera and Orthoptera (Fig. 3.10). These data suggest that there may be an increase in the proportion of Diptera, a decrease in the proportion of Hymenoptera, and various smaller seasonal differences in the other groups collected, but none of these changes were statistically significant.

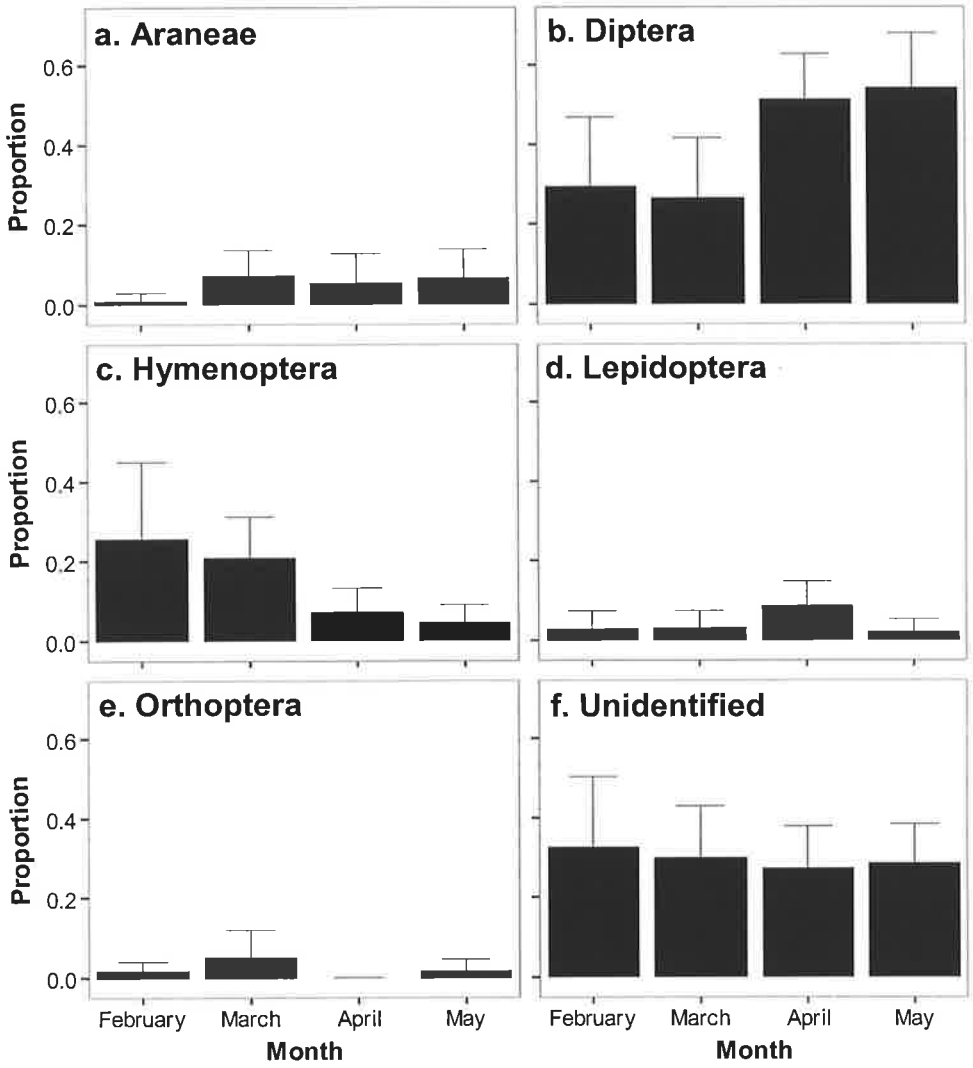

Fig. 3.10: Seasonal changes in the five most abundant prey types: (a) Araneae, (b) Diptera, (c) Hymenoptera, (d) Lepidoptera, (e) Orthoptera, and (f) the proportion of unidentified items over four months of the wasps' season. Error bars represent 95% confidence intervals.

3.3.3 Changes in sucrose concentration

A total of 68% (1,746) of foragers not carrying PREY or PULP back to the nest had liquid in their crops. Ninety-five percent of these had varying sucrose concentrations above zero (Fig. 3.11). Although there was a hint of increasing sucrose concentration with season, and an interaction between season and time period was observed, the overall effect of month was not significant

($F_{3,1633} = 0.358$, $p = 0.785$). Temperature, humidity, light levels, and average traffic similarly had no statistically significant effects on sucrose concentration.

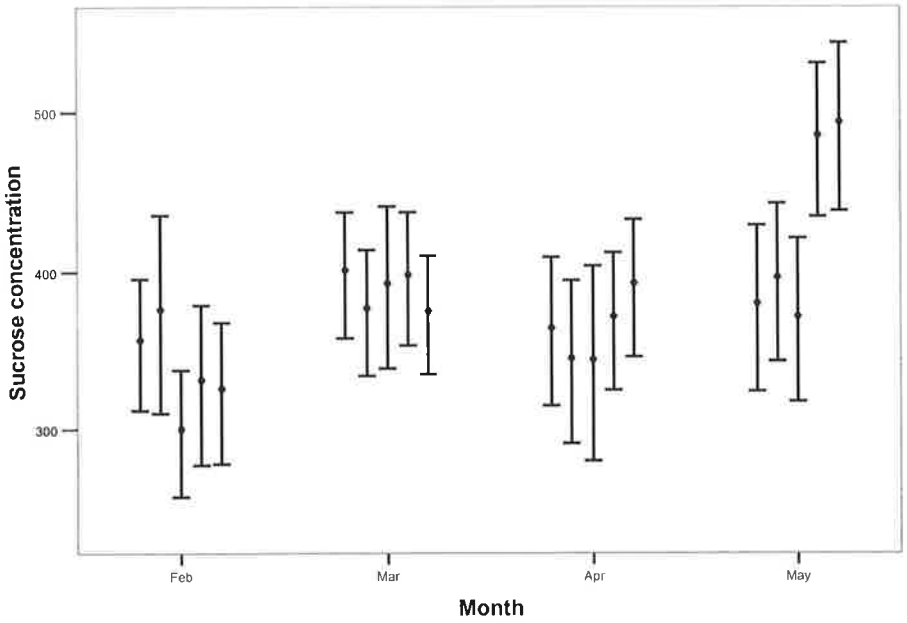

Fig. 3.11: Carbohydrate concentration (g/L) change in wasp crops from February to May. Different points represent consecutive sampling periods during the day. Bars represent mean sucrose concentration ± 95% confidence interval.

3.4 Discussion

3.4.1 Overall resource proportions

In this study, the overall relative proportions of resources foraged for were broadly similar to those noted elsewhere. For example, 12-23% of incoming *V. germanica* and *V. vulgaris* workers were previously found to forage on PREY, while PULP constituted 5-8% (e.g. Harris 1991; Harris and Oliver 1993; Sackmann *et al.* 2000). Studies that have examined crops for the presence of fluid also found that 50-65 % contained fluid (Harris 1991; Archer 2000). The only noticeable difference exists in the proportion of NOTHING foragers found in this study and that reported from a study of *V. vulgaris* in England (Archer 2000). While the current study found an average of 26% of wasps returning

with NOTHING, Archer found this to be much lower (5-10%). The function of NOTHING foragers, if any, is unclear; they could be cleaners and excavators, new (naïve) foragers, guards, or unsuccessful foragers (Archer 2000). As there are no other comparable studies, the observed differences are difficult to explain, and could be due to wasp species (*V. germanica* versus *V. vulgaris*), location (Australia versus England), or habitat (semi-urban versus rural).

3.4.2 Daily changes in resource proportions

The results of this study indicate that the relative proportions of PREY, PULP, CARBOHYDRATE, WATER and NOTHING foragers are fairly constant throughout the day. This is in contrast to previous studies that have suggested that diurnal changes do occur. For example, Potter (1964) observed a gradual decrease in FLUID foragers (approximately 20%; WATER and CARBOHYDRATE were not distinguished) between morning and evening, and a contrasting 35% increase in PREY foragers in a study of *V. vulgaris* in England. The proportion of PULP foragers increased until mid-day, and then decreased, with a total variation of about 20%. A study of *V. vulgaris* and *V. germanica* in France by Roland (1976, in Edwards 1980) also suggested daily changes in these three types of foragers, but these fluctuated greatly and did not show any definite trends.

Differences in findings may be partially attributed to methodologies used. Previously, an observation box separating the incoming and outgoing workers was used, and PULP, FLESH and FLUID carriers were scored visually (Potter 1964; Edwards 1980). The methods and sampling protocol for these studies are unclear but appear to be based on one day of observations of a single nest. In the current study, wasps were individually captured and examined. Samples were taken at five distinct periods throughout the day, from a variety of nests and across four months, providing a solid foundation for statistical analyses. Perhaps the patterns described by Potter (1964) and Roland (1976, in Edwards 1980) are equivalent to comparison of individual data points in this study, and whatever differences might exist disappear when results are compared across a number of colonies. As foraging activity reflects the needs of the colony, these are not likely to change within a day, and thus the finding that the relative proportions of resources remain constant is not surprising. It is also possible,

although not as likely, that increasing the number of sampling periods during the day would show greater variation. Future studies could address this.

3.4.3 Seasonal changes in relative resource proportions

Although variation in the relative amount of resources collected over the season has long been acknowledged (Edwards 1980), relatively little prior research has been carried out to examine this. The studies of Archer (1998a) on *V. vulgaris* in England and Sackmann *et al.* (2000) on *V. germanica* in Argentina compared the relative proportions of PREY and PULP foragers over time. Both concluded that PREY intake increases with season, a result not dissimilar to the one obtained in this study. The only known comprehensive study that examined seasonal changes of not only PREY and PULP but also FLUID (CARBOHYDRATE and WATER) and NOTHING incomers, was that of Archer (2000) on *V. vulgaris* in England. The trends from that study indicate that the number of FLUID foragers increased with season, whereas the number of PREY foragers first decreased, then increased, then decreased again. Further, PULP and NOTHING incomer proportions decreased seasonally for *V. vulgaris*. Archer (2000) attributed all seasonal trends in resource proportions as being influenced solely by the development of the colony, i.e. internal colony needs (but see Ch. 4).

A wasp season may be divided according to the different growth stages of the colonies (Archer 1980a; Archer 2000). Initially, only the queen and a few workers are present in the nest, and this may be referred to as the 'early colony' stage. Next, the colony undergoes rapid expansion in terms of size and the number of workers and brood present, and this corresponds to the 'small-cell colony' phase. Finally, large reproductive cells are built in the nest and the next generation queens and males are reared in them, corresponding to the 'large-cell colony' phase. As seen in Chapter 2, the actual timing of these three stages differs between England and Australia. In England, the wasp season is shorter, spanning five months at most (Spradbery 1971; Archer 1980a). The early colony stage lasts approximately two months, small-cell colonies are present for the next six weeks, while during the last six weeks large-cell colonies predominate (Archer 1980a; Archer 2000). The wasp season in Australia is longer, between 7 and 8 months, with the first one to two months

and the last two months representing early and large-cell colony stages, respectively. The main difference exists in the small-cell colony stage, which lasts at least four months in Australia, a three-fold increase in the length of this phase compared with England (Ch. 2; Archer 2000). Thus the four month period covered in this study did not include the early colony stage of the wasps' lifecycle. The first two months, February and March, correspond to the second half of the small-cell stage of Archer's colony model, while April and May correspond to the large-cell stage of the same model (Archer 2000).

Thus the increase in PREY foragers observed by Archer (2000) was attributed to the increased demands for proteins of both small-cell and large-cell larvae present in the nest, while the decrease in NOTHING incomers was suggested to represent fewer numbers of new foragers, or guards, present during the small-cell colony phase. In the present study, PREY foragers were similarly seen to increase while NOTHING foragers were seen to decrease at the large-cell colony phase, and thus these changes may be explained in terms of varying colony requirements with season. Interestingly, neither the increase in PREY, nor the increase in NOTHING foragers was significantly correlated with traffic rates. This suggests that although traffic rates may reflect the size of the colony (Potter 1964; Malham *et al.* 1991; Ch. 2), internal needs are driven by more than just numbers of brood. Variation in quality of resources brought back could also be an important factor, and this would certainly explain variation between different nests. However, the observed seasonal changes were pooled across nests, accounting to some degree for this variation. Internal colony needs may thus be influenced by colony size and possibly other factors, such as energy requirements. For example, new queen and reproductive male brood during the large-cell colony phase may require more energy in preparation for the mating flight and winter hibernation in queens than do workers.

Changes in WATER, CARBOHYDRATE and PULP observed in this study could not be explained in terms of seasonal variation. Instead, changes in CARBOHYDRATE and WATER proportions were explained by variation in temperature, especially at 30°C and above (Figs. 3.7 and 3.8). These high temperatures resulted in an increase in WATER foragers and a corresponding decrease in carbohydrate FORAGERS. Water is frequently used by wasps in nest thermoregulation (Wilson

1971). Additionally, it has also been shown that water may be regurgitated to cool down the body temperature of adult wasps (Coelho and Ross 1996). Thus, the increasing requirement for water in the nest at high temperatures is not surprising.

The decrease in PULP collection during the large-cell phase of the colony in Archer's study (Archer 2000) was explained in terms of smaller amounts of pulp required to build large cells, as these occupy the same area per comb as small cells. The present study found no differences in the amounts of PULP brought into the colony with season, traffic rates, or any environmental variables. *Vespula germanica* colonies in Australia typically contain more reproductive-bearing large cells than *V. vulgaris* colonies in England (Ch. 2), and thus it is likely that nest expansion remains constant during the small-cell and large-cell phases of the colony, requiring similar proportions of pulp.

3.4.4 Seasonal changes in prey

Workers of *Vespula* spp. are thought to be opportunistic foragers, and the prey types these wasps collect most probably reflect their relative abundance in the environment (e.g. Spradbery 1973; Edwards 1980; Harris and Oliver 1993; Sackmann *et al.* 2000). As in other studies, the current study found slight seasonal differences in the relative occurrence of prey orders collected by *V. germanica*. These were not statistically significant presumably due to the large variation within each sample relative to the number of samples. However, whereas most other studies found a shift in the predominant prey type (e.g. Gambino 1986; Harris 1991; Sackmann *et al.* 2000), this study did not, and the relative abundance of each prey type in the wasp diet remained fairly constant. A similar result was observed by Harris and Oliver (1993) in New Zealand. It is possible that in some areas wasp densities are so high that some prey orders are depleted. For example, the study of Harris (1991) was conducted in New Zealand's honeydew beech forests, where abundant energy-rich carbohydrate resources exist, and extraordinarily high densities of wasps are supported, while the study of Gambino (1986) was based on a perennial (and thus presumably very large) wasp colony. Nest densities throughout Adelaide can be 300 times

lower than those in beech forests (see Ch. 6), and thus prey depletion due to high foraging pressure is not as likely.

3.4.5 Changes in sucrose concentration

It has been suggested that a shift in dietary preferences of vespid wasps may exist towards the end of the season, with a preference for CARBOHYDRATE over protein (PREY) foods (Thomas 1960; Spurr 1996). This hypothesis has been based on the observations of larger numbers of wasps being attracted to carbohydrate food sources associated with humans in autumn (Thomas 1960), as well as preference for carbohydrate versus protein baits observed during the large-cell colony phase (Spurr 1996). The current study did not find this; in fact, prey as opposed to carbohydrate intake was seen to increase seasonally. Furthermore, examination of worker crop content did not indicate that carbohydrate collected later in the season is more concentrated than earlier on. These findings indicate that the increased numbers of wasps observed scavenging for sugary liquids should be attributed to factors other than a shift in dietary preferences of workers. For instance, male wasps were frequently seen foraging at flowers of the English ivy (*Hedera helix*) after leaving the nest (pers. obs.), and these can easily be mistaken for workers by the naïve observer. Alternatively, the apparent increase in attractiveness of carbohydrate sources could be an artefact of natural sources of carbohydrate being washed away or diluted by rain. It has previously been shown that mean carbohydrate concentration in *V. germanica* and *V. vulgaris* wasps was significantly lower after or during rain (Harris *et al.* 1991a), while the mean volume remained unchanged. Thus, a greater number of trips for carbohydrate would have to be made to obtain the same energy levels.

CHAPTER 4:

FACTORS INFLUENCING DAILY FORAGING ACTIVITY

4.0 Chapter summary

In social insect societies, workers forego reproduction in favour of performing foraging and other tasks to promote growth of the whole colony. Maximising individual work effort is contrasted by the physical constraints on foraging outside the nest. Studies of factors influencing activity in social insects suggest that light intensity, ground temperature, air temperature, relative humidity, solar radiation, wind speed and/or rainfall may be important in some species, while not in others. Previous research into the factors that influence foraging in vespids often lacks replication and is purely descriptive. This study aims to determine which environmental factors influence foraging in an invasive social insect, *Vespula germanica*. This species has been introduced to Australia, where it experiences a hotter and drier climate than in its native range. Activity was measured in terms of foraging traffic, with nests from a range of locations being monitored. Results indicate that the onset of rain reduces activity by 30%, but foraging returns to previous levels during the next dry period. Foraging time is correlated with the duration of daylight, with wasp daily activity being on average 20 min longer than the time between sunrise and sunset. Low light was found to restrict wasp activity, as were low and high temperatures. A mixed model developed to explain the influence of these variables on numbers of foragers was highly significant. Under hot conditions, *V. germanica* thermoregulates its body temperature by regurgitating water. It also uses water in evaporative cooling to keep nests at optimum temperatures. Thus, in this species, hot temperatures increase the need for water, and it may be excluded from hot areas where water is limited.

4.1 Introduction

In social insect societies, workers normally forego reproduction in favour of foraging for resources necessary to expand their natal nest. As larger colonies

produce more reproductives, foragers can enhance colony fitness by maximising their individual work effort (Michener 1964; Cole 1984; Lee and Winston 1987). Colony needs are counterbalanced by physiological constraints on workers outside the nest. For visually navigating insects, the availability and intensity of light poses the uttermost limitation on activity (e.g. Wehner 1976; Hilário *et al.* 2000). Extreme temperatures, hot and cold, have also been shown to restrict foraging in ants, bees, wasps and termites (e.g. Marsh 1985; Kleinert-Giovannini and Imperatriz-Fonseca 1986; Porter and Tschinkel 1987; Salman *et al.* 1988). In ants, ground temperature and the availability of thermal refuges was found to be important (e.g. Rhoades and Davis 1967; Porter and Tschinkel 1987; Wehner *et al.* 1992), while in bees and wasps, air temperature was more important (e.g. Heinrich 1984; Hilário *et al.* 2000). Other environmental factors, such as humidity, rainfall, barometric pressure and wind speed, have also been observed to influence activity in some hymenopterans, but not in others (e.g. Rhoades and Davis 1967; Southwick and Moritz 1987; Riessberger and Crailsheim 1997; Human *et al.* 1998).

Apart from providing an insight into the mechanisms responsible for simulating or inhibiting colony activity, determining the factors affecting foraging may have additional implications when considering invasive species. Often, an invasive species adapts to its new environment, and understanding how the two interact is necessary to develop and revise control methods (Salman *et al.* 1988). This knowledge may also aid in assessing the impact the species has on the invaded community.

Vespula germanica is an example of a highly invasive social insect. It has expanded its native range of Europe and parts of Asia to include four other continents, and the species is now considered a pest in the USA, Chile, South Africa, New Zealand and Australia (Edwards 1976; Akre *et al.* 1981; Spradbery and Maywald 1992; Tribe and Richardson 1994). Like other vespids, the life cycle of *V. germanica* is typically annual, with colonies headed by a single queen. Nests are founded in early spring, and continuously expand until early autumn, when reproductive forms are produced (Spradbery 1973; Edwards 1980). With the expansion of the colony, numbers of workers foraging for

resources also increase, and thus their impact as pests and on the environment increases as well.

Despite the relative abundance of studies conducted on bees and ants, very little information is available on the foraging activity of *Vespula* spp. Most studies are based on single observations of a single nest, and have not attempted any replication. However, the general conclusion is that rates of foraging activity are not always constant - they have been shown to vary diurnally (e.g. Potter 1964; Pallett and Plowright 1979), as well as seasonally (Archer 2000). Moreover, foraging in vespids may change according to availability of resources (Ydenberg and Schmid-Hempel 1994). Some studies (Gaul 1952a; Gaul 1952b; Blackith 1958; Potter 1964) have noted the existence of a threshold in terms of light and temperature below which wasps did not leave the nest, however this varied greatly among species and between observations. For example, Gaul (1952a) found *V. maculifrons* workers did not leave the nest below 8.5°C and 5 lux, while Potter (1964) concluded that *V. vulgaris* workers started foraging at 2°C and 1.5 lux. Since all previous studies have not employed replicate observations, their data were not examined statistically.

Summaries of daily activity patterns are somewhat more similar when comparing different studies and species, although variation still exists. Examples of patterns from *V. vulgaris, V. rufa* and *V. maculifrons* exhibit a high peak in activity for 1-2 hours early in the day, followed by a drop to approximately 50% of the maximum for the remainder of the day, and a sharp decline towards the evening (see Edwards 1980, Heinrich 1984). However, two other examples of *V. vulgaris* activity show constantly high foraging levels throughout the day (see Edwards 1980, Heinrich 1984).

The study presented in this chapter attempts to examine daily activity patterns of *V. germanica* in a systematic manner. The aim is to determine the environmental variables that influence activity for this species, and summarise the daily activity patterns observed under the climatic conditions experienced in its introduced range in metropolitan South Australia.

4.2 Materials and Methods

4.2.1 Data collection

The distribution of *V. germanica* in South Australia is mostly confined to metropolitan Adelaide. Although highest wasp densities are correlated with urban centres, the species is also present in the nearby semi-rural hills (Goodall and Smith 2001). A total of 11 nests were monitored for daily foraging activity during January, February, March, and April 2000-2002, corresponding to the most intensive growth phase of colonies. As it was necessary for the nests to remain *in situ* for a few days, most nests were located away from populated areas, in parks and reserves where they would not be interfered with. This included locations in the hills and on the plains. Each nest was observed on two to seven different days, typically three (Table 4.1).

Table 4.1: Locations of wasp nests used in the daily activity study and the number of days each nest was observed. The months during which the nest was sampled, as well as the maximum nest size on any day, are also presented.

