Regulation of nest construction behaviour and nest development in Vespine wasps with special reference to *Dolichovespula norwegica* and *D. sylvestris*

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To my father, who interested me in engineering and talked me into Biology, and to my mother and my wife for their help and encouragement.

Abstract

The objective of this thesis was to examine various aspects of behavioural regulation of nest construction in Vespine wasps. This was achieved by examining nest structure principally in colonies of *Dolichovespula sylvestris* and *Dolichovespula norwegica* at various developmental stages. Some aspects of nest construction behaviour were also examined in *Vespula vulgaris*.

The construction of the envelope requires a large investment in the time and resources of the colony. As the principal function of envelope is nest insulation, the amount constructed should reflect the requirement of the colony for thermoregulation. The thickness and number of layers of envelope constructed in nests of *D. sylvestris* and *D. norwegica* was found to increase with colony development, reaching a peak near the end of the lifecycle and when production of reproductives is at a maximum.

Spradbery (1973) and Edwards (1980) claimed that small Vespine nests have proportionally thicker envelopes than large nests. The findings of this project did not agree with this claim and envelope thickness was found to increase linearly with nest diameter. This resulted from the allocation of a constant proportion of material to comb and envelope construction through colony development. The increase in envelope thickness is achieved by adding additional layers, while maintaining a constant gap between them.

As the principal function of the envelope is insulation, temperature may act as a cue regulating its construction. Potter (1964) found evidence that the rate of foraging for pulp in *V. vulgaris* was affected by nest temperature. He did not, however, determine if this pulp was used in the construction of comb or envelope. A heated nest box and entrance trap were therefore developed to determine if environmental factors, such as temperature, affect the rate at which envelope is constructed. The nest box was successful in maintaining a colony of *D. sylvestris* transferred from the field. It was also capable of maintaining a range of temperatures selected by the experimenter of up to 35°C. The entrance trap was designed to allow foragers returning to the nest to be sampled and the type of forage carried to be determined. The entrance trap was based on a design by Harris (1989) for subterranean nests of *V. vulgaris* and *V. germanica* and was successfully adapted for separating and sampling foragers of *D. sylvestris*.

Workers were found to exhibit a difference in behaviour when producing comb and envelope paper. Comb paper was found to be thinner and consisted of shorter fibres

than that of envelope. In *D. sylvestris* and *D. norwegica*, comb paper was also found to be denser than that of envelope. *D. sylvestris* and *D. norwegica* were found to have very similar behaviour in fibre selection, pulp processing and paper manufacture. The *Dolichovespula* species were, however, found to exhibit several behavioural differences in paper manufacture to *V. vulgaris*. Comb and envelope fibres in *V. vulgaris* were found to be shorter and thicker than those of the *Dolichovespula* species. Comb and envelope paper was also found to be thicker in *V. vulgaris* than in *D. sylvestris* and *D. norwegica*. The use of short, thick fibres in *V. vulgaris* led to envelope with a lower tensile strength than that of *D. sylvestris* and *D. norwegica*.

The ability of the colony to elevate its temperature was found to increase during development, reaching a peak at the start of the production of the reproductives. The colony showed its greatest ability to thermoregulate shortly before the maximum envelope thickness was reached in the nest.

Several factors were examined which may limit the ability of the colony to elevate nest temperature. These included the number of workers, eggs, small larvae, large larvae and pupae. Differences between colonies in their ability to elevate nest temperature were only significantly explained by the number of old larvae present.

Spradbery (1973) claimed that there is a higher density of comb supports on the upper combs than the lower combs. The findings of this thesis confirm this claim. In both *D. sylvestris* and *D. norwegica*, there was a higher density of supports on the upper comb in the nest than on any other comb. In constructing additional comb supports, workers appear to use a cue originating from a change in the size of the combs both directly and indirectly suspended. The cue for the construction of comb supports appeared to result from a change in the mass or size of comb suspended. The cue regulating the placement of the supports is, however, unknown. In *D. sylvestris* and *D. norwegica*, workers do not use the distance to neighbouring supports as a cue for initiating new supports.

The results presented in this thesis indicate that workers use simple behavioural rules in the regulation of construction of comb, comb supports and envelope. The use of very simple behavioural rules may have penalties to the colony in terms of the adaptability of the nest structure. However they reduce the time spent by workers surveying the nest and processing information.

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1.1. Introduction to thesis

Nest construction in vespine wasps is a complex task, involving many individual builders engaged in different construction tasks simultaneously. Mechanisms must therefore exist to regulate the behaviour of the individual, and to co-ordinate this construction behaviour. The objective of this thesis is to examine some of the rules regulating nest construction. This is achieved through an examination of the changes in nest structure with colony development in vespine colonies collected from the field.

The classification of vespines is somewhat confused in the literature, in particular that of the genus *Vespula* Thompson. This chapter will therefore first review vespine classification. The basic biology, lifecycle and major architectural features of vespine wasps will then be described. Nest site preferences and the geographical range of British vespines will then be presented. This chapter will then examine how simple behavioural rules at the level of the individual, can explain the complex organised nest construction at the level of the colony. Finally, this chapter will outline the objectives of the thesis, and introduce the Chapters.

1.2. Classification of the Vespinae

The classification of vespine genera has recently been intensively reviewed by Carpenter (1987, 1991). The nomenclature for vespine genera proposed by Carpenter (1987) was therefore adopted in this thesis (Table 1.1). The nomenclature of the *Vespula* Thompson genus has been particularly dynamic in the literature. Matsuura and Yamane (1990) consider the monophyly of the genus to be uncertain, but suggested the division of the genus into the subgenus *Paravespula* Blüthgen consisting of three species groups and the subgenus *Vespula* Thompson consisting of two species groups. Archer (1982) described a further subgenus *Rugovespula* Archer. Carpenter (1987, 1991), however, found the *Vespula* genus to be monophyletic by seven characters and did not therefore divide it into sub-genera. The genus was, however, divided into two species groups; the *V. vulgaris* (Linnaeus) and *V. rufa* (Linnaeus) sister groups. The *V. rufa* and *V. vulgaris*

Table 1.1. Current classification of the Vespinae genera (excluding *Provespa*) following that of Carpenter 1987, 1991 with a complete species list (taken from Edwards 1980).

***Dolichovespula* Rohwer, 1916**	***Vespula* Thompson, 1869**	***Vespa* Linnaeus 1758**
D. adulterina (du Buysson)	***Vespula vulgaris* (Linnaeus) species group**	*Vespa analis* Fabricius, 1775
D. albida (Sladen, 1918)	*V. flaviceps* (Smith, 1870)	*Vespa affinis* (Linnaeus, 1764)
D. aranaria (Fabricius, 1775)	*V. flavopilosa* Jacobson, 1978	*Vespa basilis* Smith, 1852
D. artica (Rower, 1916)	*V. germanica* (Fabricius, 1793)	*Vespa binghami* du Buysson, 1905
D. ingrica (Birula, 1930)	*V. koreensis* (Radoszkowski, 1887)	*Vespa bellicosa* de Saussure, 1854
D. lama (du Buysson, 1903)	*V. maculifrons* (du Buysson, 1905)	*Vespa bicolor* Fabricius, 1787
D. maculata (Linnaeus, 1763)	*V. pensylvanica* (de Saussure, 1857)	*Vespa crabro* Linnaeus, 1758
D. media (Retzius, 1783)	*V. shidia* Ishikawa, Sk. Yamane & Wagner, 1980	*Vespa dyboskii* André, 1884
D. norwegica (Fabricius, 1781)	*V. structor* (Smith, 1870)	*Vespa fervida* Smith, 1858
D. norwegicoides (Sladen, 1918)	*V. vulgaris* (Linnaeus, 1758)	*Vespa luctuosa* Saussure, 1854
D. pacifica (Birula, 1930)		*Vespa mandarina* Smith, 1852
D. saxonica (Fabricius, 1793)	***Vespula rufa* (Linnaeus) species group**	*Vespa mocsaryana* du Buysson, 1905
D. sylvestris (Scopoli, 1763)	*V. acadica* (Sladen, 1918)	*Vespa multimaculata* Pérez, 1910
D. tibialis (Olivier, 1791)	*V. atropilosa* (Sladen, 1918)	*Vespa orientalis* Linnaeus, 1771
	V. austriaca (Panzer, 1799)	*Vespa philipinensis* Saussure
	V. consobrina (de Saussure, 1854)	*Vespa similima* Smith, 1868
	V. intermedia (du Buysson, 1905)	*Vespa tropica* (Linnaeus, 1758)
	V. rufa (Linnaeus, 1758)	*Vespa variabilis* du Buysson, 1905
	V. schrenckii (Radoszkowski, 1861)	*Vespa veluntina* Lepeltier, 1836
	V. squamosa (Drury, 1770)	*Vespa vivax* Smith, 1870
	V. sulpphurea (de Saussure, 1854)	*Vespa walkeri* du Buysson, 1905
	V. vidua (de Saussure, 1854)	*Vespa wilemani* Meade Waldo, 1911

species groups are divided on the basis of morphology and behaviour (Macdonald *et al.* 1976).

Matsuura and Yamane (1990) divide the *Vespa* Linnaeus genus into the sub-genera *Nyctovespula* Van der Vecht and *Vespa* Linnaeus. Carpenter (1987), however, did not divide *Vespa* into subgenera. The *Dolichovespula* Rower genus was divided by Matsuura and Yamane (1990), into the subgenera *Dolichovespula* Rower, *Boreovespula* Blüthgen and *Metavespula* Blüthgen. Carpenter (1987, 1991) did not feel it was necessary to divide *Dolichovespula* into sub-genera or species groups. For the purpose of the thesis, genera will be abbreviated following Edwards (1980). *Vespula* and *Dolichovespula* are therefore abbreviated to '*V.*' and '*D.*' respectively, while *Vespa* is unabbreviated.

1.3. Biology, lifecycle and architecture of vespines

The lifecycle of vespine colonies begins in late summer or early autumn with the emergence of the new queens and males (the reproductives) from colonies. Reproductives appear to feed for a while on nectar or other carbohydrate sources to increase their weight before finally leaving the nest to mate (Edwards 1980). It is not known whether they mate primarily with siblings or adults from other colonies. Each queen, however, appears to mate with only one male. Following copulation, the male eventually dies but the new queen selects a site to diapause (Edwards 1980). *Vespula* queens generally emerge in April in Britain, while *Dolichovespula* queens emerge later, in May. *V. austriaca* (Panzer), the obligate social parasite of *V. rufa* (Linnaeus), does not make its appearance until June when the host nest is ready to occupy (Edwards 1980). Warm spells in autumn and winter will often rouse vespine queens early from diapause. These warm spells probably deplete the energy reserves of the queen leading to her death (Edwards 1980).

Following emergence from diapause, the queen must find a suitable nest site. *Vespa crabro* Linnaeus, *D. sylvestris* (Scopoli) and *D. norwegica* (Fabricius) appear to construct their nest near the diapause site. They may therefore select the diapause site based on its suitability for nest construction. *V. rufa, V. germanica* (Fabricius) and *V. vulgaris* search away from the diapause site, and seek dark cavities with narrow entrances (Edwards 1980). There is some debate as to the ability of the queen to select a

suitable nest site. Nests are often constructed in cavities that are too small to prevent nest expansion (Edwards 1980). Once the queen has selected a nest site she performs a characteristic orientation flight.

The nest is initiated with the construction of a hanging sheet or pedicel, which is attached to the substrate. Vespine wasps collect fibre from wood and other plant sources, which is chewed and mixed with saliva. The resulting pulp is drawn out into thin strips forming wasp paper from which the nest is constructed. At the end of the pedicel the queen constructs two or three cells. An umbrella like envelope is then constructed over the comb. The queen continues nest construction alone, until worker emergence, constructing around 35-60 cells in *Vespa* and *Vespula* and 35-40 in *Dolichovespula* (Matsuura and Yamane 1990). An egg is deposited in each cell soon after its construction has started. This is the most vulnerable stage of the colony due to accidental death or infertility of the founder queen (Brian and Brian 1948). The layers of envelope are constructed in a spherical shape with an entrance hole at the bottom. In *D. sylvestris* the queen takes about two days to construct each layer of envelope.

The envelope has a variety of functions including colony defence (Matsuura and Yamane 1990), protection against intense light (Brian and Brian 1952) and weatherproofing. The primary function of the multi-layered envelope in vespine wasps is nest insulation (see Chapter 2). The ability of the colony to thermoregulate appears shortly after the construction of the first layer of envelope. When the brood are small, the queen can only heat the nest by around 1°C above ambient. This ability increases with colony development. As the brood increases in size, they are capable of contributing to nest heating, and nest temperature is raised by up to 4°C above ambient (Gibo *et al.* 1977; see Chapter 6).

When the workers emerge, the queen undergoes a physiological change and she begins to perform less of the nest construction and maintenance (Matsuura and Yamane 1990). The queen no longer leaves the nest and the workers assume most of the duties in the nest. At this point, her main responsibility is egg laying. The queen, however, seems to exert control over the colony through a pheromone. The presence of the queen has several effects on the workers. In the absence of the queen, workers of *Vespa orientalis* Linnaeus begin to oviposit in the cells, show increased aggression, and may leave the nest completely (Ishay *et al.* 1965; Ikan *et al.* 1969).

The workers continue to expand the nest, enlarging the comb by adding cells to its periphery. The larvae pass through five instars. During the first three instars, the larvae remain attached to the cell and face outwards, away from the centre of the comb. At instar four, however, the larvae are free to move in the cell and turn to face the centre of the comb (Potter 1964). Prior to pupation, the larvae produce a silk cocoon which is attached to the side of the cell. Following the formation of the cocoon, the larvae void faeces accumulated during development (Edwards 1980). This is pushed to the bottom of the cell with the last pupal cast, and hardens forming the *meconium*.

To accommodate comb expansion paper is removed from the inner layers of envelope while new sheets are constructed on the outside. The structure of the nest is therefore dynamic through colony development. Material removed from the inside of the envelope is re-used in the nest (Akre *et al.* 1976). Recycled envelope material may be used selectively for the construction of envelope (Makino 1980). The source of material for comb and envelope construction is discussed in Chapter 4.

With the emergence of the workers, and an increase in the number of brood present, the ability of the colony to thermoregulate improves. Thermoregulation is achieved by both the workers (Milani 1982, Heinrich 1984) and brood (Gibo *et al.* 1974; Maschwitz 1966; Ishay and Ikan 1966). The ability of the colony to thermoregulate reaches a peak when the large cell brood is produced. At the peak of colony development, temperature is closely maintained at around 32°C (Himmer 1932; Sailer 1950; Potter 1964; Martin 1988). The decline of temperature regulation in the colony similarly coincides with the loss of the brood (Martin 1990). A thorough review of nest thermoregulation is presented in Chapter 6.

Each cell can be used for rearing several broods, and when an adult emerges from a cell the pupal cap is trimmed away from the top of the cell and the queen oviposits in the bottom. The cells may be used to rear up to three generations of brood. In examining colony composition, the number of generations reared in each cell can be estimated by counting the meconia (Edwards 1980). In *D. sylvestris* and *V. vulgaris*, small cells are used for rearing up to three generations, while large cells are normally used for rearing one (Archer 1981). As the comb is expanded, the brood is at different stages of development. Initially the youngest brood is at the edge of the comb while the older larvae and pupae are at the centre. Following the emergence of the adults from the

centre of the comb, the second generation of brood is reared at the centre. This leads to a characteristic ring structure of the brood in the comb. The deposition of meconia at the base of the comb, and the accumulation of silk lining the cell, are thought to function in strengthening the comb (Matsuura and Yamane 1990).

The colony first produces workers; then later workers and males; and finally new queens. There is a considerable overlap in the production of workers, males and queen. In general, workers are produced in small cells and queens in large cells while males can be produced in either. In *V. vulgaris* there is a clear distinction between small and large cells, in *D. sylvestris*, however, there is not (Archer 1981). In *V. vulgaris* the first few combs in the nest consist of small cells with subsequent combs containing large cells. In *Dolichovespula* and the *V. rufa* group, however, the first, or upper comb, is used for rearing workers and males, while the second and subsequent combs are used for rearing males and new queens. About five percent of the cells around the periphery of the upper comb, however, are used for rearing queens (Archer 1981). In *V. vulgaris*, however, several of the upper combs are used for rearing workers and males while the lower combs are used for rearing queens.

The cue for the change in construction of cells from small to large is unclear, but in *Vespa orientalis* it appears to result from photoperiodicity (Ishay *et al.* 1983). The development of the larvae into workers or queens is dependent on cell size. Ishay (1975) found that if eggs of young larvae are transferred from small cells to large cells at the end of the season, they develop into queens. Similarly, eggs or young larvae transferred from queen cells to worker cells at the end of the season, resulted in workers. Ishay *et al.* (1983) noted that in *Vespa orientalis*, cell size changed gradually from small to large, and males were reared in medium sized cells. As males result from unfertilised eggs, the queen must use cell size as a cue for ovipositing fertilised or unfertilised eggs.

Following the emergence of the reproductives the colony goes into decline. The males and new queens leave the nest and mate, then the new queens find a place to diapause. With the loss of brood in the nest, the ability of the colony to thermoregulate rapidly declines. Martin (1992) found that the deterioration in the ability of a colony of *Vespa simillima* to thermoregulate coincided with a decline in the number of brood present. Workers were found to be present in the colony for one to two months after the loss of thermoregulation.

The length and strategy of the colony lifecycle varies between genera of vespines. Archer (1980, 1981) found a difference in the lifecycle strategy of *D. sylvestris* and *V. vulgaris*. *V. vulgaris* can be characterised as a long lifecycle wasp, whereas *D. sylvestris* is a short lifecycle wasp. The long lifecycle strategy is typified with a long colony life span, large nest size and a high ratio of small to large cells. This appears to be characteristic of the *V. vulgaris* species group (Archer 1980). The *V. rufa* group appears to similarly exhibit an intermediate lifecycle while hornets, seem to exhibit long-cycle characteristics (Archer 1980; Reed and Akre 1983).

The strategy of the long-cycle wasp is to reach a large colony size in order to produce more queens at the end of the season. The short-cycle wasps, however, need to compress development by rearing reproductives with the minimum number of workers. Members of the *V. vulgaris* group construct a large proportion of worker cells and have a ratio of large to small cells of 1.9-7.7. *Dolichovespula* species on the other hand rear their reproductives with very few workers and have a cell ratio of 0.4-0.6. The *V. rufa* group has an intermediate ratio close to unity of 0.7-1.5 (Archer 1980). Lifecycle strategies in vespine wasps may have diverged to avoided competition when these groups were evolving (Archer 1980). More specifically these groups may have avoided competition by divergent forage preference. Members of the *V. rufa* group will forage only on live prey, while members of the *V. vulgaris* group will also scavenge carrion (Akre and Davies 1978). *Dolichovespula* wasps will normally only forage for live prey (Akre and Davies 1978), although *D. maculata* (Linnaeus) has been noted to scavenge (Matsuura and Yamane 1990).

1.4. Nest sites and geographical range of British vespines

There are differences both between genera and between species in nest site preference. *Dolichovespula* species are normally aerial nesters, whereas *Vespula* species are subterranean nesters. *Vespa* species, however, are generally classified into those which prefer open sites above ground and those which prefer covered sites under or above ground (Matsuura and Yamane 1990).

Nest site preferences of the British wasps were studied by Archer (1989), and are presented in Table 1.2. *Vespa crabro* always nests in cavities such as trees, bird boxes

and buildings (Edwards 1980. Of the *Dolichovespula* genus, *D. norwegica* nests are mainly aerial and exposed, although a few are constructed at or below ground level (Edwards 1980). Archer (1989) found that nests of *D. norwegica* were normally constructed in trees, hedges or other vegetation. *D. sylvestris* nests at aerial sites which are normally partially or completely enclosed. Typical nest sites for *D. sylvestris* include bird boxes, wall cavities and tree hollows (Bunn 1982). Some nests are constructed underground in pre-existing cavities.

Table 1.2. The number of nests constructed at various types of nest site sampled in England in the period 1957-1985 (Archer 1989).

Species	Building over-hang	Cavity	Building/ structure	Dense veget-ation	Open veget-ation	Ground level	Subter-ranean
Vespa crabro	1	31	15				
V. vulgaris		1	26	2			
V. germanica			6	1			38
V. rufa	2	2	1				43
D. sylvestris	6		55	4		9	23
D. norwegica					79	6	85

In the *V. vulgaris* group, *V. vulgaris* nests are predominantly subterranean, although some are constructed in other cavities or at aerial sites (Akre and Davies 1978). *V. germanica* is typically subterranean (Akre and Davies 1978), but may also nest aerially or in cavity walls (Spradbery 1973). Nearly all members of the *V. rufa* group are subterranean (MacDonald *et al.* 1974; MacDonald 1975), although *V. rufa* tends to nest at shallow depths.

There are currently nine species of the Vespinae present in Britain. *Vespa crabro* is the only member of the *Vespa* genus represented in Britain, and is only found in southern half of England (Edwards 1980). In the *V. vulgaris* group, *V. vulgaris* is present in most parts of Scotland (Laidlaw 1930) and throughout Britain (Edwards 1980). *V. germanica* is common in most of Britain, but is relatively uncommon in Scotland (Laidlaw 1930) and completely absent from Northwest Scotland (Edwards 1980). Of the *V. rufa* group, *V. rufa* is distributed throughout Britain, preferring open hilly or rural areas (Edwards 1980). *V. austriaca*, the obligate social parasite of *V. rufa*, is rare throughout Britain.

There are currently four established species of *Dolichovespula* in Britain. *D. sylvestris* is common throughout Britain (Edwards 1980), although in the North and Scotland is not

as common as *D. norwegica* (Laidlaw 1930; Edwards 1980). *D. norwegica* is more common in the northern half of Britain (Edwards 1980). *D. media* (Retzius) has recently become established in the southern regions of Britain but is uncommon (Colvin 1992). A nest of *D. saxonica* (Fabricius) was noted in the London area by Archer (1992), and has now become widely established in the South of England.

1.5. Changes in nest architecture with colony development, and architectural differences between genera.

In vespine wasps, the architecture of the nest changes greatly following the emergence of the workers. Although there are some differences between genera in nest architecture in the embryo nest, they are very similar at this stage. The embryo nest is suspended from the substrate by a structure called the pedicel. There are differences between genera in the shape of the pedicel, with *Vespa* constructing a club shaped pedicel, while in *Vespula* and *Dolichovespula* the pedicel is triangular in shape with a spiral twist (Matsuura and Yamane 1990). There is very little variation in the structure of the comb between genera in embryo nests; aside from the number of cells constructed (Matsuura and Yamane 1990). The envelope, however, shows variation both in the number of layers and structure between genera. *D. sylvestris* constructs between 2-5 envelope layers (Brian and Brian 1948) while *V. vulgaris* typically has 4-5 (Potter 1964). In *Vespula*, the first envelope sheet is suspended from the pedicel, and subsequent sheets are constructed at the base of the preceding sheet. In *Dolichovespula*, however, new sheets are independent of each other and are attached directly to the pedicel above the preceding sheet (Matsuura and Yamane 1990).

There are some architectural features only present in the embryo nests that appear to function in thermoregulation. In the embryo nest of some species of *Dolichovespula* and *Vespa*, the entrance is extended down into a tube-like structure, the vestibule (Matsuura and Yamane 1990). This may have some function in nest defence. However, in *D. maculata*, the vestibule is constructed shortly before pupation of the first brood, and dismantled shortly afterwards (Greene 1979). Heating the nest at the pupal stage appears to have a direct effect on the quality of the emerging adults (Ishay 1972, 1973). The construction of the vestibule may therefore reduce heat loss through the entrance at this stage. A further architectural feature in the embryo nest that appears to have a

thermoregulatory function, is the disc. This consists of a circular sheet of paper constructed between the first layer of envelope and the twist of the pedicel, and is only present in *Vespula* and *Dolichovespula*. In *Dolichovespula*, however, the disc seems to be the remnants of the first layer of envelope, which is trimmed back shortly after the subsequent layers are constructed. In *Vespula* this structure is smooth at the sides, and is therefore thought to be constructed in that form (Matsuura and Yamane 1990). In vespine wasps, the queen has been observed to curl around the pedicel beneath the disc when warming the nest (Spradbery 1973). The disc is therefore thought to function as a baffle, directing heat produced by the queen down toward the brood (Matsuura and Yamane 1990).

Differences between genera in the form and number of combs constructed become apparent following the queen nest stage. The *V. vulgaris* species group constructs a large number of worker combs (Greene 1979). *V. vulgaris* and *V. germanica* produce eight to nine combs during the season. Between two and four of the lowest combs contain large cells (Spradbery 1973). During the season they may produce between 3,500 and 15,000 cells in total (Akre and Davies 1978). Species of *Dolichovespula* and the *V. rufa* group may produce as few as four combs, only one of which is used for rearing workers (Greene 1979; Edwards 1980). *Dolichovespula* nests normally have up to 3,000 cells, while the *V. rufa* group has 500 to 2,500 cells (Akre and Davies 1978).

Differences in the shape of the comb between genera also become apparent following the embryo stage. The comb of *Vespula* is raised slightly around the central support but is generally flat. In *Dolichovespula*, the comb is raised at both the centre and the edge (Matsuura and Yamane 1990). The *Vespa* genus seems to be divided into species with flat combs, and species with curved combs (Matsuura and Yamane 1990).

There are clear differences in the form of the comb supports between genera. *Dolichovespula* species generally have ribbon-like comb supports, which are in the form of long, thin strips with a rod-like comb support at the centre of the comb (Matsuura and Yamane 1990). As *Dolichovespula* species mainly nest at aerial sites, they are subject to wind and movements of the substrate to which they are attached. The flexible ribbon type comb supports would allow combs to twist and move with respect to each other. Species of the *V. vulgaris* group generally have rod-like comb supports, although in some species the rod-like supports are extended and joined together in the latter stages becoming ribbon-like (Matsuura and Yamane 1990). The *V. rufa* group has ribbon-like

supports on the upper comb and chord-like supports on subsequent combs (Greene 1979). *V. vulgaris* and *V. rufa* tend to nest at subterranean sites where the nest would be subject to little movement. The rod-like supports of the group are less flexible, and would tend to fail rather than twisting with the combs.

The construction of the envelope in large continuous sheets in the embryo nest is termed *laminar construction*. Following the emergence of the workers, *Dolichovespula* and members of the *V. rufa* group generally continue to construct laminar envelopes (Matsuura and Yamane 1990). In the *V. vulgaris* species group, however, workers begin to construct the envelope in small sections of tight shell-like shapes (Matsuura and Yamane 1990), which are constructed by adding loads of pulp in an arc. This is termed *cellular construction*, and each shell of envelope is self-contained. The shell shape results from the arc being flatter towards the top and tighter towards the bottom. However, some *Dolichovespula* species have been noted to have varying degrees of cellular construction, although they are more like the elongate shells of *Vespa* than the small scallop shapes of *V. vulgaris*. The tightly scalloped envelope of the *V. vulgaris* group is also apparent in some *Vespa* (Matsuura 1971). Greene (1979) noted that *D. maculata* exhibits this cellular form of envelope construction to varying degrees, although some nests are entirely laminar. This form of envelope is also common in the upper parts of *D. norwegica* nests (personal observation).

Little is known about the adaptive significance of the different forms of envelope. In Britain though, aerial nesters have the laminar construction while the subterranean nesters have the cellular construction. The cellular construction may be more suited to cavity nesting. Each cell encloses a volume of air so the nest can expand unevenly to fill any shape of cavity. In laminar construction, however, a much larger section of envelope must be constructed before any extra volume of air is enclosed. In addition, the laminar type of construction would seem to be more suited to weatherproofing providing less resistance against wind and allowing rain to run-off.

There are notable differences between species and genera in the texture and quality of nest paper. Some members of the *V. vulgaris* group such as *V. flavopilosa* Jacobson and *V. maculifrons* (du Buyson), generally construct their nest from fibre collected from rotten wood which produce a very fragile paper. Other members of the group, such as *V. germanica* and *V. pensylvanica* (de Saussure) select fibre from sound wood, and have a more robust carton (MacDonald *et al.* 1980). *Dolichovespula*, and the *V. rufa* group

generally construct their envelope from sound wood, although they are known to occasionally use rotten sources (Greene 1979). This selection leads to stronger, more flexible nest paper than in the *V. vulgaris* group (Greene 1979). The quality of nest paper is discussed in more detail in Chapter 4.

1.6. Regulation of nest construction behaviour and the *stigmergy* hypothesis.

There are several possible mechanisms to explain how individual construction behaviour is regulated. One hypothesis is that individuals construct the nest to a final form or *blueprint*. This idea presents several difficulties. Firstly, each individual would have to hold a large amount of detailed information on the structure of the nest. In order to work efficiently towards a final plan, the worker would also have to continually survey the entire construction to compare it to the inherent plan. Social insect nests show a great variety of nest forms within species and appear to be able to adapt the nest structure to environmental constraints (Downing and Jeanne 1988). The worker would therefore have to posses many alternative blueprints. There would also be constructional problems; a blueprint would not, by definition, carry information about the individual steps required to arrive at a final form. This is especially important in structures in tension, whereby the nest has to be expanded in specific ways to avoid failure. Finally in social insects, the nest continually expands through the development of the colony. The structure must therefore be usable throughout its construction. In vespine wasps, for example, the envelope has vital functions in defence, insulation and weatherproofing, and in most species must enclose the nest from the earliest stage of colony development.

Nest construction behaviour must be regulated at two distinct levels, the level of the individual and the level of the colony. The study of behaviour in social insects, especially in wasps, has followed a reductionist approach in determining the organisation of the colony through an examination of the behaviour of the individual. There are, however, properties of colony behaviour resulting from the interaction between individuals at the colony level which cannot be predicted by thorough knowledge of individual behaviour. This is known as *emergent behaviour* (Goodwin 1998). Behaviour only exhibited at the level of the colony is therefore meaningless at the level of the individual (Wenzel 1996). Similarly, it is difficult to determine individual behaviour based only on knowledge of colony behaviour. Emergent behaviour, which results from simple interactions in the

colony, is often termed *order for free*, and Goodwin (1988) considers that the emergence of complexity from simple interactions may be hard to avoid. A complete understanding of social insect behaviour can only be obtained through the study of both individual and colony behaviour.

Goodwin (1998) investigated complex patterns of activity in ant colonies. The ant *Leptothorax tuberointerruptus*, forms small colonies of between 40 and 80 members. From observations of the colony behaviour, these ants were known to exhibit cyclic periods of activity and inactivity. The pattern of this activity changed, depending on the density of ants in the colony. Goodwin (1998) modelled this behaviour with a computer simulation of colony behaviour. In the model, individual ants interacted very simply. When an active ant comes into contact with an inactive ant, it was stimulated into activity. The result of this simulation was that the simple pattern of interaction produced the same complex patterns of behaviour observed at the colony level. In the simulation, ants exhibited the same pattern of behaviour observed in real colonies, behaving chaotically at low densities and more rhythmically at high densities.

Grassé (1959) tested the hypothesis that the termite *Macrotermes natallensis* constructs the nest to an inherent design or blueprint, and co-ordinates construction through communication. He did not find any evidence of either, but instead proposed a new hypothesis termed *stigmergy*. According to the stigmergy hypothesis, the builder inherits a linear programme of steps with cues originating from previous construction. Once a builder has identified the present step of construction, it only has to decide whether to continue with the current stage of construction or move to the next. Wilson (1975) proposed the term *sematectonic communication* for the process in which information is gained from previous construction.

Downing and Jeanne (1988) highlighted several problems with stigmergy theory. Firstly, Grassé's theory only dealt with the construction of one structure within the nest that followed a linear sequence of events. Therefore there was no mechanism for construction to be switched from one linear sequence to another. It did not explain how workers correct for construction errors or cope with nest repairs. There was also no mechanism by which construction could be regulated by external factors such as environmental conditions. The stigmergy hypothesis therefore could only explain behaviour that followed a linear sequence, and did not explain non-linear switching between sequences.

The stigmergy theory has been demonstrated in several animals such as weaverbirds (Collias and Collias 1962) and eumenid wasps (Smith 1978). These species, however, constructed their nest in a linear sequence of events. Downing and Jeanne (1988) studied individual construction behaviour in *Polistes*, which exhibited both linear, and non-linear construction behaviour.

Downing and Jeanne have extensively studied the regulation of nest construction behaviour in *Polistes fuscatus* (Fabricius) a primitively eusocial wasp which constructs relatively simple nests (Downing and Jeanne, 1988, 1990, 1994; Downing, 1985). The nest consists of a single comb attached to the substrate by one or more petioles. The initial phase of nest construction is linear. The builders begin nest construction preparing the substrate by antennating and rubbing the surface with thier prothoracic tarsi. A strip of wood pulp is then added to the surface and drawn into a spike. One or two more loads of pulp are added to the spike to construct a petiole. A load of pulp is then added to one side of the petiole and is drawn down and out from the petiole. A second load is then added on the opposite side to complete a flat sheet. This forms the shared wall between the first and second cell. The first cell is constructed along the distal edge of one side, and at 90° to, the flat sheet. After the first cell is initiated, construction becomes non-linear as the workers have a choice of various linear programmes to engage in. The builder can start the second cell, lengthen the first or add pulp to thicken the petiole (Downing and Jeanne 1988).

The length of the petiole was the cue for moving from construction of the petiole to construction of the flat sheet. Its construction is therefore in accordance with the stigmergy theory. The cue for the placement of the flat sheet, however, does not come from the previous construction. In initiating the flat sheet, the workers do not simply attach the sheet to the distal end of the petiole, but construct the sheet at a measured distance from the substrate. The cue for the construction of the flat sheet is therefore the previous stage of construction in accordance with the stigmergy hypothesis and absolute measurements, which is not stigmergic. This extends the idea of the stigmergy hypothesis as wasps are using cues from both the previous stage of construction and from absolute measurements. The use of absolute measurements allows the workers to correct for construction errors arising in the previous stage.

The cues used in construction of the first cell further extended the hypothesis. The width of the flat sheet acted as a cue for the workers to progress to the next step: construction

of the first cell. The cue for the placement of the first cell, however, was the distance to the petiole. The builder is not therefore using only cues from the previous stage of construction, but from other stages also.

The cues regulating the progress of linear construction in *P. fuscatus* can be explained partly by stigmergy, but also by absolute measurement of the structure. In the non-linear stage of construction, a builder may be engaged in construction at one of several locations around the nest and also at various stages of construction. A mechanism must therefore exist to allow workers to move from one linear construction sequence to another.

Downing and Jeanne (1990) have described in more detail the non-linear stage of nest construction following initiation of the second cell. Following construction of the first two cells, subsequent cells are added to the circumference of the comb. As the comb is supported at the centre, mechanisms must exist to ensure that the comb is enlarged evenly. When a new row of cells is started, a *side cell* is constructed across the junction of two cells in the previous row. Workers then show a greater tendency to add cells to the side of the first cell than in any other location. Further cells are then added to the side of this first cell of the new row until it is complete, the *corner cell* completing the row. When initiating a new row, workers showed no preference as to where to construct the side cell. When a row was almost complete, builders had a choice of constructing a corner cell or a side cell. Builders showed a greater tendency to add corner cells hence completing the row, than to add a side cell to start a new row.

When placing a new cell, the worker must decide where along the length of an old cell to initiate it. The position of the neighbouring cells is the only cue used in initiating a new cell - providing the comb is a minimum distance from the substrate. A new cell is initiated as an arch symmetrically around the groove of two existing cells. The worker antennates this groove and uses it to direct cell construction. Gravity and the position of the groove between existing cells, direct construction of the cell walls as they are lengthened. In lengthening the cell, the wasp continually reassesses construction. It is likely that the wasps use their antennae to place the cell around a groove and to assess the width of the cell. If the antennae are part or completely removed the perception of the wasps is altered and they construct cells that are not centred on a groove. In addition, the width of the cells is inconsistent along their length

When initiating a new cell, wasps use the groove between two existing cells as a cue for construction. When elongating the cell, however, wasp use gravity. A primary cue was therefore used to initiate construction, and other cues allowed the workers to correct problems in previous construction. The primary cue therefore formed the basis of construction, but in its absence other cues were used. This hierarchical use of cues allowed construction to be more flexible coping with a range of building problems.

Cell size in vespine wasps appears to be controlled by an environmental factor, but may also be controlled by cues originating from the brood. Ishay *et al.* (1983) found that the cell size in *Vespa orientalis* changes gradually from small to large. Workers seem to measure cells and construct them to a threshold size, which appears to be regulated by photoperiodicity. Workers, however, appear to use a hierarchical system of cues to determine cell size. Ishay (1975) found that when eggs or young instar larvae of *Vespa orientalis* and *V. germanica* are transferred from small cells to large cells at the start of the season, workers narrow the cell opening and the brood will develop into workers. Workers therefore appear to use larvae as a redundant cue to correct for construction problems in cell size. Workers also appear to construct cells to a width threshold across the opening. This threshold appears to be different depending on whether the worker is constructing a small or large cell.

Downing and Jeanne (1990) described many of the cues that stimulate a worker to engage on a particular building programme. They did not, however, determine how builders evaluate cues arising from these different types of construction in order to decide which area to construct next. Stigmergy alone only describes the behaviour of the individual, it is not sufficient to explain how construction is co-ordinated when several individuals are involved in construction simultaneously.

Karsai and Pénzes (1993) have proposed a model to show how individual construction decisions contribute to co-ordinated nest construction. To co-ordinate behaviour individuals must communicate. In construction behaviour, the cue arises from the structure itself. This can therefore be regarded as a form of indirect communication between individuals, and may be sufficient to account for the co-ordination of construction behaviour. The co-ordination of construction through stigmergy is termed *self-organisation*. Karsai and Penzés (1993) investigated how stigmergic script can explain co-ordinated construction behaviour based only on self-organisation. They simulated comb construction behaviour in wasps, with each wasp using only simple

behavioural rules. The cue for construction came from previous construction, and the wasp only had to survey the local area and make simple yes/no decisions.

A worker returning to the nest with construction material would first have to decide whether the cell in front of it was small or not (i.e. in need of enlargement). The worker would measured the cell, and if it was below a certain depth threshold it was considered a small cell, and the answer was yes. If the answer to this first question was yes, the worker would then make a second decision, whether to enlarge the cell or move to another position. This decision was based on a simple probability level pre-determined by the programmer. These probabilities were based on observed tendencies in wasps, including those described by Downing and Jeanne (1988, 1990) for *Polistes*. These were necessary to ensure that workers were more likely to engage in some types of construction activities than others. For example, wasps were more likely to initiate cell construction if the cell was next to another. This avoided new rows being started before others were complete which would lead to uneven comb growth. Using only simply stigmergic rules, wasps constructed comb that grew evenly with respect to the initial cells without the wasps having any concept of where the centre of the comb was.

Downing and Jeanne (1990) noted that one problem with the stigmergy hypothesis was that it did not provide a mechanism for workers to evaluate several cues in deciding which part of the nest to construct. They observed workers moving around the nest with pulp loads antennating areas of comb in a seemingly random way. The self-organisation model of Karsai and Penzés (1993) predicts this random aspect of behaviour. In their model the wasps had ten possible situations in which to make a yes/no decision. As the outcomes of several of these decisions were based on a balance of probability, the individual wasps exhibited a degree of random behaviour.

Karsai and Penzés (1998) have further shown how simple behavioural rules can lead to the variety of comb forms exhibited in Polistine wasps simply by altering their preference as to which side of the comb to initiate cell construction. This could explain how wasps cope with environmental constraints such as nest site restrictions. If a wasp is denied from initiating a cell at one side of the comb due to spatial restrictions, the workers may change their preference as to which side of the comb to initiate construction

Self-organisation has also been demonstrated to explain organised construction behaviour in ants. A similar model to that of Karsai and Penzés (1993) successfully

modelled construction behaviour in the ant *Leptothorax tuberointerruptus* (Franks *et al.* 1992: Franks and Deneubourg 1997)

1.7. Objectives of thesis and introduction to chapters

The construction of envelope places considerable demands on the time of the workers. It is therefore likely that its construction is regulated such that it closely matches the requirements of the colony for thermoregulation. The requirements of the colony for thermoregulation, and therefore insulation, may depend on the relative proportions of types of brood present in the nest. As very little is known about the effects of thermoregulation on brood development, it is difficult to predict the requirements of the colony for insulation. Thermoregulation may be beneficial throughout colony development decreasing the development time of the brood (Martin 1990). Warming the nest may, however, be more beneficial to brood at specific developmental stages. Ishay (1973) found that thermoregulation has a direct effect on the success of pupal development in *Vespa crabro*. The envelope may therefore be thickest when a large proportion of the brood is at the pupal stage. **Chapter 2** will therefore examine the relationship between the developmental stage of the colony and the amount of envelope constructed.

Pulp as a resource, can be allocated to the construction of comb or envelope. One way in which the allocation of material to these two components may be regulated is to employ a simple behavioural rule in which a fixed proportion of time is spent constructing them. **Chapter 2** will examine the allocation of material to comb and envelope by comparing the ratio of comb to envelope mass between developmental stages. If workers spent a fixed proportion of their time constructing comb and envelope, there would be no difference between developmental stages in the ratio of comb to envelope.

From a consideration of colony biomass and the surface area to volume ratio, it would be predicted that as colonies increase in size, their ability to thermoregulate also increases. Large colonies should therefore require proportionally less envelope to maintain the same temperature. Potter (1964) and Spradbery (1973) claimed that small vespine nests

have proportionally thicker envelopes than large nests. As neither author presented data to support this observation, one of the objectives of **Chapter 2** is to determine the relationship between nest diameter and envelope thickness.

Variation in envelope construction is also apparent between nest sites. There is much evidence in the literature that envelope construction is affected by nest site. Potter (1964) noted that nests of *V. vulgaris* constructed in exposed situations have thick envelopes, while those constructed at sheltered nest sites have thin ones. Edwards (1980) and Archer (1981) found that when nests of *D. sylvestris* are constructed in bird nest boxes, the envelope is often missing at the sides. Nest site restrictions may have an effect on both the decision of the builder to construct envelope and the placement of the envelope. **Chapter 2** will examine if there are differences in the total amount of envelope constructed at restricted and unrestricted nest sites.

The regulation of nest construction behaviour can also be examined experimentally (Downing and Jeanne 1988, 1990). The construction of the envelope requires a great investment in the time and resources of the colony. As its principal function is in nest insulation, then the amount constructed should be regulated to meet the needs of the colony. One way in which its construction could be regulated is through the use of nest temperature as a cue. Potter (1964) found some evidence that the proportion of forage trips made for pulp in *V. vulgaris* were regulated by temperature. Temperature may therefore effect the rate at which the envelope is constructed. Potter did not, however, determine whether the pulp returned to the nest was used in the construction of comb or envelope. **Chapter 3** presents a nest box and entrance trap, which were designed to determine the effect of temperature on the rate of envelope construction.

The two major nest components comb and envelope, perform very different structural functions in the nest. The comb functions as a beam or cantilever, and supports the mass of the brood and as such is subject to both tensile and compressive forces. The envelope, however, must principally support its own weight in tension. As comb and envelope material perform different functions in the nest it is likely that they are constructed to different specification. **Chapter 4** will examine the mechanical properties of the two materials. Differences in the properties of the two materials may result during the selection and processing of fibres, or in the manufacture. **Chapter 4** will therefore

determine if there are differences in the selection and processing of fibres used in the construction of comb and envelope paper.

As *Dolichovespula* and the *V. vulgaris* species group have different nest site preferences, the envelope must perform different tasks. At subterranean sites the envelope insulates the nest but is not required to resist wind and rain. *Dolichovespula* species, however, are principally aerial nesters and as such the envelope must perform additional weatherproofing functions. As the envelope in *Dolichovespula* nests performs additional functions to *Vespula* nests, it would be expected that the material from which they are constructed would have different mechanical properties. There are several qualitative descriptions of differences in paper quality between species. McGovern *et al.* (1988) provided quantitative information on the properties of comb and envelope paper in different species. Their results were, however, obtained from relatively few nests and were not subject to statistical analysis. A further objective of **Chapter 4** will therefore be to compare the mechanical properties of *D. sylvestris*, *D. norwegica* and *V. vulgaris* paper. It will then examine whether differences in comb and envelope result from differences in fibre selection and processing, or in paper manufacture behaviour.

The comb supports (or suspensoria) hold the weight of the comb in tension. The amount of comb supports constructed and their position on the comb is therefore essential to avoid mechanical failure. The combs are suspended below each other, with the load supported by the suspensoria depending on the position of the nest. The position and number of suspensoria should therefore reflect the amount of mass supported. Spradbery (1973) claimed that in vespine wasps "*the number of such pillars is variable, but their quantity and robustness are related to the area of comb supported below them so that there are more pillars per unit area in the upper combs compared to the lower combs*". **Chapter 5** examines this claim by comparing the density of comb supports between combs in *D. sylvestris* and *D. norwegica*.

Although the regulation of comb support construction has not been extensively examined in vespines, Downing and Jeanne (1990) have investigated regulation in *Polistes fuscatus*. They found a significant positive relationship between the number of cells in the comb, and both the thickness of the petiole and the number of secondary comb supports constructed. They therefore investigated cues which workers may use in the construction of supports and found evidence that the thickness of the petiole was

influenced by comb mass. They also found that adding an off-centre weight to the comb did not significantly effect the number of secondary supports constructed. The cue for the placement of comb supports could be the distance to neighbouring supports. The worker would therefore only have to survey the local area. If the supports were more than a threshold distance apart, the worker could construct a new support. **Chapter 5** will therefore determine if comb supports are constructed at a minimum distance apart or are randomly positioned with respect to nearest neighbours. Alternatively, the cue for comb support construction could result from an increase in the mass or size of the combs suspended. **Chapter 5** therefore examines the relationship between the length of comb supports constructed and various factors related to amount of comb supported (e.g. number of brood reared and comb surface area).

The ability of the colony to thermoregulate is well-documented (Sailer 1950; Potter 1964; Roland 1969; Ishay and Ruttner 1971). The function and mechanisms of thermoregulation, however, are not. The queen and workers are capable of directly raising the temperature of the nest through the action of their flight muscles (Milani 1982; Heinrich 1983). Older larvae (instars 4 and 5) are capable of moving in their cells and as such can raise the temperature of the nest (Ishay and Ruttner 1971; Ishay 1972, 1973). They can also contribute indirectly to nest heating by providing the adults with carbohydrate-rich saliva, which can be respired to heat the nest (Maschwitz 1986; Ishay and Ikan 1966, 1968a). The relative importance of the brood and the adults in nest thermoregulation is, however, poorly understood. Heating the nest may have general benefits to the brood in reducing development time (Himmer 1932, Martin 1990). The only specific evidence of its benefits, however, is on the success rate of pupation (Ishay 1972, 1973).

As the principal function of the multi-layered envelope in vespine wasps is in nest insulation, the amount constructed at a particular developmental stage should reflect the requirement of the colony for thermoregulation and its ability to raise nest temperature. **Chapter 6** will first examine the ability of the colony to thermoregulate. It will then examine the factors limiting thermoregulation such as the number of eggs, larvae and brood in the nest. The pattern of envelope construction observed in Chapter 2 will then be discussed in relation to the ability and requirement for nest thermoregulation.

Finally, **Chapter 7** will summarise the main findings of the thesis. The evidence obtained on the behavioural regulation of nest construction will be discussed in the context of the stigmergy, and self-organisation.

Chapter 2. Regulation of envelope construction

2.1. Introduction

This chapter will examine the regulation and development of envelope structure in *D. sylvestris* and *D. norwegica*. In particular, the relationship between developmental stage of the colony and the amount of envelope constructed will be examined. The allocation of pulp, as a resource, to the manufacture of the two major nest components, comb and envelope will also be considered. The species *D. sylvestris* and *D. norwegica* were examined as they are closely related and have similar lifecycles and can therefore provide a useful verification of findings. In addition, there are small differences between these species, particularly in nest site preference, which could provide clues about the regulation of envelope construction.

The envelope has several important functions in the nest. Its two principal functions are insulation and defence against parasitoids and predators (Spradbery 1973; Matsuura and Yamane 1990). Although the envelope is undoubtedly essential in defence of the colony, Matsuura and Yamane (1990) consider that the construction of a multi-layered envelope in most vespines provides little more defence against predators than does a single layer. In tropical and subtropical regions where daily temperature fluctuates very little, several species of *Vespa* construct very little envelope. *Vespa crabro*, and *Vespa tropica* (Linnaeus) for example, construct only a single layer of envelope, and its primary function is likely to be in colony defence (Matsuura and Yamane 1990). Species that nest in temperate climates, however, such as those of the *Vespula* and *Dolichovespula* genera, often construct a thick, multi-layered envelope (Edwards 1980; Matsuura and Yamane 1990). In these species the most important function of the envelope is in providing a closed, insulated environment, allowing the active maintenance of an elevated nest temperature.

The multiple layers of the envelope trap air, and as such function like double glazing, providing excellent insulation with minimum increase in nest weight. Envelope is therefore integral to the process of thermoregulation; "*Aside from their body heat generated when inside the nest, the principal contribution of most workers to warming the brood is in envelope construction*"(Greene 1991). Vogt (1986) has demonstrated the importance of insulation in bumblebees. Colonies that were not insulated produced

fewer adults than insulated colonies. The envelope must therefore be regarded as an integral part of temperature regulation, and its construction may be regarded as a passive mechanism of thermoregulation.

Weatherproofing is another important function of the envelope, protecting the brood and brood combs from intense sunlight, wind and rain. The exclusion of light is likely to be important for the brood as they do not have the highly sclerotised cuticle of the adults, and are likely to be damaged by UV light entering the nest. Little is known about the water proofing qualities of the envelope. Edwards (1980) considers that they are due to both the oral secretions used in the construction of envelope, and to the binding properties of the hyphae of the blue-stain fungus, *Aureobasideum pullulans*. It is unlikely that the weather proofing functions of the envelope (other than insulation) are greatly improved by the construction of a multi-layered envelope. Although in cavity nesters weatherproofing is a relatively unimportant function of the envelope, in open nesters it is essential.

Potter (1964) proposed that in limiting the circulation of air, envelope might also function in the regulation of humidity. Potter measured humidity in a laboratory colony of *V. vulgaris* maintained at 32°C and found it to be fairly constant; between 85 and 95%. Humidity outside the nest was not, however, recorded, and as humidity is closely dependent on temperature, this result is unlikely to be a result of behavioural regulation. Although it is reasonable to assume that the brood may benefit from elevated nest humidity, the envelope is porous and hygroscopic (Biermann 1993) and so is unlikely to be suited to maintaining an elevated humidity within the nest.

The construction of a multi-layered envelope by cavity nesting vespines provides further evidence that the principal function of the multi-layered envelope is in nest insulation. In temperate climates, cavity nesters such as *V. vulgaris*, construct a large amount of envelope. It is unlikely that at these nest sites, the envelope has significant functions in protection against UV or in weatherproofing. At underground sites the envelope is less important in colony defence, and the entrance to the cavity can be controlled by the workers. As a multi-layered envelope is unlikely to be significantly more effective in colony defence, exclusion of light or weatherproofing than a single layered envelope, its construction (apart from in the roof cone of some *Vespa* species) can only be explained in terms of thermoregulation.

Envelope structure changes during colony development, and in the process of nest enlargement the inner layers of envelope must be removed as the combs increase in diameter, whilst layers are added to the outside. During the period of rapid colony expansion, the thickness of the envelope will therefore be dynamic. The construction of envelope places considerable demands on the time of the workers. It is therefore likely that its construction is regulated such that it closely matches the requirements of the colony for thermoregulation. One way in which the construction of envelope may be regulated is using external cues such as light intensity, nest temperature and wind speed. As the principal function of the envelope is in insulation, it is possible that temperature is one cue regulating its construction. A drop in the temperature of the nest for example, may stimulate workers to construct envelope. Construction may continue until the cue (i.e. a drop in temperature) is diminished. This would be consistent with the negative feedback mechanism proposed by Stuart (1967) in which construction in termites is stimulated by an environmental stimulus which in turn results in the elimination of that stimulus.

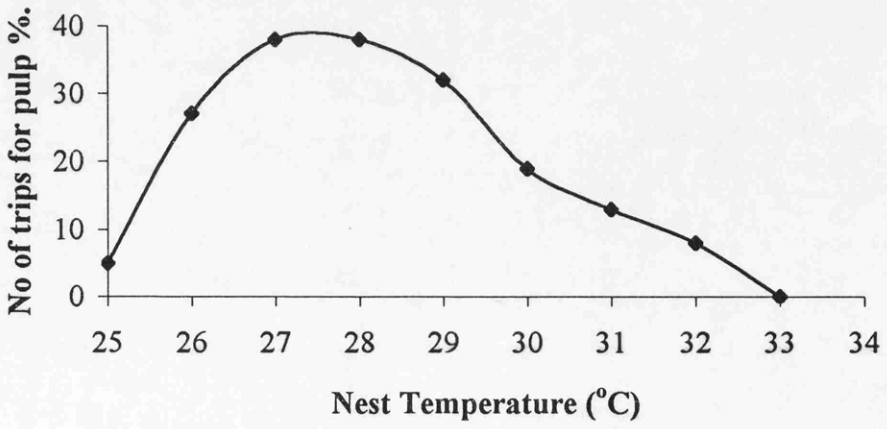
Brian and Brian (1948) suggested that light intensity at the nest site may be an important factor regulating envelope construction. This was based on observations of two embryo nests of *D. sylvestris* constructed in a similar location within one day of each other. One nest was exposed to a light intensity 20 times higher than the other, and after 7 days the queen was constructing her 5th layer of envelope compared to 3 layers at the darker site. Potter (1964), however, found that light only influenced the rate of construction of the first layer of envelope. The envelope of a mature nest of *V. vulgaris* was removed and the nest placed in a nest box heated to 32°C. When the nest was exposed to light one layer of envelope was quickly constructed covering the nest, after which envelope construction was very slow. Although the influence of light on envelope construction needs further experimental clarification it is likely that it only acts as a cue in nest repair. Light may be a useful cue to the workers to so that the nest can be secured against predators following damage. It may also more directly stimulate construction in order to exclude UV light which may harm the brood.

As the primary function of the envelope is thermal insulation of the nest, it is likely ambient temperature has a direct effect on the rate of envelope construction. Greene (1991) appreciated this relationship, "*rate of envelope construction as a function of nest temperature has not been well studied. In one intriguing series of tests, foraging for*

pulp was stimulated by temperatures slightly lower than optimum but then was inhibited at 25°C, a puzzle that awaits further experimental clarification''. Potter (1964) looked at the effect of temperature on envelope construction by recording the number of trips for pulp made by workers in a captive colony of *V. vulgaris*. The nest was artificially heated in its nest box with a paraffin lamp then gradually cooled by leaving the box door open. Potter (1964) found that the proportion of trips made for pulp was highest when the nest temperature was kept at a temperature below the nest optimum of 32°C (Figure 2.1.). Further experiments were carried out in a temperature controlled nest box. When the nest was kept at the optimum temperature, less than 10% of forage trips were for pulp. At 28°C between 30 and 40% of trips were made for pulp with a decrease in both fluid and flesh collection. At 26°C the number of trips decreased to 20-30% with a reduction in the number of trips for flesh but an increase in the trips for fluid.

There are several difficulties in interpreting Potter's results. Firstly although Potter found an effect of temperature on number of foraging trips for pulp in *V. vulgaris*, it was not determined if pulp forage was used in the construction of comb or envelope. A second difficulty is that material is recycled within the nest, and so comb, for example, may be constructed mostly from recycled envelope. A further problem is that workers must divide their time between many colony activities including foraging for water, pulp, carbohydrates and carrion. The proportion of time spent on foraging for pulp may therefore reflect the effect of temperature on one or more other colony activities.

Figure 2.1. The amount of pulp (% of total trips) collected at different nest temperatures by workers of *Vespula vulgaris* L. Redrawn from Potter (1964).



More evidence on the effect of environmental factors on the rate of envelope construction was provided in further work by Potter (1964). In this study a nest of *V. vulgaris* was kept at 32°C in the dark and the envelope removed. The wasps made very little envelope, and recovered the nest very slowly. Although this strongly suggests that ambient temperature is an environmental cue modulating the rate of envelope construction, the author did not establish controls for temperature or for removal of the envelope. The rate of envelope reconstruction cannot therefore be compared to that of an undamaged nest. In addition, this experiment examines the effect of temperature on nest repair rather than nest construction that may result from a different behaviour.

The regulation of envelope construction can be examined experimentally (e.g. Potter 1964). This approach has the benefit of providing direct information on the regulation of envelope construction and of allowing experimental manipulation of the nest structure (e.g. Downing & Jeanne 1990). There are, however, problems in this approach. Firstly, it is difficult to maintain a suitable number of captive colonies of vespine wasps to allow meaningful statistical analysis. Secondly, as activity inside the nest is obscured by the envelope, it is difficult to determine directly whether pulp brought into the nest is used in the manufacture of comb or envelope. The heated nest box and entrance trap presented in Chapter 3 were developed for this purpose, to allow the experimental manipulation of ambient temperature, and to monitor pulp foraging. For reasons beyond the control of the experimenter, however, the techniques and equipment presented in Chapter 3 could not be used for detailed experimental work that would provide further evidence on the regulation of envelope construction.

An alternative approach to the study of behavioural regulation of envelope construction is to examine envelope structure in a large number of colonies at different developmental stages collected from the field. This allows the allocation of nest material to the major nest components to be examined with respect to nest size and colony development. Although this approach requires the collection of a large number of colonies, it provides sufficient replication for analysis and was therefore adopted to address the questions outlined below.

The requirements of the colony for thermoregulation and therefore insulation may depend on the relative proportions of types of brood present in the nest. As very little is known about the effects of thermoregulation on brood development, it is difficult to predict the requirements of the colony for insulation. Thermoregulation may be

beneficial throughout colony development decreasing the development time of the brood Martin (1990). Warming the nest may, however, be more beneficial to brood at specific developmental stages. Ishay (1973) found that thermoregulation has a direct effect on the success of pupal development in *Vespa crabro*. Nest temperatures of below 20°C resulted in adults with malformed wings, especially during the latter stages of pupal development. The envelope may therefore be thickest when a large proportion of the brood is at the pupal stage. As the success of the colony is directly dependent on the quality of the reproductives, thermoregulation and therefore nest insulation may be most beneficial when the sealed brood consists mainly of reproductives. In this chapter, the relationship between colony development and the amount of envelope constructed will therefore be examined (**Question 1**).

The ability of the colony to actively regulate its temperature will depend on the relative rates of heat production and heat loss from the nest. The rate of heat production in the colony is related to colony biomass (Gibo *et al.* 1974), whereas the rate of heat loss is related to the nest size and insulation. Gibo *et al.* (1974) found that colonies of *D. maculata* had a higher biomass and were more efficient at regulating nest temperature than similarly sized colonies of *D. arenaria* (Fabricius). The surface area to volume ratio of the nest will decrease as the nest diameter increases, and therefore large nests should require relatively less insulation to achieve the same rate of heat loss and thus nest temperature (Spradbery 1973).

Pulp as a resource can be allocated to the construction of the two major nest components comb or envelope. One way in which the allocation of material to these two components may be regulated is to employ a simple behavioural rule in which a fixed proportion of time is spent constructing them. Akre *et al.* (1976) observed that workers of *V. pensylvanica* constructing envelope earlier in life than comb. The preference for constructing comb or envelope may therefore be dependent on the age of the worker. **Question 3** will examine the relationship between comb and envelope mass. A linear relationship would indicate a fixed allocation rule.

From a consideration of colony biomass and the surface area to volume ratio, it would be predicted that as colonies increase in size their ability to thermoregulate also increases and they would require proportionally less envelope to maintain the same temperature. Potter (1964) and Spradbery (1973) claimed that small vespine nests have proportionally thicker envelopes than large nests. As neither author, however, presented data to

support this observation, one of the objectives of this chapter is to determine the relationship between nest diameter and envelope thickness (**Question 4**).

The developmental stage of the colony is also important in determining the ratio of workers to brood. Although the workers principally are responsible for heat production, they rely on the supply of carbohydrates from the larvae (Ishay and Ruttner 1971; Ishay 1972, 1973: see Chapter 6.). The ability of the colony to thermoregulate the nest will therefore depend on the ratio of workers to brood present (Martin 1990). The ability of the nest to actively regulate its temperature will therefore determine the requirement for the construction of envelope.

An increase in envelope thickness may be achieved through the construction of a greater number of layers, or by leaving a greater gap between layers. Adding new layers of envelope would increase its insulating properties but would require the collection of a large amount of pulp. This could also be achieved by increasing the gap between layers of envelope and thus the volume of air enclosed. It is possible therefore that in smaller nests, workers compensate for the higher rate of heat loss by increasing the mean gap left between layers. This, however, has the disadvantage, that increasing the gap between layers of envelope would also increase the diameter of any new layers of envelope, and thus the amount of material required in its construction. This strategy may therefore have only limited benefits in terms of increasing nest insulation using the minimum amount of material. One objective of this chapter is therefore to determine if there are any differences between developmental stages and between regions in the mean gap between layers of envelope (**Question 2**).

The distribution of the envelope around the nest is important in terms of its ability to retain heat. Heat generated in the nest will tend to rise and escape through the top and upper regions of the nest. It would therefore be expected that envelope construction is regulated such that more envelope would be constructed in the upper parts of the nest than the lower parts. The distribution of the envelope will therefore be examined in this chapter (**Question 1**).

The architecture of the envelope varies greatly between species depending on nest site preference. This variation is particularly obvious between members of the *Vespa* genus where there is a great difference in structure between open nesters and cavity nesters. In those species which nest in open sites such as *Vespa analis* Fabricius and *Vespa affinis* Linnaeus, the envelope is often relatively thick and consists of aerial chambers of the type

constructed by *V. vulgaris* (Masuura and Yamane 1990; Starr and Jacobson 1990). However, the envelope is often thin and does not completely cover the combs in cavity nesters such as *Vespa crabro* and *Vespa tropica*. Martin (1990) found that nests of *Vespa affinis*, a tropical/subtropical species, had a thinner envelope than *Vespa simillima*, a temperate species, and consequently a reduced ability to elevate its temperature above that of the surroundings.

Matsuura and Yamane (1990) proposed that species of *Vespa* nesting in covered spaces have very few layers of envelope as these nest sites are 'thermoregulated' (insulated). This, however, does not seem to be the case in *Vespula* species which are predominantly cavity nesters and construct a large amount of envelope. Subterranean sites offer some degree of thermoregulation as diurnal temperature fluctuations decrease with depth (Spradbery 1973). The average temperature at these sites would, however, be much lower than the optimum. In tropical and sub tropical regions, however, covered nest sites would protect the nest from some of the temperature fluctuations, while having a mean temperature close to the ideal.

Variations have also been noted within species depending on nest site. Duncan (1939) observed that when the nests of *Vespa crabro* are constructed in the open or in soft earth the nest has a similar structure to those of *Dolichovespula* species whereas nests constructed on hard or stony ground lack envelopes. Potter (1964) noted that nests of *V. vulgaris* constructed in exposed situations have thick envelopes while those constructed in sheltered positions have thin ones. Spradbery (1973) similarly noted that a subterranean nest of *V. germanica* had an envelope 2cm thick while a nest from an aerial site had an envelope 6cm thick. Neither author, however, mentioned the relative size of these aerial and subterranean nests.

When predominantly aerial nesting species nest in cavities the nest often lacks envelope. Edwards (1980) and Archer (1981) noted that when nests of *D. sylvestris* were constructed in bird boxes, the envelope was absent from the sides of the nest with only a few layers of envelope remaining at the top and bottom. Duncan (1939) similarly notes that nests of *Vespa crabro* constructed in hollow trees often have no envelope apart from a few rudiments above the first comb and a few sheets to narrow the entrance hole in the tree. Edward's (1980) suggested that the lack of envelope in *Dolichovespula* nests constructed in cavities results from both a lack of available space and the insulation provided by the site. It is unlikely, however, that nest sites such as bird boxes provide

sufficient insulation, and that the construction of envelope is unnecessary. Cavity nesters in sub-terranean sites often build a large amount of envelope. When the same species construct their nest at a site where the cavity cannot be extended envelope is often lacking in the mature nest. Matsuura (1984) noted that in nests of *Vespula* constructed in wall cavities, part of the envelope adjacent to the walls is removed during the later nesting period.

It is likely then that at these sites the lack of envelope is simply a result of a lack of space for expansion of the nest. When the volume of the nest approaches that of the cavity, it may be more profitable to expand the combs at the expense of the envelope, than to retain nest insulation. No statistical evidence is available on the effects of nest site restrictions on envelope construction. Although there is plenty of evidence to suggest that envelope may be reduced or absent when nests are constructed in cavities, there is no evidence that the total amount of envelope manufactured is reduced. One of the objectives of this chapter is therefore to investigate the effect of nest site restrictions on the proportion of material allocated to comb and envelope construction **(Question 5)**.