Nest ID	Location	Days observed	Month(s) sampled	Nest size *
SLP	35° 00.972'S, 138° 42.941'E	6	Jan	50-100, >100
Ins	34° 58.185'S, 138° 38.095'E	7	Jan, Feb, Mar	<50, 50-100
K2	35° 00.980'S, 138° 43.685'E	3	Feb	<50
Sad	34° 56.298'S, 138° 34.676'E	3	Feb	>100
K3	35° 00.999'S, 138° 43.595'E	3	Feb	<50
K5	35° 00.989'S, 138° 43.623'E	3	Feb	50-100
WI1	34° 58.032'S, 138° 37.012'E	3	Mar	<50, 50-100
WI2	34° 58.078'S, 138° 37.091'E	2	Mar	>100
WI3	34° 58.041'S, 138° 37.068'E	2	Feb	50-100
Ar1	34° 58.228'S, 138° 37.762'E	3	Apr	50-100
Ar2	34° 58.099'S, 138° 37.695'E	3	Apr	<50

* maximum daily traffic range; expressed as wasps/min

Wasps forage for food, water and pulp (Edwards 1980; Raveret Richter 2000; Ch. 3). In order for foraging to take place, workers must leave the nest, and thus wasp activity can be assessed at the nest entrance. All nests used in this study were subterranean, with a single entrance hole, making visual monitoring of wasp activity straightforward. Preliminary observations indicated that wasp activity commences up to 30 min before sunrise, and concludes approximately

30 min after sunset. Thus, the first daily measurements were taken between 5:30 and 6:45 am, 15-30 min before any wasps left the nest, and similarly, final observations took place between 21:00 and 19:30, 15-30 min after the last wasp entered the nest. In the first and final hours of activity, measurements were taken every 15-30 min; and every hour for the remainder of the foraging day

Traffic rates, in terms of wasps entering and leaving the nest, were counted visually. Each count consisted of the number of wasps entering the nest during five one-minute periods (in), and the number of wasps leaving the nest in five one-minute periods (out). The 'in' and 'out' counts were done pair-wise in random order. The average of all ten observations was then taken and this is referred to as 'average traffic rate' for a particular nest at a particular time. Throughout this study, traffic rates are used as a measure of wasp activity. In order to take into account the varying numbers of foragers among nests, wasp foraging activity is represented as a percentage of the mean (over 10 observations) maximum daily traffic rate for each nest on a particular day.

Several localised weather conditions were measured at the same time as traffic rates. Air temperature in the nest vicinity was recorded to the nearest 0.5°C with a hand held thermometer, suspended at least 1m from the soil and protected from direct sunlight. Light readings were taken using a LI-COR Inc. quantum meter (Model LI 185B). Relative humidity readings were obtained using a Dick Smith Electronics hygrometer (Cat. No. Y5189). Rainfall was scored as present or absent, but was not quantified as it could not be accurately measured on a scale comparable to wasp traffic observations (i.e. mm rain per min).

4.2.2 Modelling factors influencing foraging

Because observations were repeated on the same nests but not always for the same number of days, a normal repeated-measures linear model could not be applied to the data. Instead, the effects of air temperature and light intensity on daily activity were modelled using a mixed-effects model (Pinheiro and Bates 2000). To incorporate the possible differences between colonies, the variation among individual nests was modelled as a random effect.

Further, the presence of a relationship between sunrise and commencement of activity and sunset and conclusion of activity was examined. Sunrise and sunset times were computed using the SUNRISENSET program, version 2.2, and were accessed from http://www.ga.gov.au/nmd/geodesy/astro/ (National Mapping Division 2003).

4.2.3 Summary of daily activity pattern

A daily activity pattern was produced by summarising wasp traffic rates during different parts of the day. Possible seasonal differences were examined by producing separate graphs for each study month, while size differences were examined by separating nests based on maximum daily traffic. To accommodate for the possible effects of temperature, days with three different maximum daily temperature ranges were compared (<25, 25-35, >35°C).

Because the study was conducted over a four-month period, traffic counts could not simply be averaged at each hour as total daily foraging time decreased with the onset of autumn. That is, the length of daily foraging was correlated with day length (i.e. the time between sunrise and sunset; see results), and thus instead of depicting wasp activity as a function of time, it was standardised using day length. For each observational day, day length was divided into ten periods ('foraging periods'), and observations of wasp activity were reassigned to these. As changes in traffic were greatest in the morning and the evening, activity during the foraging period just before sunrise and just after sunset was further categorised as having occurred within 0.3 and 0.5 of that foraging period, i.e. two additional points were summarised for each of these two periods.

In addition to constructing a daily activity summary, rates of foragers returning to the nest were compared to ones leaving the nest. Observations were summarised by foraging period as described above.

4.3 Results

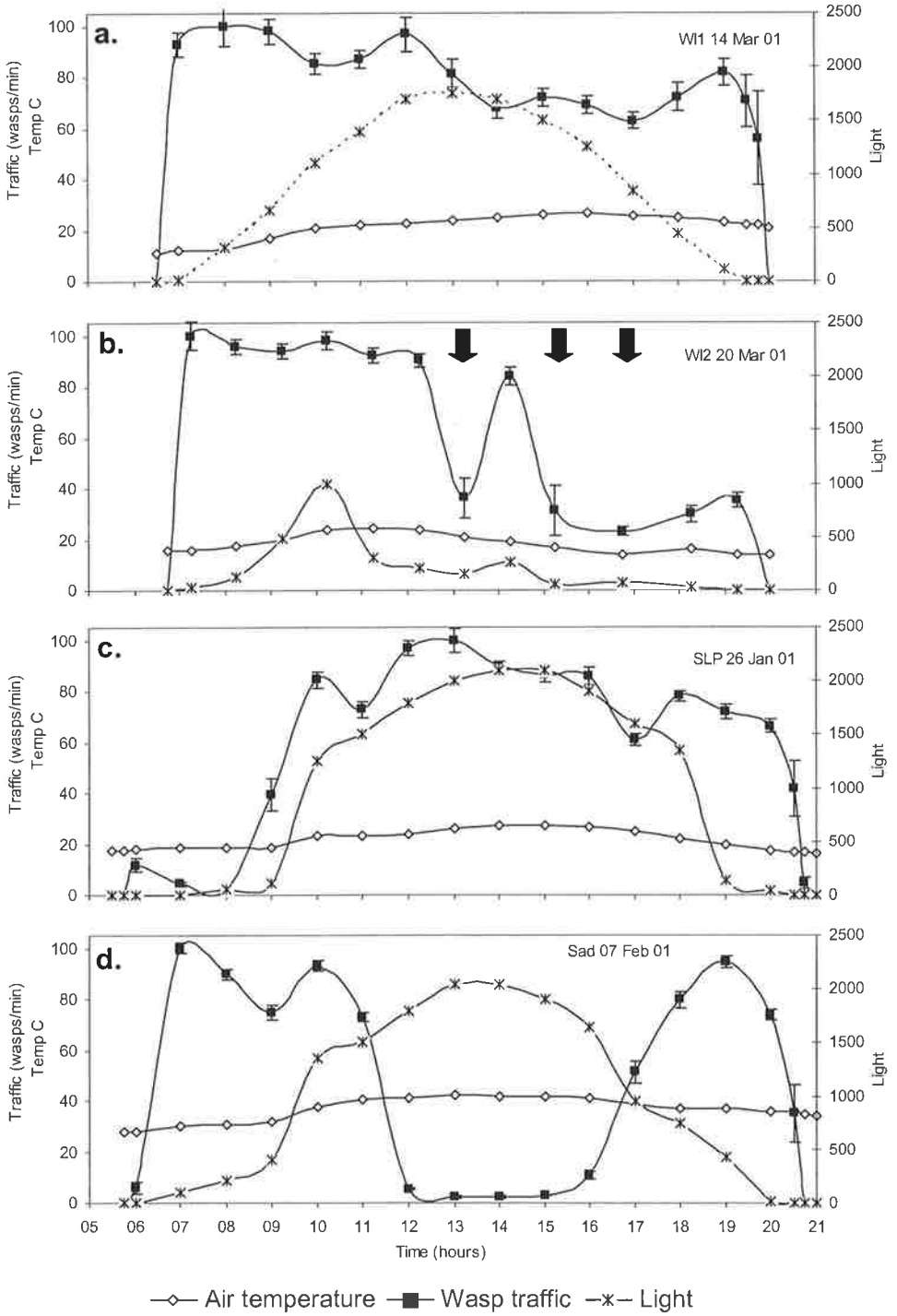

Fig. 4.1: Examples of *V. germanica* daily foraging activity patterns, highlighting the environmental variables associated with the differences associated with them. (a) Foraging activity on a clear, sunny day, (b) cloudy day with three rain events at 13:00, 15:00 and 17:00; marked by arrows), (c) foraging when there was a morning fog, obscuring visibility and reducing light intensity until 09:00, and (d) hot sunny day when the maximum daily temperature reached over 40°C. Traffic counts represent means and standard errors of ten one-minute counts. Temperature was measured in degrees Celsius, while light intensity was measured in μmols photons sec^{-1} m^{-2}.

Over the four month study period, nests were monitored for a total of 38 days, with 679 individual observations made. Although foraging activity patterns, including the time that foraging begun and ceased, differed between days and nests, generally wasp activity began rapidly in the morning, stayed fairly constant at high levels throughout the day, and ended in the evening (Fig. 4.1 a-d). Deviations from this pattern were attributed to three of the four environmental factors examined.

4.3.1 Effect of rain

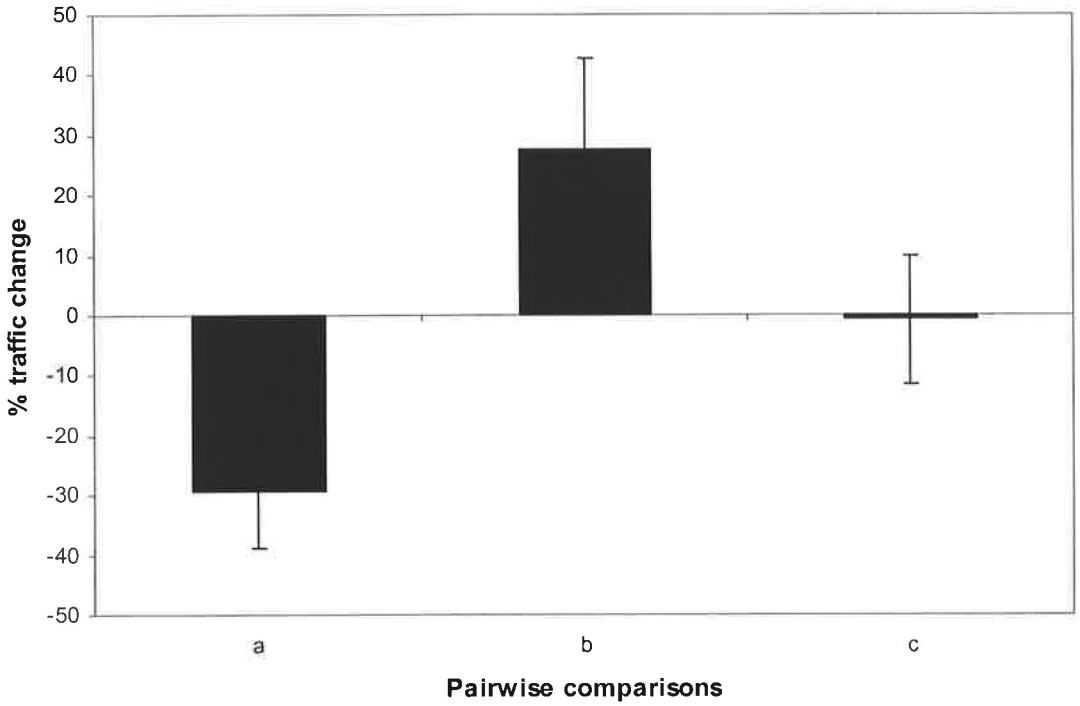

Fig. 4.2: The effect of rain on traffic rates: (a) onset of rain, (b) cessation of rain, and (c) overall change between the period before and the period after rain. The bars indicate the mean % traffic change and CI between consecutive observations.

Rainfall events decreased wasp activity (Fig. 4.1b). Rain was relatively rare during the study period, with only 37 of the 679 observations including rainfall events. For these, foraging activity was compared before, during and after rainfall events. Figure 4.2 indicates that average traffic rate dropped by 30% (from 77 to 48%) during rain, but increased again in the observation period immediately after. The overall change in foraging activity before and after rainfall was not significantly different from zero, suggesting rain did not have

any 'after-effects' on wasp activity. As rainfall was not quantified during the study but only scored as 'present' or 'absent', observations taken during rainy periods were omitted from subsequent analyses.

4.3.2 Effect of light

Foraging started prior to sunrise and continued until after sunset. A fit of day length (measured as the time between sunrise and sunset) versus foraging time (between first forager leaving the nest and last one returning) suggested that a strong linear relationship exists between these factors ($R^2 = 0.96$, $F_{1,36} = 777.57$, $p < 0.001$; Fig. 4.3).

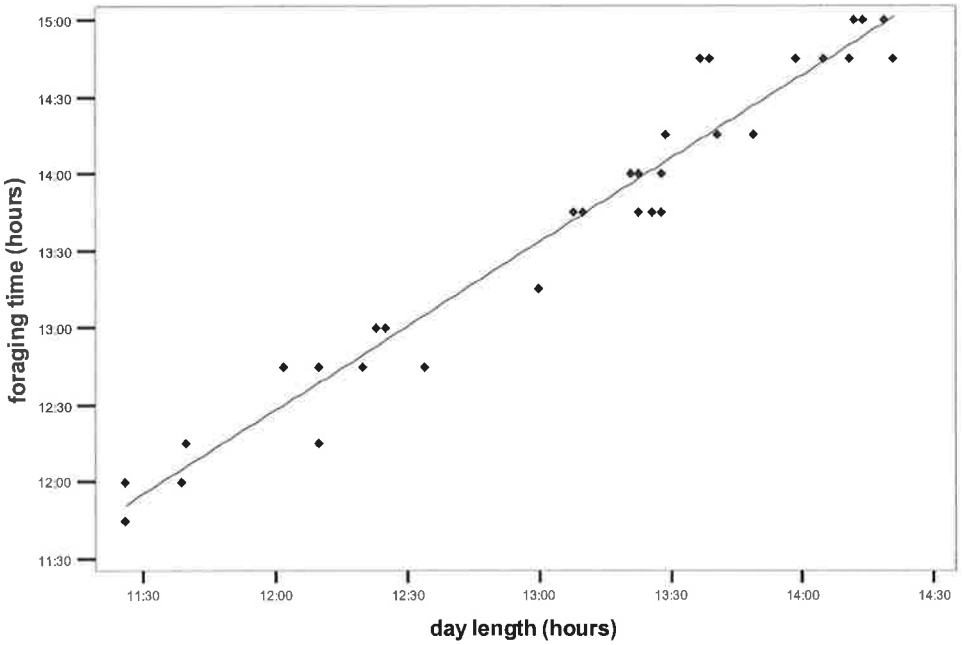

Fig. 4.3: Relationship between day length (sunrise minus sunset) and wasp foraging time (first wasp out minus last wasp in). The start and end of daily wasp foraging were recorded to within 15 min. Foraging time = -0.47 + 1.08 x day length, R^2 = 0.96, where the foraging time is expressed in decimal hours.

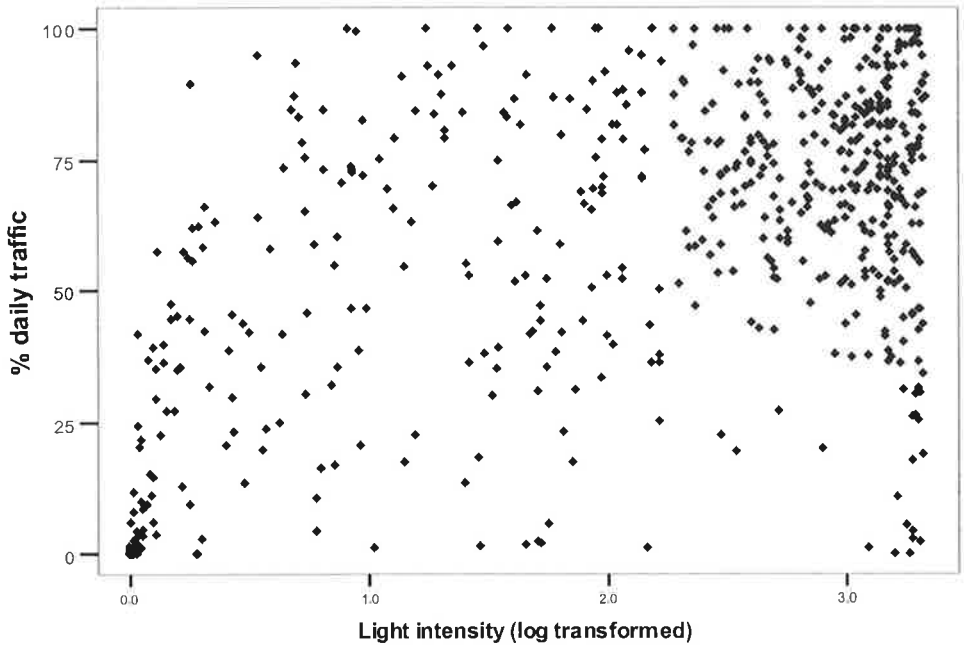

Fig. 4.4: Scatter plot showing pattern between the percent mean maximum activity and log (light intensity +1). Each point represents a single observation; all nests and observation days are pooled. Light intensity was measured in μmols photons sec^{-1} m^{-2}.

A plot of traffic rate as a function of light intensity shows activity started at extremely low light levels (50% daily activity was reached at 0.1 μmols photons sec^{-1} m^{-2}, 0.04 log), and wasps foraged at all higher light intensities (Fig. 4.4). Generally, low activity levels were not observed at higher light intensities, and conversely, high activity levels were not common under low light conditions. The reduction of activity at low light levels was particularly evident on foggy mornings (Fig. 4.1c).

4.3.3 Effect of temperature

The temperatures recorded during this study ranged from 6 to 42.5°C. Activity occurred at all temperatures above 7°C and below 41°C (Fig. 4.5). However, some distinct patterns are evident. Below 20°C, traffic rates increased with temperature. Between 20 and 35°C, wasp activity levels varied, but were generally at least 50% of the daily maximum. At temperatures above 35°C traffic rates decreased again, and activity virtually ceased above 40°C (Fig.

4.1d). This pattern suggests a quadratic relationship between foraging activity and temperature (see 4.3.5 below).

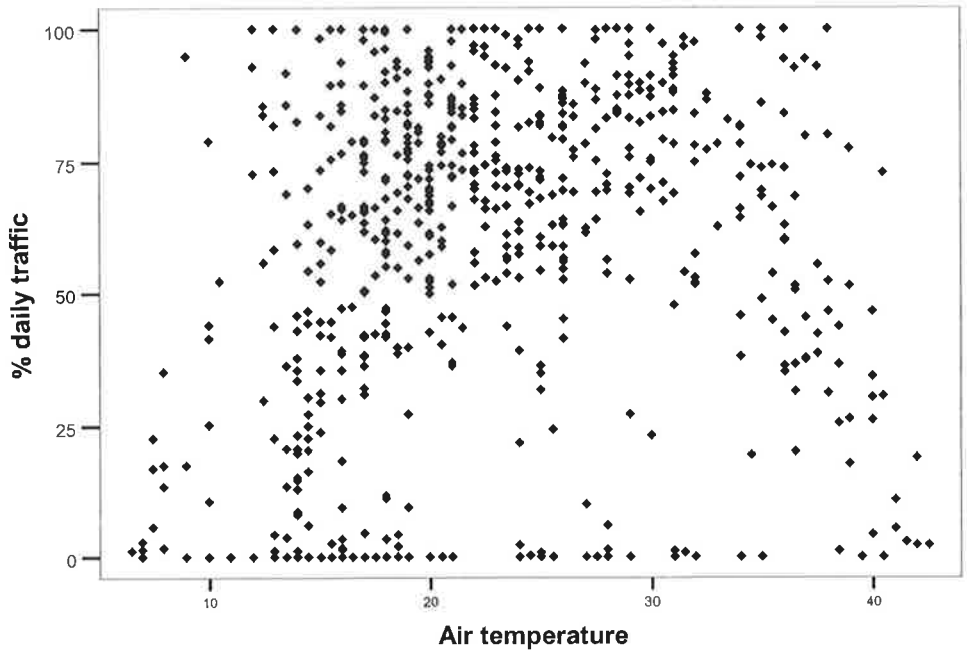

Fig. 4.5: Scatter plot of percent mean maximum activity and ambient air temperature. Each point represents a single observation. Air temperature was measured in degrees Celsius.

4.3.4 Effect of humidity

A plot of traffic rates versus humidity did not reveal any trends (R^2 = 0.005, $F_{1,289}$ = 1.39, p = 0.24). Thus, foraging activity did not appear to be influenced by humidity.

4.3.5 Modelling factors influencing wasp activity

A linear mixed effects model confirmed that traffic rates were a function of both air temperature and light intensity. The analysis indicated air temperature, air temperature squared, and light intensity (log transformed) were all significant predictors of daily traffic (Table 4.2). The final model was:

$$\%\ daily\ traffic = -47.5 + 6.71 \times air\ temperature - 0.15 \times (air\ temperature)^2 + 18.3 \times \log(light\ intensity)$$

Table 4.2: Results of the linear mixed effects model fitted to explain variations in daily wasp activity. All factors were highly significant (all $p<0.01$, 628 d.f.).