Principal questions to be addressed in this chapter are listed below.

1. Are there differences between developmental stages and between regions of the nest in the relative amount of envelope constructed?
2. Are there differences between developmental stages and between regions of the nest in the mean gap between layers of envelope?
3. Do workers have the same behavioural rule through colony development for the allocation of material to comb and envelope?
4. Do small nests have proportionally thicker envelopes than large nests?
5. What is the effect of restrictions in the amount of space available at the nest site on the amount of envelope constructed?

2.2. Methods

Nest Collection

All nests of *D. sylvestris* and *D. norwegica* were collected from the Central region of Scotland and were principally located in the grounds of private houses. Nests were located with help from West Lothian Council pest control service, although poster

adverts were also placed in public places such as parks and garden centres and some nests were located by 'word of mouth'.

Vespine nests are normally collected in the evening or at night when workers have ceased foraging in order to ensure that most of the adults in the nest have been collected (Edwards 1980). However, as most of the nests collected for this project were located on domestic premises, it was not possible to collect them at night and they were collected during the day. The adults in the nest were killed with proprietary pyrethroid based aerosol pesticides. On arrival at the site, pesticide was sprayed into the nest entrance and any workers returning to the nest were also sprayed. The nest was not removed from the site until the last worker had returned. This was normally between 30 and 60 minutes following spraying.

The nest was removed from the site by placing a thin piece of plastic between the substrate and the envelope. It was then placed with the workers in a sealed box and transferred to a freezer for latter examination. Details on the location, date of collection and address were also recorded and enclosed with the nest. The number of nests collected of each species and nest sites are listed in Table 2.1. As the nests examined in this chapter were selected from those reported by the public, they were not randomly sampled, and therefore do not accurately represent nest site preferences in the two species.

Table 2.1. The location, numbers and sites of nests of *D. sylvestris* and *D. norwegica* examined in this chapter.

Location	Number of nests	
	D. sylvestris	*D. norwegica*
Overhang	8	4
Nest box	1	0
Bush	0	10
Hedge	0	20
Tree coniferous	1	4
Tree deciduous	0	1
Garden ornament	2	2
Bird box	8	1
Shed/ garage	39	2
Attic	4	0
Wall cavity	1	0
Glass house	2	0
Total	**66**	**44**

Nest sites were also classified according to the degree to which they restricted the expansion of the nest. Locations in which nest expansion was not impaired were classed as *unrestricted* sites. Those in which envelope expansion was prevented on one or two sides were classed as *semi-restricted.* Nests in vegetation such as hedges, bushes and trees were impeded by branches and foliage, but not prevented from expansion were also classed as semi-restricted. Nests in locations such as bird boxes where expansion was prevented on three or four sides were classed as *restricted.*

Nest size

Prior to examination nests were allowed to defrost. Nest diameter was then recorded from ten measurements recorded from three different regions of the nest. These nest regions were: height measured from the nest entrance to the attachment point at the top of the nest, diameter at the equator of the nest taken across the middle of the nest, and various other points around the nest (termed pole-pole).

Envelope measurements

The envelope was then separated into two halves by cutting it from the entrance hole to the top of the nest with dissecting scissors and scalpel. The combs were carefully removed by cutting through envelope attachment points and put to one side. This allowed thickness to be measured and the number of its layers to be counted in five regions of the envelope. These regions were the top (attachment point to substrate), the equator, the upper interval (between the top and equator), the lower interval (between the equator and the bottom of the nest) and the bottom of the nest (around nest entrance). At each region an average of ten counts and measurements was calculated. Envelope thickness was measured using a vernier calliper accurate to 0.1mm. The envelope was then placed in a drying oven for 24 hours at 60°C before being re-weighed.

Comb measurements

Prior to examination, the combs were separated by carefully cutting the comb supports with a scalpel and fine dissecting scissors. The combs were then traced around with a pencil and a piece of paper, in order to measure comb surface area and information on the length and spacing of comb supports was recorded at this time (Chapter 5).

In the process of removing the brood to record colony statistics, the number of large and small cells was counted in the comb. In *D. sylvestris*, small cells are generally used in the rearing of workers and large cells for rearing new queens, while males are reared in both small and large cells (Edwards 1980; Archer 1981). Although in nests of *V. vulgaris* there is a clear distinction between large and small cells, in *D. sylvestris* and *D. norwegica* there is not. In general, however, the first or upper comb in *D. sylvestris* consists of predominantly small cells with a few large cells around the periphery, the subsequent combs consist entirely of large cells (Archer 1981). Archer (1981) estimated that large cells constitute only 5% of the upper comb in mature nests. For the purpose of this project, cells in the upper comb were assumed to be small cells unless they contained queen pupae. It was found that *D. norwegica* had a similar distribution of small and large cells.

The mass of the comb material was recorded following the removal of the brood. One of the aims of the project was to examine the allocation of pulp as a resource to comb and envelope. It was therefore necessary to remove meconia from the bases of the cells as it formed a significant proportion of the mass of the comb. The meconia were cut from the base of the cells individually with a scalpel blade carefully removing any attached paper. The remaining material was then placed in a drying oven at 60°C for 24 hours before being weighed.

Colony statistics

The number of workers, males and new queens was recorded from the nest. The presence of the founder was also noted. She can normally be distinguished from the new queens by her duller more ragged appearance.

The combs were then examined and information on colony composition recorded. For each comb, the cell contents were noted on a *comb map*, which consisted of hexagonal patterned paper. The use of the comb map provided an easy way to record data and avoided cells being omitted or examined twice. The contents of the cells were categorised as eggs, larvae and pupae. Pupae were identified as workers, males and queens. Queen pupae were readily distinguished by their size and they protruded noticeably from the cells (Edwards 1980). Male pupae were distinguished from worker pupae, as they appear to have thick cap and the pupae cannot be seen through the cap. In worker pupae, however, the cap is thinner and therefore more visible. In pupae close

to eclosion, the genitalia of males and workers were readily distinguished. As the thickness of the pupal cap was not always clearly distinct, where possible, males and worker pupae were identified by this character.

Data recorded on the comb maps was then transferred to nest summary tables and generation summary tables as described by Archer (1981). These summary tables allowed the colonies to be categorised into the developmental stages devised by Archer (1981). These developmental stages describe colonies with a similar composition and numerical characteristics, and are limited in number to allow reasonable sample sizes in each group.

Some modifications were made to Archer's classification of developmental stages. The sub-periods CDL and CDS described by Archer were merged to form stage CDL/S. At the CDL stage the large cell brood was present up to the egg or larvae, while at the CDS stage the large cell brood were present up to the pupal stage. As the architecture of the nest could not change greatly between these stages, they did not differ sufficiently to be of relevance in this chapter. The sub-periods CDAB and CDAC were also merged to stage CDAB/C. During the sub-period CDAB the colony is at the peak of production of reproductives. Sub-period CDAC, however, represents the period of decline in the colony following the emergence of most of the reproductives. It is therefore unlikely that the structure of the envelope differs from that during the peak production rate of reproductives. Archer's classification of developmental stages were also adapted such that more precise numerical characteristics could be used to separate developmental stages. The nomenclature used for developmental stages in this chapter was similar to that of Archer for easy comparison.

Stage I. Queen nest (QN)

This stage is the same as that described by Archer as *Period 0 colonies, queen nest, QN.* This consisted of nests constructed entirely by the queen prior to the emergence of the first workers. The nests had only one comb containing brood of the first generation, any sealed brood consisting of only workers. In Archer's description this category included nests in which some workers had emerged. For the purpose of clarity where workers had emerged, colonies were classified as Stage I.

Stage II. Small cell nest (SCN)

This stage is the same as that described by Archer as *Period 1, SCN*. The nest consisted of only small cells and both the founder queen and one or more workers tended the nest. Only the first (upper) comb constructed. In some nests males were reared.

Stage III. CDL/S

This stage included both Archer's sub-periods *CDL* and *CDS* of *Period 2*. The large cell brood was present up to the sealed brood stage. No large cell adults had yet emerged.

Stage IV. CDAA

This was the same as Archer's sub period *CDAA* of *Period 2*. Between 1 and 100 males and queens had emerged from large cells.

Stage V. CDAB/C

This included the subperiods *CDAB* and *CDAB/C* of *Period 2*. Over 100 large cell adults had emerged. This included nests up to the end of colony development.

Colonies were separated into developmental stages by computer using logical functions on a spreadsheet (Microsoft Excel). The numerical criteria outlined in Table 2.2 were used to separate developmental stages.

Table 2.2. Summary of the characteristics used to separate colonies into developmental stages in using logical functions on a spreadsheet (Microsoft Excel).

Developmental stage	No. of combs	No. of workers	No. of small cell adults reared	No. of large cell adults reared	No. of large cell sealed brood	No. of large cells
QN	1	0	0	–	–	–
SCN	1	≥1	–	0	0	–
CDL/S	–	–	–	0	–	≥1
CDAA	–	–	–	1-100	–	–
CDAB/C	–	–	–	>100	–	–

The five developmental stages were well represented in *D. sylvestris*. In *D. norwegica*, however, colonies at an early stage of development were not located due to differences in nesting habit (Table 2.3). As colonies of *D. sylvestris* will nest on overhangs or in garden sheds, they are relatively easily located at an early stage. Colonies of *D.*

norwegica, however, are normally located in trees and hedges where small nests are often obscured by vegetation and are not observed.

Table 2.3. Summary of the number of nests of *D. sylvestris* and *D. norwegica* studied in each developmental category.

Developmental period	Number of nests	
	D. sylvestris	*D. norwegica*
Queen nest (QN)	8	0
Small cell nest (SCN)	7	0
Large cell initiation (CDL/S)	18	9
Large cell expansion (CDAA)	23	16
Large cell peak /senescence (CDAB/C)	10	19
Total	66	44

Question 1. Are there differences between developmental stages and between regions of the nest in the relative amount of envelope constructed?

This question will be answered by comparing the mean thickness and number of layers of envelope between regions and between developmental stages. In *D. sylvestris* the thickness and number of layers of envelope will be compared in all five developmental stages, while in *D. norwegica* they will be compared in the three latter stages represented.

Question 2. Are there differences between developmental stages and between regions of the nest in the mean gap between layers of envelope?

The mean gap between layers of envelope was estimated by dividing the total thickness of the envelope by the number of layers at each of the five regions measured. The total thickness of the envelope includes the thickness of the envelope paper. However, as the thickness of the paper is very small in relation to the total thickness of the envelope it was ignored (see chapter 4.).

Question 3. Do workers have the same behavioural rule through colony development for the allocation of material to comb and envelope?

This question was addressed by examining the structural and statistical relationship between the dry mass of comb and the dry mass of envelope. Caution must be taken in

interpreting the results, however, as the silk lining was not removed from the cells. It would therefore be expected that the comb is proportionally heavier in larger older cells. This question will also examine if there is a difference between *D. sylvestris* and *D. norwegica* in the allocation of material to comb and envelope.

Question 4. Do small nests have proportionally thicker envelopes than large nests?

This was addressed by examining the relationship between envelope thickness and nest diameter. The nest diameter was taken as a mean of the three measures of diameter (height, equator and pole-pole). Envelope thickness was calculated as the mean of the thickness measured from five regions of the nest. As the diameter of the nest includes the envelope thickness this was removed by subtracting twice the envelope thickness from the nest diameter. The statistical and structural relationship between envelope thickness and nest diameter was examined.

Question 5. What is the effect of restrictions in the amount of space available at the nest site on the amount of envelope constructed?

The effect of nest site restrictions on the amount of envelope constructed was determined by comparing the ratio of comb to envelope by dry mass at the three categories of nest; unrestricted, semi-restricted and restricted.

Statistical analysis

The normality of all data was checked with a frequency histogram prior to analysis. Ratios were normalised by arcsine transformation (Sokal and Rohlf 1995). Envelope thickness and corrected nest diameter in Question 4 were normally distributed and were untransformed. All other data was not normally distributed and the square root transformation was found to be most effective in normalising the data. For all Analysis of Variance (ANOVA) the homogeneity of variances was tested with the F_{max} test (Fowler and Cohen 1992).

Differences in the thickness and number of layers (Question 1) and mean gap between layers of envelope (Question 2) between developmental stages and between regions of the nest were examined by two-way ANOVA. A General Linear Model (GLM) was fitted which allowed unequal data to be analysed with unequal numbers of nests in each developmental category. Differences between means were located with the Tukey-Krammer test for unequal sample sizes (Sokal and Rohlf 1995).

Differences between developmental stages in the allocation of material to comb and envelope were examined with a one-way ANOVA using a GLM (Question 3). Differences between means were located using a Tukey-Krammer test. The Tukey-Krammer test allows means to be compared where there are unequal sample sizes.

Differences between categories of nest sites in the ratio of comb to envelope were examined by one-way ANOVA (Question 5). Differences between means were located with a standard Tukey test (Fowler and Cohen 1992).

Note that methods for multiple unplanned comparisons among means for both equal and unequal sample sizes are conservative with respect to Type Two Errors. There is therefore an increased chance of wrongly accepting the null hypothesis. The statistical relationship of comb to envelope by dry mass (square root transformed) was determined by product moment correlation (Question 3). Model II lines were fitted to illustrate the structural relationship and fitted with a 95% confidence zone (Sokal and Rohlf 1995).

The ratio of comb to envelope by dry mass in *D. sylvestris* (developmental categories SCN-CDAB/C) was compared to that in *D. norwegica* (categories CDL/S and CDAB/C) with a Z-test (Question 3.).

The statistical relationship between corrected nest diameter and envelope thickness (Question 4) was examined by product moment correlation and fitted with a model II regression line with 95% confidence zone (Sokal and Rohlf 1995). A model II regression line was fitted, as there was no *a priori* reason to assume causality between nest diameter and envelope thickness.

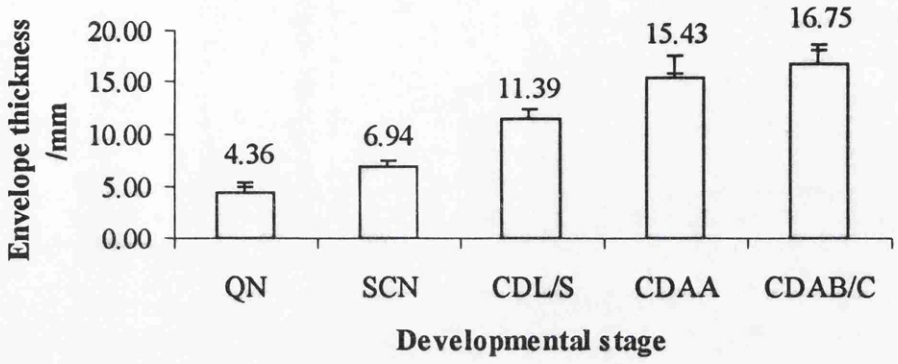
2.3. Results

Question 1. Are there differences between developmental stages and between regions of the nest in the relative amount of envelope constructed?

Dolichovespula sylvestris

The two-way ANOVA (GLM) indicated that there was a significant difference in the thickness of the envelope between developmental stages in *D. sylvestris* ($F=37.41$ at df 4, 305: $P<0.01$). There was, however, no significant difference in envelope thickness between regions of the nest ($F=1.36$ at df 4,305). No significant interaction was found between developmental stage and nest region ($F=0.69$ at df 16, 305). Results are presented in Figure 2.2.

Figure 2.2. The mean envelope thickness in nests of *D. sylvestris* at various developmental stages with 95% confidence intervals (calculated from the square-root transformed data and back transformed).



A Tukey-Krammer test was used to locate differences between developmental stages and between regions of the nest. The Tukey test indicated that there was no significant difference between stages CDAA and CDAB/C in the thickness of envelope. There were however significant differences between all other pairs. Envelope thickness increases from one developmental stage to the next, reaching a peak at CDAA after which there is no significant increase (Table 2.4.).

Table 2.4. Tukey-Krammer pairwise comparison of envelope thickness between developmental stages in nests of *D. sylvestris* (square-root transformed)

Comparison	Difference between means	$q_{0.05}$, a=5, v=305	n	T	P<0.05
QN vs SCN	0.737	3.86	37.5	0.613	signif.
QN vs CDL/S	1.428	3.86	65.0	0.503	signif.
QN vs CDAA	1.898	3.86	77.5	0.486	signif.
QN vs CDAB/C	2.015	3.86	45.0	0.562	signif.
SCN vs CDLS	0.691	3.86	62.5	0.527	signif.
SCN vs CDAA	1.160	3.86	75.0	0.511	signif.
SCN vs CDAB/C	1.278	3.86	42.5	0.583	signif.
CDLS vs CDAA	0.470	3.86	102.5	0.373	signif.
CDLS vs CDAB/C	0.587	3.86	70.0	0.467	signif.
CDAA vs CDAB/C	0.117	3.86	16.5	0.449	n.s.

The two-way ANOVA indicated that there was a significant difference between developmental stages (F=44.85 at df 4, 305: P<0.01) and between regions of the nest (F=4.18 at df 4, 305: P<0.01) in the number of layers of envelope. There was no significant difference between developmental stages and nest regions (F=0.83, df 4, 305). These results are presented in Figures 2.3 and 2.4 respectively.

Figure 2.3. The mean number of layers of envelope in various regions of nests of *D. sylvestris*, with 95% confidence intervals (calculated from the square-root transformed data and back transformed).

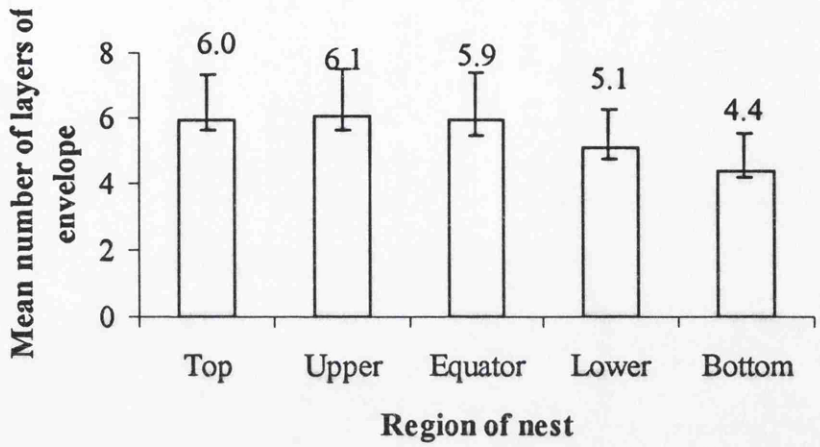
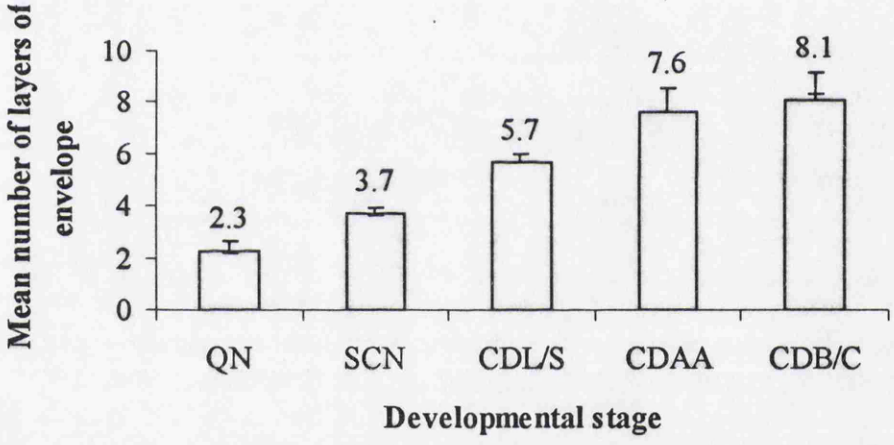


Figure 2.4. The mean number of layers of envelope in nests of *D. sylvestris* at various developmental stages, with 95% confidence intervals (calculated from the square-root transformed data and back transformed).



The Tukey test was used to determine whether there were differences in the number of layers of envelope between stages and between nest regions. The number of layers of envelope increased significantly between stages up to stage CDAA. The number of envelope constructed did not differ significantly between the stages CDAA and CDAB/C in of layers (Table 2.5.). The bottom of the nest was found to have significantly fewer layers of envelope than the top, upper and equator of the nest. There was however no significant difference in the number of layers between any other pair of nest regions (Table 2.6.).

Table 2.5. Tukey-Krammer pairwise comparison of the number of layers of envelope (square-root transformed) between developmental stages in nests of *D. sylvestris*

Comparison	Difference between means	$q_{0.05}$, a=5, v=305	n	T	P<0.05
QN vs SCN	0.481	3.86	37.5	0.361	signif.
QN vs CDL/S	0.907	3.86	65.0	0.296	signif.
QN vs CDAA	1.233	3.86	77.5	0.287	signif.
QN vs CDAB/C	1.291	3.86	45.0	0.331	signif.
SCN vs CDLS	0.426	3.86	62.5	0.311	signif.
SCN vs CDAA	0.752	3.86	75.0	0.301	signif.
SCN vs CDAB/C	0.810	3.86	42.5	0.344	signif.
CDLS vs CDAA	0.326	3.86	102.5	0.220	signif.
CDLS vs CDAB/C	0.384	3.86	70.0	0.275	signif.
CDAA vs CDAB/C	0.058	3.86	16.5	0.265	n.s.

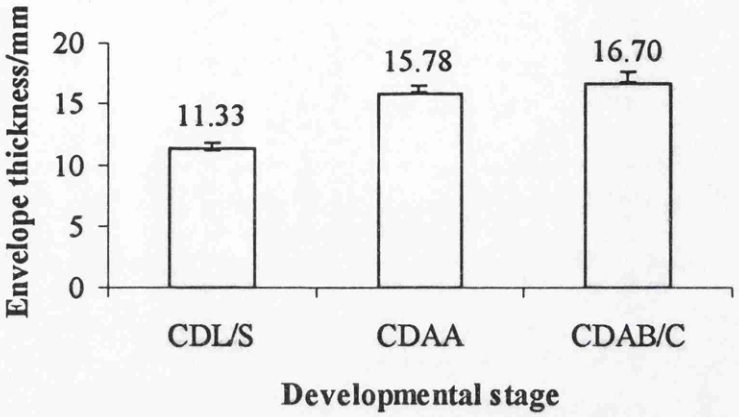
Table 2.6. Tukey-Krammer pairwise comparison of the number of layers of envelope (square-root transformed) between nest regions in *D. sylvestris*

Comparison	Difference between means	$q_{0.05}$, a=5, v=305	n	T	P<0.05
Top vs Upper	0.020	3.86	66.0	0.256	n.s.
Top vs Equator	0.000	3.86	66.0	0.256	n.s.
Top vs Lower	0.150	3.86	66.0	0.256	n.s.
Top vs Bottom	0.356	3.86	66.0	0.256	signif.
Upper vs Equator	0.021	3.86	66.0	0.256	n.s.
Upper vs Lower	0.170	3.86	66.0	0.256	n.s.
Upper vs Bottom	0.376	3.86	66.0	0.256	signif.
Equator vs Lower	0.149	3.86	66.0	0.256	n.s.
Equator vs Bottom	0.355	3.86	66.0	0.256	signif.
Lower vs Bottom	0.205	3.86	66.0	0.256	n.s.

Dolichovespula norwegica

In *D. norwegica* the two-way ANOVA indicated a significant difference between developmental stages in the thickness of envelope (F=17.32 at df 2, 205: P<0.01). Results are presented in Figure 2.5. There was however no significant difference between nest regions (F=1.92 at df 4, 205). There was no significant interaction between developmental stage and region (F=0.05 at df 8, 205).

Figure 2.5. The mean envelope thickness in nests of *D. norwegica* at various developmental stages with 95% confidence intervals (calculated from the square-root transformed data and back transformed).



A tukey test was then performed to locate differences between developmental stages in the mean thickness of nest envelope. No significant difference was found between stages CDAA and CDAB/C in mean envelope thickness. Stage CDL/S was however found to have a significantly thinner envelope than stages CDAA or CDAB/C (Table 2.7.).

Table 2.7. Tukey-Krammer pairwise comparison of envelope thickness between developmental stages in nests of *D. norwegica* (square-root transformed)

Comparison	Difference between means	$q_{0.05}$, a=3, v=205	n	T	P<0.05
CDLS vs CDAA	0.576	3.31	57.5	0.295	signif.
CDLS vs CDAB/C	0.681	3.31	75.0	0.275	signif.
CDAA vs CDAB/C	0.105	3.31	87.5	0.238	n.s.

The two-way ANOVA indicated that there was a significant difference between stages in the number of layers of envelope in *D. norwegica* (F=17.92 at df 2, 205: P<0.01). There was also a significant difference between regions of the nest in the number of layers of envelope (F=17.92 at df 4, 205: P<0.01). There was no significant interaction between developmental stages and nest region (F=1.79 at df 8, 205). The results are presented in Figures 2.6. and 2.7.

Figure 2.6. The mean number of layers of envelope at various developmental stages in nests of *D. norwegica*, irrespective of nest region. Fitted with 95% confidence intervals (calculated from the square-root transformed data and back transformed).

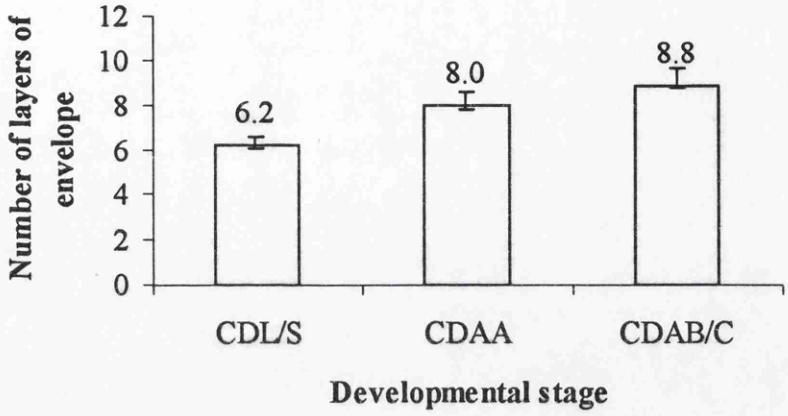
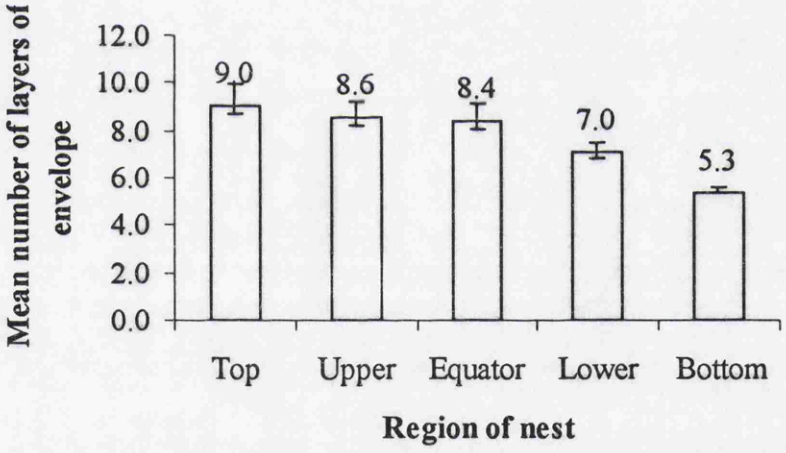


Figure 2.7. The mean number of layers of envelope in various regions of nests of *D. norwegica*, with 95% confidence intervals, irrespective of developmental stage (calculated from the square-root transformed data and back transformed).



The Tukey test indicated that nests of stages CDAA and CDB/C had significantly more layers of envelope than stage CDL/S. There was however, no significant difference between the number of layers of envelope between stages CDAA and CDAB/C (Table 2.8.). The tukey test also indicated that there were significantly fewer layers of envelope in the lower region of the nest than in the upper, equator of top of the nest. The bottom region of the nest had significantly fewer layers of envelope than any other region of the nest. The lower region had significantly fewer layers of envelope than the top and upper regions. There was no significant difference between any other pair of regions (Table 2.9.).

Table 2.8. Tukey-Krammer pairwise comparison of envelope layers between developmental stages in nests of *D. norwegica* (square-root transformed)

Comparison	Difference between means	$q_{0.05}$, a=3, v=205	n	T	P<0.05
CDLS vs CDAA	0.315	3.31	57.5	0.215	signif.
CDLS vs CDAB/C	0.449	3.31	74.5	0.197	signif.
CDAA vs CDAB/C	0.134	3.31	87.5	0.171	n.s.

Table 2.9. Tukey-Krammer pairwise comparison of envelope layers between nest regions in *D. norwegica* (square-root transformed)

Comparison	Difference between means	$q_{0.05}$, a=5, v=205	n	T	P<0.05
Top vs Upper	0.052	3.86	44	0.245	n.s.
Top vs Equator	0.079	3.86	44	0.245	n.s.
Top vs Lower	0.317	3.86	44	0.245	signif.
Top vs Bottom	0.680	3.86	44	0.245	signif.
Upper vs Equator	0.027	3.86	44	0.245	n.s
Upper vs Lower	0.264	3.86	44	0.245	signif.
Upper vs Bottom	0.628	3.86	44	0.245	signif.
Equator vs Lower	0.238	3.86	44	0.245	n.s
Equator vs Bottom	0.601	3.86	44	0.245	signif.
Lower vs Bottom	0.364	3.86	44	0.245	signif.

Question 2. Are there differences between developmental stages and between regions of the nest in the mean gap between layers of envelope?

Dolichovespula sylvestris

The two-way ANOVA indicated that there was no significant difference between developmental stages in the mean gap between envelope layers in *D. sylvestris* (F=0.82 at df 4, 295). There was however, a significant difference between nest regions (F=30.03 at df 4, 295: P<0.01). There was a significant interaction between developmental stages and nest regions (F=1.44 at df 16: 295). The results obtained are summarised in Figure 2.8.

The Tukey test indicated that there was no significant difference in the gap between envelope layers in the equator and lower region. There was a significant difference between all other pairs of regions, with the gap between layers increasing from the top to the bottom of the nest. (Table 2.10.).

Figure 2.8. The mean gap between layers of envelope in different regions of the nest in *D. sylvestris*, irrespective of developmental stage. Fitted with 95% confidence intervals calculated from the square root transformed data and back transformed.

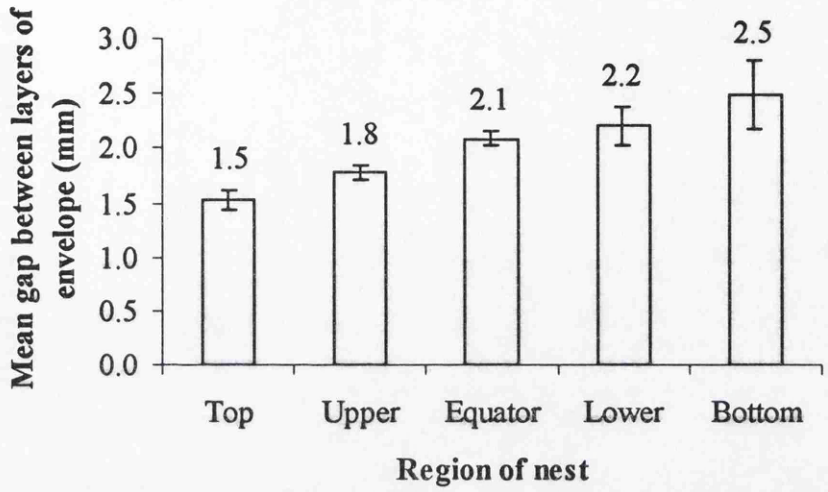


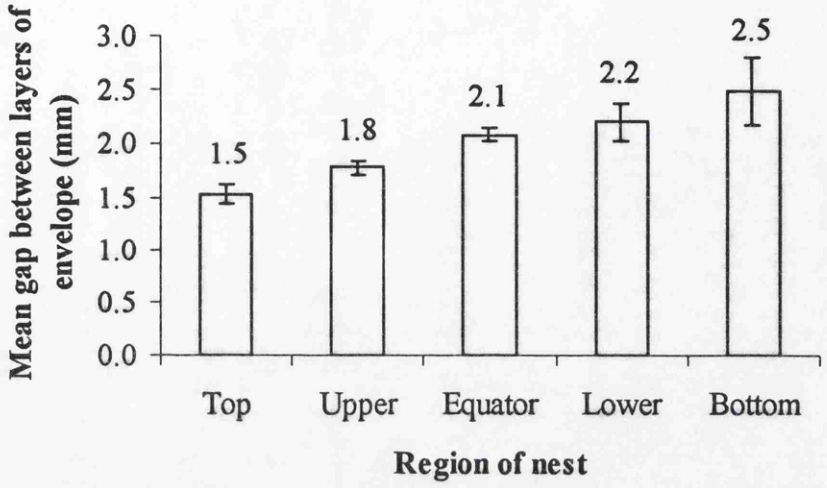
Table 2.10. The results of a Tukey multiple comparison test to locate differences between nest regions in the mean gap between layers of envelope in *D. sylvestris*.

Comparison	Difference between means	$q_{0.05}$, a=5, ν=295	n	T	P<0.05
Top vs Upper	0.248	3.86	65.0	0.234	signif.
Top vs Equator	0.556	3.86	65.0	0.234	signif.
Top vs Lower	0.673	3.86	65.0	0.234	signif.
Top vs Bottom	0.956	3.86	64.0	0.236	signif.
Upper vs Equator	0.308	3.86	64.0	0.236	signif.
Upper vs Lower	0.427	3.86	64.0	0.236	siginf.
Upper vs Bottom	0.707	3.86	63.0	0.238	signif.
Equator vs Lower	0.117	3.86	64.0	0.236	n.s.
Equator vs Bottom	0.399	3.86	63.0	0.238	signif.
Lower vs Bottom	0.283	3.86	63.0	0.238	signif.

Dolichovespula norwegica

The two-way ANOVA indicated that there was a significant difference between developmental stages in the mean gap between envelope layers in *D. norwegica* (F=3.06, at df 2, 204: P<0.05). There was also a significant difference between nest regions (F=40.89, at df 4, 204: P<0.01). There was no significant interaction between nest region and developmental stage (F=0.08 at df 8, 204). These results obtained are summarised in Figure 2.9.

Figure 2.9. The mean gap between layers of envelope in different regions of the nest in *D. norwegica*, irrespective of developmental stage. Fitted with 95% confidence intervals calculated from the square-root transformed data and back transformed.



The Tukey-Krammer test however indicated that there was no significant difference between developmental stages in the gap between envelope layers. The Tukey-Krammer test indicated that there was no significant difference between the lower region and equator or between the upper region and top of the nest. There were however, significant differences in the mean gap between envelope layers in all other pairs of regions (Table 2.11)

Table 2.11. The results of a Tukey multiple comparison test to locate differences between nest regions in the mean gap between layers of envelope in *D. norwegica*.

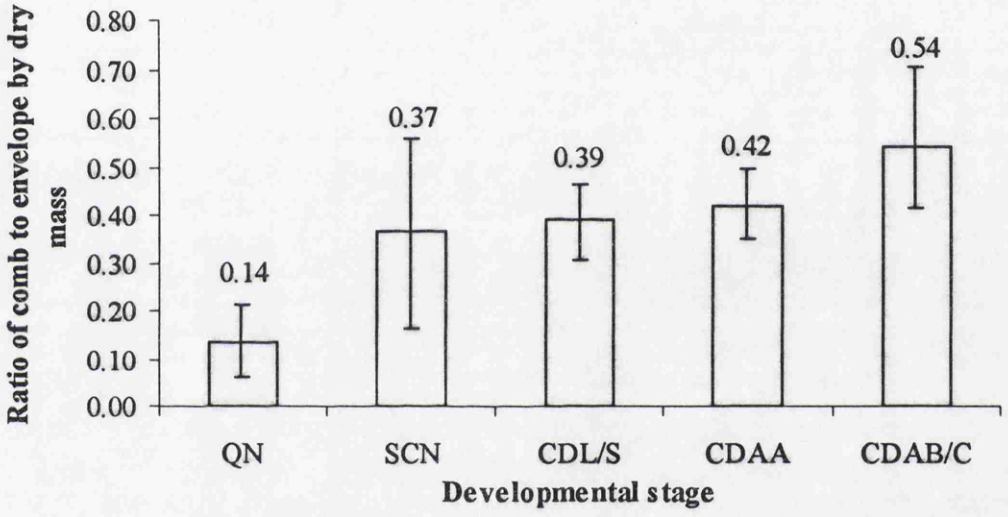
Comparison	Difference between means	$q_{0.05}$, a=5, v=204	n	T	P<0.05
Top vs Upper	0.023	3.86	43.5	0.070	n.s.
Top vs Equator	0.123	3.86	44.0	0.070	signif.
Top vs Lower	0.167	3.86	44.0	0.070	signif.
Top vs Bottom	0.309	3.86	44.0	0.070	signif.
Upper vs Equator	0.101	3.86	43.5	0.070	signif.
Upper vs Lower	0.144	3.86	43.5	0.070	signif.
Upper vs Bottom	0.286	3.86	43.5	0.070	signif.
Equator vs Lower	0.044	3.86	44.0	0.070	n.s.
Equator vs Bottom	0.186	3.86	44.0	0.070	signif.
Lower vs Bottom	0.142	3.86	44.0	0.070	signif.

Question 3. Do workers have the same behavioural rule through colony development for the allocation of material to comb and envelope?

Dolichovespula sylvestris

In *D. sylvestris* the one way ANOVA indicated that there was a significant difference between developmental stages in the ratio of comb to envelope material (dry mass arcsine transformed), ($F=7.73$ at df 4,61: $P<0.01$). Results are presented in Figure 2.10.

Figure 2.10. The effect of developmental stage on the ratio of comb to envelope by dry mass, in nests of *D. sylvestris*. Fitted with 95% confidence intervals (calculated from arcsine-transformed data and back transformed).



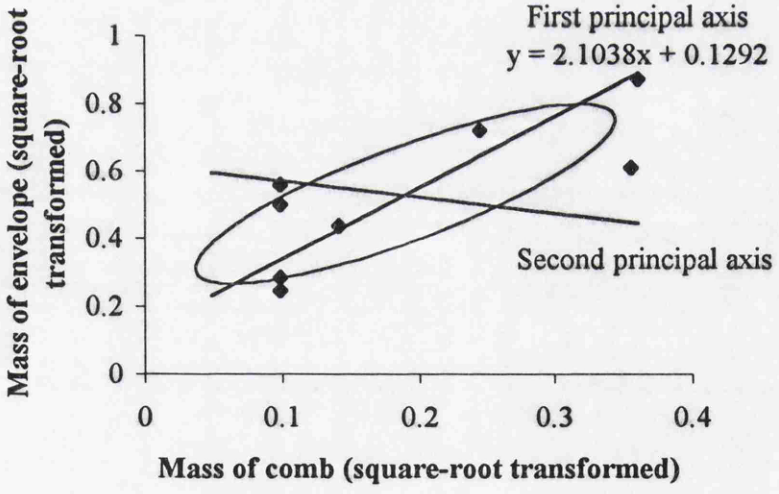
The Tukey test showed that the queen nests (QN) had a lower ratio of comb to envelope (arcsine transformed), than all other developmental stages (Table 2.12). There was no significant difference between any other pair of ratio means tested. In queen nests therefore, proportionally more material was allocated to envelope than comb, than at any other developmental stage.

From the results of the ANOVA data was divided on the basis of developmental stage. The dry mass of comb (square-root transformed) was plotted against the dry mass of envelope (square-root transformed) for the queen nests and for all the other developmental stages combined. Dry mass of comb was strongly positively correlated with dry mass of envelope in the queen nests ($r=0.784$: df 6: $P<0.05$) (Figure 2.11) and in the other developmental stages ($r=0.874$: df 56: $P<0.01$) (Figure 2.12).

Table 2.12. Tukey-Krammer pairwise comparison of the mean ratio of comb to envelope in nests of *D. sylvestris* at various developmental stages (arcsine transformed).

Comparison	Difference between means	$q_{0.05}$, a=5, v=61	n	T	P<0.05
QN vs SCN	16.18	3.91	7.5	14.99	signif.
QN vs CDL/S	18.33	3.91	13.0	12.31	signif.
QN vs CDAA	19.75	3.91	15.5	11.89	signif.
QN vs CDAB/C	25.83	3.91	9.0	13.74	signif.
SCN vs CDLS	2.15	3.91	12.5	12.91	n.s.
SCN vs CDAA	3.57	3.91	15.0	12.51	n.s.
SCN vs CDAB/C	9.65	3.91	8.5	14.28	n.s.
CDLS vs CDAA	1.48	3.91	8.5	9.11	n.s.
CDLS vs CDAB/C	7.49	3.91	15.0	11.42	n.s
CDAA vs CDAB/C	6.08	3.91	16.5	10.97	n.s.

Figure 2.11. The relationship between dry comb mass (square-root transformed and dry envelope mass (square-root transformed) in queen nests of *D. sylvestris*. Fitted with 95% confidence zone. The slope of the first principal axis indicates the structural relationship between variables.



Dolichovespula norwegica

In *D. norwegica* the one way ANOVA found a significant difference between developmental stages in the ratio of comb to envelope by dry mass (square-root transformed) (F=3.81 at df 2, 41: P<0.05). Results are presented in Figure 2.13.

Figure 2.12. The relationship between dry comb mass (square-root transformed and dry envelope mass (square-root transformed) in nests of *D. sylvestris* excluding queen nests. Fitted with 95% confidence zone. The slope of the first principal axis indicates the structural relationship between variables

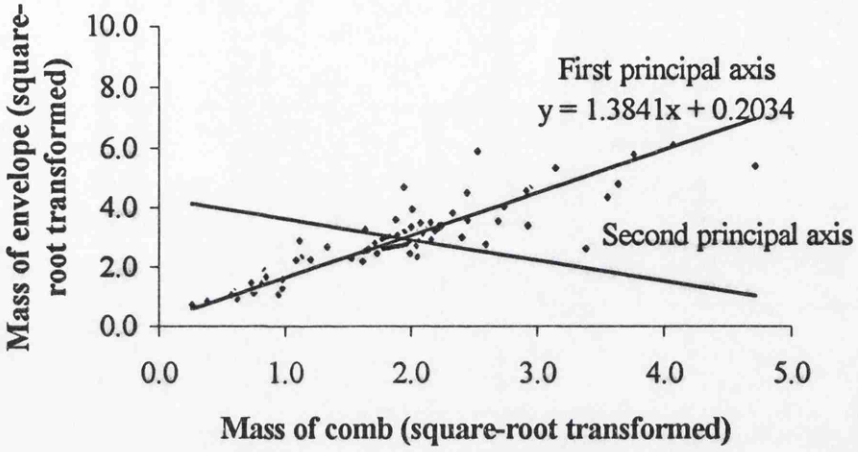
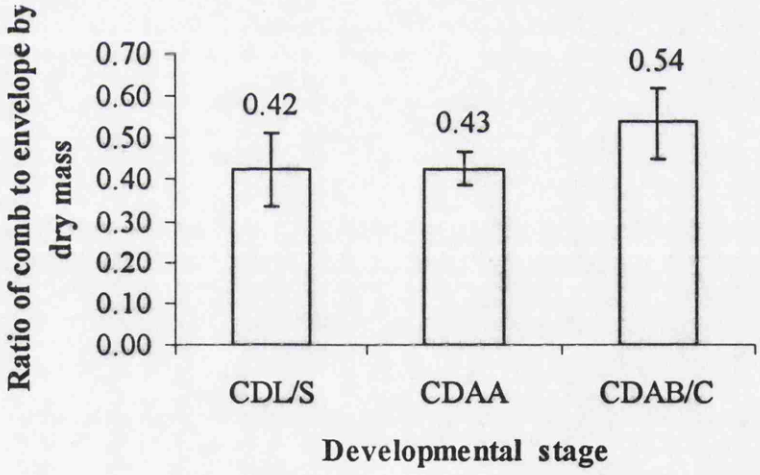


Figure 2.13. The effect of developmental stage on the ratio of comb to envelope by dry mass, in nests of *D. norwegica*. Fitted with 95% confidence intervals (calculated from arcsine-transformed data and back transformed).



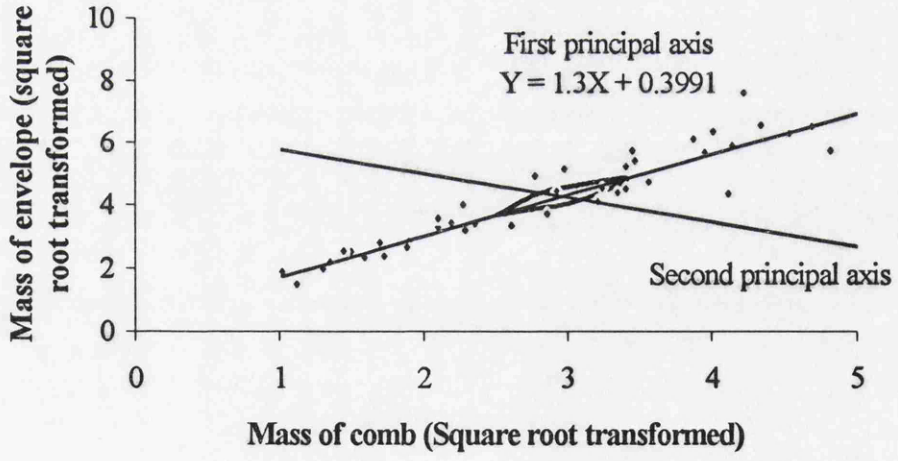
The Tukey-Krammer multiple comparison test however, failed to locate any significant difference between developmental stages.

The developmental stages were also pooled in *D. norwegica* and the dry mass of comb (square-root transformed) was plotted against the dry mass of envelope (square-root transformed) (Figure 2.14.). Dry mass of comb was strongly positively correlated with dry mass of envelope ($r=0.893$ at df 42: $P<0.01$).

It can be seen from the above charts that *D. sylvestris* and *D. norwegica* allocate a similar proportion of material to comb and envelope. The ratios of comb to envelope by

dry mass could not be compared within the analysis of variance unequal numbers of developmental stages represented in *D. sylvestris* and *D. norwegica* were unequal. However, as there was no significant difference in the ratio of comb to envelope between stages SCN, CDL/S, CDAA and CDAB/C in *D. sylvestris* these results could be pooled. Similarly as there was no significant difference in the ratio of comb to envelope between the developmental stages represented in *D. norwegica* and the results could be pooled. It was therefore possible to compare the ratio of comb to envelope in *D. sylvestris* with that in *D. norwegica* using a Z-test. No significant difference was found between the mean ratio of comb to envelope by dry mass in *D. sylvestris* and that of *D. norwegica*. There was no need to include queen nests of *D. sylvestris* in the comparison as this had been tested in the ANOVA. It can be concluded therefore that a higher proportion of material was allocated to the construction of envelope in queen nests of *D. sylvestris* than in other developmental periods or in the developmental stages tested in *D. norwegica*.

Figure 2.14. The relationship between dry comb mass (square-root transformed) and dry envelope mass (square-root transformed) in nests of *D. norwegica*. Fitted with 95% confidence zone. The slope of the first principal axis indicates the structural relationship between variables



Question 4. Do small nests have proportionally thicker envelopes than large nests?

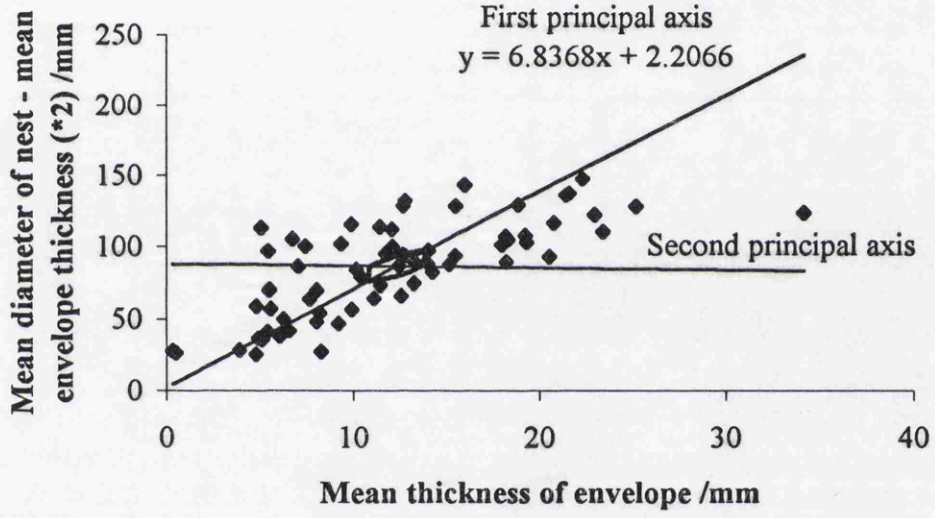
This question was addressed by examining the relationship between envelope thickness and nest diameter. Nest diameter was calculated from a mean of the three measurements (nest height, diameter and pole-pole), while envelope thickness was the mean the five nest regions measured (top, upper, equator, lower and bottom). As the relationship between diameter and envelope thickness was examined it was necessary to

remove the envelope thickness from the mean diameter. The corrected diameter was therefore calculated by subtracting twice the mean envelope thickness.

Dolichovespula sylvestris

There was a strong positive correlation between envelope thickness and the corrected mean nest diameter ($r=0.729$ for df 64: $P<0.01$). The structural relationship between envelope thickness and corrected mean nest diameter is shown in Figure 2.15. It can be seen that there is a linear relationship between envelope thickness and nest diameter.

Figure 2.15. The structural relationship between mean nest diameter (corrected for envelope thickness) and envelope thickness in *D. sylvestris*. Fitted with a 95% confidence zone.



Dolichovespula norwegica

There was also a strong positive correlation between envelope thickness and corrected mean nest diameter in *D. norwegica* ($r=0.626$ for df 42: $P<0.01$). The structural relationship between corrected nest diameter and envelope thickness is illustrated in Figure 2.16.

Question 5. What is the effect of restrictions in the amount of space available at the nest site on the amount of envelope constructed?

Dolichovespula sylvestris

The effect of nest site restrictions on the amount of envelope constructed was examined by comparing the ratio of comb to envelope in nests constructed at unrestricted, semi-

restricted and restricted sites. As the proportion of material allocated to comb and envelope material has already been shown to be significantly different in queen nests of *D. sylvestris* than other developmental stages, this category was excluded from the analysis. The one-way ANOVA indicated that there was no significant difference in the ratio of comb to envelope (arcsine transformed) between unrestricted, semi-restricted and restricted nest sites. The data are presented in figure 2.17.

Figure 2.16. The structural relationship between mean nest diameter (corrected for envelope thickness) and envelope thickness in *D. norwegica*. Fitted with a 95% confidence zone.

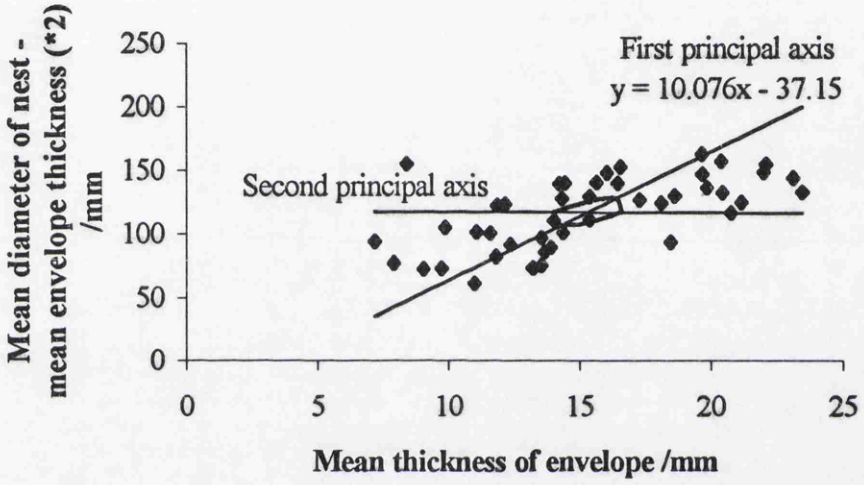
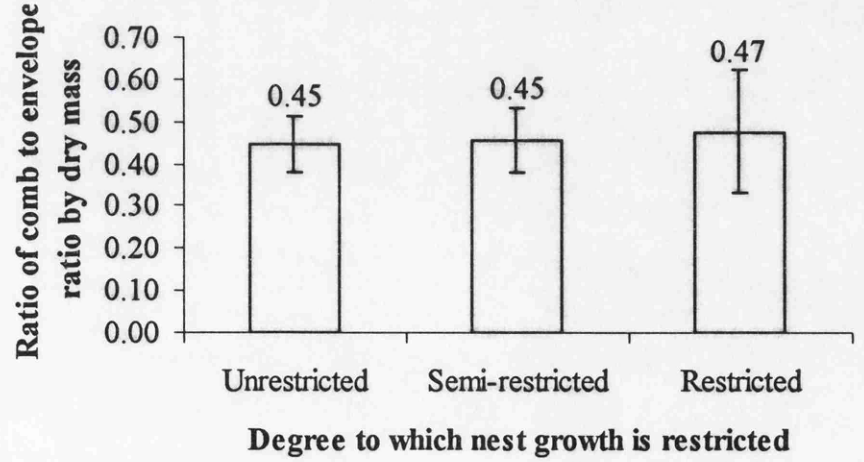


Figure 2.17. The ratio of comb to envelope in nests of *D. sylvestris* constructed at unrestricted, semi-restricted and restricted locations, with 95% confidence intervals (calculated from the arcsine transformed data and back-transformed).

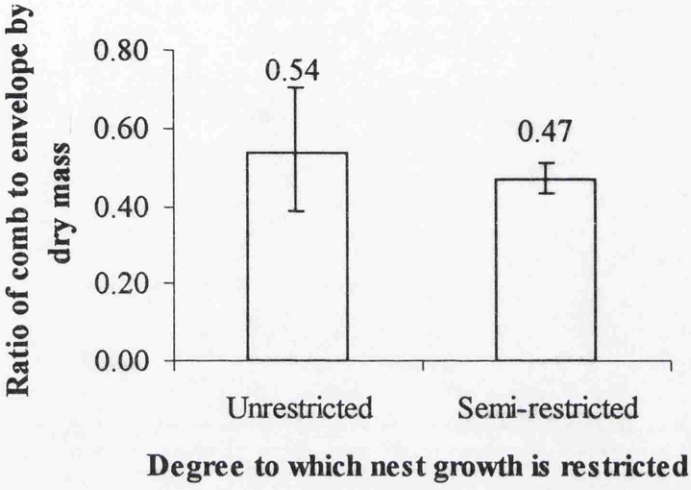


Dolichovespula norwegica

In *D. norwegica* only one nest was constructed at a site categorised as restricted. This category of nest site was therefore excluded from the analysis. The one-way ANOVA

indicated that there was no significant difference between unrestricted and semi restricted nest sites in the ratio of comb to envelope (arcsine transformed). The untransformed data is presented in Figure 2.18.

Figure 2.18. The ratio of comb to envelope in nests of *D. norwegica* constructed at unrestricted and semi-restricted locations, with 95% confidence intervals (calculated from the arcsine transformed data and back-transformed).



2.4. Discussion

Question 1. Are there differences between developmental stages and between regions of the nest in the relative amount of envelope constructed?

A significant difference was found between developmental stages in both the thickness and number of layers of envelope constructed in both *D. sylvestris* and *D. norwegica*. Both the thickness and number of layers increased significantly from one developmental stage to the next reaching a peak at the CDAA stage.

The amount of envelope constructed may peak at this time for one of two reasons. Firstly, the reproductives begin to emerge during this developmental stage, and secondly a large number of brood will be present as pupae. Ishay (1973) has shown that in *Vespa crabro* the success of pupation is particularly dependent on thermoregulation, it is therefore important that the nest is heated during this period. During this developmental period the reproductives are just starting to emerge and a large number of pupae will be present in the cells. The quality of the emerging reproductives will have a direct effect on the reproductive fitness of the colony. The thickness and number of layers of envelope constructed may therefore reach its peak at this stage as a large

number of male and queen pupae are present in the nest and the colony would have most to benefit from thermoregulation.

As heat tends to rise in the nest it will be lost through the top and the upper regions of the envelope. It was therefore predicted that envelope would be significantly different between regions with the top of the nest having a thicker envelope than the bottom. There was however very little variation between regions of the nest in the thickness and number of layers of envelope constructed in both *D. sylvestris* and *D. norwegica.* The only significant difference between regions was the bottom of nests of *D sylvestris*, and the bottom and lower regions in *D. norwegica* had fewer envelope layers. The envelope therefore would appear to be relatively uniformly distributed around the nest. The differences observed between regions of the nest are concentrated at the bottom of the nest. During periods of rapid comb expansion, envelope may be removed at a faster rate from these regions. The lower quantity of envelope in the lower regions of the nest may be due to the more rapid removal of envelope from these regions as new combs are constructed. Differences between regions may however be in part masked by the inclusion in the data set of nests constructed at restricted nest sites (see question 5).

Question 2. Are there differences between developmental stages and between regions of the nest in the mean gap between layers of envelope?

In both *D. sylvestris* and *D. norwegica* there was no difference between developmental stages in the gap between envelope layers. It appears that the gap between envelope layers is relatively constant and is closely regulated. Workers do not therefore appear to compensate for a lack of envelope at earlier developmental stages by increasing the gap between envelope layers. As discussed in the introduction increasing the gap between layers increases the insulation provided by the additional layer but more materials are required to construct it. The specific gap between layers of envelope might provide therefore give optimum increase in nest insulation for the amount of material required in its construction.

The gap did however vary between regions of the nest, and in both species increased from the top to the bottom of the nest. There was a difference in the gap between envelope layers in different regions of the nest. The difference in gap between nest regions may however be simply due to the effect of gravity on the envelope. In *Dolichovespula* nests, although layers of envelope join in places, over much of their

area there is little connection between them. At the sides and bottom of the nest therefore the envelope will hang freely. In the upper regions of the nest however, gravity will tend to move the layers of envelope closer together.

Question 3. Do workers have the same rule through colony development for the allocation of material to comb and envelope?

No significant difference was found in the ratio of comb to envelope between developmental stages in *D. norwegica*. However in *D. sylvestris*, the queen nest stage was found to have a significantly different ratio of comb to envelope to that in all other stages. In queen nests of *D. sylvestris* a significantly higher proportion of material was allocated to the construction of comb, than to envelope. There seems therefore to be a difference in the regulation of envelope construction behaviour between nests constructed by queens and the nests constructed by workers. Workers appear to have a simple rule for the allocation of material to comb and envelope with a relatively constant proportion of material allocated to the two major nest components.

The allocation of material to comb and envelope shows great similarity between species as no significant difference was found in the proportion of material allocated to comb and envelope between post QN nests of *D. sylvestris* and *D. norwegica*. This may be a convergent trait due to the similarity in the nesting habits and lifecycles of these species. Alternatively this may provide additional evidence of the degree of phylogenetic association between the two species.

Yamane *et al.* (1981) also found evidence of a fixed allocation rule for material to comb and envelope. They found a linear relationship between the number of cells and the number of envelope sheets constructed in embryo nests of *Vespula*.

Question 4. Do small nests have proportionally thicker envelopes than large nests?

The relative amount of envelope constructed did not decrease with nest size as predicted from a consideration of surface area to volume ratio of the nest, and of colony biomass. In both *D. sylvestris* and *D. norwegica* envelope thickness increased linearly with nest diameter. Small nests did not therefore have proportionally thicker envelope than large nests as claimed by Potter (1964) and Spradbery (1973).

The linear relationship between nest diameter and envelope thickness would suggest that envelope thickness is regulated by a factor directly resulting from nest diameter. The cue for envelope construction may therefore result from the measurement by workers of its thickness in relation to nest diameter. Alternatively, this relationship may be a result of the allocation of a constant proportion of material to the construction of comb and envelope (see Question 3).

The allocation of a relatively constant proportion of material could result from workers performing different tasks at different ages. There is evidence that the type of forage collect by workers changes with worker age; *V. vulgaris* workers first collecting fluid, pulp and then flesh (Potter 1964). More specifically, Akre *et al.* (1976) observed that envelope construction in workers of *V. pensylvanica* began earlier in their life than comb construction. Workers may therefore spend a relatively constant proportion of their time constructing comb and envelope.

Question 5. What is the effect of nest site restrictions in the space available at the nest site on the amount of envelope constructed?

The degree to which expansion of the nest at the nest site was restricted appeared to have little effect on the ratio of comb to envelope, although in restricted nests the envelope was clearly thinner or absent from the parts of the nest which were in contact with restrictions. Therefore it appears that the total amount of envelope constructed is not effected by restrictions. This would suggest that workers are stimulated to construct envelope before identifying a specific area of envelope to extend. Workers may simply add envelope material to other regions of the nest if access to one area is restricted.

Archer (1981) investigated the effect of nest site restrictions on the size of *D. sylvestris* nests constructed in bird boxes and found that although there appeared to be some effect on nest size it was not statistically significant.

General discussion

The thickness and number of layers of envelope appears to gradually increase through colony development reaching a peak during the production of the reproductives. From

a consideration of colony biomass and the surface area to volume ratio, it would be predicted that the ability of the colony to thermoregulate the nest would be greatest at this point.

Thermoregulation has a direct effect on the quality of adults emerging from pupae (Ishay 1973). As the fitness of the colony is directly effected by the quality of the reproductives, the colony may achieve the greatest benefit from heating the nest when the greatest numbers of reproductives are at the pupal stage. The construction of nest insulation would therefore be most profitable at this stage.

The linear relationship between comb and envelope mass indicates that the workers spend a relatively constant proportion of their time constructing comb and envelope material. This may result from workers simply specialising in construction of comb an envelope during different periods of their life.

The results indicate that there is no difference between small and large nests in the relative thickness of envelope constructed. Furthermore they indicate that envelope thickness increases linearly with nest diameter which could result from workers using nest diameter directly as a cue for the construction of envelope. Alternatively it may also result form workers spending a relatively constant proportion of their time constructing comb and envelope material. This would simplify the construction process, as workers would not need to spend a large proportion of their time making decisions about whether to construct comb, comb supports or envelope. As there appears to be a rigid regulation of envelope construction behaviour through colony development, it seems unlikely that external cues such as temperature at the nest site play a significant role in its construction.

Although Potter (1964) found that temperature at the nest site had a direct effect on the proportion of trips made for pulp (Figure 2.1), temperature did not necessarily have an effect on the allocation of material to the construction of comb and envelope. The type of forage collected by workers at any particular time will depend on a number of factors including the availability of the forage and the needs of the colony. There is a peak for example, in the number of trips made for water in the morning and following rain storms (Potter 1964). During these periods water is easily obtained close to the nest through condensation (dew). Potter (1964) also found that when he removed the envelope of a mature nest of *V. vulgaris* and maintained it in a nest box heated to 32°C,

following the reconstruction of the first layer, envelope construction was very slow. This however is an example of nest repair rather than a model of nest construction.

Chapter 3. Development of a temperature regulated nest box and entrance trap for behavioural studies.

3.1. Introduction

A heated nest box and entrance trap are presented in this chapter. Pilot trials have shown that they are effective. The nest box and entrance trap were intended for experimental work on the regulation of nest construction. They were developed in order to study the effect of ambient temperature on foraging for pulp and on the rate of envelope construction in colonies of *D. sylvestris*. Although it was not possible to use the nest box and entrance trap for this purpose within the project, the techniques and equipment should be useful for further investigations. Due to circumstances, however, experimental work could not be continued beyond piloting the nest box and entrance trap. They are presented, however, as they could assist in future research.

Many authors have presented a variety of designs for nest boxes for vespine wasps (Potter 1964; Ishay *et al.* 1967; MacDonald *et al.* 1976; Edwards 1980; Bunn 1982; Martin 1990). Roland (1969) for example, maintained a colony of *V. vulgaris* in a glass case in the laboratory. MacDonald *et al.* (1976) constructed nest boxes consisting of a simple wooden box with a glass bottom for the study of *V. pensylvanica* and *V. atropilosa* (Sladen). Many of these designs are for laboratory colonies or colonies situated in vespiaries. The design presented in this chapter is intended to be free standing and is based on the shape of a tit-box similar to that used by Bunn (1982) for housing colonies of *D. sylvestris*.

Heated nest boxes have been employed by other researchers for housing colonies of vespine wasps. Potter (1964) constructed a heated nest box for *V. vulgaris*. This design incorporated a thermostatically controlled water jacket. The water in the jacket and the air in the nest box were circulated by propellers. This design appears unnecessarily complex, and did not appear very portable or flexible for use in the field. Martin (1990) maintained a colony of *Vespa simillima* Smith in a forest in a temperature-controlled cabinet at 30°C. This was not designed for use in the field and would be prohibitively expensive for the maintenance of several experimental colonies. It was therefore decided to develop a new, simpler design specifically for housing colonies of *Dolichovespula* species

Several designs for entrance traps have been developed for vespine wasps, which allow outgoing and incoming wasps to be separated, in order to sample the type of forage

returned to the nest. Potter (1964) and Archer (1977) presented similar entrance trap designs for workers of *V. vulgaris*. Although Potter's trap was designed for use with a nest box and Archer's design was for subterranean nests, they both consisted of a simple oblong box with a diagonal partition separating outgoing and incoming wasps. Various entrance traps are presented in the literature for sampling foragers of *Vespa* and *Vespula* nests. Edwards (1980) presented a design for an entrance trap consisting of a system of tubes and funnels to separate outgoing and incoming foragers. He did not, however, state which genera of wasps it was intended for use with.