Fixed effects	Value	Std. Error	*t*-value
Intercept	-47.54	7.02	-6.77
Air temperature	6.71	0.62	10.88
(Air temperature)^2	-0.15	0.01	-12.28
Log (light intensity)	18.30	0.81	22.61
Random effects	**Standard deviation**		
Intercept	4.17		
Residual	21.14		

4.3.6 Daily foraging activity pattern

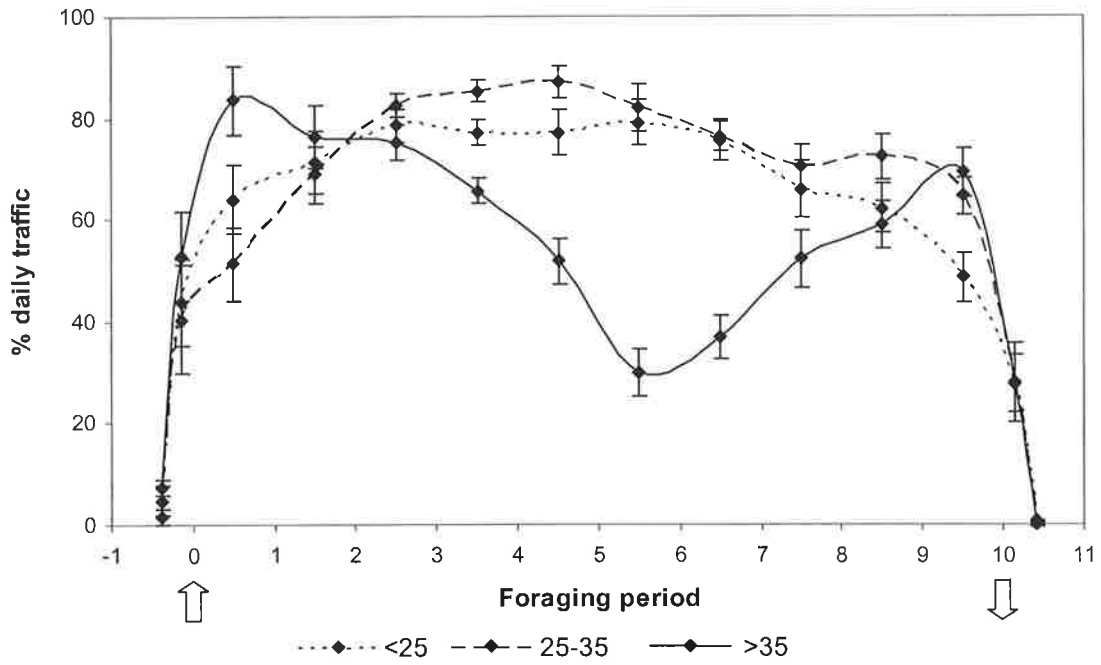

Fig. 4.6: Daily activity pattern at a *V. germanica* nest entrance at three different maximum daily temperatures <25°C, between 25 and 35°C, and >35°C. Each foraging period corresponds to 10% of day length. Sunset and sunrise are indicated by the arrows. Error bars represent standard error of mean.

Figure 4.6 shows data pooled across all months but separated by three maximum daily temperature ranges (<25, 25-35, >35), while figure 4.7 summarises daily wasp activity patterns during the different months of the

study. Overall, it is clear that wasp activity began rapidly in the morning, and similarly, ends in the evening, regardless of temperature. On days with maximum daily temperatures below 35°C, activity was relatively constant throughout the day. The pattern differed on hot days, when activity was substantially reduced in the middle of the day, corresponding with the hottest periods (Fig. 4.6). Patterns were not significantly different between months (Fig. 4.7). Patterns also did not differ between different sized colonies (data not shown).

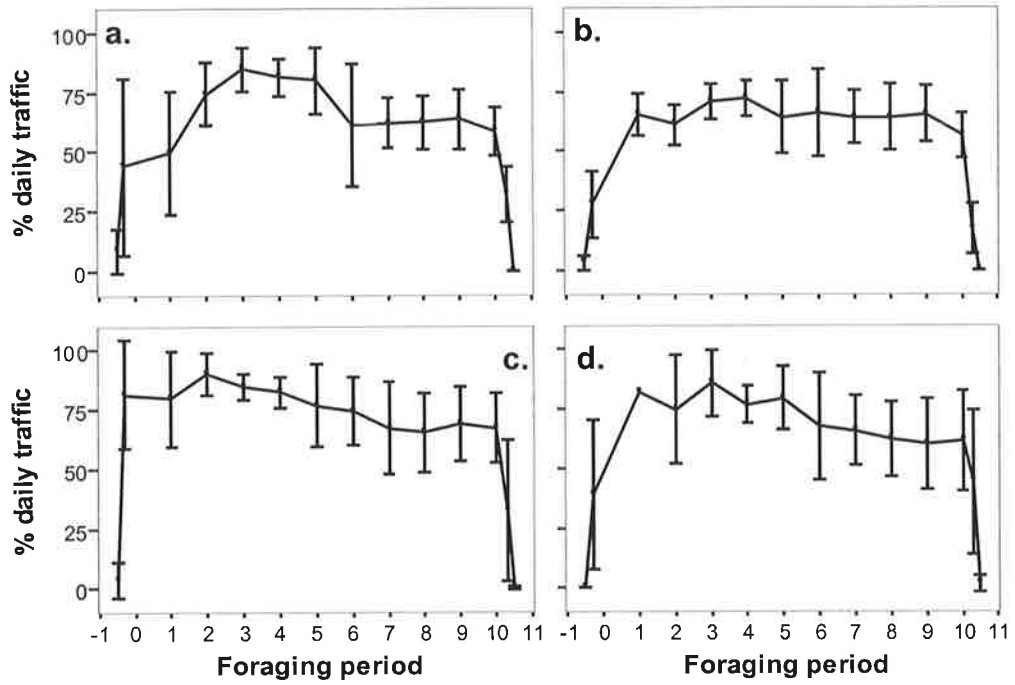

Fig. 4.7: Changes in daily wasp activity during the four months of the study (a) January, (b) February, (c) March, (d) April. Error bars represent 95% CI.

4.3.6 Changes in incoming vs. outgoing traffic

As Fig. 4.8 indicates, proportions of incoming and outgoing workers were the same at all time periods. The only exception was the period immediately after sunset, when the incomers outnumbered the outgoing workers. A converse pattern was observed in the morning but this was not statistically significant.

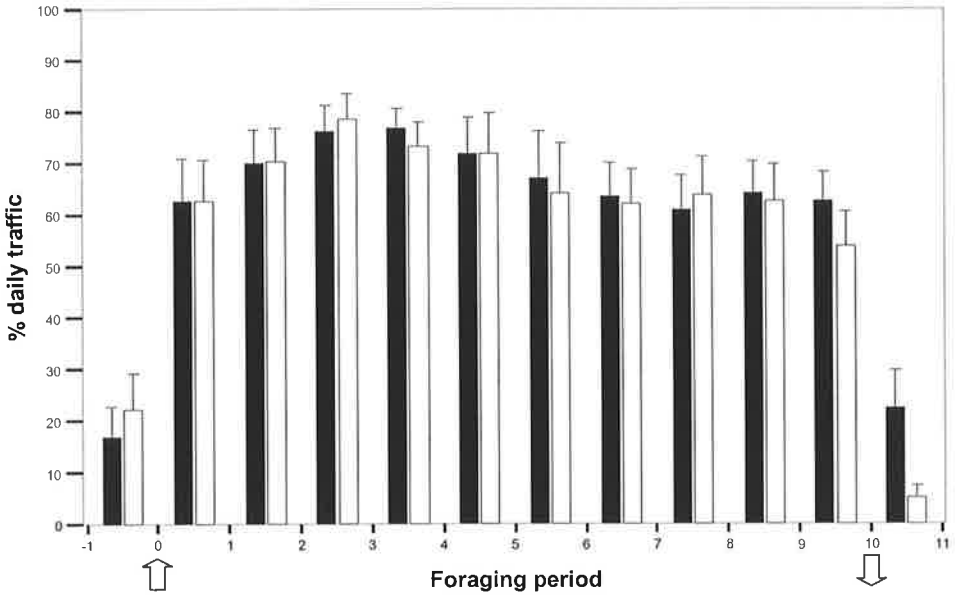

Fig. 4.8: Daily foraging pattern for incoming (black) and outgoing (white) *Vespula germanica* workers. Each foraging period corresponds to 10% of day length. Sunrise and sunset are indicated by the arrows. Error bars represent standard error of the mean.

4.4 Discussion

4.4.1 Daily activity pattern

The summarised daily activity pattern for *V. germanica* generated by this study, indicating that foraging remains fairly constant throughout the day, differs from the 'typical' (Spradbery 1973; Edwards 1980) pattern previously described for *Vespula* spp. This 'typical' pattern is characterised by a sharp peak of activity in the morning, when traffic rates are nearly twice those for the remainder of the day, followed by a decline to a constant level, and a small increase in traffic shortly before activity ceases for the day (Gaul 1952a; Potter 1964; Edwards 1968 in Edwards 1980). The morning peak in activity has been explained in terms of an influx of outgoing workers, due to an increase in larval food demand after the night (Gaul 1952a), or the high abundance and thus ease of obtaining fluid from dew (Potter 1964). The evening high was attributed to workers returning to the nest *en masse* prior to the setting darkness (Gaul 1952a; Potter 1964; Edwards 1968 in Edwards 1980). The patterns presented in these three

studies were very similar despite being conducted on different *Vespula* species and in different locations (*V. maculifrons* in the U.S.A., and *V. vulgaris* and *V. rufa* in England).

The current study found no differences between outgoing and incoming worker proportions, except for the period just after sunset, when more foragers were returning to the nest. Despite this, the summarised activity pattern does not show a spike in overall activity at that time, suggesting that the influx of incomers was compensated by severely reduced rates of outgoing traffic. Due to the lack of any increased activity (in the morning or evening), the pattern of daily foraging observed in this study is more similar to two other reported studies of *V. vulgaris* that did not find any activity spikes (in France, Roland 1976 in Edwards 1980; and in U.S.A., Heinrich 1984). The same pattern was also described by Potter (1964) for *V. vulgaris* colonies implanted with extra larvae. Although the current study did not find any differences in activity patterns for nests of different sizes, the study period did not cover the early colony stage, when only the queen and a few workers are present in the nest, or the first part of the small-cell stage, when rapid colony expansion occurs (Potter 1964; Archer 1980a; see Ch. 2), and foraging patterns may well differ between early (small) and large colonies. Alternatively, variations in patterns could exist due to behavioural differences between un-manipulated and *in situ* colonies (Heinrich 1984; current study), and ones transplanted into artificial nest boxes (Gaul 1952a; Potter 1964). In any case, the activity pattern observed for *V. germanica* in this study was explained mainly in terms of environmental conditions outside the nest rather than by any diurnal pattern of changes in colony needs (as suggested by Archer 2000). This finding complements the results described in Chapter 3, that resource intake does not change on a daily basis.

4.4.2 Environmental influences on wasp activity

That weather influences activity in social insects, and insects in general, is not a new finding. Numerous studies on bees and ants suggest that light, temperature, humidity or rainfall may regulate foraging (e.g. Kalmus 1954; Szabo 1980; Burrill and Dietz 1981; Porter and Tschinkel 1987). In *Vespula*

spp., knowledge of environmental factors influencing activity is mainly based on early observations by three researchers: Gaul (1952a; 1952b) on *V. maculifrons* in U.S.A, Blackith (1958) on *V. rufa* in England, and Potter (1964) on *V. vulgaris*, also in England. All three authors agree that vespid activity is dependant on light and temperature thresholds, and to a lesser extent on rain. The current study builds on these observations, utilising a comprehensive sampling strategy, replicating nests, days, and months, to produce a quantitative model explaining the factors influencing foraging activity of *V. germanica* in South Australia. For ease of interpretation and comparison, the factors are discussed separately (4.4.3 - 4.4.5 below); however, they do not act independently, as shown in the model (section 4.3.5). This study also differs from previous accounts of *Vespula* foraging in that it has examined the relationship of light and temperature with activity, and not simply made note of thresholds as has been done previously.

4.4.3 Effect of rain

Perhaps the least studied variable to influence activity is rainfall. Previous studies of bees conclude that activity is reduced or stopped during rain (e.g. *Apis mellifera*, Riessberger and Crailsheim 1997; *Melipona marginata*, Kleinert-Giovannini and Imperatriz-Fonseca 1986). A similar effect for *V. vulgaris* in England was observed by Potter (1964), who noted that storms and very heavy rain stopped all activity. During his observation, activity during rain dropped to zero from 65-75% of daily maximum, and increased after the rain to 25-30% of the daily maximum. The results obtained in the present study show that activity did not stop completely during rain but instead decreased in all nests by ~30%, and returned to previous levels straight afterwards. Although the patterns are different between these two studies, both suggest that precipitation reduces activity. The finding here that activity was not dependant on humidity gives further support to the hypothesis that it is not moisture in the air but the physical impact of raindrops that suppresses wasp traffic (Potter 1964). Future studies could concentrate on quantifying the relationship between the amount and duration of precipitation and foraging activity.

4.4.4 Effect of light

The relationship between the initiation and cessation of activity and light has previously been well documented in bees (e.g. *Apis dorsata*, Abrol 1987; *A. mellifera*, Vicens and Bosch 2000). Like bees, vespids navigate by visual cues, and a similar light threshold below which wasps did not forage has been observed for *V. vulgaris* (Potter 1964), and *V. maculifrons* (Gaul 1952a). This threshold was substantially different between the two species, but this difference was attributed mostly to an inability to measure light intensity accurately at low levels. The results presented here also suggest a light threshold for foraging, as evidenced by the relationship found between foraging time and day length. Sunrise and sunset are events defined by the angle of the sun over the horizon, and thus coincide with the amount of light present. The linear relationship found in this study (Fig. 4.3) differs from that found by Edwards (1968 in Edwards 1980), who noted an increasing difference between sunrise and first sorties, and sunset and last returns of *V. rufa* in England. The variation observed during Edward's three week study was explained in terms of moon phases, and longer foraging was attributed to a full moon providing more light to forage by. The data obtained in the current study shows no evidence for this.

Older studies, such as those of Gaul (1952a) and Potter (1964), have measured light in terms of lux, or the illuminance of a surface as seen by the human eye. Research has since shown that insect vision spans wavelengths 350-550 nm, and thus measuring it in terms of lux is not as meaningful (Shields 1989). The present study has used a quantum meter to measure light, and found that a direct relationship exists with wasp activity. A similar relationship has also been found in bees (e.g. *A. mellifera*, Szabo 1980; Burrill and Dietz 1981; Abrol 1988; *A. cerana indica and A. dorsata*, Abrol 1992). Although the minimum levels of light required for flight cannot be directly compared due to differences in units used (photons $m^{-2}s^{-1}$ versus watts m^{-2}), these studies indicate that *V. germanica* activity starts earlier, at lower light, and finishes later than bees. These differences may exist because bees forage for nectar and thus their activity should correspond with times when this resource is available from flowers, while *Vespula* also need to supplement their diet with other insects, and need to find

prey at the lowest possible light levels. Also, it is possible that a carbohydrate diet provides bees with a higher energy resource, while the lower energy obtained from a prey diet requires *Vespula* to replenish resources earlier in the day.

4.4.5 Effect of temperature

Temperature is perhaps the most widely studied environmental factor influencing foraging activity in social insects. Previous studies of *Vespula* suggested a minimum threshold below which wasps will not forage (Gaul 1952a; Potter 1964). However, this was very low: 2°C for *V. vulgaris* observed by Potter in England, and 5°C observed by Gaul for *V. maculifrons* in U.S.A. Temperatures that low were not recorded in the present study. Adelaide experiences a Mediterranean climate, with temperatures ≤5°C only recorded from April to October (Commonwealth Bureau of Meteorology 2004). However, temperatures ≥35°C, not often recorded in England or the U.S.A., occur regularly in Adelaide from October to April (Commonwealth Bureau of Meteorology 2004), and are thus more likely to affect activity of *V. germanica* here.

An increase in activity with temperature until a maximum is reached has also been described for other social insects. A similar pattern was found in desert bees (e.g. *Melipona marginata*, Kleinert-Giovannini and Imperatriz-Fonseca 1986; Hilário *et al.* 2000), several species of desert ants (e.g. *Cataglyphis* sp. and *Ocymyrmex barbiger*; Marsh 1985; Cerdá and Retana 2000), and the red imported fire ant (*Solenopsis invicta*, Porter and Tschinkel 1987). These studies found that while ground foraging ants were influenced by ground temperatures, flying bees were more likely to be affected by air temperatures. In both cases, the insects are vulnerable to desiccation. The ants can withstand extremely high ground temperatures, with activity only reducing at 46°C in some thermophilic desert ant species (Marsh 1985; Cerdá and Retana 2000). While ant body temperatures are mainly determined by ambient and soil temperatures, flying insects produce excess heat from their flight muscles, increasing their body temperatures further during flight (Ellington 1985). Thus, winged insects reach critical temperatures sooner, and their foraging decreases

at lower air temperatures than non-winged insects (Willmer and Stone 1997). For example, in the *Melipona* bee species mentioned above, the maximum air temperatures at which activity took place were 26 and 27°C (Kleinert-Giovannini and Imperatriz-Fonseca 1986; Hilário *et al.* 2000). This study found foraging in *V. germanica* wasps to still increase at these temperatures, to a maximum of approximately 35°C.

It is widely accepted that many insects are capable of thermoregulation (Stone and Willmer 1989). Flight will not occur until a certain thoracic temperature is reached (e.g. Stone and Willmer 1989; Coelho and Ross 1996). Coelho and Ross (1996) investigated the mechanisms responsible for thermoregulation and found that *V. germanica* can reduce their body temperature by regurgitating fluids, reducing it by as much as 4°C. Lethal body temperature for *V. germanica* was 50.7°C. As a flying wasp's lethal body temperature has to be higher than ambient temperature, it is possible that the reduction in activity of *Vespula* at 35°C corresponds to foraging at temperatures that are near lethal for most individuals. Conversely, vespids also thermoregulate the temperature inside their nests. This temperature varies between species and colony size, but is kept constant at between 28 and 31°C (Gibo *et al.* 1974; Harcourt 2002). On hot days, temperature inside the nest is reduced by evaporative cooling, using regurgitated water and wing fanning (Wilson 1971; Spradbery 1973). It is therefore possible that the decrease in foraging activity at high temperatures is not due to physiological restraints on the individual wasps, but rather on the nest. Either way, the distribution of *V. germanica* in Australia might be limited to areas experiencing cooler temperatures, and to places with nearby water.

A recent study of *Polistes humilis*, a native Australian paper wasp, revealed that this species does not start foraging until air temperature has reached 17°C, however, temperatures of 35°C and above do not reduce its activity (Perry 2001). This variation in temperature tolerance between *P. humilis* and *V. germanica* could arise due to the difference in nest architecture between the two species. While *V. germanica* has mostly subterranean nests that are thermoregulated to a constant temperature, *P. humilis* builds small, open nests. Cooling of larvae during hot weather is achieved by evaporative cooling in both species. However, while enclosed underground nests may require workers to

remain in the colony to help with wing-fanning, open aerial nests of *P. humilis* may require more water instead, satisfied by increasing their foraging trip frequency (Perry 2001). Apart from behavioural adaptations, physical and physiological dissimilarities between *V. germanica* and *P. humilis* may also cause differences in temperature tolerance limits. The world distribution of the genus *Polistes* is more tropical rather than Holarctic (Greene 1991; Reeve 1991), and thus the body shape or the cuticular transpiration rate in *P. humilis* may perhaps be more favourable in hot climates than those of *V. germanica*.

CHAPTER 5:

DIET OF *V. GERMANICA* AND PREY OVERLAP WITH A NATIVE PAPER WASP *POLISTES HUMILIS*

5.0 Chapter summary

In newly invaded communities, interspecific competition is thought to play an important role in determining the success of the invader and its impact on the native community. In southern Australia, the native *Polistes humilis* was the predominant social wasp prior to the arrival of the exotic *Vespula germanica* (Hymenoptera: Vespidae). Both species forage for similar resources (water, pulp, carbohydrate and protein prey), and concerns have arisen about potential competition between them. The aim of this study was to identify the protein foods that these wasps feed on. As many prey items are masticated by these wasps to the degree that they cannot be identified using conventional means, morphological identification was complemented by sequencing fragments of the mitochondrial 16S rRNA gene. GenBank searches using BLAST and phylogenetic analyses were used to identify prey items to at least order level. The results were used to construct complete prey inventories for the two species. These indicate that while *P. humilis* is restricted to feeding on lepidopteran larvae, *V. germanica* collects a variety of prey of invertebrate and vertebrate origin. Calculated values of prey overlap between the two species are used to discuss the implications of *V. germanica* impacting on *P. humilis*. Results obtained are compared to those gained by solely 'conventional' methods, and the advantages of using DNA-based taxonomy in ecological studies are emphasised.

5.1 Introduction

When an exotic species has been introduced into the range of an ecologically similar species niche overlap often occurs and this may initially result in competition (Connell 1961). Although the existence of interspecific competition in natural populations at equilibrium has been heavily debated (Connell 1983;

Schoener 1983; Gurevitch *et al.* 1992), evidence shows that in communities driven by density dependent processes, species sharing the same ecological niche are often separated by differences in temporal activity patterns, habitat usage or foraging behaviour (MacArthur and Levins 1964; May 1973; Schoener 1974). This suggests that competition is still occurring, or may have occurred in the past and has acted on the organisms to partition their resources to minimise overlap (Schoener 1974). Where this does not occur, and species' resources overlap, competition may cause a reduction in fecundity, survivorship or growth of one or both species. Thus an introduced species has the ability to restructure the receiving community not only through predation, but if the alien species has a competitive advantage, this may also lead to the suppression or even extinction of native species (Brown *et al.* 1994).

Certain classes of species are more likely to establish and consequently become environmental pests than others. Social insects, especially ants and wasps, pose a particular threat as they are polyphagous and their dispersal and reproductive characteristics enable them to establish and expand rapidly in new habitats (Moller 1996; Tsutsui *et al.* 2000). Their long life, large colony size, and versatile nesting gives them an advantage as competitors (Moller 1996).

The current study focuses on a social wasp, *Vespula germanica*, the European or German wasp. The species is native to the Palaearctic region but has successfully invaded many other parts of the world, including New Zealand, Chile, Argentina, U.S.A. and Australia (Edwards 1976; Akre *et al.* 1981; Spradbery and Maywald 1992). *Vespula germanica* displays all the characteristics thought to make organisms efficient invaders, including polyphagy, colony initiation by a single inseminated queen and an annual life cycle (Akre *et al.* 1981).