There are differences in the nest site preferences of the genera, *Vespula* is predominantly a subterranean nester and *Dolichovespula* is predominantly an aerial nester. As subterranean nesters construct long tunnels, they are likely to be more adaptable to an entrance trap system than aerial nesters. No trap design could be located in the literature, specifically designed for sampling *Dolichovespula* workers. The design of nest trap in the present study was based on that of Harris (1989) for the sampling of workers from subterranean colonies of *V. vulgaris* and *V. germanica*. This trap combines the funnel and tube type design, but also allows incoming foragers to be sampled to study the type of load being transported (see Figure 3.3).

3.2. Temperature controlled nest box and entrance trap

Temperature regulated nest box

The nest box was constructed from marine plywood of (12mm thickness) which is suitable for field use. A hinged door was fitted to one side of the box for access to the nest (Figure 3.1e). At the other side of the box was a sliding Perspex panel for easy inspection of the nest and access (Figure 3.1d). The box was fitted with aluminium brackets (Figure 3.1a) for fixing to an exterior wall. The entrance to the nest box had an internal diameter of 25mm and was in the form of a boss (Figure 3.1f) in order to attach the entrance trap (Figures 3.3 and 3.4). The lid of the box (not illustrated) was a simple oblong sheet of plastic and was secured with silicone sealant.

The nest box was heated via a 50W 'Ultratherm Power Plate' fixed to the inner side of the rear panel of the box (Figure 3.1b). This was regulated via a Honeywell room thermostat of the type used in domestic heating systems accurate to +/- 1°C (Figure 3.1c). This was attached to the inner side of the rear panel of the box. The heater and thermostat allowed the box to be maintained at a variety of temperatures up to 35°C.

Figure 3.2 shows the temperature maintained in the nest box during a 5 day period following the transfer of a colony of *D. sylvestris*. The nest box temperature was maintained at a mean temperature of 31.8°C (SD 0.42) while the mean temperature outside the box was 15°C (SD 2.96).

Figure 3.2 illustrates that the nest box was able to maintain a constant temperature in a wide range of ambient temperatures.

Figure 3.1. Nest box **a.** aluminium wall brackets, **b.** power plate, **c.** thermostat, **d.** Perspex sliding door, **e.** inspection door, **f.** entrance trap boss.

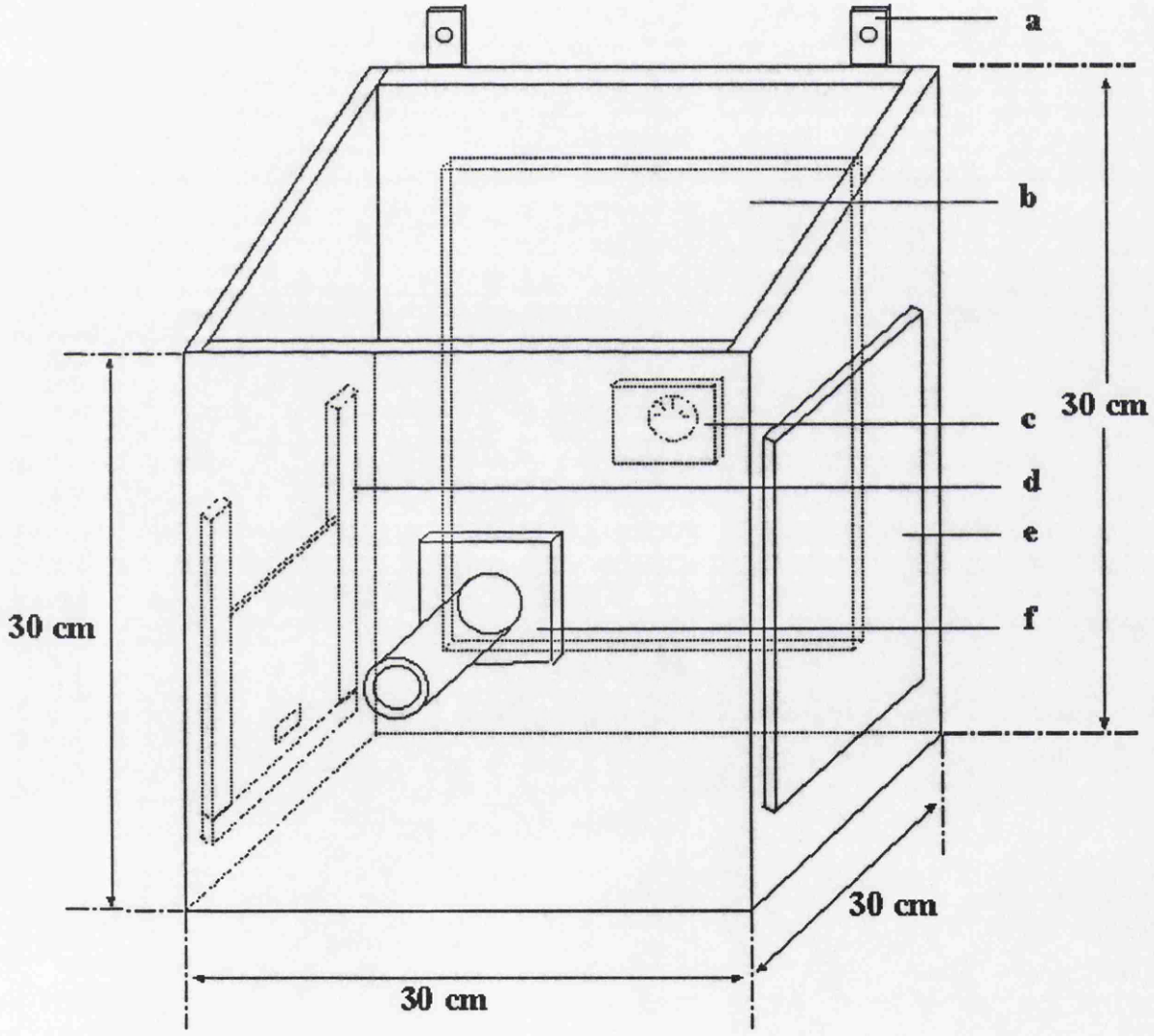
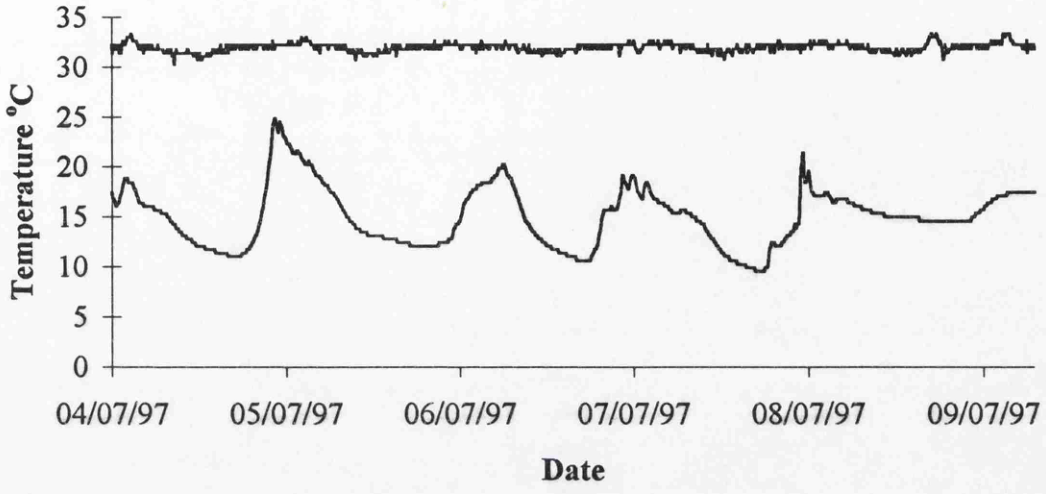


Figure 3.2. Nest box temperature recorded over five days. Upper line shows the temperature inside the nest box, lower line shows the temperature outside the nest box. The abscissa runs from 00:00 hours on 4/7/97 to 00:00 hours on 9/7/97.



The entrance trap

The entrance trap was very similar to the design presented by Harris (1989) for the *V. vulgaris* and *V. germanica* colonies (Figure 3.3). Several problems were encountered with the installation and design of the initial trap. These problems arose from differences in the behaviour of *Dolichovespula* workers and the *Vespula* workers for which Harris (1989) had designed the trap. Several modifications were therefore necessary to the installation and design of the trap illustrated in Figure 3.3. This section will therefore describe the difficulties and subsequent modifications, which led to the modified trap illustrated in Figure 3.4.

The entrance trap was modified to improve its efficiency for colonies of *D. sylvestris* (Figure 3.4). The exterior funnel of the unmodified trap (Figure 3.3c) was constructed from an acrylic laboratory funnel. The exterior funnel of the modified trap, and the interior funnels were constructed from two acrylic laboratory funnels with their openings stuck together (Figure 3.5). The nest box connecting tube (Figure 3.3i) was constructed from ducting of the type used in vacuum cleaners. The constriction tubes (Figure 3.3l) and (Figure 3.3m) and anaesthetising tubing was of 10mm internal diameter clear plastic. All other tubing was of clear plastic with a 25mm internal diameter. Tubing and funnels, with the exception of the sampling tube, anaesthetising tube and the entrance and exit windows, were covered in black gaffer tape to exclude light. The trap was mounted on plywood in order to anchor the valve and tubing. The valve was a 'Whale' diverter valve

designed for use with a boat pump. This valve can be turned to allow workers entering the trap to be diverted into the sampling tube

The intended operation of the entrance trap was as follows. The entrance trap was connected to the boss of the nest box (Figure 3.1f) via the connecting tube (Figure 3.3i). Workers enter the trap via external funnel (Figure 3.3c) and into the more accessible of the two tubes which is the entrance connecting tube (Figure 3.3d). They then move through the diverter valve (Figure 3.3g), the internal funnel connector tube (Figure 3.3h) and the internal funnel (Figure 3.3a) and into the nest box via the nest box connecting tube (Figure 3.3i). Workers exiting the nest enter the trap via the nest box connecting tube and into the internal funnel. Workers then exit the internal funnel via the funnel connector tube (Figure 3.3b) and into the external funnel before leaving through the entrance tube. To sample workers returning to the nest a collection jar is connected to the sampling tube (Figure 3.3e). A hose from a CO^2 canister is then attached to the anaesthetising tube (Figure 3.3f). The direction of the diverter valve is then changed to direct workers into the collection jar via the sampling tube where they were anaesthetised.

3.3. Evaluation of nest box and development of entrance trap.

Nest box installation and transplantation of a colony of *D. sylvestris*

The heated nest box was set up at the council offices in Linlithgow, West Lothian on the roof of a single story out building. Prior to transferral of the nest, the heater was turned on and the thermostat set at 32°C.

A mature nest of *D. sylvestris* was located on 27/6/97 in a bird nesting box in the garden of a private property in West Lothian. The nest was taken from the bird box between 12-30p.m. and 13-30 p.m. Prior to removal of the nest, the workers were captured in perforated plastic jars with screw lids. To capture a worker the lid of the jar was removed and the neck of the bottle placed over the entrance hole of the bird box. When a worker had entered the bottle, a piece of paper was placed between the bottle and the box. The lid could then be placed on the bottle. This was repeated until all returning workers had been captured. A total of 75 workers were captured from the nest. A further two workers were lost when capturing the nest and could not be retrieved.

Figure 3.3. Nest box entrance trap before modifications (of a similar design to that of Harris 1989) **a.** internal funnel, **b.** funnel connecting tube, **c.** external funnel, **d.** entrance connecting tube, **e.** sampling tube, **f.** anaesthetising tube, **g.** diverter valve, **h.** internal funnel connector tube, **i.** nest box connecting tube.

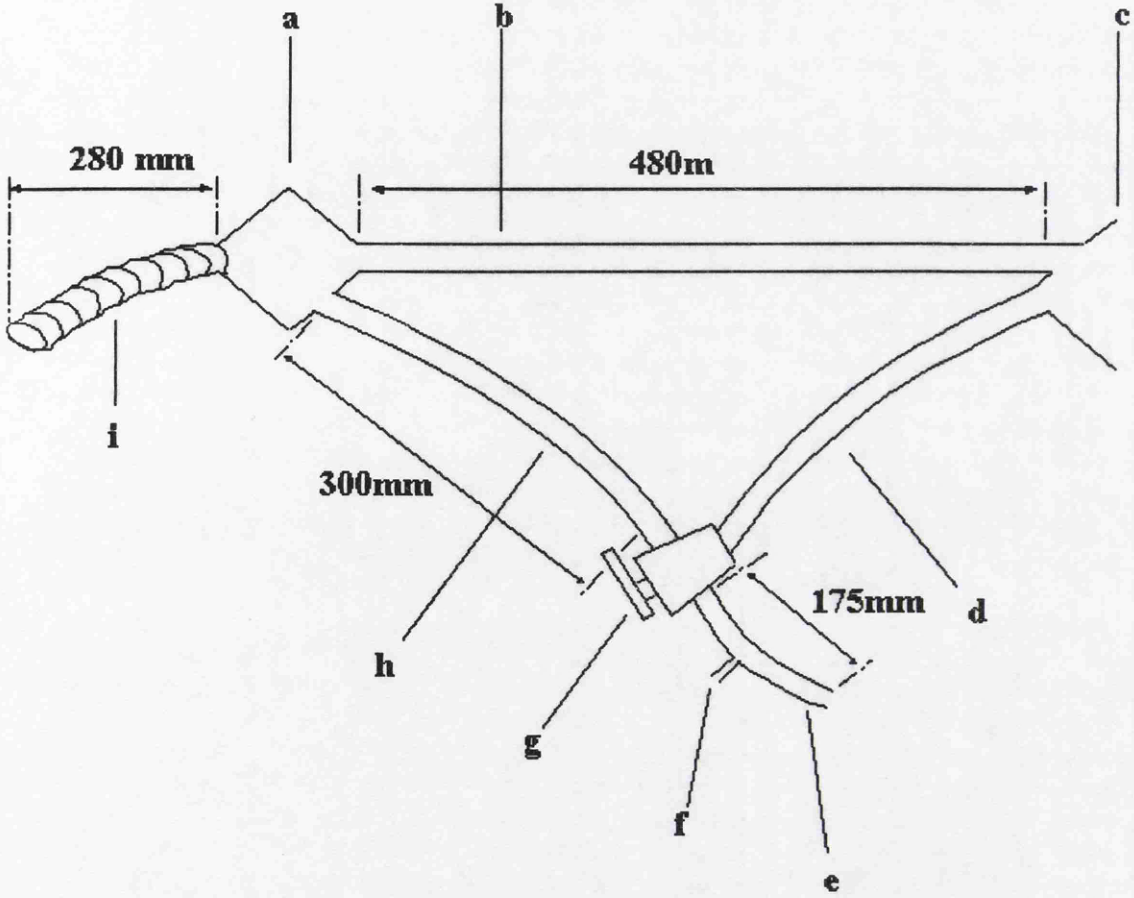
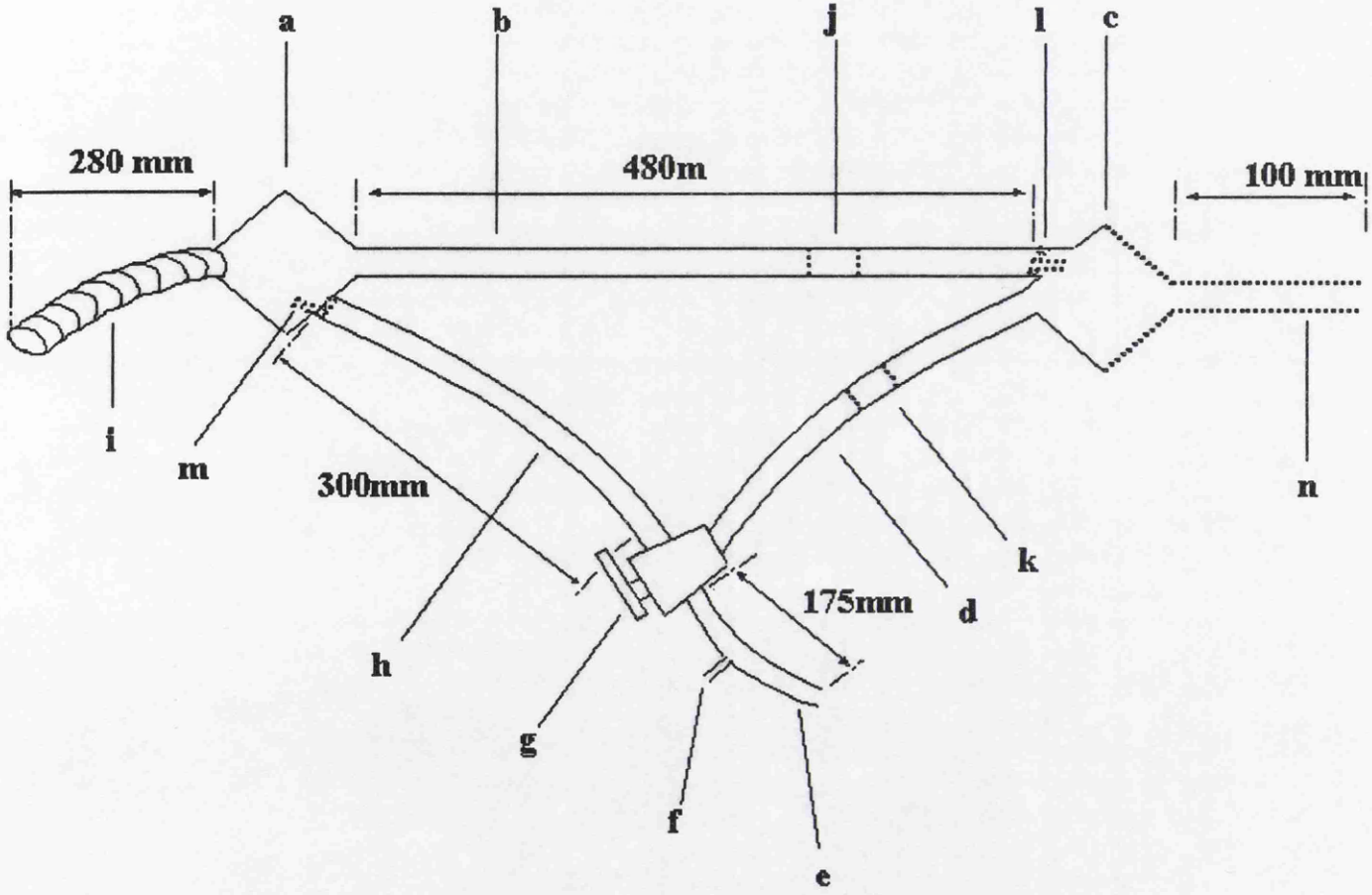


Figure 3.4. Nest box entrance trap with modifications (indicated as dotted lines) for *D. sylvestris* **a.** internal funnel, **b.** funnel connecting tube, **c.** external funnel, **d.** entrance connecting tube, **e.** sampling tube, **f.** anaesthetising tube, **g.** diverter valve, **h.** internal funnel connector tube, **i.** nest box connecting tube, **j.** exit window, **k.** entrance window, **l.** external funnel constriction, **m.** internal funnel constriction, **n.** entrance tube



The nest was removed by breaking open the bird box. It was noted that the queen was still inside the nest. The nest had expanded to fill the bird box and the envelope was incomplete, consisting of several layers at the top and bottom of the nest, but only one or two layers at the sides. Large gaps were present at the sides of the envelope. Once removed, the nest containing the queen was transferred to a sealed cardboard box.

The nest and workers were then transported to the nest box site in Linlithgow. The nest containing the queen was removed from its box and was glued onto the side of the nest box with contact adhesive as used by Pallet *et al.* (1983) for the transfer of colonies of *D. arenaria*. The lid of the nest box was then secured in place with silicone sealant and the entrance hole of the nest box was secured by placing a cap over it. The jars containing the workers were placed inside the nest box through the Perspex sliding door (Figure 3.1d) and the jar lids loosened. The loosened lids of the jars were removed quickly and the Perspex door closed liberating the workers within the box. The workers were not allowed to forage for 24 hours. Confining workers to the nest box for a period of time prior to release seems to stimulate orientation behaviour in workers (Akre *et al.* 1976). Water and sugar cubes were left in the nest box to reduce the impact of starvation which can have an effect on colony development (Harris 1995). Roland (1969) found that when a captive colony of *Vespa crabro* was prevented from foraging the ability of the colony to regulate its temperature quickly declined. After Roland provided the wasps with honey, however, thermoregulation quickly resumed.

The cap was removed from the nest box on 28/6/97 allowing the workers to forage. Workers immediately left the box and orientated to the new nest site. The jars were removed from the nest box on the following day and it was found that 17 workers had died in transfer. Shortly after commencing foraging, the workers began to expand the envelope and it assumed a more normal, spherical shape. A temperature logger ('Tinytalk') was placed on the floor of the nest box to monitor the box temperature, while a probe of a second logger was inserted into the nest to record nest temperature. To monitor the effectiveness of the heated nest box initially a third logger was placed outside the nest box to record ambient temperature.

Although the principal development of the nest box was conducted on the colony of *D. sylvestris*, a second heated nest box was deployed at a site in Kilmacolm. In this box a colony of *D. norwegica* was successfully established.

Trap installation

It was found by trial that workers were not able to use the complete entrance trap illustrated in Figure 3.3 when installed intact. Workers therefore had to be adjusted to the trap system in stages. To train workers to the complete system, the external funnel only (Figure 3.3c) was initially connected directly to the entrance trap boss (Figure 3.1f) on 3/7/97 at 1p.m. Within 24 hours, workers had adjusted to this modification and were entering and exiting as before. The nest box connecting tube (Figure 3.3i) was then fitted between the boss and the external funnel and workers adapted to this very quickly. After 1 hour, the full trap was installed. Workers, however, did not successfully use the trap and searched for the entrance funnel in its previous location. The full trap was therefore removed and the single funnel and nest box connecting tube re-attached. The box was then moved forward so that when the full trap was attached the box could be pushed back allowing the external funnel to remain in the same location. After one hour the wasps had adjusted to the new position of the single entrance funnel and the full funnel was re-attached. This time workers successfully orientated to the new position of the funnel and within 24 hours were successfully entering and exiting the trap.

The trap, however, was not successfully separating incoming and outgoing workers. The number of wasps entering and exiting the trap was observed over 37 minutes. Workers were using the entrance tube correctly as 16 wasps had entered the entrance tube, but none had exited. They did not, however, use the exit tube successfully as 84 wasps had entered the exit tube and 99 had exited via that tube. It was therefore concluded that the internal funnel system was functioning correctly as no workers exited via the entrance tube. The external funnel did not, however, appear to be functioning properly.

Constrictions (Figure 3.4l, 3.4m) were added to the system on 24/7/97 in order to make it more difficult for workers to enter the incorrect tube. These consisted of smaller diameter tubes (10mm) fixed into the tubes with silicone sealant. This, however, only had limited success and a proportion of workers still entered by the wrong tube. The efficiency of the internal funnel did not appear to be affected. In a half-hour traffic count 8 workers entered via the entrance but no workers exited. The efficiency of the exit tube, however, was not sufficiently improved as 11 workers exited and 3 entered.

The external funnel did not appear to be functioning correctly as workers could easily orientate to the exit tube after leaving it. This may be due to differences in the behaviour of *Vespula* and *Dolichovespula* as the trap was originally designed for use

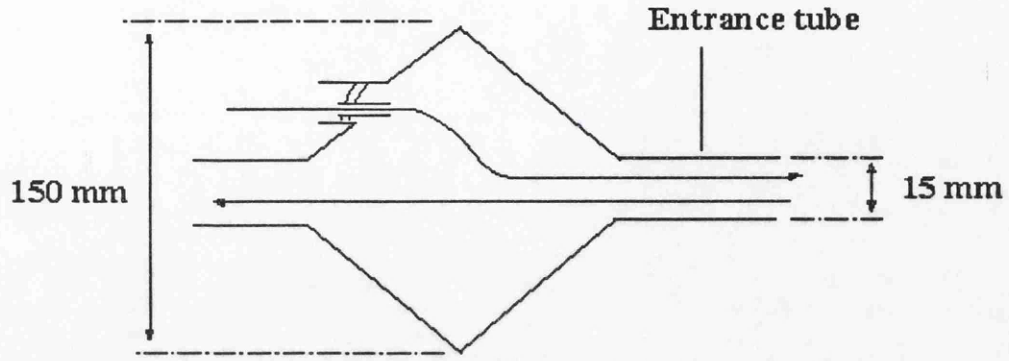
with underground nests of *V. vulgaris* and *V. germanica*. Underground nesters tend to land a few centimetres from the entrance tunnel and run-in. Aerial nesters such as *D. sylvestris* tend to land near the top of the nest and run down to the envelope (Edwards 1980). As the exit tube of the trap is raised above that of the entrance tube, *V. vulgaris* and *V. germanica* workers would tend to walk toward the entrance tube. Subterranean nests frequently have several entrances and several entrance tunnels, and so workers may enter via a different tunnel from the one they exited. They may therefore not orientate to one precise entrance and may rely more on colony odour for example. In *D. sylvestris*, however, the workers frequently nest in open situations and the nest only has one entrance.

It was therefore decided that the external funnel should be adapted to resemble the internal funnel (Figure 3.4c, Figure 3.5). In the modified funnel workers orientate to the entrance tube and could not orientate to the outlet of the funnel connecting tube as insufficient light was available. It was now, however, impossible to asses the efficiency of this modification as it could not be seen if workers were entering and exiting via the correct tubes. In addition workers occasionally entered then exited the external funnel without entering the nest. It was therefore decided to remove a section of tape covering the funnel connecting tube and the external funnel connecting tube to create windows through which workers could be seen passing (Figure 3.4j and 3.4k). It was anticipated that light entering the centre of the trap would disorientate workers. This, however, was not the case and the windows proved successful for monitoring traffic.

The fully modified trap could not be completely assessed as the nest was already in a state of rapid decline. However, with the full system installed a half-hour count was made and eleven workers were observed to exit via the correct tube and nine workers were observed to enter via the correct tube. No workers were observed to enter or exit via the wrong tubes.

The sampling tube was not trialled in the set-up described above. The trap illustrated in Figure 3.3 was, however, trialled in the 1996 with a colony of *D. norwegica*. The funnel system was not sufficiently developed in the 1996 season to allow workers entering and exiting the nest to be separated. The diverter valve was trialled and found to be effective in sampling workers returning via the entrance connecting tube.

Figure 3.5. External funnel of the entrance trap. Lines through the funnel with arrowheads indicate the direction of wasps through the trap.



To sample workers returning to the nest, the diverter valve was turned redirecting workers entering the valve from the internal funnel connecting tube to the sampling tube. Prior to the collection of workers, a glass jar was connected to the collecting tube, and a hose from a carbon dioxide gas cylinder connected to the anaesthetising tube. Workers could be seen entering the sampling tube, and after a sufficient number had entered, they were anaesthetised with carbon dioxide.

3.4. Discussion

A mature colony of *D. sylvestris* was successfully transferred to the nest box. The nest box functioned well and was successful in maintaining the temperature of the nest at 32°C. The entrance trap presented by Harris (1989) for use with subterranean colonies of *V. vulgaris* and *V. germanica* required several modifications for use with the colony of *D. sylvestris* due to behavioural differences between the genera. The modified trap, however, functioned well and should be useful in future studies on foraging behaviour in this species.

The box would also be of use for studies involving *Vespula* species as it is simple and comparatively inexpensive design compared to those presented by Potter (1964) and Martin (1990). It would, however, be necessary to increase the internal volume of the box for species such as *V. vulgaris*, which produce very large nests.

Some problems were found with the nest box and modifications could be made to improve the efficiency of the nest box and trap. The temperature of the nest box could not be adjusted without opening the box and disturbing the nest. This would be inconvenient for experimental work such as investigating the effects of nest temperature

on foraging. An improvement would be to replace the central heating style thermostat with a digital thermostat with a remote or external control. A further improvement would be to line the box with insulation material to improve the temperature regulation of the box and to reduce the energy consumption of the unit.

Other forms of electrical heater are available which could be used with the nest box. The Ultratherm power plate, however, is designed for home vivaria, and to be fitted on to wood. As it is in the form of a flat plate, the heat is relatively diffuse and is therefore less likely to harm workers coming into direct contact with it. In addition, as it has relatively low power consumption it would be possible to power it for several hours from a portable source such as a car battery (fitted with an inverter) in the field.

An alternative to maintaining the colony in a heated nest box for studying the effect of temperature on foraging is to measure the natural variation in ambient temperature (Martin 1988, 1990, 1992; Gibo *et al.* 1974). This, however, gives the experimenter no control over temperature and it is more difficult to replicate experiments.

Although the colony of *D. sylvestris* was at a mature state of development the method of transfer was generally successful. Of the 77 workers known to be in the nest two workers were lost in the transfer and a further 17 died immediately following the transfer. Colonies are normally moved at night (Pallet *et al.* 1983; Gibo *et al.* 1974). The colony of *D. sylvestris* in the present study was captured during the day, as it was located on domestic premises. This method is more time consuming, as foragers returning to the nest must be captured. The transfer, however, resulted in relatively minor loss of workers. This may be partly due to the heating of the nest box, which allowed continuity of thermoregulation during the transfer. Transplanted colonies are normally supplied with a carbohydrate source (Akre *et al.* 1976; Gibo *et al.* 1974) to maintain thermoregulation in the nest hence reducing the loss of brood.

Pallet *et al.* (1983) devised a method for capturing colonies of aerial nesting Vespids. The method was devised in order to transfer colonies without damaging nest. Queen nests were located and removed from their substrate and re-attached to a square of Plexiglas with contact adhesive. The Plexiglas base was then returned to the original position until the nest was more developed and contained around 50 adults, at which point the nest was captured and the Plexiglas square attached directly to the roof of a cage in the laboratory. Colonies were collected after dark and the Plexiglas square formed the lid of a collection box. The lid of the box was then attached to the lid of a nesting cage.

Colonies could alternatively be established in nest boxes from overwintered queens (Gibo 1977; Ross *et al.* 1981; Mathews *et al.* 1982). This, however, has only limited success due to the highly variable quality of the queens. Brian and Brian (1948) found that only 1 in 10 queens of *D. sylvestris* produce successful colonies. Ross *et al.* (1981) established colonies of various *Vespula* from queens in the laboratory. The success rate, however, was low, and no colony constructed more than one comb or produced reproductives. Establishing nests from queens is most successful when queens that have emerged from diapause are searching for nest sites (Mathews *et al.* 1982; Ross *et al.* 1981). This can be achieved by catching queens in spring or by obtaining mated queens at the end of the winter and storing them at a low temperature over winter (Ross *et al.* 1981; Mathews *et al.* 1982).

Heated nest boxes are only of use in examining the effects of elevating the temperature above ambient. They are of less use in regions where ambient temperature is frequently at or above the nest optimum, or where the aim of the investigation is to examine the effects of low temperatures on colonies. Gibo *et al.* (1974) investigated the effect of cold stress on colonies of *D. arenaria* and *D. maculata*. This was achieved by maintaining colonies for several hours in an environmentally controlled room, or in an ice chest at ambient temperatures of 5°C.

In the nest box situation, many aspects of colony activity can be monitored. Akre (1991) suggests that with advances in electronics many of the activities can be recorded automatically throughout the entire life of the colony. Various electronic counters for example have been used for counting traffic in wasps (Edwards 1980; Potter 1964) while others have been developed for bees (Spangler 1969). A counter was developed for use with the entrance trap in this study. The counter was fitted on the entrance tube as workers tend to patrol the exit tube. The counter used an infrared emitter and receiver as wasps do not perceive light at this end of the spectrum (Edwards 1980). This was, however, found to be unsuccessful, and workers frequently triggered multiple counts. Although insufficient time was available to develop the device, it was found that the frequency of traffic from the colony of *D. sylvestris* was so small that events could be adequately monitored by eye. A counter would, however, be very useful for mature colonies of *V. vulgaris* and *V. germanica* where the traffic rate is too high to be effectively monitored by eye.

The unmodified trap based on the design of Harris (1989) did not function successfully in monitoring nest traffic in *D. sylvestris*. The various modifications presented in this

chapter, however, allowed it to be used successfully. Harris similarly found that with his design 98% of foragers left the nest by the correct tube, but only 90% used the correct entrance. Therefore the internal funnel in his trap also appeared to be functioning very well, while the external funnel was less effective. The modifications presented in this chapter may also improve the efficiency of the trap when monitoring colonies of *V. vulgaris* and *V. germanica.*

Chapter 4. Inter and intraspecific differences in comb and envelope construction behaviour.

4.1. Introduction

Vespine wasps construct their nests from plant fibre (principally wood) that is macerated, mixed with saliva and drawn into thin strips, producing 'wasp paper'. Production of paper by wasps is similar to that of manmade paper. The use of wood fibre in paper manufacture apparently resulted from a study by the French naturalist R.A.F. de Reamer on wasps nests in 1719, when he showed that they were made from wood particles held together with protein from wasp saliva (Biermann 1993). The advantages of paper as a construction material, are that plant fibres are easily obtained close to the nest (Matsuura and Yamane 1990), it is a light material with strength in tension (Hansell 1984), and is easily worked and modified (Matsuura and Yamane 1990; Akre and Davis 1978).

Wasp paper can be regarded as a composite material consisting of plant fibres embedded in a saliva matrix. Composite materials are particularly strong in tension, the matrix functioning to transfer load between the fibres (Gordon 1991a). Paper fibres are held together through direct inter-fibre hydrogen bonding (Biermann 1993) although in wasps they are also held together with saliva. The strength of composite materials increases with fibre length and amount of matrix, and is dependant on fibre alignment. They are strongest when the fibres are aligned in the direction in which a tensile load is applied, and weakest when fibres are aligned perpendicular to load direction. (Gordon 1991a).

Relatively little information is available in the literature on the mechanical qualities of wasp nest paper. The production of paper in wasps shows many similarities to that of manufactured paper from wood pulp. There is a considerable body of scientific literature on the production of manufactured paper which can be utilised in the study of paper produced by wasps. Many of these sources are listed in Biermann's (1993) comprehensive textbook on paper manufacture. In investigating the properties of wasps nest paper therefore, the literature on manufactured paper will be frequently referred to.

The production of wood pulp for paper in wasps is similar to mechanical pulping in manufactured paper, such as the use of grindstones. The grindstones have a groove pattern on their surface, and separate the fibres using only mechanical attrition, applying repeated shear stress to the wood (Biermann 1993). In vespine wasps fibres are removed from a wood source by scraping its surface with the toothed mandibles. In manufacture, paper strength is a compromise between the amount of inter-fibre bonding

and the strength and length of individual fibres. Refining pulp increases the flexibility of fibres allowing them to form around each other and capillary action pulls the fibres together. This increases the area of contact between fibres so that inter-fibre bonds can form. Refining, however, reduces the strength of individual fibres (Biermann 1993).

When vespine workers collect fibre for pulp production, the wasp alights on a wood source and aligns itself parallel to the direction of the grain. The wasp then grips the wood with its legs spread widely apart and stretches its head forward scraping fibres from the surface with 'an alternate closing and downwards movement of the mandibles' (Edwards 1980). Saliva may be applied to the surface of the wood to hold fibres together as they are scraped off (Spradbery 1973; Edwards 1980). Once the fibre has been collected the wasp leans back on its mid and hind legs and grips it between the palps, mandibles and neck, before flying back to the nest. Workers probably collect fibre from a variety of sources as bands of many different colours are apparent in nest paper. The use of several different fibre sources by colonies has been noted in *Vespa orientalis* by Ishay *et al.* (1967). Wasps may spend some time when selecting a new pulp site, and once at a site, may move off if the wood is of the incorrect consistency (Edwards 1980). *V. pensylvanica* and *V. atropilosa* workers have been noted to visit more than one site to collect a single pulp load (Akre *et al.* 1976).

The use of saliva and water is very important in the processing of pulp. Ishay *et al.* (1967) noted that on the way to pulp collection, workers of the hornet *Vespa orientalis* collected water. Water is often added to the surface of the wood prior to scraping fibres from its surface in vespine wasps (Spradbery 1973; Edwards 1980) and at the nest the pulp is further chewed and mixed with more saliva (Matsuura and Yamane 1990). The origins of the liquid used in pulp collection and paper production are unclear. Although wasps have been noted to use crop water in the production of paper they also seem to use saliva (Edwards 1980).

The use of water is essential in the production of wood pulp, but it is possible that saliva contains other substances which aid in pulping. In paper manufacturing wood can be pulped by chemical methods such as the 'Kraft' method involving strong alkali (pH 13-14) or the 'sulfite', 'acid' or 'bisulfite' processes which are acidic (pH 1.5-5) (Biermann 1993). Wasps saliva may therefore be mildly acidic or alkaline to aid in pulping. Their saliva may also have an enzymatic action containing cellulases to aid in wood

degradation. There is, however, no detailed information available in the literature on the chemical content of wasp saliva.

Several glands have been described which open to the oral cavity, including the mandibular, hypopharyngeal, and thoracic gland. Although the function of the mandibular and hypopharyngeal is unclear, Edwards (1980) and Spradberry (1973) have suggested that the thoracic gland may function to bind together fibres in nest paper. The thoracic or salivary gland is situated in the prothorax, the ducts of which open at the apex of the hypopharynx and base of the lingua. There is, however, no indication as to whether the secretions of this gland are protenacous or whether they contain acid, alkali or enzymatic secretions.

In producing paper the worker applies the pulp load to the edge of the comb or envelope. The wasp then holds onto the edge of the paper with its forelegs and repeatedly moves backwards working the paper thinner with its mandibles constantly antennating both sides of the paper edge (Akre *et al.* 1976). This may have some function in gauging the final thickness of the paper. Typically three or four passes are made to thin the pulp (Edwards 1980; Akre *et al.* 1976).

Vespine wasps collect pulp for the construction of three basic components of the nest comb, comb supports and envelope. Although all three components are made of paper, they perform different structural functions in the nest, and differ in appearance. It is therefore possible that there are differences in the manufacture and composition of the material used in their construction. These differences could result from behavioural differences in manufacture. Of these three components this chapter will examine the allocation of material to the two largest components; comb and envelope.

In vespine wasps the production of comb paper differs to that of envelope. Comb is enlarged at the edge by adding new cells to the junction of two existing cells. Pulp loads are first formed into a cup shape producing the base of the cell and subsequently added in thin arc shaped horizontal strips forming the circumference (Spradbery 1973). There are no obvious differences between species in the production of comb paper. There is, however, great variation in envelope manufacture between species. In *V. vulgaris* the envelope is cellular, consisting of shell-like pockets. Pulp loads are added in an arc to existing envelope with the ends of the arc facing downwards. *Dolichovespula sylvestris* and *D. norwegica* have a laminar envelope consisting of large pliable sheets of smooth

overlapping paper. Pulp loads are added in more or less horizontal strips to the outside of the nest.

Although the structure of comb and envelope is very different, they are similar in texture, and appear to be constructed from the same fibre source. *Dolichovespula norwegica* and *D. sylvestris* construct a strong high quality paper from long thin woody fibres forming an interwoven mat, similar in texture to course tissue paper. The high quality paper of *D. sylvestris* and *D. norwegica* arises from the collection of fibre from sound to well weathered wood (Table 4.1), with *D. norwegica* selecting more weathered wood than *D. sylvestris* (Weyrauch 1935). The fibres of *Dolichovespula* are relatively strong, and consist mostly of individual plant cells which are scraped from the surface of the wood. In *V. vulgaris* paper is constructed from wood chips and has a fragile, crumbly texture. The crumb-like texture of *V. vulgaris* paper results from the collection of pulp from rotten or semi rotten, wood (Table 4.1). In *V. vulgaris* paper fibres are short and relatively weak resulting in the fragile texture of the paper. They consist of chunks of plant cells, which are cut from surface of wood.

Comb is structurally more complex than envelope, and it is difficult to predict the types of load on the material. As the combs are supported from above, they are structures in tension, carrying their own weight and that of the brood. They are, however, supported unevenly, and so function as a beam or cantilever (Hansell 1984). The cell walls are therefore subject to both tension and compression. In addition, the cell walls are subject to tension around the circumference caused by the larvae pushing against the sides (Hansell 1984).

Matsuura and Yamane (1990) suggested that combs are strengthened by the deposition of meconia at the base of the cell, and addition of silk to the cell wall during pupation. Silk has a high tensile strength (*c.f.* commercial silk 350 MN/m^2, Gordon 1991b), and in mature comb may function to strengthen cell walls. In addition the deposition of meconia at the base of the cell may function to bear compressive loads.

The functions of the envelope are principally in defence, thermal insulation and weather proofing. The envelope does not normally carry the weight of the combs, and as it is supported from above it is principally subject to its own weight in tension. In species such as *D. norwegica* and *D. sylvestris*, which nest in open or semi-open situations, the envelope is also subject to lateral force from wind and rain.

Differences in comb and envelope paper could arise when selecting pulp sources, or in the processing of pulp. As comb and envelope perform different structural functions in the nest it is likely there are differences in specifications to which they are manufactured. Very little information is available on the differences in the manufacture of comb and envelope paper. One of the objectives of this chapter is therefore to examine the specifications to which comb and envelope are manufactured **(see Question 1)**.

The selection of fibre sources can have an effect on the length and mechanical properties of fibres. Fibre length varies greatly between genus and even species of tree; softwoods for example have much longer fibres (typically 3-3.6mm) than hard woods (typically 0.9-1.5mm) (Biermann 1993). In composite materials, tensile strength increases with fibre length (Gordon 1991a). The second objective of this chapter therefore is to determine if there are differences in the fibres from which comb and envelope are composed in particular fibre length **(see Question 2)**.

Mastication of fibres reduces the strength of fibres, making them more compliant and allowing more inter-fibre bonds to form (Biermann 1993). The amount of time spent masticating paper is therefore likely to increase the strength of the paper. The use of water or saliva in pulp production also has an effect on fibre strength. In addition, mastication may cut some fibres resulting in a difference in fibre length.

Differences in comb and envelope material could result from the re-cycling of materials within the nest. As the nest expands, material is removed from the inner layers of the envelope (Edwards 1980). This material can either be used to construct comb, comb supports or envelope or may be discarded. It is not clear to what extent comb and envelope are constructed from pulp brought in from outside the nest or from materials re-cycled in the nest.

Akre *et al.* (1976) examined pulp collection, and comb and envelope construction, in laboratory colonies of *V. pensylvanica* and *V. atropilosa*. Pulp foragers were normally observed to add new loads of pulp to the envelope. Paper was later removed from the envelope, then masticated and added to cells or envelope. Paper was never removed from the cells and added to the envelope. This prompted Akre *et al.* (1976) to propose that envelope may also function as a '*storage area for fibre*'. The reuse of envelope material for comb construction has also been noted in embryo nests of *D. media*, and *D. saxonica nipponica* (Makino 1980), and *V. flaviceps lewisii, Vespa simillima, Vespa mandarina* Smith and *Vespa tropica* (Matsuuara 1990). In laboratory embryo nests of

V. maculifrons, however, queens have been observed to discard dismantled envelope (Mathews *et al.* 1982).

If envelope is selectively recycled for the production of comb this would have an effect on mechanical properties of the two materials. The additional paper mastication would increase the strength of paper through inter-fibre bonding. It may also cut the fibres leading to a difference in the length of comb and envelope fibres. The extent to which re-cycled paper is used in comb and envelope would therefore affect the properties of the paper.

There are noticeable differences between species in the quality of nest paper. The paper of *D. sylvestris* and *D. norwegica* species appears to be stronger and of higher quality than that of *V. vulgaris*. This may be explained by differences in nesting habit. *D. sylvestris* and *D. norwegica* nest in open or semi-open sites where as *V. vulgaris* is predominantly a cavity nester (Archer 1989; Spradbery 1973; Edwards 1980). In all three species the envelope functions in insulation and defence. In *D. norwegica* and *D. sylvestris*, however, the nest is also subject to movement of the substrate (in hedges and trees) and forces from wind and rain. The final objective of the chapter is therefore to quantify differences between species in paper quality and to relate this to differences in colony lifecycle and nesting habit (**Question 3**).

The collection of fibre and paper manufacture requires a major investment of colony time and labour. It therefore is important to understand how fibre, as a resource, is allocated to comb and envelope material. The aim of this chapter is therefore to determine if there are differences in comb and envelope material with respect to the fibre source and the specifications to which they are manufactured. It is therefore necessary to quantify differences in paper structure and fibres composition of comb and envelope paper. In this chapter inter and intraspecific differences in comb and envelope paper will be addressed with three principal questions:

1. Are comb and envelope manufactured to the same specifications?
2. Are there differences in the fibres from which comb and envelope are composed?
3. Are there differences between species in the mechanical properties of comb and envelope paper?

The first of these questions was examined through a comparison of the thickness and density of comb and envelope paper; the second through an examination of the fibres in

comb and envelope paper, and the third through a comparison of paper thickness, density, tensile strength, Young's modulus and fibre composition.

As there is an overlap in the experimental evidence required to answer these questions, the methods used to provide this information will be described individually before returning to the three principal questions in the discussion (section 4.5.). The experimental methods chosen are therefore introduced under their own headings together with a comparison of techniques employed by other authors (where relevant).

Measurement of paper thickness

Relatively little information is available on the measurement of nest material thickness in social wasps. Martin (1992) measured the thickness of envelope paper in *Vespa affinis* with a thickness gauge (Teclock) accurate to 0.01 mm. The use of an engineering thickness gauge is obviously a simpler, less time consuming technique. Gauges of this accuracy are, however, of less use in measuring comb material as they have relatively large contact surfaces. In paper manufacture, for example, thickness is measured using a micrometer with circular contact surfaces of 16mm diameter (Biermann 1993). This would lead to unacceptable inaccuracies when measuring small pieces of comb material.

Hansell and Turillazii (1995) measured the thickness of brood cell material in various species of *Anischnogaster* (Stenogastrinae) by taking histological sections, which were subsequently measured under a compound microscope. Taking histological sections of a material is particularly useful in measuring the thickness of comb material, as it requires very small amounts of material. Preparation of material may, however, effect the thickness of sectioned material especially in aqueous media, as paper is hygroscopic (Biermann 1993). Although this is unlikely to have a different effect on comb and envelope material, care must be taken in interpreting absolute values.

In the present work the thickness of comb and envelope material of *D. sylvestris*, *D, norwegica* and *V. vulgaris* was measured from histological sections. Comb and envelope were initially embedded in wax and sectioned with a microtome. This was, however, found to be ineffective as the woody fibres tore out of the wax in sectioning. Samples were therefore embed in resin and sectioned with an ultratome.

Measurement of paper density

Paper density was determined by weighing samples of comb and envelope of known dimensions. Density was calculated by dividing mass per unit area by the paper thickness measured from histological sections.

Analysis of fibre composition

The nest paper of *D. sylvestris* and *D. norwegica* consists of an interwoven mat of long, thin fibres, which obscure each other. Fibres must therefore be separated out in order to measure their length. Hansell & Turillazii (1990, 1995) examined the composition of nests material in stenogastrine wasps by making a squash preparation of material cleared in histoclear. *D. sylvestris* and *D. norwegica* paper fibres do not, however, separate easily, and when fibres are separated manually they tend to break. It was therefore necessary to first soak samples in a general biological solvent to disperse the matrix and release inter-fibre bonds. Fibres could then be separated, stained and mounted on a slide for separation following a method described by Purvis *et al.* (1966) for the preparation of plant macerations.

The paper of *V. vulgaris*, however, consists of short 'chunks' of woody material, or 'wood chips'. They could not be adequately prepared using the method developed for the examination of *Dolicovespula* fibres as wood chips tend to disintegrate in the process of separation. However, as *V. vulgaris* paper consists of short, thick chunks of wood, a proportion of the fibres are visible at the surface of the paper. Samples of comb and envelope paper were therefore examined whole with a scanning electron microscope.

Aqueous slide preparations used in the examination of *Dolichovespula* material may have affected the dimensions of fibres. Although fibre length is not appreciably affected by moisture content, fibre width can be greatly affected (Biermann 1993). It should also be noted that as different techniques were used in the preparation of *Dolichovespula* and *Vespula* material, caution must also be taken in comparing fibre width and length between these groups.

Tensile strength and Young's modulus

Tensile strength and Young's modulus describe two important properties of a material. The tensile strength of a material is a measure of the amount of force required to pull it apart. The strength of a material is normally measured by applying an increasing load in

tension until it breaks. In order to compare the behaviour of samples of different sizes the force applied to a material is normally divided by its cross sectional area termed stress;

$$Stress\ in\ MN/m^2 = \frac{Load\ in\ MN}{Cross\ sectional\ area\ in\ m^2}$$

The tensile strength of a material is simply the stress required to break a material;

$$Tensile\ strength\ in\ MN/m^2 = \frac{Maximum\ load\ in\ MN}{Cross\ sectional\ area\ in\ m^2}$$

The Young's modulus of a material is a measure of its stiffness while strain is a measure of how much a material will stretch under load per unit of original length;

$$Strain\ (dimensionless) = \frac{Extension\ under\ load\ in\ m}{Original\ length\ in\ m}$$

In order to compare materials of different dimensions, stress is divided by strain to give the Young's modulus (E). This is a constant of a material and is calculated as follows;

$$Young's\ Modulus\ in\ MN/m^2 = \frac{Stress}{Strain}$$

In tensile strength tests the extension of the specimen was measured. As the original length of the specimen was known, the Young's modulus could be calculated by dividing the tensile strength by the strain at maximum load. Care must be taken, however, in interpreting the Young's modulus calculated from a tensile strength test. Paper does not break evenly under load and tears can appear before the maximum load is reached which then gape, extending the specimen. This can lower the calculated Young's modulus. Young's modulus therefore describes how much a material will stretch under load. Young's modulus and tensile strength are not dependent on sample size, and as such are constant properties of a material.

In the present study tensile strength tests were conducted only on envelope material. This is partly due to the limitations of the test equipment. The largest sample size that can be prepared from comb material is limited to the dimensions of one cell wall (approx. 3 mm × 5 mm). Preliminary tests showed that the breaking load of comb specimens was below that which could be accurately tested by the 50N load cell fitted to the materials testing machine. In addition, the small size samples required the jaws of the machine to be too close together.

The strength and stiffness of materials, particularly composite materials is highly dependent on the direction in which load is applied. Samples were therefore tested in

Table 4.1. List of typical fibre sources commonly noted in the literature for British Vespine wasps (not comprehensive).

Fibre source		**Species**					
		V. crabro	***D. norwegica***	***D. sylvestris***	***V. rufa***	***V. vulgaris***	***V. germanica***
Rotten wood	General	Newport 1842; Weyrauch 1935; Bunn 1880				Weyrauch 1935; Akre and Davis 1978	
Well Weathered wood	General		Weyrauch 1935		Weyrauch 1935		Spradbery 1973
	Semi-rotten elm	Rennie 1840					
Weathered wood	General		Arnold 1966	Laidlaw 1930	Weyrauch 1935		
	Weathered willow				Arnold 1966		
Sound wood	General		Nicholson 1917		Weyrauch 1935		Greene, 1979
Other wood	Ash	Donisthorpe 1929; Edwards 1980; Kemper & Döhring 1967; Simpson 1948					
	Birch	Bromley 1931					
	Spruce				Sandeman 1936		
	Pine		Kaynard 1957				

Fibre source		Species					
		V. crabro	*D. norwegica*	*D. sylvestris*	*V. rufa*	*V. vulgaris*	*V. germanica*
Bark	General	Walker 1901	Arnold 1966; Wayrauch 1935				
	Poplar bark		Arnold 1966; Wayrauch 1935				
	Bark from weathered umbellifers		Arnold 1966	Arnold 1966	Arnold 1966		
Other plant Material	Living lilac	Donisthorpe 1929; Wray 1954					
	Rhodedendron	Bromley 1931					
	Trometa from underside of Rhododendron leaf		Laidlaw 1930				
Man made fibres	Paper		Walsh 1929	Walsh 1929		Guiglia 1946	
	Cotton or wool form fabrics			Lith 1956			Benson 1945
Other	Sand	Omerod 1868					

two directions; in the direction of the pulp loads, 'direction A', and perpendicular to the pulp loads, 'direction B' (Figures 4.2a and 4.2b respectively).

4.2. Methods

Paper thickness

Paper thickness was determined by embedding samples in resin, which were sectioned and examined under a compound microscope. Five comb and five envelope samples were taken from each of five nests of *D. sylvestris*, *D. norwegica* and *V. vulgaris* (total of 25 samples of each material type per species). Envelope samples were taken randomly from the nest, while comb samples were taken only from cells of the second and subsequent combs (i.e. large cells only) in which pupation had not occurred (i.e. no silk lining). Each sample was trimmed to approximately 3 mm × 6 mm for embedding.

Prior to embedding, samples were dehydrated by placing into plate wells half filled with absolute alcohol for 2 hours. Wells were then topped up with LR White medium grade acrylic, to make a 50:50 resin alcohol mix and left overnight for the alcohol to evaporate. The plate containing the samples was then placed under vacuum for 3 days to remove air bubbles, aiding resin penetration.

The specimens were then transferred to fresh resin in TAAB capsules (polythene 8mm diameter) with lids on. They were then placed into an oven at 60°C for 3 days to polymerise. The resin blocks were then removed from the capsules and trimmed to an appropriate size for sectioning.

Samples were sectioned to a thickness of 2μm using an ultratome (L.K.B. Ultratome III). Sections taken from the ultratome were placed in a drop of water on a microscope slide. The slide was then placed on a hot plate to evaporate the water. The section was then covered with a drop of stain solution (1% Toluene Blue, 1% Borax) and placed back on a hot plate. The stain was immediately rinsed off with distilled water and the slide placed back on the hot plate to evaporate excess water. Finally the section was mounted in D.P.X..

Paper thickness was measured using a compound microscope fitted with an eyepiece graticule. From each sample, forty measurements of thickness were taken randomly. Measurements were calibrated using a slide graticule.

Paper density

The mass per unit area of comb and envelope samples taken from nests of *D. norwegica*, *D. sylvestris* and *V. vulgaris* was calculated by weighing samples of known surface area. Ten comb and five envelope samples were taken from each of five nests (total of 50 samples of each material type per species). Samples were prepared by cutting around a cardboard template with a scalpel. Envelope samples were cut to approximately 10 mm × 10 mm while comb samples to approximately 3 mm × 3 mm. Envelope samples were taken randomly from any area of the nest, while comb samples were taken from only large cells form which adults had not emerged (to avoid the inclusion of silk).

Samples of comb and envelope were weighed on a Sartorius-research balance, accurate to 1/100000 g. The mass of comb and envelope samples was recorded together with a measurement of the sample dimensions measured with a vernier calliper so that a more accurate surface area could be calculated. From the results obtained the mass per unit area was calculated. This was converted to density using the mean nest comb and envelope paper thickness.

Fibre analysis

Five comb and five envelope samples were taken from each of five nests of *D. norwegica* and *D. sylvestris* (total of 25 samples of each material type per species). Envelope samples were taken randomly from any area of the nest while comb samples were taken only from the second and subsequent combs (i.e. large cells only) in which pupation had not occurred (i.e. no silk lining).

To separate the paper fibres, each sample was placed in a watch glass containing a general-purpose biological solvent for 24 hours ('Stain Remover 2: Dylon International Ltd London). This process softened the fibres and dispersed the matrix. The fibres were then transferred to a watch glass of distilled water for 5 minutes to remove excess solvent. Individual fibres are relatively colourless, and were therefore stained in a third watch glass containing Methylene blue (0.1% aq.) to make them more visible. After 60 minutes, excess solvent was removed by placing the samples in a watch glass of distilled water for 5 minutes. A small quantity of the fibres was then placed in a few drops of glycerol on a microscope slide. The fibres were then examined under a dissecting microscope and gently separated using a pair of flattened mounted needles. When the

majority of fibres could be distinguished for the purpose of measuring their length and width, a cover slip was then placed on the slide and sealed with nail varnish.

The paper fibre samples were examined with a compound microscope fitted with an eye piece graticule. From each sample forty fibres were selected randomly. The length and width of each of these fibres was recorded (Figure 4.1). Fibres were generally of three types; those consisting of single complete plant cells, those consisting of single incomplete plant cells and those consisting of bundles of two or more plant cells. Fibres were therefore categorised as:

1. Single complete fibres
2. Single incomplete fibres
3. Multiple fibres

In *V. vulgaris* five comb and five envelope samples (of size 3 mm × 3 mm) were taken from each of five nests. Envelope samples were taken randomly from any area of the nest, while comb samples were taken only from large cells combs in which pupation had not occurred (i.e. no silk lining). The samples were then attached to stubs with carbon tape and coated with gold for Scanning Electron Microscopy. From each sample two S.E.M.s were taken at a magnification of ×50 (Figure 4.2).

Images from the electron microscope were printed out on a P.C. which was linked to the video output of the S.E.M. via a video capture card. The length and width of ten fibres was measured from each micrograph with a vernier calliper (a total of 20 fibres per sample). This was calibrated using micrographs of a mesh of known size examined at the same magnification in the S.E.M.

The fibres of *V. vulgaris* consisted of large bundles of cut plant cells. It was not therefore possible to accurately determine the number of individual plant cells in each bundle and so this data was not recorded. Similarly, single plant cells were not observed in the paper of *V. vulgaris*.

Tensile strength tests and Young's modulus.

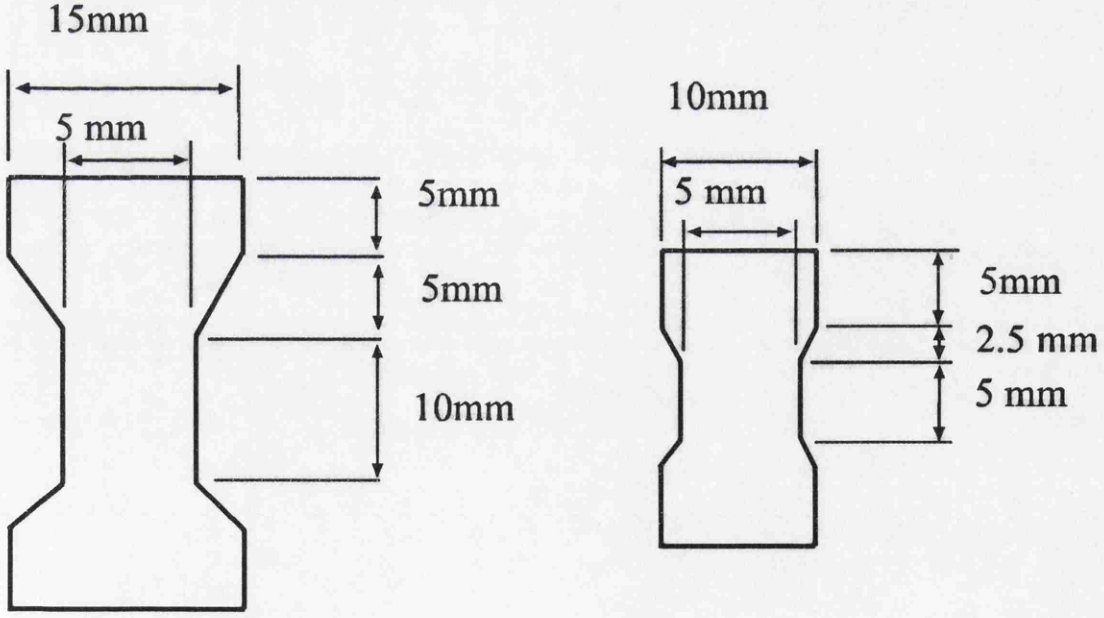
The tensile strength and Young's modulus of envelope material were determined by placing dumbbell shaped specimens under a tensile load and measuring the extension of

the specimen. Samples were tested with a 'Lloyd Instruments 1000' materials testing machine, fitted with a 50N load cell.

Three envelope samples were taken randomly from each of five nests of *D. norwegica*, *D. sylvestris* and *V. vulgaris* (total of 15 samples of each material type per species). Dumbbell shaped samples were prepared for testing by cutting around a cardboard template with a scalpel. Samples were prepared in this shape, as they tend to fail near the clamp in testing as a result of damage when inserting the specimen. By making the specimen wider near the clamp, the specimen is more likely to fail near the centre of the specimen which is less likely to be damaged. Despite these precautions some specimens failed near the clamp during testing. This was assumed to be due to damage during clamping and these results were rejected.

Samples were cut to the largest size that could be practically obtained from the envelope. *D. sylvestris* and *D. norwegica* samples were cut to the dimensions noted in Figure 4.3a, while *V. vulgaris* samples were smaller (Figure 4.3b).

Figure 4.3. Dimensions of templates for envelope samples of *D. sylvestris* and *D. norwegica* (**a.**), and *V. vulgaris* (**b.**) used in tensile strength testing



a. **b.**

Envelope samples were first clamped into the lower jaw of the machine such that the lower 5mm of the sample was not visible. The upper jaw of the machine was then lowered so that the upper 5mm of the sample could be clamped. The sample was then loaded at a constant rate of elongation of 1 mm/min. Load was applied until the sample

Figure 4.1. Photograph of typical fibres of *Dolichovespula* nests. Taken from the envelope of a mature nest of *D. norwegica*. Some fibres consist of single plant cells while others consist of bundles of fibres. Fibres are typically 1.2mm long and 0.03mm wide.

Figure 4.2. Scanning Electron Micrograph of typical nest material of *V. vulgaris*, taken from the envelope of a mature nest. Fibres consist of cut bundles of plant cells. Fibres typically 0.3mm * 0.1mm.

fractured. The breaking load was taken as the maximum load supported by the specimen prior to failing. The testing machine was linked to a P.C., which gave a print out of the test results. This included the maximum load supported by the specimen, and the extension at maximum load.

The Young's modulus was calculated from the extension at the maximum load. Tensile strength is therefore the stress at the point of maximum load. The cross sectional surface area of the material was calculated from the width of the central section of the dumbbell shape (5mm) and the mean nest envelope paper thickness.

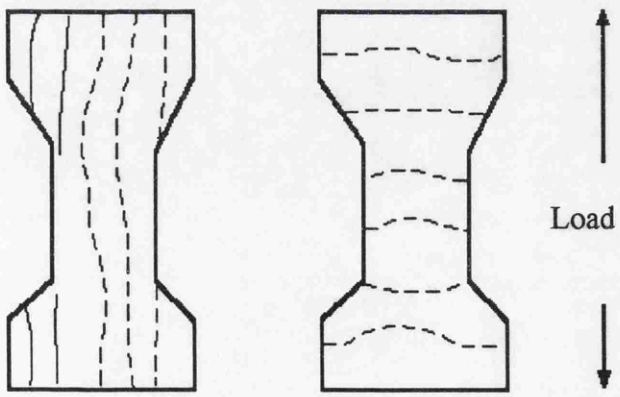
Strain was calculated from the original length of the specimen and the extension of the specimen at maximum load. Therefore;

$$YoungsModulus\ in\ MNm^2 = \frac{Tensile\ strength\ in\ MN/m^2}{Strain\ at\ \max imum\ load\ (\dim ensionless)}$$

The magnitude of the Young's modulus indicates the stiffness of the material. The higher the Young's modulus the stiffer the material, the lower the Young's modulus the more flexible the material.

Envelope samples were tested in the direction of the pulp loads (figure 4.4 a) and perpendicular to the bands of pulp (Figure 4.4 b). A total of 30 samples of each material type per species were therefore tested.

Figure 4.4. Diagram showing the two directions in which paper samples were tested. Dotted lines indicate the joins between pulp loads.



a. In the direction of pulp loads

b. Perpendicular to direction of pulp loads

Statistical analysis

Statistical analysis was performed on the means of nest samples. For tensile strength tests and Young's modulus, the mean of three samples from each nest was taken, and the five nest means were compared for each species. For paper thickness, fibre processing, fibre length and fibre width results, the mean of the five sample means per nest was taken so that five nest means for comb and envelope were compared in each of the three species. For the density results the mean of ten comb samples per nest and five envelope samples per nest were was taken.

The normality of the data was checked graphically with a frequency histogram, and the homogeneity of the variances was examined with the F_{max} test (Fowler & Cohen 1996). Percentages were not transformed. Although data involving percentages or proportions are normally arcsine, when percentages fall between 30 and 70% it is not necessary to apply the arcsine transformation (Sokal and Rohlf 1995). The means of tensile strength tests were found to be homoscedastic and normally distributed. All other results were log transformed to normalise and reduce the heteroscedasticity of the data.