Throughout much of its introduced range *V. germanica* has spread rapidly and established in great numbers, and concerns have arisen that it may have a detrimental impact on the native ecosystems. Such an impact has already been documented in New Zealand, where *Vespula vulgaris*, a related species, has now spread throughout most of the country's native honeydew beech forests (Harris *et al.* 1991b). Comprehensive diet studies have shown this wasp species feeds on a range of native invertebrates, mostly flies and spiders,

removing 1-8 kg invertebrates/ha/year, as well as up to 99% of honeydew produced by scale insects in these forests (Harris 1991; Moller *et al.* 1991b). These resources are also utilised by many native insects and birds and thus competition for food is likely occur (Beggs and Wilson 1991; Harris 1991; Beggs 2001). However, little information exists about the actual extent of food overlap between *V. vulgaris* and native species.

Vespula germanica is thought to have established in southern Australia in the late 1970s (Spradbery and Maywald 1992). Prior to the arrival of this highly eusocial wasp, a native primitively eusocial paper wasp, *Polistes humilis humilis* was the predominant social wasp species in this region (Richards 1978). While *V. germanica* typically has large subterranean colonies, supporting several thousand individuals by late summer (Ch. 2), *P. humilis* builds its small, open nests, supporting up to 150 individuals, on man-made structures as well as in vegetation (Richards 1978; pers. obs.).

The food resources collected by all social wasps include carbohydrates (nectar) and proteins (predominantly other invertebrates, but also scavenged vertebrate flesh; Edwards 1980). The narrow petiole of wasps means that adults are unable to ingest solid foods. After capturing prey, foragers either leave it intact, chew off certain appendages, or masticate it extensively (Archer 1977). They then take the item back to the nest, where worker-larvae trophallaxis occurs. Workers feed portions of prey to the larvae, while the larvae regurgitate a solution containing sugars, protein and free amino acids (Hunt 1991).

Traditionally, the masticated items retrieved from wasps have been identified visually using morphological characters. This can require a high level of taxonomic knowledge, depending on the level of identification required. Identification can be further complicated by the degree to which prey items have been masticated. Often only a small proportion of items can be identified to order level [e.g. 97% identified by Harris and Oliver (1993), 70% by Harris (1991), 69% by Madden (1981), but only 65% identified by Archer (1977), 39% by Sackmann *et al.* (2000), and 30% by Gambino (1986)]. In these studies, all unidentified items have been grouped into a single category and thus made no allowance that unidentified prey may not represent the same proportions as the visually identified items. For instance, small or soft bodied prey may be

particularly difficult to identify after chewing. Therefore studies which rely on visual identification will not necessarily provide a complete prey profile.

An alternative method of identification that has recently become widely available is nucleotide sequencing. Unlike visual identification, DNA can be extracted from highly masticated items, PCR-amplified and sequenced. Using bioinformatic techniques and known DNA sequences available in public databases such as GenBank (http://www.ncbi.nlm.nih.gov/Genbank/), the prey can be identified at least to order, or in many cases family, genus and species levels. Bioinformatic techniques that can be used to identify the systematic origin of a target sequence include the standard BLAST® search (Altschul *et al.* 1990) and phylogenetic methods (reviewed by Swofford *et al.* 1996). BLAST searches use a local alignment algorithm that can rapidly search the nucleotide database, with sequence similarity being converted into a score that is used to rank sequence matches. The output from such an analysis is a list of taxa/sequences and their alignments with the sequence of interest. Assignment of the target to a particular taxonomic group can be based on which taxa in the list show closest sequence similarity or the highest BLAST score. However, currently there are no criteria to assess the confidence of BLAST scores to enable accurate assigning of sequences to a specific systematic group. In addition, BLAST scores may not necessarily reflect true species relationships because they do not take account of variation in rates of evolution of DNA in different lineages and gene regions. A phylogenetic approach may be used to overcome these. One advantage of this approach is that it is possible, using techniques such as bootstrap analysis (Felsenstein 1985) or Bayesian posterior probabilities (Huelsenbeck and Ronquist 2001), to assess the robustness of phylogenetic clades, allowing a measure of confidence of group identification. The disadvantage of this approach is that it is much more time consuming than a BLAST search, particularly if there are multiple unknown target sequences from a broad range of taxonomic groups.

The purpose of the present work was two-fold. First, the prey inventories of *V. germanica* and *P. humilis* were studied to determine the degree of prey overlap between the introduced and the native social wasps. This was done using a DNA sequencing/bioinformatic method of identification to complement

prey identified visually. The second aim was to assess the use of molecular methods in this diet study, including comparing the reliability of a BLAST search approach for systematic identifications with one based on phylogenetic analyses.

5.2 Materials and Methods

5.2.1 Prey collection and visual identification

Polistes humilis and *V. germanica* foragers were collected from nests throughout metropolitan Adelaide, South Australia, and surrounding suburbs, during the summer of 2000 and 2001. Wasps were captured at the nest with a hand net. For *Vespula*, a small amount of ether was poured into the nest to anaesthetise workers inside and ensure that only the incoming foragers were sampled. This was not necessary for *Polistes* as it has much smaller nest sizes and thus distinguishing incoming foragers was straightforward.

Captured wasps were killed and any items they were carrying were removed from the net and placed on ice. These were subsequently stored in 70% ethanol until analysed. Items were visually identified using distinguishing morphological characters as far as possible (i.e. to order level and sometimes to family and genus) under a stereo-microscope.

All unidentified *Polistes* prey items (n = 27), and a sub-sample of *Vespula* items (n = 44, 24% of all unidentified prey items), were subjected to further identification by nucleotide sequencing. Additionally, seven 'test' items were also sequenced. These were prey collected from *Vespula* and *Polistes* wasps which were visually identified as representatives of major arthropod orders known to be wasp prey, namely Hemiptera, Diptera, Lepidoptera, Orthoptera, Araneae, Odonata, and Hymenoptera.

5.2.2 Prey identification by nucleotide sequencing

Each sample was washed with 10mM Tris buffer before DNA was extracted using the commercial Puregene DNA isolation kit (Gentra Systems).

Although numerous loci could be used for the amplification of DNA, the mitochondrial 16S r-DNA was selected for several reasons. First, mitochondrial DNA is easy to extract and PCR-amplify because it is present in hundreds of copies in each cell; second, mitochondrial genes have been widely used in DNA sequencing and phylogenetic studies and, therefore, a substantial database exists (Moritz *et al.* 1987; Simon *et al.* 1994; Saccone *et al.* 1999). 16S was chosen as it is one of the three mitochondrial genes most commonly studied in arthropods (Olsen and Woese 1993; Caterino *et al.* 2000). It also possesses highly conserved as well as more rapidly evolving regions associated with its secondary structure, enabling it to be useful at more than one level of phylogenetic divergence (Hillis and Dixon 1991; Buckley *et al.* 2000). The conserved regions also enable the use of 'universal' primers that work well on a range of taxa (Kocher *et al.* 1989; Simon *et al.* 1994). The primers used, LR-J-12887 (5'-CCG GTC TGA ACT CAG ATC ACG T-3') and LR-N-13398 (5'-CGC CTG TTT ATC AAA AAC AT-3') target a 500-650 base pair region on the 16S gene, and have previously been shown to work on a range of invertebrates, as well as some vertebrates (see Simon *et al.* 1994).

Polymerase chain reaction (PCR) was conducted in a final volume of 25µl containing 10-100ng DNA extract, 0.2 µM of each primer, 0.2mM of each dNTP, 3mM $MgCl_2$, 1X reaction buffer and 0.5 unit of *TaqGold* DNA polymerase (Promega).

Amplification was carried out using a thermal cycler (Eppendorf Mastercycler) under the following optimised conditions: initial denaturation at 94°C for 9 min; 35 cycles of 94°C for 45 sec, 52°C for 45 sec, 72°C for 1 min; and a final elongation at 72°C for 6 min.

Prior to sequencing, the PCR reactions were cleaned up using an UltraClean™ PCR Clean-up Kit (MoBio Laboratories Inc). Sequencing reactions were set up in a final volume of 10 µl, using 5 µl cleaned up amplified DNA, 1 µl (5 pmol) of one of the primers and 4 µl of BigDye™ (Applied Biosystems) and cycle sequenced using procedures specified by Applied Biosystems. Sequencing fragments were purified using isopropanol precipitation and analysed using an ABI 3700 DNA sequencer.

Sequences were checked and edited using SeqEd (version 1.0.3, Applied Biosystems), and aligned with those in the International Nucleotide Sequence Databases (INSD) (Benson *et al.* 2003; Miyazaki *et al.* 2003; Stoesser *et al.* 2003) using BLAST searches (Altschul *et al.* 1990). Although the numbers of sequences in the INSD are continuously increasing, few sequences obtained in this study provided exact matches, and many showed low levels of similarity. Thus, phylogenetic trees were constructed in order to further confirm the identity of items.

5.2.3 Phylogenetic analysis

All sequences were entered unaligned into BioEdit (version 5.0.9, Hall 1999). The 16S secondary structure model of Buckley *et al.* (2000) was used as a template for aligning sequences obtained from wasp prey items. Additional sequences corresponding to the closest BLAST search matches were obtained from INSD and aligned to this set of aligned sequences by hand. Four regions were very poorly conserved making assessment of homology difficult, and were thus excluded from phylogenetic analyses (helix 75, terminal loops of helices 68 and 88, and the unpaired region between helix 81' and 88; see Buckley *et al.* 2000). The final data set, comprising of all *V. germanica* and *P. humilis* insect prey as well as additional sequences, contained 141 insect taxa. The aligned data matrix is available on the web from:
(http://www.ees.adelaide.edu.au/research/cebb/).

ModelTest (version 3.06; Posada and Crandall 1998) was used on the aligned sequences to determine the most appropriate nucleotide substitution model to use in the subsequent analysis. The likelihood ratio tests, conducted using ModelTest, indicated that the General Time Reversible model (Rodríguez *et al.* 1990), with a proportion of invariant sites and unequal rates among sites (Yang 1996) modelled with a gamma distribution (GTR+I+Γ) was the most appropriate model for the phylogenetic analyses. Phylogenies were generated using the Bayesian method in MRBAYES (version 3.0, Huelsenbeck and Ronquist 2001). The settings were: nst=6, rates=gamma, nchains=4, ngen=1,500,000, samplefreq=100, burnin=1,000 and default uninformative priors were used. The likelihood and parameter values converged to relatively stationary values after

about 40,000-60,000 generations and, therefore, a burn-in of 1,000 trees was chosen. A 50% majority rule consensus tree was produced from the remaining 14,000 trees.

Prey items were assigned to order groups based on the result of the BLAST searches and the phylogenetic analysis. For the latter, identification of prey items was based on the posterior probability support for monophyletic clades that contained the prey species, or for clades that were the sister group to the prey species.

5.2.4 Complete prey inventories

Complete prey inventories for both wasps species were derived by combining the visual and molecular prey identities:

$$n_i(adj) = n_i(raw) + n_i(mol) \qquad \text{eq. 5.1}$$

where $n_i(adj)$ is the adjusted number of prey items in category *i*, $n_i(raw)$ is the morphologically identified number of prey items in category *i*, and $n_i(mol)$ is the number of prey items in category *i* as identified by nucleotide sequencing. The $n_i(mol)$ was rounded to the nearest integer after deriving it from:

$$n_i(mol) = p_i(mol) * u_i(raw) \qquad \text{eq. 5.2}$$

where p_i is the proportion of prey items in category *i* successfully identified by sequencing, and $u_i(raw)$ is the number of prey items in category *i* that were morphologically unidentifiable.

5.2.5 Prey overlap values

Two indices were used to obtain values of prey overlap - Schoener's index (Schoener 1970) and Levins' asymmetric index (Levins 1968). Schoener's index has been shown to be the best suited to use with small sample sizes and the most accurate of four commonly used indices (Linton *et al.* 1981). This index varies from 0 when diets are completely different to 1 when they are identical. Overlaps >0.6 are considered high and may be indicative of competition if resources are limiting, whereas overlaps <0.5 are considered low and may represent a lack of competition. Levins' asymmetric index gives two

values that indicate the relative degree of overlap of each species on the other to suggest the most intense direction of competition.

5.3 Results

5.3.1 Morphologically identified prey

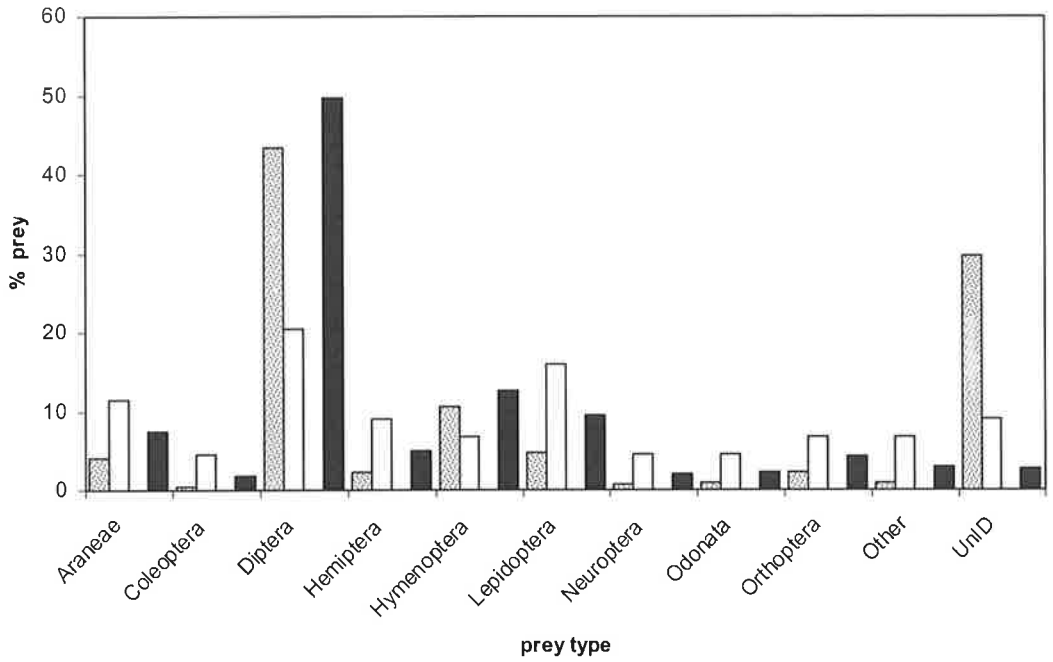

Fig. 5.1: Proportions of various prey types collected from *V. germanica* identified to order level. The shaded bars represent results obtained from solely visual identification, the white bars are for morphologically unidentifiable items as recognised by nucleotide sequencing, while the black bars show the complete prey inventory of *V. germanica* based on the visually identified items plus unknowns identified using molecular means (see text). The 'Other' category includes visually identified Amphipoda, reptile and Mollusca, as well as sequenced chicken and kangaroo. 'UnID' are prey which could not be identified using each method.

A total of 605 prey were collected from 4,360 *V. germanica* foragers (see Appendix 3 for complete list). As Figure 5.1 indicates, the largest proportion of items was identified as Diptera (43% of total prey). Many of these were identified to family level, and were found to be mostly Calliphoridae (at least 40% total flies) and Muscidae (7% total flies). The next most abundant prey items were Hymenoptera (11%), 95% of these comprising *Apis mellifera*; Lepidoptera (5%); and Araneae (4%). Other prey items included Orthoptera,

Amphipoda, Coleoptera, Neuroptera, Hemiptera, Odonata, as well as some non-arthropod items (parts of mollusc and a skink foot). 30% of all prey were so badly masticated that they could not be identified visually.

In comparison, only 39% of all *Polistes* prey (44 total prey collected from 514 foragers) could be visually identified. These were all lepidopteran larvae. The remaining 27 prey were identified by nucleotide sequencing.

5.3.2 Nucleotide identified prey inventories

All the nine morphologically identified 'test' prey items were PCR-amplified and sequenced. These included one representative each of Hemiptera (V.HEM.TEST), Diptera (V.DIPT.TEST), Orthoptera (V.ORTH.TEST), Araneae (V.ARA.TEST), Odonata (V.ODO.TEST), and two Lepidoptera (V.LEP.TEST and P.LEP.TEST) and Hymenoptera (V.HYM.TEST1 and V.HYM.TEST2). All the test items with the exception of one lepidopteran (P.LEP.TEST) were obtained from *V. germanica*. BLAST analysis of these sequences gave the highest scores with exemplars from the expected taxonomic groups (Table 5.1).

Similarly, all 27 *P. humilis* prey items were amplified and sequenced. All were classified as Lepidoptera based on BLAST and phylogenetic analyses (Table 5.1 and Fig. 5.3). The scores from BLAST analyses for these ranged from 309 to 718 (85-93% sequence similarity), while the posterior probability for the single clade containing all Lepidoptera and prey items assessed to be lepidopteran species was 97% (Table 5.1).

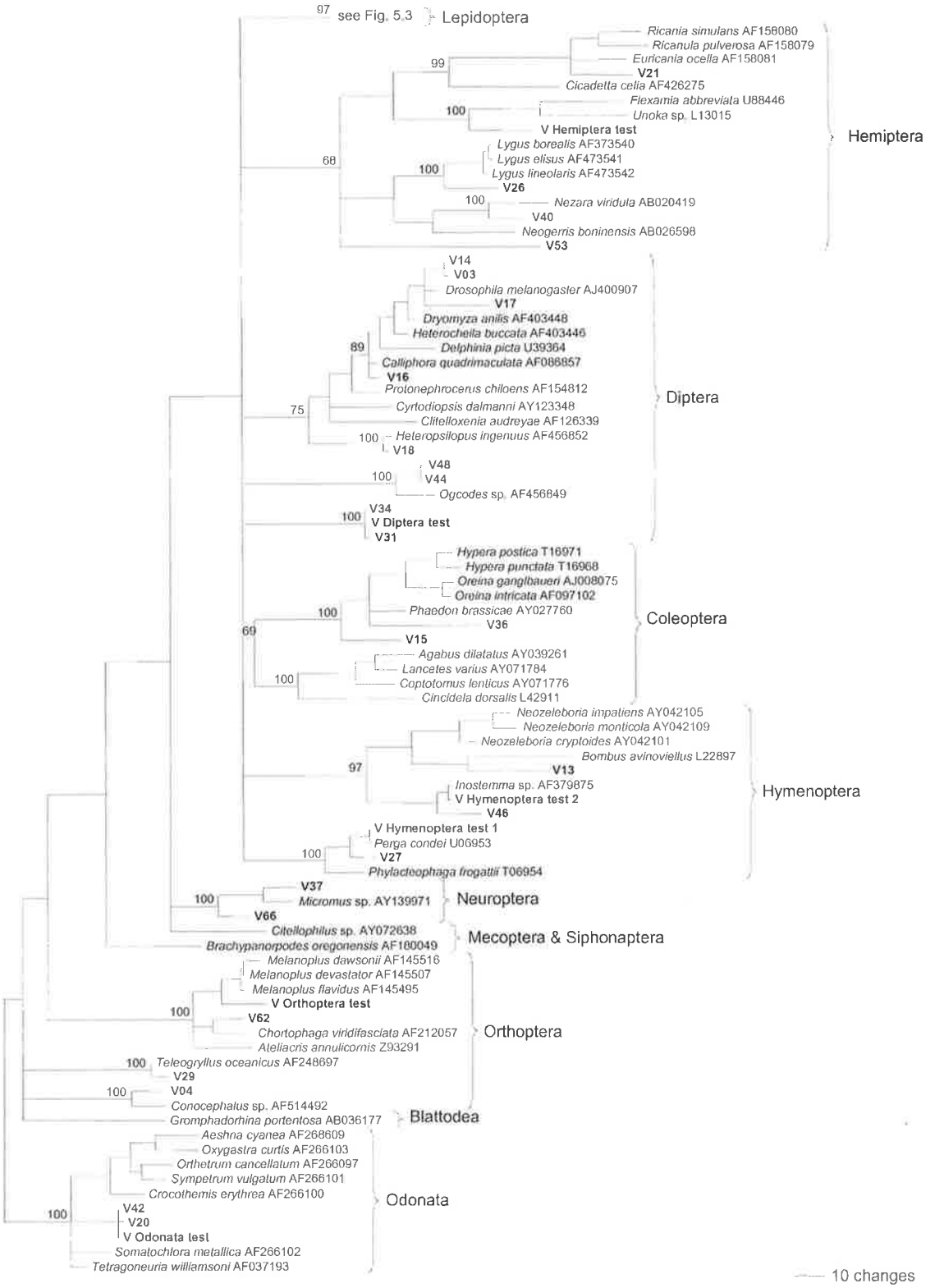

Fig. 5.2: 50% majority rule consensus tree showing positions of sequenced prey within known taxonomic groups. Morphologically unidentifiable prey obtained from *V. germanica* are marked as V, test items are labelled TEST, and sequences obtained from GenBank show their corresponding accession numbers. The phylogram was rooted using Odonata as an outgroup. Bayesian posterior probability values, calculated from 14,000 trees, are shown for each order clade as well as for those internal nodes used to assess the placement of unknown prey items. All orders except Lepidoptera are shown.

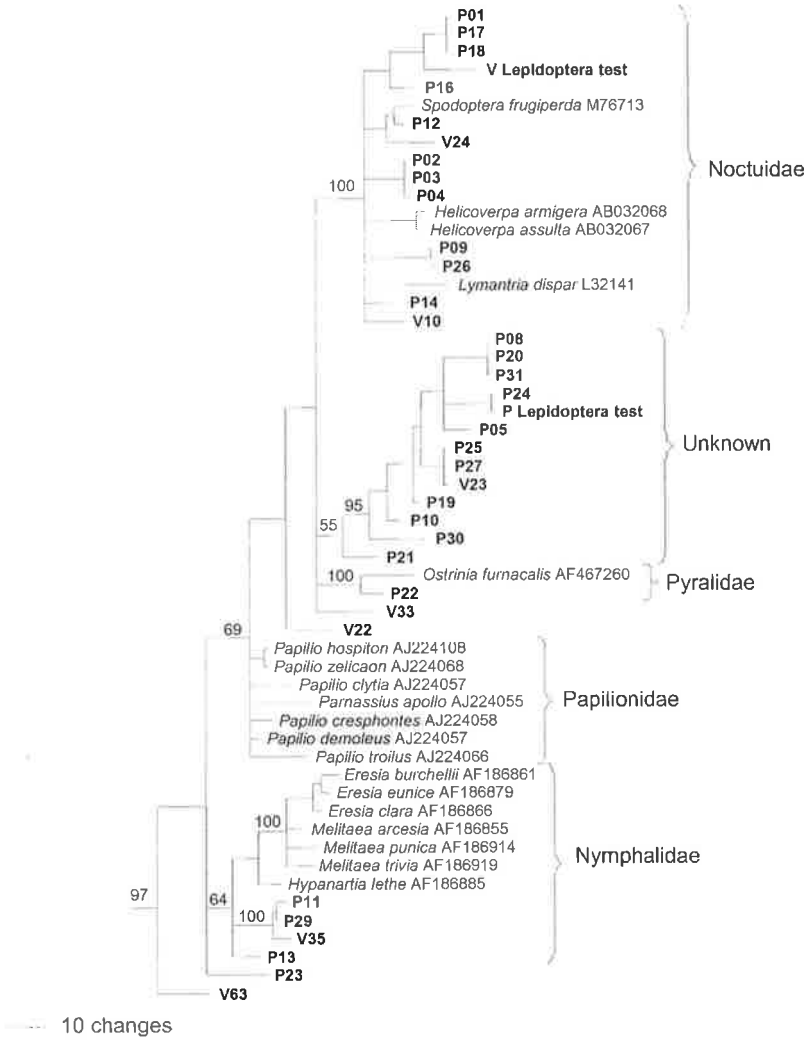

Fig. 5.3: 50% majority rule consensus tree showing positions of sequenced prey within Lepidoptera (from Fig. 5.2). Morphologically unidentifiable prey obtained from *V. germanica* are marked as V, from *P. humilis* are marked as P, test items are labelled TEST, and sequences obtained from GenBank show their corresponding accession numbers. Four known families of Lepidoptera are highlighted.

Table 5.1: All sequenced *V. germanica* (prefix V) and *P. humilis* (prefix P) prey and test items (suffix .TEST) as identified by BLAST and phylogenetic analysis. The final classification is based on the results of the phylogeny. Shown are the nearest BLAST match, its accession number, and BLAST score, as well as the posterior probability as obtained from the phylogeny. These values are for ordinal level support unless this was less than 95%, in which case a value for an internal node within the order clade giving the strongest support is shown. Samples with an asterisk were classified differently using the two methods, and the second closest BLAST match is also shown for these. The identity of items in italics was only obtained from the BLAST search.

Final classification	Sample	Nearest BLAST match	Accession	BLAST score	Posterior probability
Chicken	V02	*Gallus gallus*	AY235571	1,108	N/A
	V65	*Gallus gallus*	AY235571	1,100	N/A
kangaroo	V28	*Macropus parryi*	AF187887	926	N/A
	V28	*Macropus giganteus*	AF187885	926	N/A
Araneae	V25	*Frontinella pyramitela*	AY078661	161	N/A
	V43	*Agelena limbata*	AF145271	133	N/A
	V45	*Pardosa milvina*	AF223233	379	N/A
	V55	*Neriene radiata*	AY078710	168	N/A
	V57	*Frontinella pyramitela*	AY078661	167	N/A
	V.ARA.TEST	*Frontinella pyramitela*	AY078661	157	N/A
Coleoptera	V15	*Phaedon brassicae*	AY027760	218	100
	V36	*Oreina ganglbaueri*	A00J8075	317	100
Diptera	V03	*Dryomyza anilis*	AF403448	684	89
	V14	*Dryomyza anilis*	AF403448	230	89
	V16	*Chrysomya chloropyga*	AF352790	833	89
	V17	*Drosophila subsilvestris*	U07311	331	89
	V18	*Heteropsilopus ingenuus*	AF456852	462	100
	V31	*Clitelloxenia audreyae*	AF126339	214	100
	V34	*Clitelloxenia audreyae*	AF126339	214	100
	V44	*Ogcodes* sp.	AF456849	434	100
	V48	*Ogcodes* sp.	AF456849	440	100
	V.DIP.TEST	*Clitelloxenia audreyae*	AF126339	214	100
Hemiptera	V21	*Euricania ocella*	AF158081	216	99
	V26	*Lygus lineolaris*	AF473542	581	100
	V40	*Nezara viridula*	AB020419	202	100
	V53*	*Macropanesthia rhinoceros*	AB036177	131	68
	V53	*Symydobius kabae*	AF275237	127	
	V.HEM.TEST	*Flexamia abbreviata*	U88446	165	100
Hymenoptera	V13	*Camponotus* sp	U36335	200	97
	V27	*Perga condei*	U06953	577	100
	V46	*Inostemma* sp.	AF379875	892	97
	V.HYM.TEST 1	*Inostemma* sp.	AF379875	936	100
	V.HYM.TEST 2	*Perga condei*	U06953	882	97

(Continued)

Table 5.1 (Continued)

Final classification	Sample	Nearest BLAST match	Accession	BLAST score	Posterior probability
Lepidoptera	V10	*Helicoverpa armigera*	AB032068	618	97
	V22	*Papilio cresphontes*	AJ224058	345	97
	V23	*Papilio clytia*	AJ224057	280	97
	V24	*Eresia eunice*	AF186879	214	97
	V33	*Spodoptera frugiperda*	M76713	468	97
	V35	*Melitaea punica*	AF186914	577	97
	V63	*Melitaea arcesia*	AF186855	456	97
	V.LEP.TEST	*Helicoverpa assulta*	AB032067	468	97
	P01	*Helicoverpa assulta*	AB032067	593	97
	P02	*Spodoptera frugiperda*	M76713	642	97
	P03	*Spodoptera frugiperda*	M76713	626	97
	P04	*Spodoptera frugiperda*	M76713	571	97
	P05	*Battus philenor*	AJ224048	569	97
	P08	*Helicoverpa assulta*	AB032067	480	97
	P09	*Chlosyne nycteis*	AF186899	517	97
	P10	*Helicoverpa assulta*	AB032067	603	97
	P11	*Euphydryas gillettii*	AF186882	575	97
	P12	*Spodoptera frugiperda*	M76713	718	97
	P13	*Phyciodes cocyta*	AF186867	591	97
	P14	*Spodoptera frugiperda*	M76713	648	97
	P16	*Spodoptera frugiperda*	M76713	674	97
	P17	*Helicoverpa assulta*	AB032067	567	97
	P18	*Helicoverpa assulta*	AB032067	567	97
	P19	*Helicoverpa assulta*	AB032067	539	97
	P20	*Helicoverpa assulta*	AB032067	529	97
	P21	*Parnassius apollo*	AJ224055	325	97
	P22	*Ostrinia furnacalis*	AF467260	605	97
	P23	*Hypolimnas bolina*	AF186886	345	97
	P24	*Eresia plaginota*	AF186912	327	97
	P25	*Papilio demoleus*	AJ224061	321	97
	P26	*Helicoverpa assulta*	AB032067	609	97
	P27	*Papilio demoleus*	AJ224061	321	97
	P.LEP.TEST	*Eresia plaginota*	AF186912	309	97
	P29	*Melitaea trivia*	AF186919	585	97
	P30	*Papilio alexanor*	AJ224052	519	97
	P31	*Helicoverpa assulta*	AB032067	529	97
Neuroptera	V37	*Micromus* sp.	AY139971	345	100
	V66*	*Brachypanorpodes oregonensis*	AF180049	315	100
	V66	*Micromus* sp.	AY139971	311	
Odonata	V20	*Tetragoneuria williamsoni*	AF037193	303	100
	V42	*Somatochlora metallica*	AF266102	660	100
	V.ODO.TEST	*Somatochlora metallica*	AF266102	668	100
Orthoptera	V04	*Conocephalus* sp	AF514492	329	100
	V29	*Teleogryllus oceanicus*	AF248697	940	100
	V62	*Chortophaga viridifasciata*	AF212057	642	100
	V.ORTH.TEST	*Melanoplus scudderi*	AF145504	634	100

Double products were amplified for four of the 44 *V. germanica* items, possibly due to presence of more than one prey species. These were classified as unidentified. The remaining 40 unknown items were successfully sequenced. The sequences were initially identified to order level using a BLAST search and showed scores between 131 and 1,108. Four items attained a score of 900 or above, equivalent to at least 95% sequence similarity. These were two *Gallus gallus* (chicken, 1,100 and 1,108); one *Macropus giganteus* (kangaroo) or *Macropus parryi* (wallaby, both 926); and one *Teleogryllus oceanicus,* (Orthoptera, 940). The scores for all other items varied between 131 and 892, but all were assessed to be of arthropod origin.

Phylogenetic analyses using exemplar sequences from within Insecta (Fig. 5.2 and 5.3) supported the BLAST identification of target sequences at ordinal level for 30 *Vespula* insect prey, with only two items placed in orders different to those predicted by BLAST (Table 5.1). One of these, V53, had the lowest overall BLAST score of 131, and was identified as most similar to *Macropanesthia rhinoceros,* Blattodea, but was classified as an hemipteran based on the phylogeny analysis. The second prey item, V66, had a relatively higher BLAST score of 315, was identified as Mecoptera by BLAST but as a Neuroptera in the phylogenetic analysis. For both items, the second closest BLAST match, with a lower score, corresponded to its identification based on the phylogeny (V53, *Symydobius kabae*, score 127 and V66, *Micromus* sp., score 311; Table 5.1). Interestingly, 5 of the 6 spiders (V25, V43, V55, V57 and V.ARA.TEST), and 1 hemipteran (V.HEM.TEST) had scores lower than 200. The spider sequences (BLAST identified) were not included in the phylogenetic analyses due to their large sequence divergence and alignment problems with insect 16S sequences, however, V.HEM.TEST was classified into the correct order using the phylogenetic approach.

As Figure 5.1 indicates, visually unidentified *V. germanica* prey represent a variety of organisms, with the most abundant group being Diptera (20% of items), followed by Lepidoptera (16%), Araneae (11%), Hemiptera (9%), Orthoptera and Hymenoptera (both 7%). Other sequenced prey include Odonata, Neuroptera, Coleoptera, chicken and kangaroo.

5.3.3 Complete prey inventories of Vespula and Polistes

When the prey inventories for both *V. germanica* and *P. humilis* were adjusted using equations 5.1 and 5.2, and values obtained from visual and molecular identification, the proportion of each prey group are seen to change (Fig. 5.1). For example, in the *Vespula* diet, the proportion for Diptera increased from 43% to 50%, for Hymenoptera from 11% to 13%, for Lepidoptera from 5% to 10%, and for Araneae from 4% to 7%. In contrast, the diet of *Polistes* remained exclusively Lepidoptera (100%).

5.3.4 Prey overlap

Schoener's index of resource overlap for the two species was 0.10. Levins' index gave a value of 0.10 overlap of *P. humilis* on *V. germanica*, and a value of 0.33 overlap of *V. germanica* on *P. humilis*.

As *P. humilis* only fed on one group of arthropods, Lepidoptera, an attempt was made to determine the overlap of individual species within that order. This was done by visual inspection of the phylogeny of overlapping prey (Fig. 5.3) as well as by examination of raw sequence divergence. The figure indicates that both *P. humilis* and *V. germanica* wasps feed on representatives of at least four major lepidopteran families, corresponding to the Noctuidae, Pyralidae, Papilionidae, and Nymphalidae. The topology of the tree indicates that the lepidopteran taxa fed on by both wasp species are dispersed throughout those clades. Pairwise sequence divergence values indicate 2.5% divergence between V23 and P27, 2.9% between V23 and P25, and a 5% divergence between V35 and P29, suggesting that both wasps feed on the same genera and potentially the same species of Lepidoptera.

5.4 Discussion

5.4.1 *Diets of* Vespula germanica *and* Polistes humilis

The results of this study, indicating that *P. humilis* specialises on lepidopteran larvae in southern Australia, while *V. germanica* is a generalist, are broadly consistent with previous studies of the two wasp genera in Australia and New

Zealand. The two predominant prey types for *V. germanica* from other locations were Diptera and Araneae, followed by Lepidoptera and Hymenoptera, and then Hemiptera, Coleoptera and Orthoptera (Madden 1981; Harris 1991; Harris 1996). A study in Argentina found *V. germanica* to feed on the same prey orders, but rather than having one predominant prey, the relative proportions of each order were similar (Sackmann *et al.* 2000). Only one study, that of Madden (1981) in Tasmania, found a large number of flesh fragments (23%) being brought back to the nest, that were likely to be of vertebrate origin, but this was not determined. Also, while Araneae was one of the two most commonly taken prey in other studies, it made up only a small proportion of the wasps' diet in the present study. These findings suggest that *V. germanica* are truly opportunistic, with their diets varying according to availability and abundance of various prey types (Sackmann *et al.* 2000).

Compared to *V. germanica*, little work has been undertaken on *P. humilis*, with only one study from New Zealand, where the species has been introduced since the 1880s (Miller 1984). There, the predominant prey type was also Lepidoptera but it also included a small number of spiders (Clapperton 1999). Other studies of related *Polistes* species from Europe (*P. dominulus*, *P. gallicus*, *P. nimphus*) indicate that the majority of their diet consists of coleopteran larvae and hemipterans (Nannoni *et al.* 2001). *Polistes* in USA (*P. anularis*, *P. fuscatus)*, and New Zealand (*P. chinensis antennalis*) show a similar pattern of feeding predominantly on Lepidoptera (i.e. 88-98 % of identified items), but also supplement their diet with other arthropods including Hymenoptera, Hemiptera, Diptera, Araneae, and Orthoptera (Rabb 1960; Clapperton 1999). The only species of *Polistes* previously recorded to have fed exclusively on Lepidoptera was *P. exclamans* in the USA (Rabb 1960).

5.4.2 The use of nucleotide sequencing for prey identification

The relatively large proportion of morphologically indistinguishable prey of *P. humilis* was identified as Lepidoptera by molecular analysis, confirming the visual finding that Lepidoptera were the only group to be utilised as a source of food by this species in South Australia.

In contrast, heavily masticated *V. germanica* prey were found to belong to at least 9 arthropod orders, and the relative proportions of these morphologically unidentified items differ from the visually identified prey (Fig. 5.1). As Diptera made up a large proportion of the morphologically identified items, but less than half of that for the sequenced items, it appears that Diptera belong to a group that are easily identified by visual inspection. Conversely, Lepidoptera, Hemiptera and Araneae made up much smaller proportions of morphologically identified items than of sequenced items. These differences presumably exist due to differential handling and degree of mastication of various arthropod groups by *Vespula*. Therefore visual identification is not fully representative of the proportions of prey that the wasps take. This may be especially true for studies where large proportions of items could not be identified [e.g. Gambino (1986), 70%; Sackmann *et al.* (2000), 68%].

Another advantage of identifying items using a DNA/bioinformatics approach is that previously unidentifiable fragments of animal protein, known to be taken by wasps to feed their developing brood (Raveret Richter 2000), could now be identified. This study enabled the identification of prey of chicken and kangaroo origin, which are likely to have been scavenged from commercial pet food, human food or carrion. These prey types are virtually impossible to identify morphologically.

Overall, 76 out of 80 samples (including test items; 95%) were successfully amplified and sequenced. The remaining samples exhibited multiple bands, and cloning may have enabled them to be sequenced. When compared to the INSD, 90% of sequences showed a BLAST score of at least 200. It is possible that better matches could have been obtained using another mitochondrial gene, such as cytochrome oxidase subunit I or II (COI or COII). COI in particular has been targeted by an increasing number of sequencing studies – a search of the GenBank nucleotide database now returns almost twice as many COI sequences as 16S for Insecta (4,741 16S vs. 8,583 COI; http://www.ncbi.nlm.nih.gov/entrez; accessed 08 Nov 2003). However, the specific region amplified in the COI gene varies between many of these studies, because so-called 'universal' primers for this gene do not always reliably amplify from a broad range of insects. In contrast, the region amplified in this

study has been widely employed in studies of insects (Caterino *et al.* 2000), and the primers utilised here appear to amplify across a very broad taxonomic range, including vertebrates. Therefore, for prey identification studies in ecology with specimens from many unknown taxonomic groups, the use of the mitochondrial 16S rRNA gene is advocated.

There has been a call for 'barcoding' species for the purpose of identification, so that every species on earth could be recognised by a specific fragment of DNA (Hebert *et al.* 2003). This concept has been widely applied by microbiologists (Theron and Cloete 2000) who commonly analyse PCR-amplified bacterial rDNA diversity using single-strand conformational polymorphism (e.g. Schwieger and Tebbe 1998) or denaturing gradient gel electrophoresis (e.g. Reeson *et al.* 2003). In forensics, this method has been called FINS (Forensically Informative Nucleotide Sequencing; Bartlett and Davidson 1992) and has been used to identify the origin of animal samples (e.g. Unseld *et al.* 1995) or to distinguish between closely related species (e.g. Vincent *et al.* 2000). The application of this method has also been extended to food science, where it has been used to test the identity of processed products such as sardines or gourmet meats (Forrest and Carnegie 1994; Jérôme *et al.* 2003). However, the use of this technique in ecology to study animal diets is still limited, and preference is given to other methods such as electrophoresis or monoclonal antibodies (reviewed in Symondson 2002). Where DNA sequencing has been used, it has normally targeted a particular species in the predator diet (e.g. Höss *et al.* 1992; Agustí *et al.* 2003). The present study has shown that, by using universal primers, DNA sequencing is an effective tool in identifying a range of taxa to at least order level. This is particularly useful in situations where lack of morphological characteristics (or taxonomic expertise) preclude identification (e.g. in stomach contents). Future developments of a more comprehensive representation of taxon sequences in the nucleotide databases should enable identification as far as species level.

In this study, identities were assigned to items based on the highest BLAST scores and tested the accuracy of these with a phylogenetic approach. The BLAST approach is very fast and has given a good level of accuracy at the order level. Only two of the samples were misidentified. One of these had a

low BLAST score, possibly reflecting the lack of taxonomic coverage of some groups in the nucleotide database. Overall, BLAST provides a good approximation of the likely taxonomic group that an unknown sequence belongs to but until the nucleotide databases are much more comprehensive (and depending on the accuracy level required after in prey identification studies), identification of taxonomic groups should use a phylogenetic approach. The advantage of this latter approach is that one can also assess prey overlap between two species within a phylogenetic context.

5.4.3 Extent of overlap between V. germanica and P. humilis

The low value of Schoener's index of food overlap between *V. germanica* and *P. humilis* suggests that competition for food may not exist between the two wasps. However, closer examination of the composition of prey implies that a single overlap value may not be suitable to use in this situation, and Levin's asymmetric index, showing a stronger impact of *V. germanica* on *P. humilis*, but not of *P. humilis* on *V. germanica,* may be more appropriate. Furthermore, unlike the mere values obtained from either index, a phylogenetic approach to prey overlap also illustrates where the overlap occurs. As the phylogeny of lepidopteran prey from both species shows, the food niche of *P. humilis* is included entirely within that of *V. germanica* (Figs. 5.2 and 5.3). In order for both species to co-exist in this type of system, the species with the included niche must be a superior competitor for the shared resource on a per capita basis (Hutchinson 1957; Schoener 1974; Colwell and Fuentes 1975). Additionally, the species with exclusive resources already has a competitive advantage as it can exist on the exclusive resources while it is also utilising the shared resources (Chase 1996). Anecdotal evidence suggests that *V. germanica* forage at lower light intensities and at wider temperature ranges than *P. humilis* (pers. obs.). Also, almost 14% of all *V. germanica* foragers return with prey compared to only 8% of *P. humilis.* This suggests that *P. humilis* may not be a superior competitor over *V. germanica*. It is therefore possible that even if Lepidoptera is not a limiting resource, *V. germanica* might still have a detrimental effect on the lepidopteran population and thus on *P. humilis,* especially if the exotic wasp reaches high densities.

CHAPTER 6:

POPULATION DYNAMICS

6.0 Chapter summary

In Australia, *Vespula germanica* (European or German wasp) is an invasive insect pest. In South Australia a nest destruction initiative has been implemented in an attempt to control this pest. Accurate records of numbers and locations of nests destroyed in the area around Adelaide have been kept for over 10 years. These data were used to model the rate of increase (r_t) of *V. germanica* densities as a function of previous nest densities as well as weather variables. A time series analysis indicates that wasp populations did not cycle, however, a partial rate correlation analysis suggests direct density-dependence. The final model, using a Ricker equation modified to allow for weather effects, explained 51% of the overall variability. Previous year's nest densities, rainfall in April as well as the number of hot days in March (when the maximum daily temperature was 35°C or above) were all significant predictors of r_t. When the model was used to predict wasp densities one year in advance, the forecast value was not significantly different to that observed. The finding that previous year's wasp densities negatively affect r_t has important implications for wasp control.

6.1 Introduction

One of the main goals in population ecology is to explain the processes responsible for fluctuating population dynamics. It has been acknowledged that populations can be regulated by endogenous (density-dependent) or exogenous (density-independent) factors (Nicholson 1933; Andrewartha and Birch 1954). Recent studies confirm that population changes can be driven by both of these factors (e.g. Leirs *et al.* 1997; Lewellen and Vessey 1998; Lima *et al.* 2002). Indeed, including an exogenous weather effect in a model frequently increases its predictive power (Rothery *et al.* 1997).

Understanding the patterns and causes of changes in population densities is often critical in resolving pest management issues. Pest density data is commonly collected as a time series, from which a simple and readily measurable predictor of future densities is required. In this chapter, the effects of previous densities and weather on population dynamics of an exotic insect pest species in Australia are examined.

Vespula germanica is a highly invasive social insect. While the species is native to the Palaearctic region, it has successfully become established in many other parts of the world, including the USA, New Zealand and Australia (Edwards 1976; Akre *et al.* 1981; Spradbery and Maywald 1992). *Vespula germanica* typically has an annual life cycle with two distinct phases. During the colony phase, a new nest is founded by a single inseminated queen in early spring (Spradbery 1973). The nest is expanded by workers and grows through summer. In early autumn, new queens and males are produced, and the foundress queen dies. Mating occurs, and the new inseminated queens begin the solitary phase of their life cycle. They hibernate outside the nest through winter, to emerge again in spring and start nests of their own (Edwards 1980).