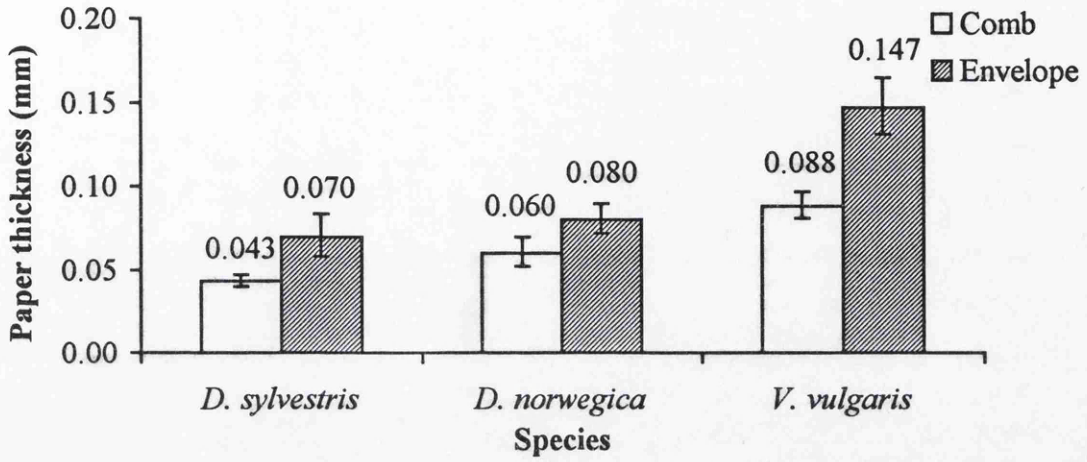
Two way analysis of variance (ANOVA) with replication was performed to test for differences in species and material (direction in tensile strength data) and for interaction. Interaction effects were further examined in more detail graphically (Fowler & Cohen 1996). A Tukey multiple comparison test was performed to locate differences found by ANOVA. Untransformed data are presented as histograms together with their 95% confidence intervals. Log transformed data are presented in the untransformed scale with their back transformed 95% confidence intervals.

4.3. Results

Thickness of comb and envelope paper

The results obtained are summarised in Figure 4.5. The 2-way ANOVA indicated that there was a significant difference in the thickness of comb and envelope paper ($F=145.22$ at df 1, 24: $P<0.01$). There was also a significant difference in paper thickness between the three species ($F=150.20$ at df 2, 24: $P<0.01$). There was a significant interaction between material and species ($F=3.75$ at df 2, 24: $P<0.05$).

Figure 4.5. Mean comb and envelope thickness in *D. sylvestris*, *D. norwegica* and *V. vulgaris* with 95% confidence intervals (calculated from log transformed means and back transformed).



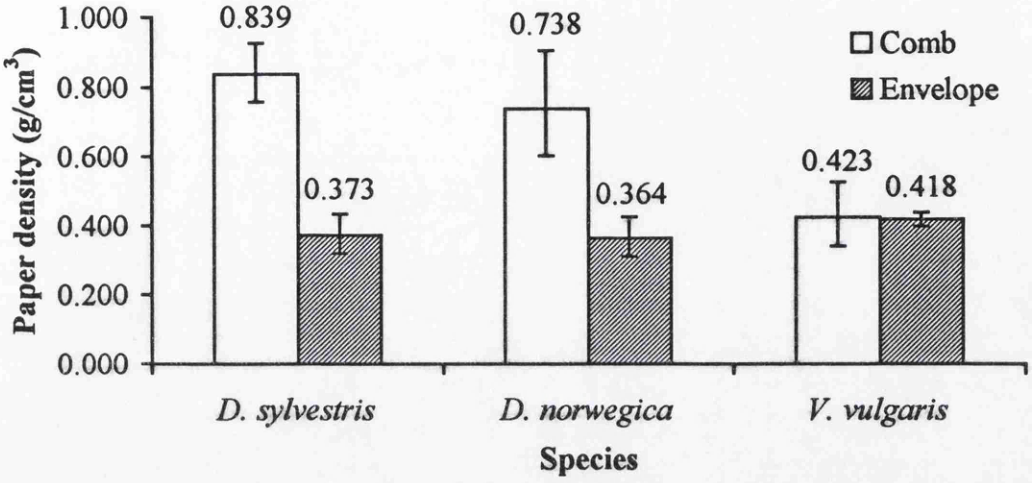
The Tukey test indicated that envelope paper is significantly thicker than comb paper in *D. sylvestris* , *D. norwegica* and *V. vulgaris*. There was a significant difference in comb thickness between species (*D. sylvestris*<*D.norwegica*<*V. vulgaris*), and in envelope thickness (*D.* sylvestris<*D. norwegica*<*V. vulgaris*). For all comparisons; T=0.081 at df=24: P<0.05, α=6).

Paper density

Results from paper density measurements are presented in Figure 4.6. The 2-way ANOVA indicated that there was a significant difference in density of comb and envelope paper (F=119.09 at df 1, 24: P<0.01). There was also a significant difference in paper density between species (F=13.53 at df 2, 24: P<0.01). A significant interaction between material type and species was found (F=30.05 at df 2, 24:P<0.01).

The Tukey test showed that comb was significantly denser than envelope in *D. sylvestris* and *D. norwegica*, but no significant difference in the density of comb and envelope paper was found in *V. vulgaris*. The Tukey results also indicated that *D. sylvestris* and *D. norwegica* comb were significantly denser than *V. vulgaris*. There was no significant difference in the density of *D. sylvestris* and *D. norwegica* comb or in envelope density in any of the three species. For all comparisons; T=0.108 at df 24: P<0.05, α=6).

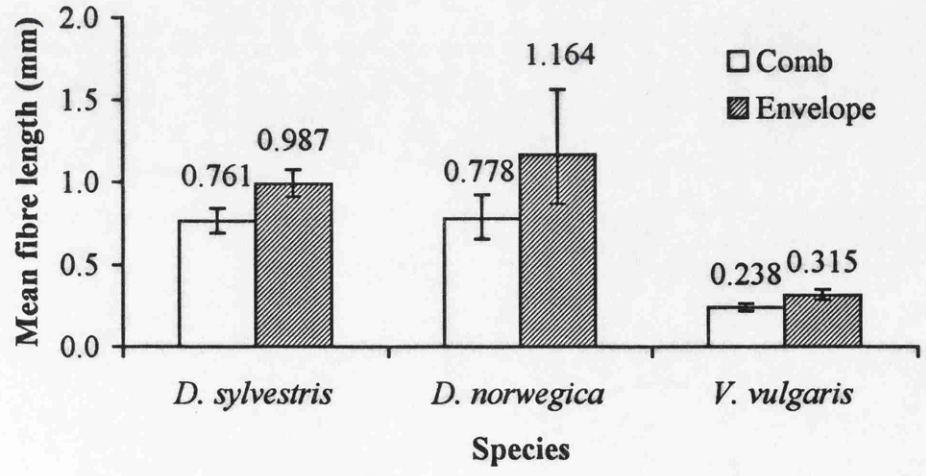
Figure 4.6. The mean density of comb and envelope paper in *D. sylvestris*, *D. norwegica* and *V. vulgaris* with 95% confidence intervals (calculated from log transformed means and back transformed).



Fibre length in comb and envelope paper

The results obtained are summarised in Figure 4.7. The 2-way ANOVA indicated that there was a significant difference in the fibre length of comb and envelope ($F=44.28$ at df 1, 24: $P<0.01$). There was also a significant difference in fibre length between species ($F=292.31$ at df 2, 24: $P<0.01$). No significant interaction between material and species was found ($F=0.72$ at df 2,24).

Figure 4.7. The mean fibre length in comb and envelope paper of *D. sylvestris*, *D. norwegica*, and *V. vulgaris* with 95% confidence intervals (calculated from log transformed means and back transformed).



The Tukey tests showed that comb fibres were significantly shorter than envelope fibres in *D. sylvestris*, *D. norwegica* and *V. vulgaris*. There was no significant difference in

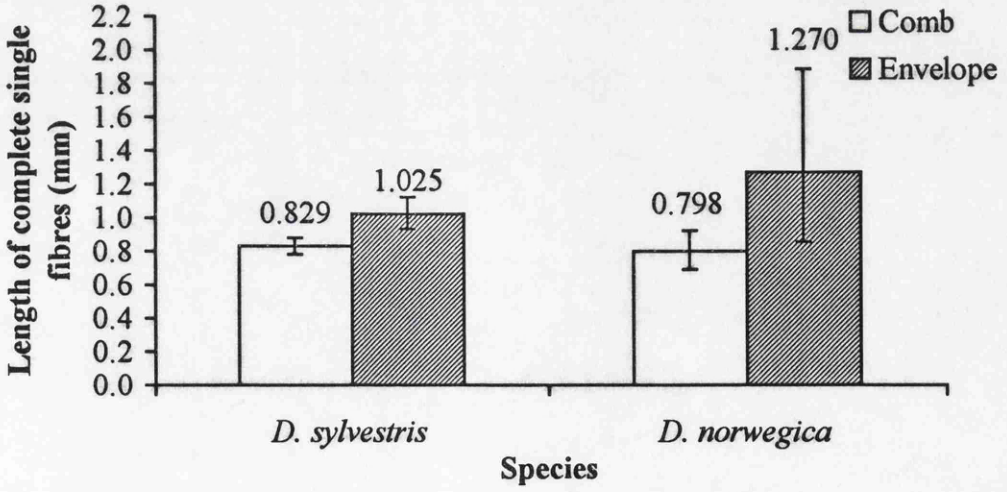
comb fibre length between *D. sylvestris* and *D. norwegica*. *V. vulgaris* had significantly shorter comb fibres than both *D. sylvestris* and *D. norwegica* respectively. There was no significant difference in envelope fibre length between *D. sylvestris* and *D. norwegica*. *V. vulgaris* had significantly shorter envelope fibres than both *D. sylvestris* and *D. norwegica*. For all comparisons; T=0.107 at df=24: P<0.05, α=6).

Length of complete single fibres in comb and envelope paper

The results obtained are summarised in Figure 4.8. The 2-way ANOVA indicated that there was a significant difference in the length of complete single fibres in comb and envelope (F=16.90 at df 1, 16: P<0.01). There was no significant difference in the length of complete single fibres between species (F=0.75 at df 1,16). No significant interaction between material and species was found (F=1.99 at df 1,16).

The Tukey tests indicated that complete single comb fibres were significantly shorter than envelope fibres in *D. norwegica*. There was no significant difference in the length of complete single fibres between comb and envelope in *D. sylvestris*. There was no significant difference in comb or envelope fibre length between *D. sylvestris* and *D. norwegica*. For all comparisons; T=0.193 at df=16: P<0.05, α=4.

Figure 4.8. The mean fibre length of complete single fibres in comb and envelope paper of *D. sylvestris* and *D. norwegica* with 95% confidence intervals (calculated from log transformed means and back transformed).

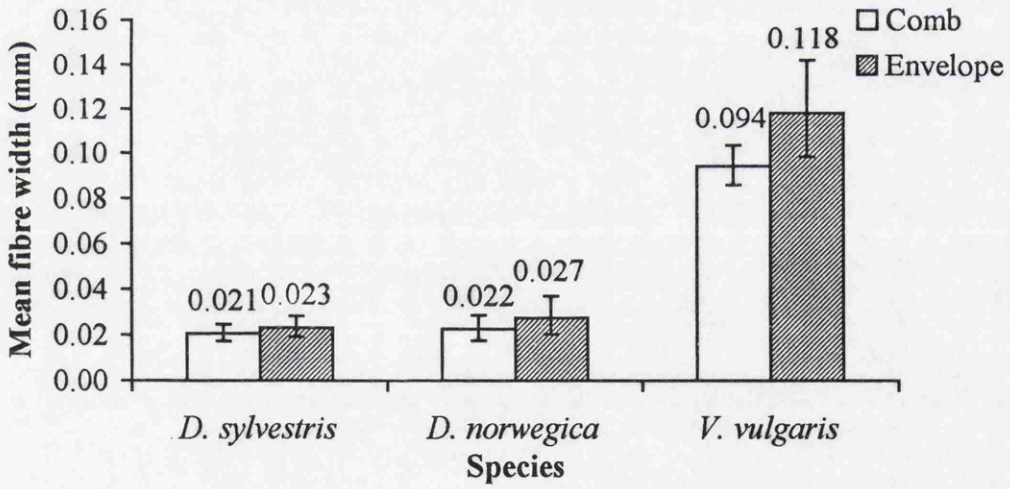


Fibre width in comb and envelope paper

Results obtained are summarised in Figure 4.9. The results of the ANOVA show that there was no significant difference in fibre width in comb and envelope paper (F=8.38 at df 1,24). There was a significant difference in fibre width between species (F=279.81 at df 2,24: P<0.01: n=10). No significant interaction between material type and species was found (F=0.23 at df 2,24).

No significant difference was found between fibre width in *D. sylvestris* comb and *D. norwegica* comb. *V. vulgaris* comb fibres were, however, significantly wider than both *D. sylvestris* and *D. norwegica* comb fibres. No significant difference was found in fibre width of *D. sylvestris* envelope and *D. norwegica* envelope. *V. vulgaris* envelope fibres were, however, significantly wider than both *D. sylvestris* and *D. norwegica* envelope fibres. For all comparisons; T=0.141 at df=24: P<0.05, α=6.

Figure 4.9. The mean fibre width in comb and envelope paper of *D. sylvestris*, *D. norwegica*, and *V. vulgaris* with 95% confidence intervals (calculated from log transformed means and back transformed).



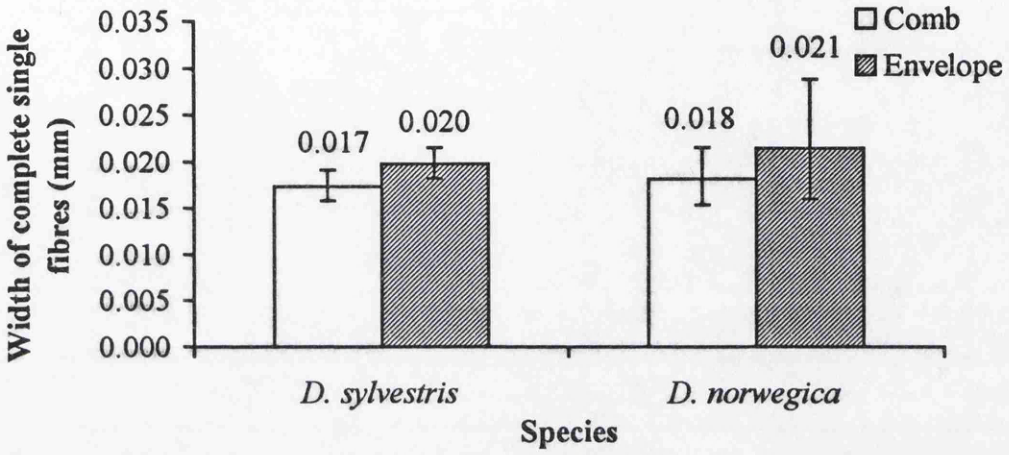
Width of complete single fibres in comb and envelope paper

Results obtained are summarised in Figure 4.10. The results of the ANOVA indicate that there was a significant difference in the width of complete single fibres in comb and envelope (F=7.35 at df 1,16: P<0.05). There was no significant difference in the width of complete single fibres between species (F=1.40 at df 1,16) and no significant interaction (F=0.13 at df 1,19).

The Tukey tests indicated that in both species there was no significant difference between the width of complete single comb and envelope fibres. No significant difference was found between fibre width in *D. sylvestris* comb and *D. norwegica* comb.

No significant difference was found in fibre width of *D. sylvestris* envelope and *D. norwegica* envelope. For all comparisons T=0.094 at df=16: P<0.05, α=4.

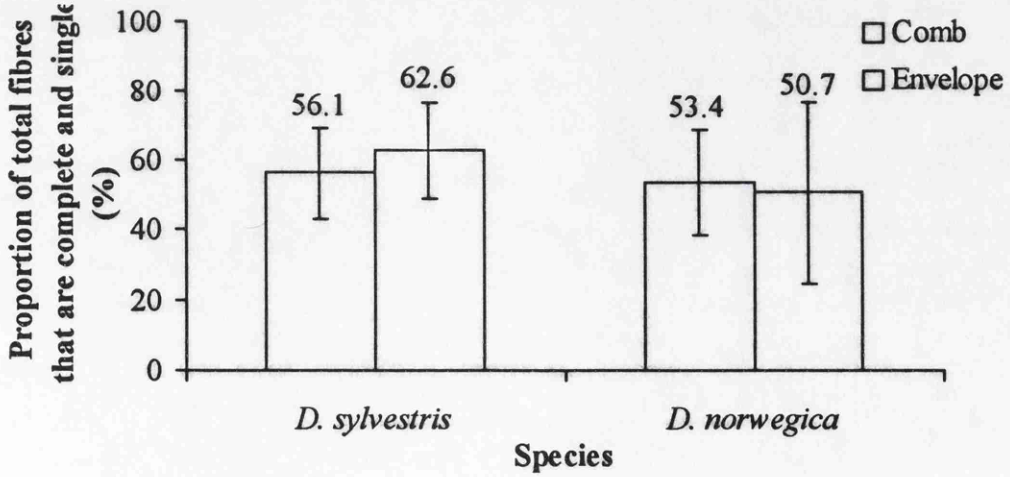
Figure 4.10. The mean fibre width of complete single fibres in comb and envelope paper of *D. sylvestris* and *D. norwegica* with 95% confidence intervals (calculated from log transformed means and back transformed).



Fibre processing

Results obtained are summarised in Figure 4.11. The 2-way ANOVA indicated that there was no significant difference in the proportion (of the total fibres) of complete single fibres between comb and envelope in both *D. sylvestris* and *D. norwegica* (F=4.10 at df 1, 16). There was also no significant difference in the proportion of complete single fibres in comb or envelope between species (F=1.24 at df 1, 16). There was no significant interaction between material and species (F=0.08 at df 1, 16).

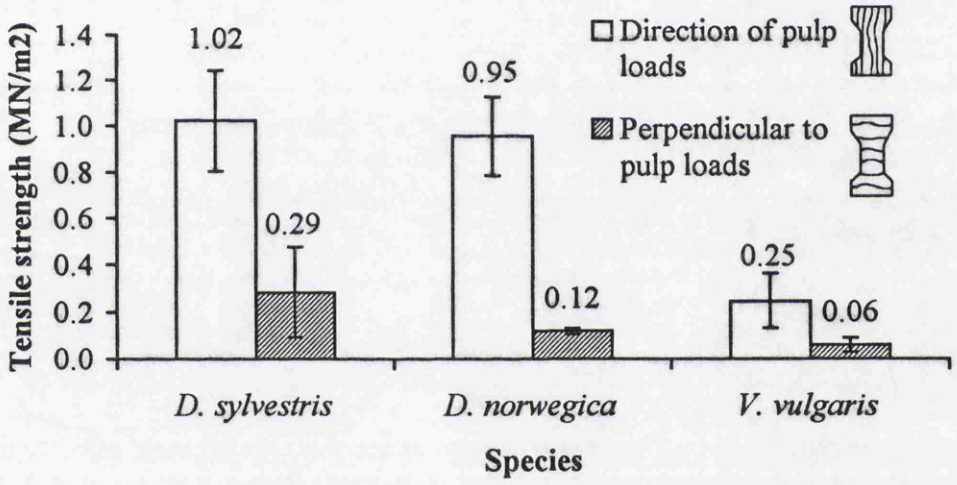
Figure 4.11. The proportion complete single fibres (of total fibres) in comb and envelope paper of *D. sylvestris* and *D. norwegica*, with 95% confidence intervals.



Tensile strength

The results obtained from the tensile strength tests are illustrated in Figure 4.12. The 2-way ANOVA indicated that there was a significant difference between the tensile strength of envelope paper when tested in the direction of the pulp loads, and perpendicular to the pulp loads ($F=184.63$ at df 1,24: $P<0.01$). There was also a significant difference in the tensile strength of envelope between species ($F=48.86$ at df 2, 24: $P<0.01$). A significant interaction was found between test direction and species ($F=21.82$ at df 2, 24: $P<0.01$).

Figure 4.12. The tensile strength of envelope paper (in *D. sylvestris*, *D. norwegica* and *V. vulgaris*) tested in the direction of the pulp loads, and perpendicular to pulp loads, with 95% confidence intervals.



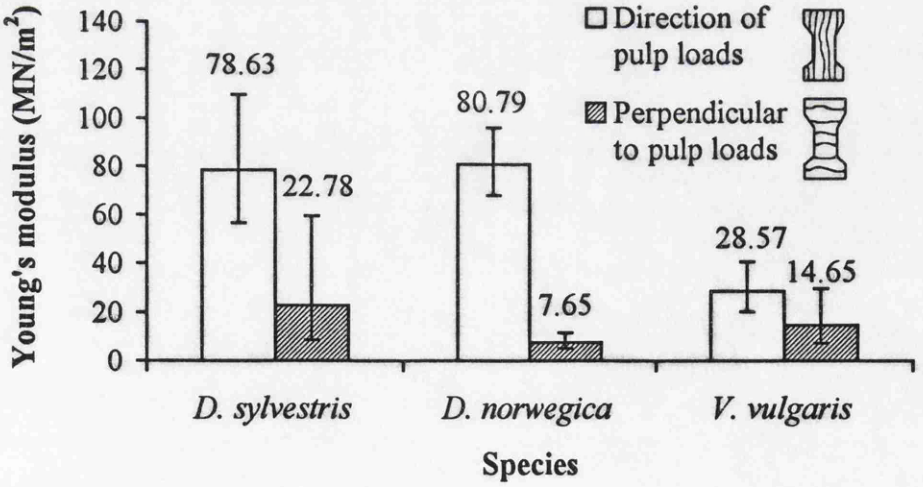
The results of the Tukey tests show that envelope had a significantly higher tensile strength when tested in direction of the pulp loads than perpendicular to the pulp loads in *D. sylvestris* and *D. norwegica*. There was no significant difference in the tensile strength of *V. vulgaris* envelope when tested in the two directions. There was also no significant difference in the tensile strength of envelope tested in the direction of the pulp loads between *D. sylvestris* and *D. norwegica*. *V. vulgaris*, however, had a significantly lower tensile strength in the direction of the pulp loads than both *D. norwegica* and *D. sylvestris*. *V. vulgaris* had a significantly lower tensile strength than *D. sylvestris* perpendicular to the pulp loads. No difference was found between *D. norwegica* and either *D. sylvestris* or *V. vulgaris* in envelope strength perpendicular to the pulp loads. For all comparisons; $T=0.232$ at $df=24$: $P<0.05$, $\alpha=6$.

Young's modulus

The results of Young's modulus tests are presented in Figure 4.13. In the 2-Way ANOVA a significant difference was found in Young's modulus in the direction of the pulp loads and perpendicular to the pulp loads ($F=89.43$ at df 1,24: $P<0.01$). There was a significant difference between species in Young's modulus ($F=5.614$, at df 2, 24: $P<0.05$). A significant interaction was found between test direction and species ($F=8.48$ at df 2, 24: $P<0.05$).

The results of the Tukey tests show that envelope was significantly stiffer in direction of the pulp loads than perpendicular to the pulp loads in *D. sylvestris* and *D. norwegica*. There was no significant difference in *V. vulgaris* envelope when tested in the two directions. There was also no significant difference in Young's modulus of envelope tested in the direction of the pulp loads between *D. sylvestris* and *D. norwegica*. *V. vulgaris* was, however, significantly more flexible in the direction of the pulp loads than both *D. norwegica* and *V. vulgaris*. No difference was found in Young's modulus between any pair of species perpendicular to the pulp loads. For all comparisons; $T=0.231$; at df 24: $P<0.05$).

Figure 4.13. The Young's modulus of envelope paper in *D. sylvestris*, *D. norwegica* and *V. vulgaris* tested in the direction of the pulp loads, and perpendicular to pulp loads. Fitted with 95% confidence intervals calculated from log transformed means and back transformed.



4.4. Discussion

With the information obtained on paper structure and fibre composition we can now address the three principal questions outlined in section 4.1.

Question 1. Are comb and envelope manufactured to the same specifications?

Comb paper was found to be thinner than envelope in all three species, and denser than envelope in *D. sylvestris* and *D. norwegica*. They are therefore manufactured to different specifications in these characteristics.

Differences in paper quality may reflect the structural requirements of comb and envelope. As comb material is strengthened with the addition of a silk lining and the deposition of meconia at the base of the cells, comb paper may be thinner than would otherwise be required. Envelope does not have these secondary structural features and may have to be thicker than comb as it performs weather proofing, defence and thermal insulation functions in the nest.

In *D. sylvestris* and *D. norwegica*, nest paper consists mostly of single plant cells, which are long and thin forming an interwoven mat. Processing of pulp makes fibres more flexible and compliant, allowing them to form around each other (Biermann 1993). The higher paper density of comb material could therefore result from workers spending more time masticating comb than envelope pulp. Although comb fibres are significantly shorter than those of envelope in *V. vulgaris*, this does not have a significant effect on paper density. Mastication is less likely to effect the density of *V. vulgaris* paper, as fibres are in the form of short chips. Additional mastication may shorten fibres and increase their flexibility, but this is unlikely to make them fit together more closely.

Few figures are available for comparison of paper thickness or density in other vespine wasps. McGovern *et al.* (1988) found that the envelope of *D. maculata* had a thickness of 0.114mm, which is thicker than that found in this project for *D. sylvestris* and *D. nowegica*, but similar to that in *V. vulgaris*. Martin (1992) measured the thickness of envelope material in *Vespa affinis* which is of cellular construction. He found that envelope material was slightly thicker in the upper part of the nest than the lower part (1mm and 0.67mm respectively). Matsuura and Yamane (1990) found that envelope paper thickness in embryo nests of hornets ranged from 0.29mm (*Vespa simillima*

xanthoptera) to 0.93mm (*Vespa mandarina japonica*). McGovern *et al.* (1988) found that envelope of *D. maculata* had a density of 0.38 g/cm^3 which is very similar to the density of envelope paper tested in all three species in this project. There are, however, some figures on density of manufactured paper presented in Table 4.2.

Question 2. Are there differences in the fibres from which comb and envelope composed?

Envelope fibres were significantly longer than comb fibres in all three species. There was, however, no significant difference in the width of comb and envelope fibres. There is therefore a difference in the fibres from which comb and envelope paper are composed with respect to fibre length.

The observed difference in fibre length between comb and envelope could result from the collection of pulp from different sources. Alternatively, as chewing is likely to result in fibres being cut, differences could result from comb being masticated longer than envelope pulp. The cause of differences observed in comb and envelope fibre length was examined further in *D. sylvestris* and *D. norwegica* as more information could be obtained on the composition of fibre types. In order to determine if differences observed in fibre length were due to the selection of different fibre types, the length and width of only those fibres which consisted of a single complete plant cell was compared. If comb and envelope fibres were selected from different sources, their fibres would be likely to have significantly different lengths and widths.

In examining complete single fibres only, no significant difference was found in the width of comb and envelope fibres. Comb fibres were, however, significantly shorter than envelope fibres in *D. norwegica*. There was, however, no significant difference in the length of comb and envelope fibres in *D. sylvestris*. This indicates that at least in *D. norwegica*, the difference observed in fibre length results from the selection of fibres from different sources. It may, however, be expected that fibres from different sources would also differ in width.

The proportion of complete fibres in comb and envelope was compared to examine further whether differences in fibre length are also due to processing of pulp. If comb pulp was masticated longer than envelope pulp, envelope paper would be expected to contain a higher proportion of complete single fibres. However, no significant difference

was found in the percentage of fibres that were complete in comb and envelope material. This suggests that differences in fibre length did not result from processing.

Insufficient information was available on the differences between comb and envelope fibres in *V. vulgaris* to draw any conclusions about the cause of differences in fibre length. In *D. sylvestris* and *D. norwegica*, however, it seems likely that differences are at least partly the result of the selection of different fibre sources. Mastication also seems to have some effect on fibre length as the difference observed in comb and envelope fibre length in *D. sylvestris* is not apparent when examining complete single fibres only.

The difference in complete fibre length in *D. norwegica* would suggest that workers collect fibre specifically for comb or envelope construction. Construction preference may be dependent on worker age or they may conduct the same construction task throughout their lives. There is evidence that the type of forage collected by workers changes with worker age, with *V. vulgaris* workers first collecting fluid, pulp then flesh (Potter 1964). More specifically, Akre *et al.* (1976) observed that envelope construction in workers of *V. pensylvanica* began earlier in their life than comb construction. Alternatively, foraging for pulp for the construction of comb and envelope may be regulated by nest ontogeny or environmental factors such as nest temperature and the amount of space available for nest expansion. Potter (1964) found that pulp foraging in captive colonies of *V. vulgaris* was highly dependent on nest temperature. The fate of the pulp was not, however, examined, and it is therefore unclear if nest temperature has a differential effect on foraging for pulp for comb and envelope construction.

More information is therefore required on the manufacture of comb and envelope construction, and on the factors which stimulate pulp selection. It is important to determine if differences in comb and envelope paper are due to differences in pulp selection or processing. In particular it would be useful in future studies to compare the amount of time spent masticating and applying pulp in the production of comb and envelope. This is difficult, however, as comb construction is obscured by the envelope shortly after the first few cells are constructed. Comb construction has been observed in colonies where the envelope has been removed (Akre *et al.* 1976), but workers rebuild the envelope quickly. There is some evidence from work on *V. vulgaris* that if the nest is maintained in the dark at 32°C, workers will reconstruct the envelope very slowly (Potter 1964). As wasps cannot see at the red end of the visible spectrum, it would be possible to examine comb construction in captured colonies under narrow spectrum red light.

It is also important to determine the source of pulp for comb and envelope production. In a nest box situation, captured colonies will build with pulp supplied by the experimenter (Akre *et al.* 1976; Gibo 1977; Mathews *et al.* 1982). Foragers could be offered artificially coloured pulp or different fibre types. The fate of these fibre types could be determined by subsequently dissecting the nest. Identification of wood sources from a microscopic examination of paper fibres could also be useful in determining the origin of pulp for comb and envelope construction. It may be possible to determine the genus of tree from which the fibre was collected. Similar information could also be obtained through a comparison of the chemical composition of comb and envelope fibres.

Question 3. Are there differences between species in the mechanical properties of comb and envelope paper?

Significant interspecific differences were found in the properties of comb and envelope paper. *D. sylvestris* showed many similarities to *D. norwegica* in the physical and mechanical properties of comb and envelope paper. *V. vulgaris*, however, differed in a number of comb and envelope characteristics from *D. sylvestris* and *D. norwegica*.

Envelope paper of *D. sylvestris* and *D. norwegica* was significantly stronger than that of *V. vulgaris*. The short crumb-like fibres of *V. vulgaris* paper produce a material with relatively low tensile strength. It may also be the result of a low amount of saliva matrix and inter-fibre bonding. There is no significant difference in the envelope paper of tensile strengths of *D. sylvestris* and *D. norwegica*. This reflects the fact that there is no significant difference in paper width and density, and fibre length and width between the two species.

Comb and envelope paper in *D. sylvestris* and *D. norwegica* was significantly thinner, and had longer, thinner fibres than in *V. vulgaris*. Envelope paper was also stronger in *D. sylvestris* and *D. norwegica* (in the direction of the pulp loads) than in *V. vulgaris*. Only two differences were found in comb and envelope material between *D. sylvestris* and *D. norwegica*. *D. norwegica* comb paper was significantly thicker than in *D. sylvestris*, and the difference in complete single fibre length between comb and envelope in *D. norwegica* was not found in *D. sylvestris*. In addition, the difference in comb and envelope density found in *D. sylvestris* and *D. norwegica* was not found in *V. vulgaris*. The similarity of paper composition in *D. sylvestris* and *D. norwegica* indicates that they

exhibit a similar behaviour in the selection and mastication of pulp and in paper manufacture. These similarities reflect nest size, colony cycle and nest site preference. Although there are some nest site differences between *D. sylvestris* and *D. norwegica*, both species nest in open or semi-open sites. The long interwoven fibres of envelope paper have strong inter-fibre bonding and are able to withstand wetting. The strength of this material also allows the envelope to withstand the forces exerted by wind and rain in open nest sites. Although pulp collected from sound or slightly weathered wood is more difficult to collect and process than rotten or semi rotten sources, it produces higher quality paper. As *D. sylvestris* and *D. norwegica* have much smaller colonies than *V. vulgaris* and must withstand weather, it is more important to invest in paper quality rather than speed of construction.