Population fluctuations in *V. germanica* as well as other *Vespula* species have previously been explained using various models emphasising either the effects of density-dependent factors or weather. For example, two year cycles have been noted for *V. maculifrons* in the U.S.A. (Roth and Lord 1987) and *V. vulgaris* in England (Archer 1985). These were explained in terms of an endogenous mechanism. In contrast, weather was found to be a major factor influencing populations of seven species of vespids in the Pacific northwest of the U.S.A. (Akre and Reed 1981), and *V. germanica* in Australia (Madden 1981; Horwood *et al.* 1993). However, these findings are often conflicting. For example, while Madden (1981) found a positive influence of spring rainfall on future wasp abundance, Akre and Reed (1981) concluded that the relationship was negative These discrepancies may exist because different studies have defined abundance in various ways, including numbers or densities of destroyed nests (e.g. MacDonald and Matthews 1981; Archer 1985; Roth and Lord 1987; Archer 2001a), densities of queens (e.g. Spradbery 1971), or numbers of foragers visiting baited traps per area (e.g. Archer 1980a). Often

the study area formed only a small proportion of the population's distribution. Moreover, none of these studies have included a combination of density-dependent and density-independent factors.

The first study of wasp population dynamics that explicitly integrated the effects of both density-dependence and weather was Barlow *et al.*'s (2002) study of *V. vulgaris* in New Zealand's native honeydew-producing beech forests. These beech forests, located in the north-western part of the South Island, are infested with scale insects (*Ultracoelostoma assimile* and *U. brittani*) that produce a high energy honeydew secretion (Moller and Tilley 1989). In their study, Barlow *et al.* (2002) monitored wasp populations at six sites by counting nests found along marked transects. Their results indicate that wasps reach extremely high densities (up to 30 nests ha^{-1}), with high and low years varying by a factor of two. These densities were found to be negatively affected by spring rainfall and also density-dependence, and the population dynamics were summarised by a Ricker model.

In South Australia, *V. germanica* was first detected in 1978, but the wasp did not become permanently established until 1984. This prompted the formation of a control program, where nests found by members of the public are destroyed free of charge by local councils. Accurate records of numbers of nests destroyed have been kept by council authorities since the early 1990's. In this study, these records are used, and an approach similar to that of Barlow *et al.* (2002) is followed to model wasp densities. In particular, a modified Ricker model is used to incorporate both weather effects and previous wasp densities in predicting the rate of increase of wasp populations. However, significant differences exist between the two studies. First, this study concentrates on a related but different wasp species. Second, and perhaps most importantly, the study environments differ greatly. New Zealand's beech forests contain a high energy carbohydrate resource (Moller and Tilley 1989), which is not available in the urban study area of Adelaide. Moreover, New Zealand's beech forests experience a mild and mostly continuous wet climate, with total annual rainfall of 600-3,000 mm and average daily maximum temperatures not exceeding 25°C (Beggs 2001; MetService 2003), while the metropolitan Adelaide region is generally drier and hotter, receiving an annual rainfall of 400-1,000 mm and

average maximum temperatures in summer reaching 30°C (Commonwealth Bureau of Meteorology 2004). Lastly, while the New Zealand study encompassed only a small proportion of the wasp's range on the south island, this study covers the entire extent of the permanent *V. germanica*'s distribution in South Australia. However, where nest sampling was unlikely to affect future wasp densities in the beech forests, the sampling method used here was destructive. Overall, these differences suggest that the factors that regulate wasp populations in urban Adelaide may not be the same as ones in New Zealand's beech forests.

Therefore, the purpose of this paper is to develop a predictive model of changes in the abundance of *V. germanica* in metropolitan Adelaide, based upon density data as well as various weather variables.

6.2 Materials and Methods

6.2.1 Study area and wasp density data

Adelaide is located between the Gulf of St. Vincent to the west, and Mt. Lofty Ranges to the east, and covers an area ~3,000 km^2. With a population of 1.1 million, the area contains highly populated urban centres on the plains, and surrounding semi- rural regions in the adjacent hills. Adelaide experiences a Mediterranean climate, with an average annual rainfall of 400 mm on the plains and up to 1,000 mm in the surrounding hills, average maximum daily temperature of 12-30°C, and an average minimum daily temperature of 7-16°C (Commonwealth Bureau of Meteorology 2004). Most of the yearly rainfall occurs in autumn and winter, between April and October.

Under the nest destruction scheme, property owners and members of general public may have *V. germanica* nests destroyed free of charge. Records of nests destroyed have been kept by individual local councils, however, these are not complete until 1992/93 onwards. Thus in this study, data was compiled from 20 metropolitan Adelaide councils for 1992/93 – 2002/03 (Table 6.1). These council areas cover the entire permanent distribution of *V. germanica* in South Australia.

Table 6.1: Local council regions used as replicates in the population dynamics study, showing land area and population at the 2001 census. Latitude and longitude values presented here are for council offices and are ones used to obtain weather data.

Council region	Area (km^2)	Human population	Lat. & Long.
Adelaide	15	13,483	34° 55'S, 138° 36'E
Adelaide Hills	795	38,718	34° 55'S, 138° 50'E
Burnside	27	42,653	34° 56'S, 138° 39'E
Campbelltown	24	46,818	34° 52'S, 138° 39'E
Charles Sturt	56	103,882	34° 52'S, 138° 32'E
Gawler	41	18,374	34° 35'S, 138° 44'E
Holdfast Bay	15	33,855	34° 58'S, 138° 30'E
Marion	56	79,223	34° 59'S, 138° 33'E
Mitcham	76	62,538	34° 58'S, 138° 37'E
Mount Barker	594	23,965	35° 40'S, 138° 51'E
Norwood Payneham and St Peters	15	33,966	34° 54'S, 138° 38'E
Onkaparinga	520	151,400	35° 16'S, 138° 33'E
Playford	345	68,840	34° 40'S, 138° 39'E
Port Adelaide Enfield	93	102,044	34° 50'S, 138° 30'E
Prospect	8	19,301	34° 53'S, 138° 35'E
Salisbury	158	115,052	34° 45'S, 138° 38'E
Tea Tree Gully	95	99,710	34° 49'S, 138° 43'E
Unley	14	36,609	34° 57'S, 138° 36'E
Walkerville	4	7,035	34° 53'S, 138° 37'E
West Torrens	37	52,370	34° 42'S, 138° 36'E

Nest densities were collated for ten wasp seasons, from 1992/93 until 2001/02. During the first five seasons, the dataset was only available from 16-19 councils. Data from 2002/03, the 11th season, was not used to compile the model but rather to test its accuracy in forecasting future densities.

Data used in this chapter are not wasp densities *per se*, but rather the densities of nests destroyed. Ninety-five percent of nests in South Australia are destroyed between November and May, with numbers peaking in January (Ch. 2). This is different to the study of Barlow *et al.* (2002), where nests were not destroyed until after the emergence of reproductive forms. The implications of this are discussed later (section 6.4.2).

Annual wasp nest density, N_t, was calculated by dividing the number of nests destroyed in each council region by the area of the council (see Table 6.1). The resulting densities are expressed as numbers of wasp nests per km^2 and are averaged across all council regions.

6.2.2 Weather data

As the total study area covered an area 80 km north to south and up to 50 km east to west, and included coastal areas, plains, foothills and hills, and thus a number of microclimates, it was considered best to obtain weather data for each council area separately. Information from weather stations does not exist on such a fine scale, and an alternate method of obtaining these data was used.

Data Drill (Jeffrey *et al.* 2001; http://www.nrm.qld.gov.au/silo/index.html) is a program provided by the Queensland Department of Natural Resources and Mines that calculates climatic variables for any given location in Australia. It uses data collected by various meteorological stations around the country and extrapolates it by splining and kriging techniques. Data are accurate to within approximately 5 km. Based on this, a representative location was chosen for each council region (Table 6.1). Values of daily minimum and maximum temperatures, and rainfall to 9am were obtained. These were then averaged on a monthly and seasonal basis.

6.2.3 Time series analysis

Univariate time series analysis were performed to examine the underlying nature of wasp dynamics (Box and Jenkins 1976; Nisbet and Gurney 1982). Specifically, the autocorrelation (ACF) and partial rate correlation (PRCF) functions were estimated for wasp densities. Prior to the analysis, wasp data were natural log transformed and de-trended by adding the difference between the expected value (computed from a linear regression through the series) and the observed data point to the mean of the series. The autocorrelation function is estimated by calculating the correlation coefficient between L_t and L_{t-1}, L_{t-2}, L_{t-3}, etc, where $L_t = \ln N_t$. These correlation coefficients are then plotted against the lag since time t. If a population undergoes periodic oscillations caused either by endogenous or exogenous effects, then its ACF also oscillates around zero at regular intervals. Alternatively, a population can gradually deviate from its initial density, often due to density regulation only at extreme levels or rates of environmental changes corresponding to population changes, in which case the ACF will become increasingly negative (Turchin and Taylor 1992).

The partial autocorrelation function (PACF) is an indicator of indirect past influences of the target variable, and can serve as a predictor of the number of years of lag to include in the final model (Box and Jenkins 1976). However, Berryman and Turchin (2001) argue that in ecological systems, population changes are brought about by changes in individuals, and thus a general regression model that takes into account *per-capita* changes may be more appropriate. The PRCF (partial rate correlation function) is similar to the PACF, except that it is a regression of the logarithmic per-capita rate of change, $r = \ln (N_{t+1}/N_t)$ against lagged population densities L_t. Like PACF, significant correlations at shorter lags followed by non-significant correlations at longer lags are indicative of the order of the autoregressive process. However, where the PACF assumes that the system operates under perfect compensation, i.e. returns immediately to equilibrium, the PRCF allows for unregulated or uncompensated dynamics. Thus the presence of a negative coefficient in the PRCF plot at lag 1 indicates direct density-dependence (Berryman and Turchin 2001).

6.2.4 Population model

Vespula germanica nest densities were modelled as a function of density-dependent and density-independent factors. A modified Ricker population growth model (discrete time logistic; Royama 1981) was used to describe the relationship between wasp rate of increase (r_t), and endogenous (N_t) and exogenous (W_t) effects. The model had the form $\ln (N_{t+1}/N_t) = a + bN_t + cW_t + e_t$, where a, b and c are regression coefficients, and e represents normally distributed noise (0, σ).

Annual nest densities were used as substitutes for endogenous factors. The exogenous component of the model included rain and temperature. A stepwise linear regression was performed to determine the important weather terms to include in the model. In addition to N_t, a total of 128 potential temperature, rainfall and extreme weather variables were tested. These were: average minimum temperature, average maximum temperature, total rainfall, proportion of days when the maximum daily temperature was above 35°C and 30°C, proportion of days when the minimum daily temperature was below 10°C and

5°C, proportion of days when the rainfall was >30 year average. All variables were summarised on a monthly as well as seasonal basis, and the discrete variables were arcsine square root transformed.

Possible site effects were initially examined by including council regions as a random effect in a linear mixed-effects model (Pinheiro and Bates 2000). However, inspection of the magnitude and nature of the variation associated with council areas in this model indicated that such explicit modelling of site effects was not warranted and so the subsequent model was based upon a simple multiple regression approach using data pooled across council areas.

Furthermore, as consecutive annual nest densities are not independent of each other, using ordinary *t*-test statistics to test the significance of model parameters may give biased results. As a check on the tests of significance of the model parameters, a bootstrap analysis was performed. In this analysis, residuals from the fitted model were repeatedly re-sampled and the *t*-statistics for the significance of the slope coefficients in the model were recalculated.

6.2.5 Forecasts

The model described in 6.2.4, generated using 10 seasons of data, was used to predict wasp densities in the next, 2002/03, season. Nest densities from the 10th season, as well as weather variables obtained from Data Drill in the same way as those for the original model, were used. Separate predictions were made for each council region, and these were then averaged to obtain a mean predicted wasp density for Adelaide.

6.3 Results

Nest densities did not differ greatly between years, with the mean nest density varying between 1.5 and 7.5 nests per km^2 (Fig. 6.1). In the first three seasons, densities appear to be solely increasing. Only one peak abundance season occurred, 1997/98, when mean wasp densities increased from 3.8 to 7.8 nests km^{-2}. Since then, wasp densities have decreased, and seem to have stabilised at around 4 nests km^{-2}.

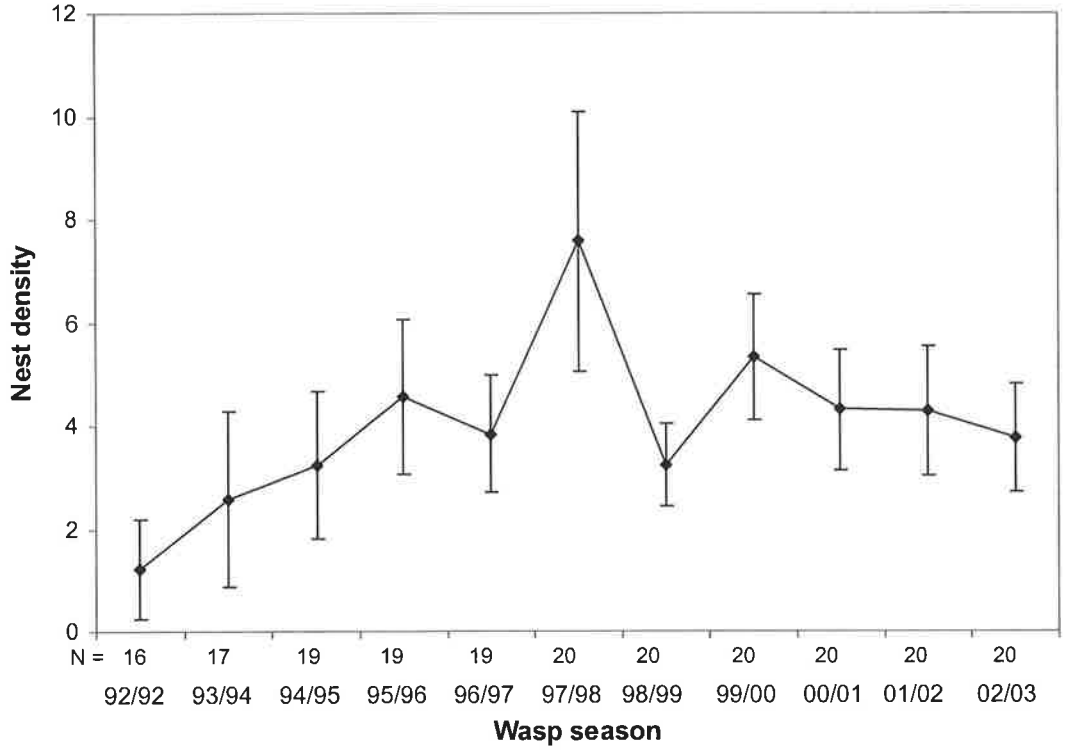

Fig. 6.1: Nest densities (number of nests destroyed per km^2) in the metropolitan Adelaide area between 1992/93 and 2001/02. Values represent mean across all councils ±95% CI. Only the first 10 seasons were used to produce the predictive model.

6.3.1 Time series analysis

There was no apparent population cycling identified by the ACF of L_t. In fact, the ACF became increasingly negative (Fig. 6.2a). This is consistent with a non-stationary, non-periodic system, where the population is thought to be undergoing a 'random walk' (Turchin and Taylor 1992). This may indicate the influence of exogenous factors on population dynamics. The PRCF on the other hand shows a negative coefficient at lag 1 (-0.81), suggesting direct density-dependence, with further autoregressive processes being non-significant (Fig. 6.2b).

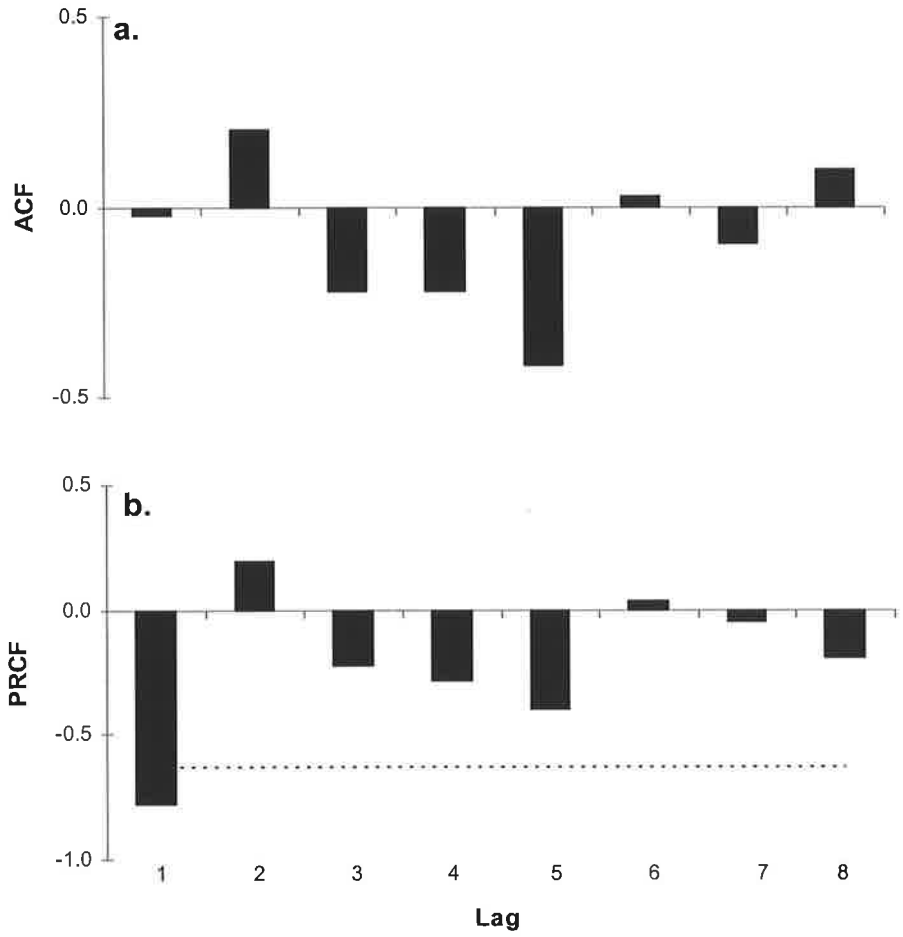

Fig. 6.2: (a) Autocorrelation and (b) Partial rate correlation structure for log density of wasp nests destroyed in metropolitan Adelaide. The dotted line indicates coefficients which fall outside Bartlett's confidence intervals.

6.3.2 *Factors affecting density*

Stepwise linear regression identified current nest density (N_t), total amount of rainfall in April (R_t), and the proportion of days in March when the maximum daily temperature was 35°C or above (E_t) as being significant predictors of wasp rate of increase ($p<0.001$ for all variables; Figs. 6.3 - 6.5, Table 6.2). The most significant predictor was R_t (R^2 = 0.34), with the model improving significantly with the addition of N_t (R^2 = 0.44), and E_t (R^2 = 0.51). The average maximum temperature in August was also identified as a significant predictor, however, it was strongly correlated with the other temperature variable and exhibited co-linearity, and was thus not included in the final model.

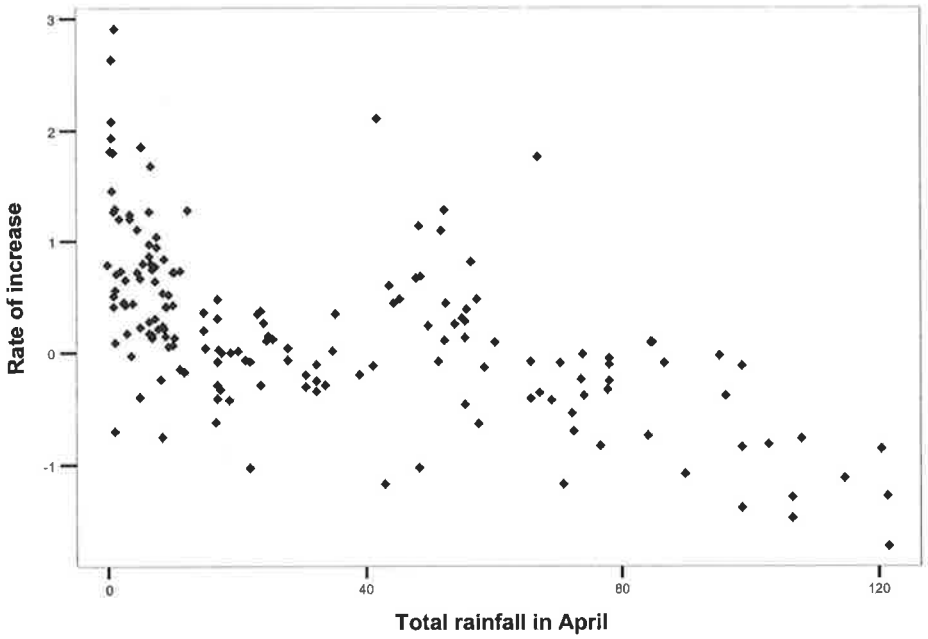

Fig. 6.3: Relationship between the exponential rate of increase, *r*, of nests destroyed from July in year *t* to June the following year (*t+1*), and total amount of rainfall in April (mm).

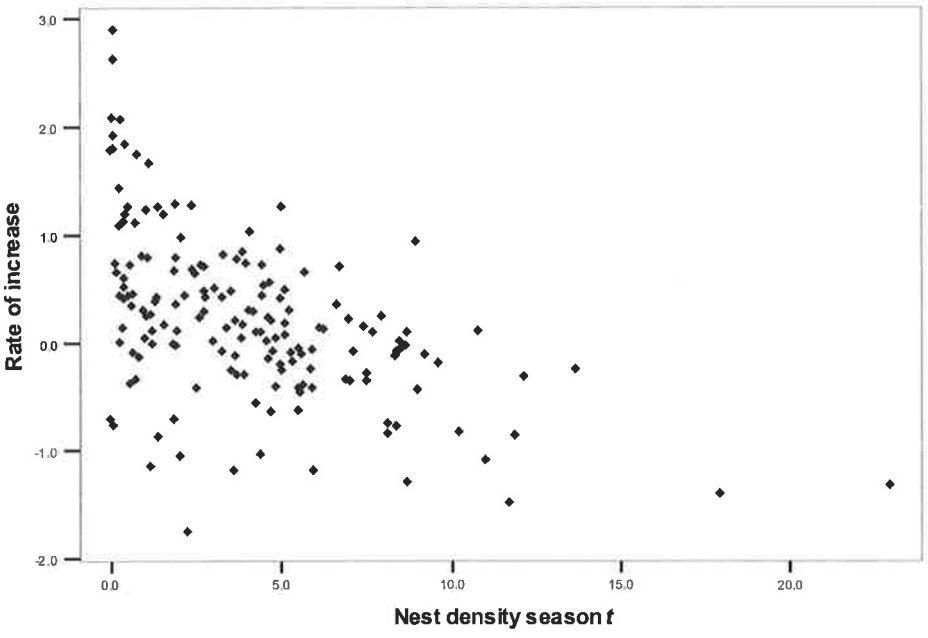

Fig. 6.4: Relationship between the exponential rate of increase, *r*, of nests destroyed from July in year *t* to June the following year (*t+1*), and number of nests destroyed in year *t*.

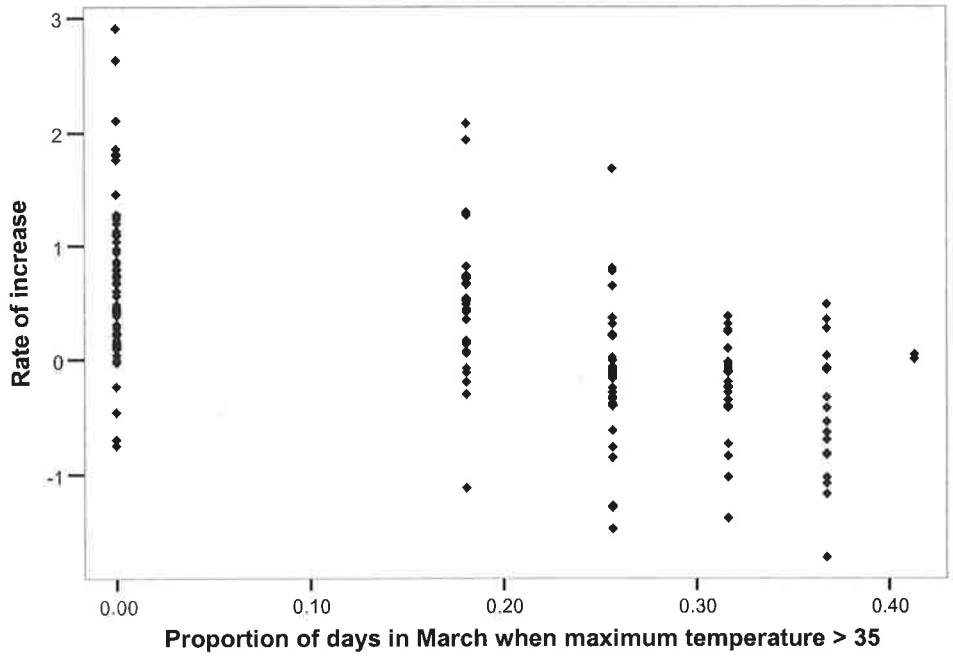

Fig. 6.5: Relationship between the exponential rate of increase, *r*, of nests destroyed from July in year *t* to June the following year (*t+1*) and proportion of days when maximum temperature in March was 35°C or above (arcsine square root transformed).

The final model ($P<0.001$, $R^2 = 0.51$; d.f. 3,167) is:

$$\ln(N_{t+1}/N_t) = 1.036 - 7.14\text{x}10^{-3}R_t - 6.75\text{x}10^{-2}N_t - 1.737E_t \qquad eq.\ 6.1$$

giving the Ricker equation:

$$N_{t+1} = N_t \exp(1.036 - 7.14\text{x}10^{-3}R_t - 6.75\text{x}10^{-2}N_t - 1.737E_t) \qquad eq.\ 6.2$$

When council region was incorporated as the random effect in a linear mixed effects model, the resulting regression was not statistically better ($p>0.05$). Thus, variability between council regions was considered of little importance, and all areas were treated as replicates. Similarly, the bootstrap analysis showed little bias in the original parameter values (bias <0.005 in all cases), confirming that the linear regression remained significant despite non-independence of the factors.

Table 6.2: Summary of the results from stepwise linear regression models of the rate of increase, $\ln(N_{t+1}/N_t)$, against 129 endogenous and exogenous variables. All models shown were highly significant ($p<0.001$). R_t represents total rainfall in April, N_t is wasp nest density in year t, E_t is the number of days in March when maximum daily temperature was 35°C or above, and T_t is average maximum daily temperature in August. The model including T_t was rejected due to its high collinearity condition index. Boldface indicates the selected model.

Model		Estimated coefficients (SE)	t	Collinearity Condition index	R^2	se
1	Constant	0.685 (0.070)	9.815	1.000	0.34	0.63
	R_t	-0.014 (0.001)	-9.266	2.464		
2	Constant	0.880 (0.074)	11.840	1.000	0.44	0.58
	R_t	-0.010 (0.002)	-6.974	2.920		
	N_t	-0.074 (0.014)	-5.323	3.194		
3	**Constant**	**1.036 (0.076)**	**13.679**	**1.000**	**0.51**	**0.54**
	R_t	**-0.007 (0.002)**	**-4.608**	**3.207**		
	N_t	**-0.068 (0.013)**	**-5.171**	**3.420**		
	E_t	**-1.737 (0.341)**	**-5.094**	**4.088**		
4	Constant	-2.085 (0.575)	-3.629	1.000	0.59	0.50
	R_t	-0.007 (0.001)	-4.869	3.167		
	N_t	-0.062 (0.012)	-5.158	3.645		
	E_t	-1.823 (0.315)	-5.789	4.385		
	T_t	0.191 (0.035)	5.472	42.052		

6.3.3 Model testing

The fit of the observed versus predicted rates of change over seasons shows a good fit (Fig. 6.6). The large discrepancies during the first three seasons occurred when initial wasp densities were low and there was more variation in the observed values as evidenced by the large confidence intervals.

Using equation 6.2, nest densities in 2002/03 were predicted based upon nest densities and weather variables in 2001/02. The predicted wasp density averaged across all council regions was 3.52 nests per km^2 (±0.41 SE). This value is within two standard errors of the value observed in the 2002/03 wasp season (3.75 ±0.52 SE; Fig. 6.1).

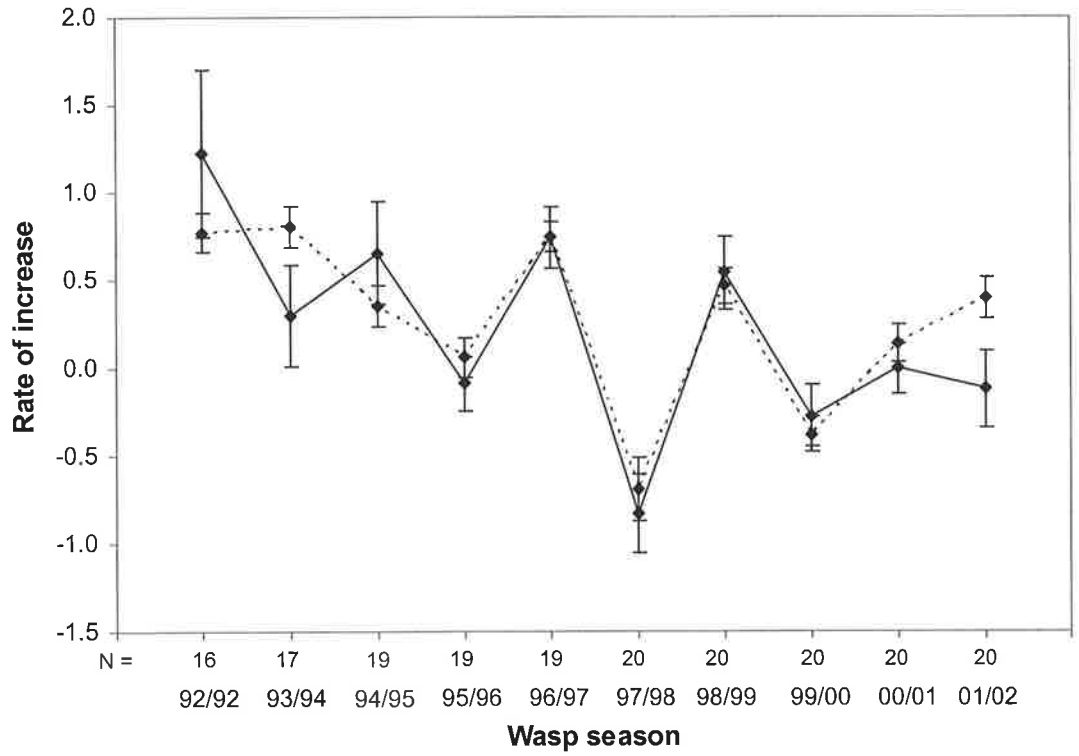

Fig. 6.6: The observed (solid) and predicted (dashed) mean rate of increase between wasp season *t* and the following season. Error bars around observed values are 95% confidence intervals representing variability among council areas in rates of increase. Error bars around predicted values are 95% confidence intervals representing variability among council areas in rates of increase predicted from equation 6.1. The tenth rate of increase, calculated for the 2002/03 wasp season, was not used in the original model and is shown to test the accuracy of the prediction.

6.4 Discussion

6.4.1 Nest densities and fluctuations

The average densities of *V. germanica* in South Australia were four nests km^{-2}, or 0.04 nests ha^{-1}, and these fluctuated between 1.5 and 7.5 nests km^{-2}, a five-fold range. These represent the lowest *Vespula* nest densities published. *Vespula vulgaris* densities at one site in England were recorded at 0.4 nests ha^{-1}, and at six honeydew forests in New Zealand at 12 ha^{-1}, while densities of *V. maculifrons* in one area in the USA were recorded to be approximately four nests ha^{-1} (Archer 1985; Roth and Lord 1987; Barlow *et al.* 2002). Other published population dynamics studies did not record nest densities.

The five-fold range in fluctuations obtained in this study falls between those previously recorded (two-fold range in New Zealand, Barlow *et al.* 2002; 20-fold range in the USA, Roth and Lord 1987; 42-fold range in England, Archer 1985).

6.4.2 Modelling annual fluctuations

Analysis of time series can give valuable insight into whether cyclicity of population densities occurs. Unfortunately, not many published accounts of population cycles exist for social insects. Using 17-27 years of data, Archer (1985; 2001a) found *V. vulgaris* and *V. germanica* wasps to oscillate in two and seven year cycles. This was further confirmed in a re-analysis of his data by Turchin and Taylor (1992). Conversely, cycles were not observed in *V. vulgaris* in New Zealand's beech forests (Barlow *et al.* 2002). No evidence of any cyclicity in annual wasp numbers was found in the current study. However, the PRCF indicated that strong density-dependence exists at lag 1. Moreover, a time series of r rather than N_t suggests that while there is no cyclicity in nest densities, rate of increase does exhibit weak bi-annual cycles (evident in Fig. 6.6). This may be mostly attributed to the dynamics in the first three seasons, when the population was only increasing. As the species has established in the Adelaide region relatively recently (early 1980s), it may still have been expanding spatially. The series is currently at the lower endpoint of adequate length for analysis, and the addition of more data may show population cycles.

A stepwise linear regression confirmed the initial predictions of the time series analyses that both endogenous and exogenous factors influence r_t. The final model indicated that April rainfall (mid-autumn), current nest density, and the number of days in March (end of summer) when the temperature reached the extreme value of 35°C or above, all had a significant negative effect on the intrinsic rate of increase.

Few previous studies have concentrated on the endogenous mechanisms underlying wasp populations. Archer (1985; 2001a) suggested a self-regulating mechanism for *V. vulgaris* in England. Barlow *et al.* (2002) also found that previous years' densities, together with weather, acted on rates of increase in a negative fashion.

The negative relationship with autumn rainfall that the present model suggests was previously proposed for nest densities of small colonies of *V. vulgaris* in England (Archer 1981). However, autumn rainfall was also found to be positively correlated with *V. germanica* densities in Tasmania, Australia (Madden 1981). The negative correlation was explained in terms of the unfavourable weather reducing new queen production, and thus fewer queens being available to start nests in the following season, while the positive one was attributed to a higher abundance of wasp prey with the wetter conditions.

Other studies of weather effects on *Vespula* populations have proposed that spring rather than autumn weather are more important determinants of next year's wasp numbers. Madden (1981) found a positive correlation with spring rainfall, as well as autumn rainfall. Akre and Reed (1981) on the other hand found a negative relationship between worker densities for seven species of vespids in the Pacific northwest of the USA with spring rainfall, and a positive relationship with spring temperature. The study of Horwood *et al.* (1993) on *V. germanica* populations in Sydney is the only one to have found a positive relationship between nest densities and total annual rainfall.

The current model was most comparable to that produced for *V. vulgaris* inhabiting New Zealand's honeydew beech forests (Barlow *et al.* 2002) in that it incorporated both density-dependent and weather factors. However, their model identified spring (September) rather than autumn (April) rainfall as having a negative influence on rate of increase. In addition, the model presented here recognised the importance of exceptionally hot days in autumn as predictors of *r*. The hot conditions of central and north-western Australia have previously been suggested to limit the potential distribution of *V. germanica* on the continent (Spradbery and Maywald 1992).

In the wasp's life cycle, the end of summer and the beginning of autumn signifies the emergence of new reproductives. Factors acting at this stage can influence queen mortality directly, e.g. by drowning of immature or newly emerged queens, or indirectly, e.g. by reducing prey abundance and hence colony food intake.

New queens require high energy foods in order to build up their fat reserves to survive winter hibernation (Harris and Beggs 1995). Both rain and extreme temperatures of ≥35°C reduce wasp foraging activity (Ch. 4). High April rainfall and extreme temperatures in March may thus prevent new queens (generation time *t*) from obtaining enough energy by preventing worker foraging and thus feeding the queen larvae. High rainfall may also simply drown nests before new queens have emerged. Alternatively, rain and hot weather may inhibit the activity of newly emerged queens, preventing them from finding optimal hibernating places, and thus experience subsequent mortality during winter. This hypothesis was supported by Archer (1980a), who postulated that the majority of queens (up to 98%) die during hibernation. Barlow *et al.* (2002) performed a key stage analysis on *V. vulgaris* populations in New Zealand, and also concluded that the variability in number of queens produced had less of an impact on future wasp densities than the survival of queens until next season. Data presented in this chapter only identify the time period but do not distinguish between mortality at the larval stage and young adult stage.

Akre and Reed (1981) identified the time from establishing the nest until the first workers emerge as the 'critical stage' of the wasp life cycle, and concluded this to be a very vulnerable time. During this period, loss of the queen when foraging (Spradbery 1991b), or usurpation by other queens (Ross *et al.* 1981; Donovan 1991) is common. However, this study found no evidence that weather variables acting on this stage of the wasp's life cycle influence population dynamics. Perhaps it is then not the stage of the wasp's life cycle that signifies a 'critical' phase, but rather the combination of significant weather variables and the different microhabitats that wasps occupy. For example, in Australia *V. germanica* is present mostly in urban areas, and its nests are often found in places that are protected from summer rain and heat by roofs or concrete (Crosland 1991; Ch. 2). Additionally, the Adelaide climate is dry, with rainfall events mostly occurring between April and October (Commonwealth Bureau of Meteorology 2004). This may enable the establishment of nests in locations that are susceptible to flooding, and the first significant rains in April may cause large mortality in those nests. In contrast, New Zealand's honeydew beech forests do not provide a protected environment for wasps, and nests

might be founded in sheltered locations to survive. Consequently, autumn rains do not cause mortality. Queen mortality occurs in the following spring, during their nest-founding period, when they are subjected to variable weather outside the protection of their nests.

In the model presented here, nest densities acted as surrogates for unobserved density-dependent factors. One of the problems with this approach is that it is unknown whether densities of nests destroyed are representative of the whole population. Numbers could differ between regions and years according to the level of media attention and public awareness, that directly influences the numbers of nests reported and therefore destroyed. In fact, the highest *V. germanica* nest densities were found close to urban centres (Goodall and Smith 2001). Similarly, this study found that human population was roughly correlated with wasp nest densities. However, the population showed no pattern when compared to changes in wasp numbers (i.e. *r*; unpublished data). Moreover, annual wasp rates of increase show little variation between council regions. This suggests that yearly differences in numbers of nests destroyed do in fact reflect changes in total nest densities.

As nests were destroyed, future nests observed in any one area did not occur as a result of queen dispersal but rather from immigration into the area. This is in contrast to most previous studies of *Vespula* populations, which employed non-destructive sampling techniques. Although it is possible that this immigration has occurred from outside the study area, it is likely to be negligible as the area sampled covers the entire permanent population of *V. germanica* in South Australia. The nearest permanent population of this wasp outside of Adelaide is in rural Victoria, at least 250 km away. While hibernating queens could be transported over this distance, the majority of new nests are more likely to have come from natural queen dispersal, thought to be up to approximately one kilometre (Thomas 1960; Edwards 1980; Crosland 1991). Molecular genetic evidence supports this hypothesis, as wasps were found to be more distantly related with increasing geographical distance (Goodisman *et al.* 2001a). Hence, all changes in annual abundances were caused by immigration into each area from neighbouring councils or less populated areas in the same council, and the system can still be considered as 'closed'.

Apart from humans, *V. germanica* does not have any known predators in Australia. Similarly, no other *Vespula* species are present at the study area. Thus, one explanation of the observed density-dependence is intra-specific competition for resources. However, as nest densities represented numbers of nests destroyed rather than the whole population, the observed 'density-dependence' likely reflects the fact that high rates of nest destruction translates to lower rates of increase in the following season. This provides evidence that the nest destruction program is effective, as it suppresses wasp numbers not only in the year that nests are destroyed, but also in the following season. Thus, in terms of management of this pest species, increased nest destruction efforts will result in lower nest densities in the following year. In the future, broad-scale control methods such as toxic baiting (Sackmann *et al.* 2001; D. Hopkins pers. comm.) should be employed to suppress wasp numbers to even lower levels.

CHAPTER 7:

GENERAL DISCUSSION

7.1 Outcomes

Human transport has immensely aided introductions of alien species into new habitats. These biological invasions often have agricultural, urban and environmental effects, sometimes leading to economic impact from damage caused by these pests (e.g. to agricultural crops) or from control efforts (e.g. eradication programs; Elton 1958; Vitousek *et al.* 1996; Williamson 1996). For example, despite Australia's isolation, at least 50 species of vertebrates, 500 species of invertebrates, and over 1,000 species of plants have become a pest or weed of environmental significance after establishing in the wild (Australian State of the Environment Committee 2001). Once established, invasive species are almost impossible to eradicate, and thus the best means of management are through prevention of establishment (Lodge 1993; Vermeij 1996). However, this is impossible without prior knowledge of the ecological characteristics of likely invaders, or the habitats they may invade. Such knowledge can only be obtained by studying successful invaders in their introduced ranges, and making comparisons to the species' biology in their native ranges (Reichard and Hamilton 1997).

This thesis brings together five inter-related studies of the biology of *V. germanica*, a highly invasive social insect, in its introduced range in South Australia.

Results indicate that substantial differences exist between the species in South Australia, and in its native range in England. For example, the milder climate in South Australia enables wasp colonies to be active for at least seven months of the year, compared with only four in England (Ch. 2). This longer period of activity has enabled *V. germanica* to build larger nests, which produce three times as many workers and queens, and twice as many males as colonies in England. The milder climate in the introduced range also enables overwintering, and may also reduce queen loss during hibernation. With the

added advantage of no native enemies, predators or parasites, *V. germanica* populations in Australia have the potential to increase at a faster rate than ones in England.

However, the climate in South Australia was also found to be detrimental to the wasp. Hot temperatures of $\geq 35^{o}C$ were seen to reduce activity levels by an average of 50% (Ch. 4), while they increased requirements for water (Ch. 3). In comparison, activity in England was only reduced at low temperatures of $2^{o}C$ or below (Potter 1964). These differences arise from variation in climate between Australia and England, as Australian summers rarely reach such low temperatures, whilst English summers are generally much cooler than in Australia.

As well as determining differences in the biology of *V. germanica* between Australia and its native range, its potential impact on the environment was also assessed in terms of predation and competition with a native species. Results indicate that *V. germanica* removes a large range of native invertebrates from the environment (Ch. 5). This may lead to reduced numbers of some species, and a possibility of local extinction in areas with high wasp densities. Moreover, as shown by a combination of morphology-based identification and molecular sequencing of wasp prey, the dietary niche of the native paper wasp *Polistes humilis* is included entirely in the much wider niche of the introduced wasp, giving *V. germanica* a competitive advantage.

Another important finding is that previous densities of destroyed nests have a negative impact on future densities, as shown by the predictive population model in Chapter 6. This finding suggests that the nest destruction program that is currently in place is an effective means of suppressing current as well as future populations. Wasp densities could possibly be reduced even further with large scale toxic baiting. Such options are currently available and appear very promising (Sackmann *et al.* 2001; D. Hopkins pers. comm.). However, it is probably impossible to eradicate the species completely, as simultaneous targeting of all nests within the Adelaide region would entail enormous effort. Moreover, additional information is required on spatial population dynamics of the species. For example, does most dispersal occur at short distance through queen flight, or is it long distance and human aided? Is the *V. germanica*

population in South Australia closed, or is there an influx from other states on an annual basis? A recent study suggests that the Adelaide population of *V. germanica* is more genetically diverse than populations in Melbourne, Canberra or Hobart, and that multiple bottlenecks have occurred during the spread of the species (Goodisman *et al.* 2001a). However, it is unknown how frequent they were, or on what spatial scale they acted. It is also unknown from where *V. germanica* populations in Australia have originated. As shown in Chapter 5, the use of molecular techniques has its place in ecology, and questions about the population genetics of *V. germanica* can be answered by applying these methods.

This study has also shown that *V. germanica* satisfies almost all criteria thought to characterise effective invaders (see Table 1.1; Lodge 1993). Differences in nest sites and duration of colony existence described in Chapter 2 indicate that *V. germanica* is able to adapt to new, more favourable conditions, suggesting a high genetic ability. The broad diet of the species, comprising of at least 9 arthropod orders plus scavenged vertebrate remains (Ch. 5), indicates the generalist feeding nature of the species. Hot temperatures experienced in South Australia were seen to restrict worker activity (Ch. 4), as well as increase its need for water (Ch. 3). This suggests that water, often only available from areas of human habitation, is necessary for the continued survival of *V. germanica*. Indeed, reported *V. germanica* nest densities were correlated with human population densities (Ch. 6), suggesting human commensalism. Examination of population dynamics of the species (Ch. 6) found that the intrinsic rate of increase was up to 1.5, which is relatively high. Previous research indicating human mediated transport (e.g. Thomas 1960; Goodall and Smith 2001) suggests *V. germanica* has a high dispersal rate. Moreover, the species has a large native Palaearctic distribution, and exhibits single parent reproduction.

These characteristics are common to many social insects, which could explain their success as invaders (Moller 1996). However, when measuring invasion success in terms of numbers of species established, ants and not wasps are the most widespread of social insects (McGlynn 1999; Holway *et al.* 2002). Well-known ant invaders include the red imported fire ant (*Solenopsis invicta*), the

Argentine ant (*Linepithema humile*), and the crazy ant (*Anoplolepis gracilipes*), although others such as the big-headed ant (*Pleidole megacephala*), the black fire ant (*Solenopsis geminata*) and the little fire ant (*Wasmannia auropunctata*) can be just as damaging (Holway and Suarez 1999; Holway *et al.* 2002; Tsutsui and Suarez 2003). There are two principal mechanisms attributed to the invasion success of ants. These are the ability to lower intra-colonial aggression, and increase inter-species aggression (Holway 1999; Holway and Suarez 1999; Tsutsui *et al.* 2000; Holway *et al.* 2002; Tsutsui and Suarez 2003). Lowering intra-colonial aggression enables ant species to direct resources away from guarding into colony growth, foraging, resource defence, and inter-specific competition (Tsutsui and Suarez 2003). Reduced intra-colonial aggression enables the formation of 'supercolonies', and is thought to have evolved as a result of a bottleneck caused at introduction. Indeed, *L. humile* colonies in their introduced range have lower genetic diversity, and only half as many alleles as colonies in their native range (Tsutsui *et al.* 2000). These ant 'supercolonies' comprise numerous queens and vast numbers of workers (e.g. *S. invicta*, *L. humile*, *A. gracilipes*; Holway and Suarez 1999; Holway *et al.* 2002; Tsutsui and Suarez 2003). It is this numerical advantage, as well as heightened inter-specific aggression, that enables unicolonial ant species to become successful invaders.

Unfortunately, most *Vespula* studies have been conducted on introduced populations in New Zealand, and now Australia, where no native *Vespula* exist. Thus little information about inter-specific aggression exists. In New Zealand, either behavioural differences or numerical dominance have been suggested to be the cause of replacement of *V. germanica* by *V. vulgaris* (Harris *et al.* 1991b; Clapperton *et al.* 1994; Harris *et al.* 1994). In the U.S.A., where *V. germanica* has also been introduced, studies found that intra-specific aggression towards *V. maculifrons* existed at meat baits (Parrish and Fowler 1983; Parrish 1984). However, little is known about intra-specific aggression in *Vespula*. In the U.S.A, aggression between conspecifics as well as nest mates was observed when *V. germanica* workers foraged on baits (Parrish and Fowler 1983; Parrish 1984). In South Australia, the mean nest density was four nests km^{-2} (Ch. 6), and thus it is possible that these low nest densities provide little opportunity for

competition. However, nests were not evenly distributed, showing a clumping pattern (Goodall and Smith 2001). Moreover, the extremely high densities of *V. vulgaris* in New Zealand's beech forests ensure opportunities for interactions between workers from multiple colonies. It is possible that like ants, these wasps also experience a decreased level of intra-specific aggression, and hence are able to reach such high densities. Future genetic studies of *Vespula* in their native and introduced ranges are necessary to address these issues. They will also aid in determining whether *Vespula* populations have suffered bottlenecks, or if re-invasion and constant mixing of populations exists.

7.2 Problems encountered

In a review of worldwide *Vespula* research, Roger Akre suggested that implementation of modern technology would benefit the field immensely (Akre 1991). Indeed, electronic counters have been used since the 1960s to record bee and wasp movements (Potter 1964; Archer 1977; Liu *et al.* 1990). However, the use of such a technique requires an elaborate artificial entrance/exit apparatus to be fitted to the nest. Previous prey studies have used simple entrance/exit traps to capture returning foragers (e.g. Harris 1989; Gambino 1992). The use of such traps was also trialled in the current study. However, it was soon apparent that the volume of wasp traffic was too large to be restricted by a narrow entrance only enabling the passage of a single wasp at a time. Moreover, wasps tended to excavate a new tunnel around the trap, while sealing the trap entrance itself with mud. Therefore, traps had to be monitored and adjusted on a daily basis, occasionally with several weeks' delay before they could be used experimentally. Due to negative media attention, the public in South Australia considers *V. germanica* to be dangerous, making it difficult to obtain nests that can be observed over a period of time. Therefore, traps were not used for either traffic counts or prey assessment studies.

Another use of modern technology suggested by Akre (1991) was computerised imaging of combs to determine cell and brood stage numbers. This was also trialled in the course of the current research. One of the major problems encountered with this approach was that imaging programs do not distinguish between empty cells and cells holding eggs or small larvae. Additionally, cells

are often built at an angle, and thus appear irregular when digitised. Optical recognition software capable of counting individual cells is still costly, while extrapolation of cell numbers from comb area is only an approximate measure, as the size of cells varies between nests, and increases during the wasp season (Spradbery 1972). Undoubtedly as technology continues to become cheaper and more sophisticated, the use of these methods will become more valuable.

Most other problems encountered during this study were similar to those already known to wasp researchers, e.g. extremely low success rate of nest initiation in captivity, and problems with transferred nests due to unfavourable ambient temperatures. Largely, these were dealt with by studying wasp nests *in situ*.

7.3 Future research

Whilst this study has provided a comprehensive picture of the basic biology and ecology of *V. germanica* in South Australia, it has raised almost as many questions as it has answered. Some of these have already been mentioned in section 7.1, e.g. the need to examine population genetics of the wasp locally in South Australia, and on a global scale, in order to understand the spread patterns and aid in control. The necessity to study *Vespula* populations in their native range has also been highlighted, as very little is known about ecological interactions between and within species, such as inter- and intra-specific aggression, diet proportions, or spread and occurrence of *Vespula* spp. on a regional scale.

Some observations pertaining to the evolution of eusociality also warrant further attention, the two most important being the production of queens in worker cells and the presence of multiple eggs (Ch. 2). Are multiple eggs being laid by new queens? If that is the case, could this be a pathway to polygynous overwintered nests? If so, why do overwintered nests not persist in higher proportions? As mentioned in section 7.1, polygyny and large colony size in ants aid some species to become successful invaders, but although overwintering is an intriguing aspect of *Vespula* biology, it has been poorly studied.

APPENDIX 1:

NESTS USED FOR SEASONAL COLONY GROWTH PATTERNS

Dates and locations of nests used to describe seasonal colony growth patterns. Note that Raymonds Rd nest (AH-002), collected on the 18th November 1999 was an overwintered nest, while all others were annual.

Season	Date	Nest	Council	Location
99/00	05 Nov 99	Oscar St	WT	34° 55.857'S, 138° 32.382'E
	18 Nov 99	Raymonds Rd*	AH	34° 55.430'S, 138° 46.043'E
	01 Dec 99	Otama Crt	PL	34° 42.155'S, 138° 42.822'E
	14 Dec 99	Nottage Tce	PR	34° 53.649'S, 138° 36.437'E
	20 Dec 99	Bosanquet Ave	PR	34° 53.103'S, 138° 35.348'E
	21 Dec 99	Albert St	PR	34° 52.792'S, 138° 35.342'E
	23 Dec 99	Hampton St	WT	34° 55.749'S, 138° 32.433'E
	06 Jan 00	Federation Ct	MI	35° 02.157'S, 138° 34.673'E
	12 Jan 00	13 Shipster St	WT	34° 55.294'S, 138° 33.760'E
	14 Jan 00	Goodwood Rd	MI	34° 59.565'S, 138° 35.488'E
	15 Jan 00	Laffers Rd	MI	35° 00.416'S, 138° 37.695'E
	18 Jan 00	TeAnau Ave	PR	34° 53.724'S, 138° 35.689'E
	21 Jan 00	154 Sheps	MI	35° 01.517'S, 138° 35.062'E
		Yelki 2	PL	34° 43.063'S, 138° 43.420'E
	24 Jan 00	Alexandra St	PR	34° 52.769'S, 138° 38.347'E
	28 Jan 00	Williams Rd 1	PL	34° 45.084'S, 138° 41.637'E
		Williams Rd 2	PL	34° 45.164'S, 138° 41.553'E
	01 Feb 00	Maud St	PR	34° 52.498'S, 138° 35.183'E
	07 Feb 00	Heysen Trail	AH	35° 01.251'S, 138° 42.775'E
	08 Feb 00	Acacia St	WT	34° 55.831'S, 138° 31.560'E
	15 Feb 00	Arthur St	MI	34° 58.237'S, 138° 34.476'E
	06 Mar 00	Wittunga 1	MI	35° 01.594'S, 138° 36.583'E
	09 Mar 00	Arbury 1	AH	35° 00.234'S, 138° 44.977'E
	10 Mar 00	Victoria St	PR	34° 52.896'S, 138° 35.500'E
	15 Mar 00	Samuel St	WT	34° 55.582'S, 138° 31.152'E
	21 Mar 00	Somerset Rd	MI	35° 01.968'S, 138° 37.435'E
	28 Mar 00	Centennial pk	MI	34° 59.918'S, 138° 35.135'E

Season	Date	Nest	Council	Location
	05 Apr 00	Upper Sturt Rd	MI	35° 01.019'S, 138° 38.387'E
	10 Apr 00	Grant Ave	WT	34° 55.080'S, 138° 34.287'E
	11 Apr 00	Spoehr Rd	AH	34° 59.572'S, 138° 48.681'E
	14 Apr 00	Warrawong	AH	35° 02.355'S, 138° 43.919'E
	19 Apr 00	Cleland 2	AH	34° 57.989'S, 138° 41.628'E
	07 May 00	Lenswood 1	AH	34° 56.971'S, 138° 42.319'E
	29 May 00	Meyer Rd	WT	34° 55.098'S, 138° 33.581'E
00/01	14 Dec 00	Howard St	PR	34° 53.331'S, 138° 36.571'E
		Lipsett Tce	WT	34° 55.898'S, 138° 32.974'E
	22 Dec 00	Ashford St	WT	34° 56.677'S, 138° 34.537'E
		East St WT	WT	34° 55.336'S, 138° 33.677'E
	18 Jan 01	Shipster St	WT	35° 00.974'S, 138° 43.730'E
	23 Jan 01	31 Turners Av	MI	35° 01.823'S, 138° 38.058'E
	08 Feb 01	Myrtle St	PR	34° 53.340'S, 138° 35.303'E
	15 Feb 01	Kemp 3	AH	35 ° 00.999'S, 138 ° 43.595'E
	20 Feb 01	Birdwood Tce	WT	34° 57.106'S, 138° 33.614'E
	12 Mar 01	Southern Ave	MI	35° 00.742'S, 138° 37.189'E
		Womens MPG	MI	35° 00.919'S, 138° 34.558'E
	13 Mar 01	Lilac pl	WT	34° 57.299'S, 138° 33.020'E
	16 Mar 01	Chapman St	MI	35° 01.032'S, 138° 37.458'E
	27 Mar 01	East Tce	MI	35° 01.291'S, 138° 37.458'E
	04 Apr 01	Wittunga 2	MI	35° 01.525'S, 138° 36.600'E
	20 Apr 01	Steeve Cooper	AH	35° 00.156'S, 138° 43.956'E
	26 Apr 01	Chookarloo	ON	35° 12.327'S, 138° 42.728'E
	03 May 01	Verdun 1	AH	35° 00.341'S, 138° 48.359'E
	15 May 01	Brickworks	WT	34° 54.956'S, 138° 33.840'E
	31 May 01	Wood St	WT	34° 57.327'S, 138° 33.812'E
01/02	28 Nov 01	Stephens Ave	WT	34° 55.237'S, 138° 33.246'E
	19 Dec 01	Guildford Ave	PR	34° 52.454'S, 138° 35.319'E
	03 Jan 02	Charles St	PR	34° 52.740'S, 138° 35.209'E
	15 Jan 02	Kegworth St	MI	34° 59.041'S, 138° 34.182'E
	31 Jan 02	Buchanan St	PR	34° 53.018'S, 138° 36.467'E
	11 Feb 02	Galway Ave	PR	34° 53.212'S, 138° 36.830'E
	17 Feb 02	Wetlands 3	MI	34° 58.041'S, 138° 37.068'E
	21 Feb 02	Pasteur Ave	MI	35° 01.449'S, 138° 37.911'E
	05 Mar 02	Botanic Gdns	ACC	34° 55.317'S, 138° 36.585'E
	09 Mar 02	Gladstone Rd	PR	34° 53.110'S, 138° 35.332'E
	22 Mar 02	Kyre Ave	PR	34° 58.253'S, 138° 36.975'E

Season	Date	Nest	Council	Location
	03 Apr 02	Brukunga 1	ON	35° 00.299'S, 138° 56.301'E
		Brukunga 2	ON	35° 00.373'S, 138° 56.277'E
	11 Apr 02	Battunga GC1	ON	35° 11.562'S, 138° 41.507'E
		Brukunga 3	ON	35° 00.317'S, 138° 56.477'E
	18 Apr 02	Lorraine Ave	WT	34° 55.334°'S, 138° 32.266'E
	23 Apr 02	Mt. Lofty Bot	AH	34° 59.397'S, 138° 42.790'E
	15 May 02	Verdun 3	AH	35° 00.341'S, 138° 48.358'E

Council codes:
ACC: Adelaide City
AH: Adelaide Hills
MI: Mitcham
ON: Onkaparinga
PL: Playford
PR: Prospect
WT: West Torrens

APPENDIX 2:

HIBERNATION IN *V. GERMANICA* QUEENS

A2.1 Background

In a closed system, population size increases with the number of births, but decreases with the number of deaths (Ch. 1; Nisbet and Gurney 1982). In England, mature colonies of *V. germanica* and *V. vulgaris* typically produce about 1,000 queens (Archer 1980a), and yet the annual rates of population increase are orders of magnitude smaller (up to 42-fold; Archer 1985). Thus, mortality rather than birth rates seem to be determining population sizes in *Vespula*. Archer (1980a) assessed queen mortality at various stages of colony development, and suggested that winter loss accounted for up to 98% of deaths. Thus better quality queens are more likely to survive winter hibernation. Harris and Beggs (1995) investigated this in more detail for queens in New Zealand. They found that, indeed, small queens were underrepresented among spring queens, suggesting that small queens had died during the winter. The aim of this experiment was to determine how queen size affects duration of hibernation. As the sample size was small (see A2.4), this experiment is included here as a preliminary study only, and is not presented in the main body of the thesis.

A2.2 Materials and Methods

The study was conducted in winter 2000 and repeated in winter 2001. Queens used in this study were obtained from two sources. In 2000, queens were collected from woodpiles, where they were already hibernating. In 2001, young queens were taken from late season nests and placed into large dark cages with pieces of old nest and newspaper until they entered hibernation.

Initial queen weights were obtained prior to commencement of the experiment. Subsequently, queens were placed into the experimental chambers. These consisted of 1.5 cm diameter holes drilled into blocks of untreated pine wood, covered with flyscreen mesh on both open sides. The chambers were kept

inside a garden shed. In 2000, the experiment started in August, while in 2001 this was July.

During the experiment, the wooden chambers were kept inside an uninsulated dry shed. The conditions experienced replicated those the queens would be subjected to during normal winter hibernation.

Queen status was checked every two to three days. Queens were recorded as being in hibernation, awake or dead. Hibernating queens assume a characteristic position, with antennae tucked under the head, and wings pressed close to the body and tucked under the gaster (Edwards 1980). Alive queens not in reproductive diapause were seen moving around in their chambers. Minimum and maximum temperature readings were also taken.

A2.3 Results

In 2000, the average minimum temperature was 5.8°C, and the average maximum was 22.1°C. Queens hibernated for up to 82 days after the commencement of the experiment (Fig. 1a). In comparison, in 2001, the average minimum temperature was 10.4°C, and the average maximum was 16.5°C. Queens were in hibernation for up to 50 days (Fig. 1b). In both years, a positive relationship could be fitted between initial queen size and length of hibernation. In 2000, this relationship was statistically significant (R^2 = 0.401, $F_{1,8}$ = 5.51, p = 0.047), while in 2001 it was not (R^2 = 0.338, $F_{1,8}$ = 4.79, p = 0.087).

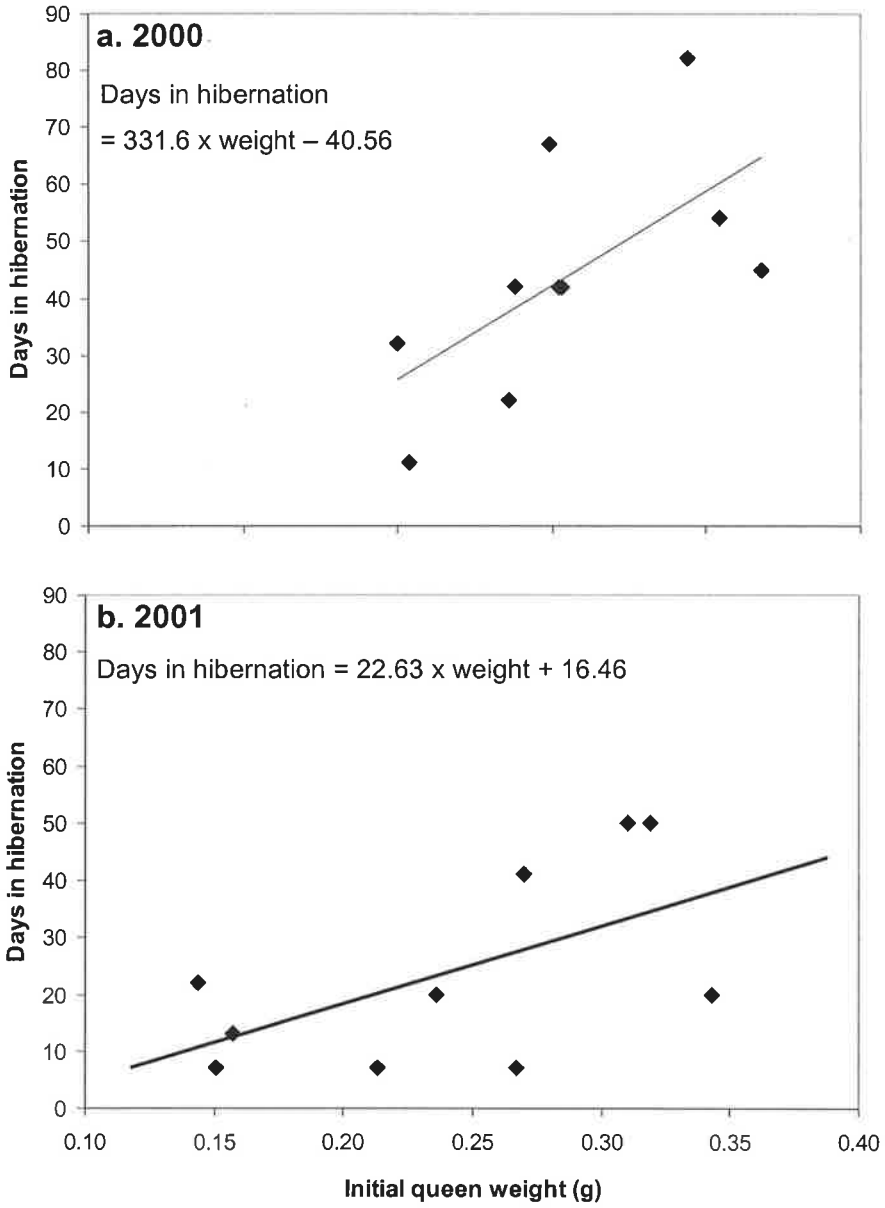

Fig. A1: Relationship between initial queen weight and number of days in hibernation in (a) 2000, and (b) 2001.

A2.4 Discussion

Although sample sizes were small in both seasons, results indicate that larger queens are capable of hibernating for longer, thus experiencing lower winter mortality. Differences in the length of hibernation and the slope of the regression between the two winters may arise from how queens were obtained. Little is known about the behaviour of new queens between emergence and

hibernation, especially if feeding outside the nest occurs. It is possible that newly emerged queens collected from nests in 2001 had not yet metabolised food received in the nest into fat. Ideally, fat content should be measured instead of weight, however, this was impossible to achieve without killing the queen. This should be considered in a future more comprehensive experiment.

Alternatively, variation in duration of hibernation could have arisen from differences in temperatures between the two winters. The higher average minimum temperature observed in 2001 could have prevented longer hibernation.

Obtaining a large sample size proved quite difficult. Searching for hibernating queens in woodpiles is opportunistic and very time consuming. Meanwhile, queens obtained from late season nests experienced high mortality without actually entering diapause. Over 300 queens were obtained using this method, but only 13 entered hibernation, and only 10 of those survived more than one day. Normally, new queens emerge from the nest and mate before entering diapause, while in this experiment they were taken out of their nests prematurely. Thus, it is unknown whether this was an artefact or represents natural mortality.

Hibernation in queens is an aspect of the wasp's biology that has largely been neglected. Future research, employing larger sample sizes and examining the effect of temperature as well as initial weight on the length of hibernation, needs to be conducted.

APPENDIX 3:

DETAILED LIST OF *V. GERMANICA* PREY

This is a list of all prey items collected from 56 *V. germanica* nests during the three study seasons (1999/2000 – 2001/2002), as identified from morphological characters.

Prey type	Number prey
Hymenoptera	**64**
Apis mellifera	61
Halictidae	1
Campanotus spp.	1
Vespidae	1
Diptera	**263**
Asilidae	1
Nematocera	1
Stratiomyidae	2
Calliphoridae	105
Muscidae	19
Syrphidae	1
Sarcophagidae	1
Other	133
Orthoptera	**14**
Lepidoptera	**29**
Odonata	**5**
Hemiptera	**14**
Neuroptera	**4**
Amphipoda	**3**
Coleoptera	**3**
Araneae	**24**
Salticidae	3
Thomisidae	1
Other	20
Skink	**1**
Mollusca	**1**
Morphologically unidentified	**180**
TOTAL	**605**

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