The use of rotten and semi-rotten wood by *V. vulgaris* in paper construction leads to short fibres and consequently low tensile strength. As *V. vulgaris* is almost exclusively a cavity nester, the envelope does not have to weatherproof the nest and can be constructed from poorer fibre sources. Edwards (1980) notes that fibre collection in *V. vulgaris* of pulp is much quicker than that in species utilising sound fibre sources, as workers can cut out a single block of rotten wood rather than scraping off single fibres. The soft fibres of rotten wood sources are also more easily worked allowing the nest to be expanded rapidly. The choice of pulp source is therefore a compromise between the rate of nest construction and paper strength.

Envelope was found to be significantly stiffer and stronger in the direction of the pulp loads than perpendicular to that direction in *D. sylvestris* and *D. norwegica*. This difference could either result from a partial alignment of the fibres in the direction of the load of pulp, or of the join between pulp loads. A similar difference in the tensile strength is apparent in manufactured paper. Manufactured paper is normally tested parallel to the direction in which it travels through machinery during manufacture (machine direction) and perpendicular to the machine direction (cross direction).

Material tested in the machine direction tends to be stiffer and stronger than material tested in the cross direction. This is often attributed to fibre orientation, but is more likely to be caused by the paper being under much higher restraint in the machine direction than the cross direction while drying Biermann (1993). This could also be a factor in the differences observed in envelope as the paper is continually worked as it dries. It is, however, more likely to be a property of the joins between pulp loads, as the

difference in tensile strength with direction in envelope paper is much higher than that measured in the machine direction and cross direction in manufactured paper (Table 4.2). The difference in tensile strength in the direction of the pulp loads and perpendicular to that direction is an important feature in nest construction. In *D. sylvestris* and *D. norwegica*, envelope is laid down in near horizontal strips around the circumference of the nest. One of the primary functions of the envelope is insulation. This is achieved by trapping air between layers of envelope. If new envelope was added to the nest in vertical strips, little additional benefit would be gained from each new strip until a complete layer of envelope had been constructed around the nest. As envelope is added in horizontal strips, however, each new pulp load will increase the volume of air enclosed by the envelope. If envelope is stronger in the direction of the layers of pulp the nest would be more able to resist lateral forces. In *V. vulgaris*, the envelope is of a cellular construction arranged in shell-like swirls and does not perform a significant weather proofing function. Air is enclosed in smaller volumes in the aerial chambers and so each additional chamber will increase the insulation provided by the envelope. This type of envelope construction has the structural benefit of allowing the nest to be expanded unevenly to fit the available space in a cavity more closely.

As the difference in tensile strength measured in the direction of the pulp loads and perpendicular to that direction is fairly constant in all three species, it is reasonable to assume that comb paper will also be stronger in the direction of the pulp loads. In comb, cells are enlarged by adding material around the circumference of the cell. Cells would therefore be expected to be stronger in this direction allowing them to resist the tensile load exerted by larvae pushing out against the cell wall as described by Hansell (1984). They would, however, be relatively weak along their length. This method of construction has an important structural benefit. As pulp loads are added around the circumference of the cell, each new pulp load will increase the useable volume enclosed by the cell. The cell can therefore be enlarged as the larvae increase in size. As a beam, however, the cell walls are subject to tension vertically and would have the structural disadvantage of being relatively weak in this direction.

There is little published information on the tensile strength on nest material in social wasps. Hansell (1987), however, has made a comparison of the mechanical properties of *Polistes exclamans* (Polistinae) nest paper with that of *Eustenogaster clayptodoma* (Stenogastrinae). Polistinae nest paper consists of long intact plant fibres, similar to that

of *D. sylvestris* and *D. norwegica*. Stenogastrinae paper is of poorer quality with that of *E. clayptodoma*, consisting of short crumb like fragments which are apparently from rotten wood and is therefore similar to that of *V. vulgaris*. *Polistes exclamans* was found to have a much higher tensile strength (0.679MN/m^2) than that of *E. clayptodoma* (0.128MN/m^2). These figures are very close similar to those found in the present study for *Dolichovespula* and *Vespula* envelope respectively (Table 4.2).

Table 4.2. A comparison of the tensile strength of envelope paper measured in *D. sylvestris*, *D. norwegica* and *V. vulgaris* with that of published figures for manufactured paper (Biermann 1993). Tensile strengths of manufactured paper are tested in the direction in which the paper travelled during manufacture (MD) and perpendicular to that direction (CD).

Material tested	Density g/cm^3	Tensile strength in test direction MN/m^2		Mean MN/m^2	Ratio
		MD or A	CD or B		CD/MD
Filter paper	0.47	1.20	0.78	0.99	0.65
Newsprint	0.68	2.67	2.13	2.40	0.80
Note pad	0.72	5.09	2.05	3.57	0.40
D. sylvestris envelope	0.37	1.02	0.29	0.65	0.28
D. norwegica envelope	0.36	0.95	0.12	0.54	0.13
V. vulgaris envelope	0.42	0.25	0.06	0.15	0.24

It is also interesting to compare tensile strength in wasp paper to that of manufactured paper (Table 4.2). It can be seen that envelope paper of all three species is relatively weak compared to manufactured paper and is most similar in density and tensile strength to filter paper.

In the present study, a limited investigation was performed on the mechanical and physical properties of vespine nest paper. More extensive work is required, in particular to investigate differences in the construction behaviour of comb, envelope and suspensoria. A whole range of industry standard tests for various aspects of paper quality are described for manufactured paper which could be applied to nest material paper such as tests for wettability, and tear resistance (Biermann 1993). The tensile strength of comb material could be tested in future studies with materials testing equipment designed for testing smaller loads and sample sizes. Some machines can be used in zero span tests which can effectively test the strength of individual fibres.

The difference in fibre type in comb and envelope is important in examining the allocation of pulp as a resource to comb, envelope and comb supports. Workers may be

stimulated to forage for pulp after surveying the nest and identifying a need for construction. As workers appear to use the same type of fibre in the production of comb and envelope, however, this would seem unlikely. Alternatively workers may be stimulated to forage for pulp by other factors such as (nest temperature), and evaluate the need for construction of comb, envelope or comb supports on returning to the nest. However, the importance of recycling of materials in the nest is unknown, and following the embryo nest stage, comb may be constructed entirely from material removed from the inside of the envelope. The reprocessing of fibre may account for the differences in fibre length in comb and envelope. It is important then to understand more about the role of material recycling of nest material.

Chapter 5. Regulation of comb support construction.

5.1. Introduction

This chapter will investigate the behavioural regulation of *comb support* construction in *D. sylvestris* and *D. norwegica*. This is achieved through an examination of the distribution of comb supports in nests collected from the field.

The comb of vespine nests function as a *beam* or *cantilever* (Hansell 1984). A beam is a structure, which is supported at both ends but loaded at the centre. A cantilever is a beam, which is supported only at one end. In the nest, the combs hang one below the other supported at several points by comb supports. The comb supports function as *tension struts* as they support the mass of the combs in tension. The mass supported by the comb supports depends on the number and size of the combs suspended below. As the construction of comb supports involves an energetic cost, it is likely that their distribution closely reflects the amount of load they support. It may therefore be expected that the lower combs should have proportionally fewer supports than the higher combs as they support less weight. The first objective of this chapter is therefore to determine if there are differences between combs in the distribution of supports (see **Question 1**).

Various terms are used for comb supports or pillars in the literature. The central support is often referred to as the *petiole* (Edwards 1980; Mathews *et al.* 1982) or *mainstay*. The term *pedicel* is often used to refer to the central support of the first or upper comb (Ross 1982; Reed and Akre 1983; Matsuura and Yamane 1990). Other comb supports are referred to as *auxiliary pillars* (Matsuura and Yamane 1990) or more frequently suspensoria (Greene 1979; Reed and Akre 1983). For the purpose of clarity in this chapter, the central comb support of all combs will be referred to as the mainstay while other supports are referred to simply as *comb supports*.

Combs are initially supported by a central support. As the comb increases in size additional supports or suspensoria are added. *'As the nest grows in size and weight, the central petiole becomes inadequate to support the combs. To strengthen the entire structure, wasps build paper suspensoria between the combs'* (Edwards 1980).

Comb supports are normally constructed at the junction of three cell walls and extended to the comb below. Others are built up from the comb below, but always join a cell wall on the comb above (Edwards 1980). Pulp loads are added in vertical strips.

Occasionally comb supports are constructed over a cell entrance with the eventual entombment of the resident (Spradbery 1973).

Comb supports are either in the form of pillars and referred to as *chord-like* or in the from of thin strips of paper and termed *ribbon-like*. The supports of *Vespa* and of the *V. vulgaris* group are normally chord-like although ribbon-like suspensoria are occasionally observed (Spradbery 1973; Greene 1979; Edwards 1980). The *V. rufa* group, have ribbon-like suspensoria between the first comb and substrate and chord like suspensoria between subsequent combs (Greene 1979; Spradbery 1973; Reed and Akre 1983). In *Dolichovespula* the comb supports are ribbon-like although the mainstay of the first comb is of the pillar type (Spradbery 1973; Makino 1982). This type of pillar is considered to be a characteristic of the *Dolichovespula* genus (Matsuura and Yamane 1990).

The predominance of chord-like comb supports in the *Vespula*, and ribbon like supports in *Dolichovespula* is probably related to their nesting habit. Nests of *Dolichovespula* are predominantly aerial (Matsuura and Yamane 1990) and are subject to the weather and hence movement of the substrate to which they are attached. Ribbon like supports are flexible which may make them more able to withstand relative movement of the combs. *Vespula* species are predominantly cavity nesters (Matsuura and Yamane 1990) and the rod like supports are rigid and would tend to fail with twisting of the combs.

Constructing several comb supports rather than one central support, also helps to distribute the weight of the comb more evenly hence preventing it from tilting. This is important in aerial nesting species where the nest is subject to movement. It is also important in cavity nesters where the nest may be prevented from expanding evenly which results in the mainstay becoming off centre. Without additional supports the comb would tilt.

Supporting the weight of the comb across several tension struts may be more efficient than with one central strut because of the weight penalty of the end fittings (Hansell 1984). The load supported by a material in tension is simply proportional to its cross sectional area. The forces on the end fittings are, however, more complex as a result of stress concentrations. The end fittings can therefore form a high proportion of the weight of a structure in tension. It can be shown that the weight of the end fittings of two tension struts operating in parallel is less than that of the end fittings of a single tension strut of equivalent cross sectional area. Although the cross sectional area of a

tension strut is proportional to the load, the volume of the end fitting increases as the power 3/2 of the load (Gordon 1991b). Although this consideration is likely to be applicable to the cord-like supports of *V vulgaris*, it would seem unlikely that this would apply to the ribbon like supports of *D. sylvestris* and *D. norwegica.*

Occasionally comb supports are constructed that connect to the envelope rather than the comb above (Spradbery 1973). These attachments, however, are unlikely to form additional support for the combs, as envelope material is relatively weak in the direction of this load (Chapter 4). It is more likely that these serve as anchorage and support for the envelope. In aerial nesters, the envelope will be subject to forces from wind and rain. The envelope is stronger in the horizontal direction in which the pulp loads are constructed, and is relatively weaker in the vertical direction (Chapter 4). The envelope attachments may serve to transfer some of the load of the envelope to the combs.

Little is known about the composition of the comb supports. MacDonald *et al.* (1975) noted that the suspensoria of *Vespula atropilosa* consisted of paper similar to that of envelope, but heavier and consisting of vertical strips. Spradbery (1973) notes that comb supports are always made of more solid, inflexible carton than the cells or envelope and that the pulp is very compacted without gaps or spaces. Edwards (1980) implies that the paper used in the manufacture of suspensoria is exclusively recycled from envelope or old cells. Silk may also be used in the manufacture of supports (Edwards 1980; Matsuura and Yamane 1990). Silk has a high tensile strength (Gordon 1991a), and would considerably strengthen the comb supports without substantially adding to their weight. In nests of *Dolichovespula* species, comb supports are occasionally constructed entirely from pupal caps (i.e. larval silk, personal observation). The pupal cap, which is normally removed from the cell following emergence, is opened up leaving an attachment at one side. The other end of the cap is then fixed to the comb below.

Comb supports may also differ from comb and envelope paper in the content of saliva matrix. The mainstay and other comb supports often have a shiny or rubbery appearance resulting from the use of oral secretions in their manufacture or subsequently added. This is particularly noticeable in the mainstay of the queen nest. Jeanne (1977) noted that the queen nest of *D. arenaria* and *D. maculata* is coated in labial gland secretion. He proposed that the function of this secretion was to allow the petiole to twist and stretch as the queen moves around the comb. This secretion is seemingly absent,

however, in the mainstay of embryo nests of *Vespula* and therefore probably functions to stabilise the nest in aerial nesters (Matsuura and Yamane 1990).

According to the 'stigmergy' hypothesis of Grassé (1959), social insects do not inherit a blue print or plan of the final nest, instead previous construction forms the cue for new construction (see general introduction). The purpose of this chapter is to determine the cues used by workers to regulate the construction of comb supports.

It is likely that workers use relatively simple cues in regulating nest construction. Although the amount of comb supports constructed may be related to the size of comb supported for example it is unlikely that workers would assess the surface area of all the combs supported before adding each load of pulp to the comb supports. This was suggested by Spradbery (1973), *'The number of such pillars is variable but their quantity and robustness are generally related to the area of comb to be supported below them so that there are more pillars per unit area in the upper compared to the lower combs'*. Although Spradbery (1973) based this statement on unpublished data, no quantitative information is available in the literature to support it. One of the objectives of this chapter is therefore to determine if there are differences in the density of supports between combs **(Question 1)**. *D. norwegica* and *D. sylvestris* have very similar nest site preferences and lifecycle. They should therefore have a similar distribution of mass in the nest. Question one will also compare the density of comb supports between species to determine if they have similar behavioural regulation of support construction

The chapter will then determine the behavioural rules, that result in the differences observed between combs. If the density of supports constructed is related to the amount of combs supported, then workers could use a cue resulting from a change in the mass or size of combs supported. **Question 2** will determine if workers are using a cue resulting from comb size, or a cue resulting from comb mass in regulating the construction of comb supports.

In adding comb supports, workers must decide whether to extend existing supports, or construct new supports. If workers constructed comb supports to a threshold length, then in adding a pulp load they would have to survey the length of surrounding supports. The third objective of this chapter is therefore to determine if differences observed in total support length between combs, are due to variation in the mean length, or number of supports constructed (see **Question 3**).

The comb supports should be constructed such that the load is supported evenly across the comb to avoid tilting and failure of the structure. The cue for the construction of comb supports may arise from an increase in the size or mass of the combs supported. In the placement of constructing comb supports wasps may take cues from neighbouring supports. Workers may therefore construct new supports at a minimum distance from neighbouring supports. The mass or size of the combs suspended may regulate this distance threshold. Comb supports could therefore be constructed in response to a change in the combs supported, but could be evenly distributed. In **Question 4** it will be examined if wasps use a cue resulting from differences in the size or mass of combs suspended in regulating the distance between supports.

Alternatively workers may not use a cue arising from the mass or size of comb suspended. Comb supports could be evenly spaced simply by maintaining a minimum distance between new supports and their neighbours. If supports were constructed at a minimum distance from neighbouring supports, they would show a non-random spacing. Alternatively if workers did not take cues from neighbouring supports, they would be randomly spaced with respect to each other. The final objective of the chapter is therefore to determine if the spacing between supports is random or regular (see **Question 4**).

Comb supports were examined in nests of *D. sylvestris* and *D. norwegica*. These species were selected as a large number of nests were collected for colony statistics and nest morphometrics. As these species exhibit many similarities in nesting habit, lifecycle and nest structure, it would therefore be expected that they would have similar regulation of nest construction.

The principal questions to be addressed in this chapter are as follows:

1. Are there differences in the distribution of supports between combs and between species?

2. What factors regulate the quantity of comb supports constructed?

3. Does the variation in the total length of comb supports result from differences in the length or number of comb supports?

4. Are comb supports randomly spaced or regularly spaced?

The first question will be answered through a comparison of the density of supports between combs and between species. The second question will be answered by explaining the variation in the total length of comb supports in terms of various morphometric characteristics of the comb. The third question will be addressed by explaining the variation in total length of comb supports in terms of the number and mean length of supports. The final question will be answered by comparing the distribution observed in the comb with that of a random distribution.

5.2. Methods

General methods

Information on comb supports and comb surface area was recorded at the same time as nest morphometrics and colony information. The combs were separated by carefully cutting the comb supports from the comb above using a scalpel and fine dissecting scissors. To determine the surface area of combs, they were placed on a piece of paper with the cell opening facing downwards and traced around with a pencil. From these tracings the surface area of the comb was determined with a BBC microcomputer fitted with a digitiser. To avoid replication, the position of the comb supports was noted on the traced comb outlines. Lengths and distances were measured with a vernier calliper accurate to 0.1mm.

The following information was recorded from each comb:

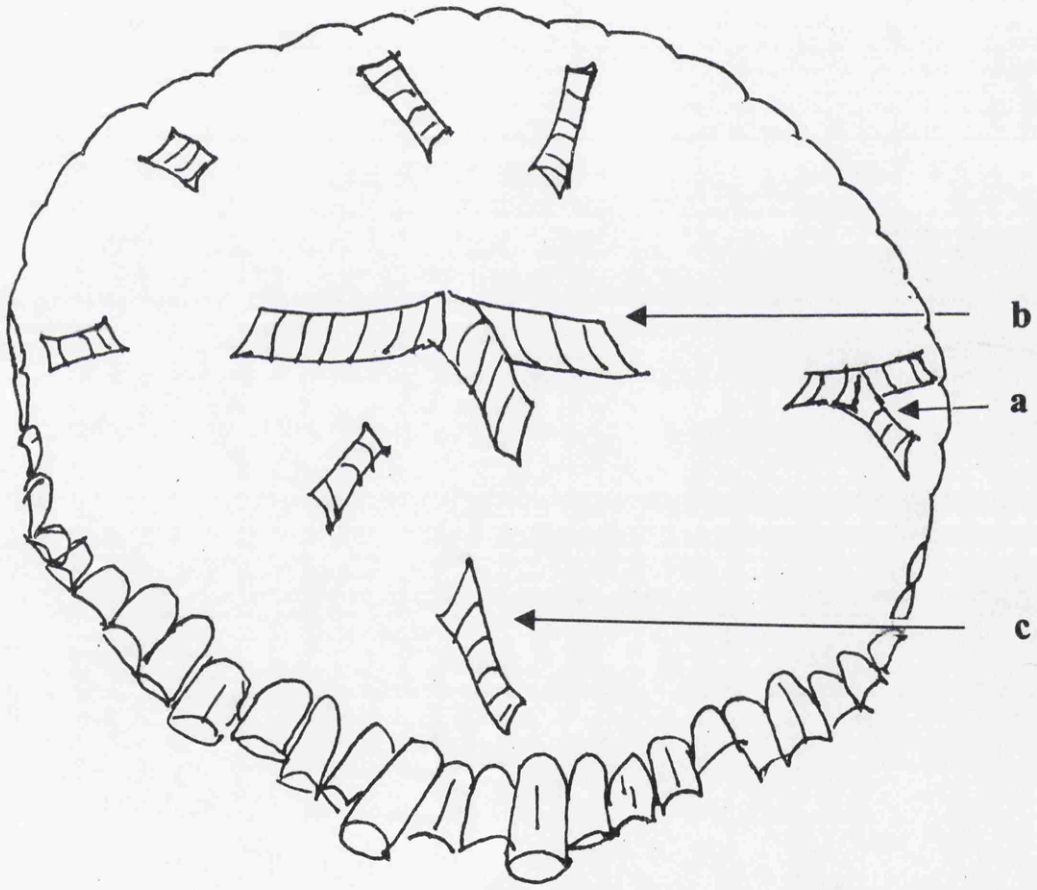
a. The surface area of the comb (mm)
b. The length of each comb support (mm). Where comb supports were curved their length was estimated by taking several smaller measurements with the callipers.
c. The distance from each comb support to its nearest neighbour, 'NN1' (mm).
d. The distance from each comb support to the second nearest neighbour, 'NN2' (mm).

In addition the following data on colony statistics were used:

a. The number of cells in each comb.
b. The number of adults which had been reared from each comb. This was estimated from the number of meconia (faecal pellets) voided at the base of each cell.
c. The total number of brood in each comb. This included eggs, larvae and pupae.

Comb supports consist either as single strips (Figure 5.1c), or as a complex of several supports joined (Figure 5.1a). For nearest neighbour analysis these support complexes were treated as single supports. For all other comparisons, the components of support complexes were considered as separate supports. No distinction was made between the mainstay (Figure 5.1b) and other comb supports.

Figure 5.1. The upper surface of a typical large comb of *D. sylvestris* or *D. norwegica*. **a.** two adjoining comb supports, **b.** mainstay with adjoining comb supports, **c.** single comb support. The typical comb of a nest of *D. sylvestris* and *D. norwegica* is approximately 100 to 150 mm in diameter.



1. Are there differences in the distribution of supports between combs and between species?

If the distribution of the suspensoria were proportional to the amount of load that they support there would be a difference in the distribution of supports between combs. This question was addressed by a comparison of the density of supports between combs. The density of the comb supports was simply the mean length of comb support per unit area

of comb (mm/mm^2 or mm). The density of supports was compared between the first four combs in 51 nests of *D. sylvestris* and 42 nests of *D. norwegica*. The number of combs compared of each species is presented in Table 5.1. Although several nests had a fifth comb, their density was not included due to the small sample size.

Table 5.1. The number of combs of different positions in the nest, used to compare comb support density between positions and between species. Taken from 51 nests of *D. sylvestris* and 42 nests of *D. norwegica*

Position	Number of combs	
	D. sylvestris	*D. norwegica*
Comb 1	51	42
Comb 2	47	42
Comb 3	38	37
Comb 4	10	28

2. What factors regulate the quantity of comb supports constructed?

The variation in the total length of comb supports was explained in terms of seven morphometric characteristics of the comb in 51 nests of *D. sylvestris* and 42 nests of *D. norwegica* (Table 5.1). If the variation in the total length of comb supports is explained mainly by the surface area of the comb, directly or indirectly supported, then it is likely that the construction of comb supports is regulated by comb size. If, however, the variation is explained by the number of brood reared in the cells directly or indirectly supported, this would suggest that the construction of comb supports is mainly regulated by cues arising from the mass of comb supported. The morphometric characters were as follows;

a. The surface area of the comb directly supported (measured in mm^2)
b. The total brood reared in the comb directly supported (estimated from the number of meconia at the base of the cells).
c. Total brood in the comb directly supported (including eggs, larvae and pupae)
d. Total surface area supported (comb directly supported plus subsequent combs measured in mm^2).
e. Total brood reared in comb supported directly and subsequent combs

f. Total cells below (in comb supported directly and subsequent combs)

g. Total brood below (eggs, larvae and pupae in comb supported directly and subsequent combs)

3. Does the variation in the total length of comb supports result from differences in the length or number of comb supports.

In adding comb supports, workers can either elongate existing supports or construct new ones. Variation in the length of comb supports could therefore either result from an increase in the number or mean length of comb supports. The variation in the total length of comb supports was explained in terms of the number of comb supports on each comb and their total length in 51 nests of *D. sylvestris* and 42 nests of *D. norwegica* (Table 5.1).

4. Are comb supports randomly spaced or regularly spaced?

Distance to first nearest neighbour

The spread of the comb supports was examined by looking at the distance from supports to their first nearest neighbour. Variation in the distance to first nearest neighbour was explained in terms of the seven comb morphometric characters listed. Workers may construct comb supports at a minimum distance from the nearest neighbour. If workers use a cue resulting from changes in comb size or mass, then one or more of the predictor variables should explain a large amount of the variation in distance to nearest neighbour.

Nearest neighbour ratio

The ratio between the distance to the first nearest neighbour and the distance to the second nearest neighbour was examined, to determine if the suspensoria are constructed with a random distance between them, or with at a minimum distance from the nearest neighbour. The nearest neighbour ratios of combs one and two, and of 5 nests of each species (selected randomly) were pooled. The nearest neighbour ratio of a total of 147 comb supports of *D. sylvestris* and 127 comb supports of *D. norwegica* were compared. The mean and variance of the nearest neighbour ratio was measured from the comb supports and compared to that in a random scatter of 50 points. If the comb supports are regularly spaced, the data will have a narrow variance and the mean of the neighbour ratio will approach one. The mean and the variance would be expected to be

significantly different to that of a random scatter. If alternatively the distance between the comb supports is irregular the neighbour ratio will have a larger variance and a smaller mean. The mean and variance would not be expected to be significantly different from that of a random scatter.

Many methods of nearest neighbour analysis generally compare observed measurements to nearest neighbours with a density dependant estimate of expected distance to nearest neighbours. Most of these are based on the model of Clark and Evans (1954). This is particularly useful when examining spatial dispersion of objects which are relatively small in comparison to the area in which they exist such as bird nests or with easily definable centres such as ant nests. They are, however, impractical for examining the spacing of comb supports in *Dolichovespula* species as they are generally in the form of long thin strips and as such have no easily discernible centre. In addition, when constructing comb supports workers could construct new suspensoria at a minimum distance from existing suspensoria. They are unlikely, however, to use the centre of the comb supports as a reference.

The ratio between the first and second nearest neighbour is more versatile and does not involve comparisons with density estimates (Pontin 1997). The ratio of the first nearest neighbour to the second nearest neighbour is compared to that in a random scatter of points.

Statistical analysis

For analysis of variance, multiple regression, and product moment correlation the normality of data was checked graphically with a frequency histogram prior to analysis. Most data was normalised by square root or cube root transformation. Nearest neighbour ratios were arcsine transformed, as both tails of proportion distributions were truncated (Fowler and Cohen 1996).

Most analysis of variance was of two-way with replication. Nearest neighbour ratios were, however, compared with one-way analysis of variance. As all designs were unbalanced (i.e. unequal numbers in each group, see Table 5.1), a general linear model was fitted as suggested in Sokal and Rholf (1995). The heteroscedasticity of the data was checked using the F_{max} test (Fowler and Cohen 1996). Differences between means were located using the Tukey-Krammer method for unequal sample sizes (Sokal and Rholf 1995). The means of the untransformed data were presented as histograms

together with the 95% confidence interval calculated from transformed data and back transformed.

Multiple regression analysis was used to explain the variation in the length of comb supports, and the distance to the first nearest neighbour, in terms of the seven, predictor variables. Multiple regression was also used to explain the variation in comb support length in terms of the total number and mean length per comb. Analysis was conducted using the MINITAB (Version 10.1) statistical package. The overall significance of the multiple regressions were tested by analysis of variance. The significance of partial regression coefficients was determined by T-tests.

Multiple regression is based on Model I techniques and therefore assumes causality between the dependent variable and the predictor variables. Model II methods for handling simple linear regression have not yet been developed for multivariate comparisons. As this assumption was not met in this case caution must be exercised in interpreting the results (Sokal and Rohlf 1995).

Nearest neighbour analysis

The variance and mean of the first to second nearest neighbour in *D. sylvestris* and *D. norwegica* were compared to that of a random scatter of 50 points. The random scatter was generated from 100 random numbers with a Microsoft Excel spreadsheet. Half of these points were used for the X co-ordinates and half for Y co-ordinates of the random scatter. The distance of each of these points to their first and second nearest neighbour was calculated.

The variance of the ratio of the first to second nearest neighbours in *D. sylvestris* and *D. norwegica* was compared to the ratio measured in a random scatter of 50 points using the F-test (two tailed, Fowler and Cohen 1996). This was conducted on the untransformed ratios.

The mean of the nearest neighbour ratios of *D. sylvestris* and *D. norwegica* were compared to those of a random scatter by one way analysis of variance with replication (general linear model). The nearest neighbour ratios of the first and second comb, and of five nests, were compared in each species by two-way analysis of variance with replication. The ratio of the first to second nearest neighbour (untransformed data) was presented graphically as a scattergram fitted with a model II regression line to illustrate

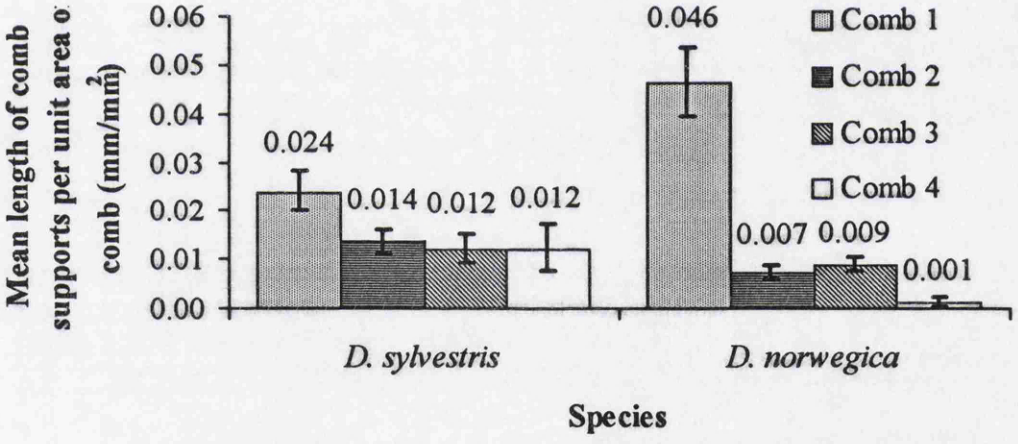
the functional relationship between the two. These lines were fitted with a 95% confidence zone (Sokal and Rohlf 1995).

5.3. Results

Question 1. Are there differences in the distribution of supports between combs and between species?

The results obtained are summarised in Figure 5.2. The two-way ANOVA indicated that there was a significant difference in the density of supports between combs ($F=77.54$ at df 287, 3: $P<0.01$) and between species ($F=5.68$ at df 1, 287: $P<0.01$). There was a significant interaction between species and combs ($F=23.01$ at df 3,287: $P<0.01$).

Figure 5.2. A comparison of the density of comb supports between combs and between species with 95% confidence intervals (calculated from square-root transformed means and back transformed).



The Tukey test indicated that comb one had a significantly higher density of comb supports than combs two, three and four in both *D. sylvestris* and *D. norwegica* ($T=0.038$, 0.044, 0.046 and $T=0.013$, 0.120 and 0.180 respectively for df=287: $P<0.05$, $\alpha=8$). In *D. sylvestris* there was no significant difference in comb support density between any of the other three combs (C2 vs C3, C2vs C4 and C3 vs C4: $T=0.052$, 0.019 and 0.017 at df=287: $\alpha=8$). In *D. norwegica* there was no significant difference between comb two and three ($T=0.016$ at df=287: $P<0.05$, $\alpha=8$). Comb four, however, had a significantly lower density of comb supports than comb two and three ($T=0.049$, 0.058, at df=287: $P<0.05$, $\alpha=8$).

D. norwegica had a significantly higher density of supports on comb one than *D. sylvestris* (T=0.060, df=287: P<0.05, α=8). *D. sylvestris*, however, had a significantly higher density of supports on combs two and four than *D. norwegica* (T=0.031, 0.072 df=287: P<0.05, α=8). There was no significant difference in the density of comb supports on comb three between *D. sylvestris* and *D. norwegica* (T=0.016 at df 287, a=8).

Question 2. What factors regulate the quantity of comb supports constructed?

The variation in the total length of suspensoria on each comb (Y) was explained with the seven predictor variables (X_1 to X_7 listed below);

Y. Square-root of total length of comb supports on comb (measured in mm)

X_1. Square-root of the surface area of the comb directly supported (measured in mm^2)

X_2. Square-root of the total brood reared in the comb directly supported (estimated from the number of meconia at the base of the cells).

X_3. Square-root of the total brood in the comb directly supported (including eggs, larvae and pupae)

X_4. Square-root of the total surface area supported (comb directly supported plus subsequent combs measured in mm^2).

X_5. Cube-root of the total brood reared in comb supported directly and subsequent combs

X_6. Cube-root of the total cells below (in comb supported directly and subsequent combs)

X_7. Cube-root of the total brood below (eggs, larvae and pupae in comb supported directly and subsequent combs)

In *D. sylvestris*, the multiple regression was found to be highly significant (F=78.56, P<0.01, d.f. 7,140). The coefficient of multiple determination was 0.787 (adjusted). Therefore an estimated 79% of the variation in total length of comb supports can be explained by the seven predictor variables.

Regression equation:

$$\textit{Total length of comb supports (SQRT)} = -2.11 + 0.0017 X_1 + 0.296 X_2 - 0.056 X_3 + 0.0149 X_4 - 0.469 X_5 + 1.39 X_6 + 0.005 X_7$$

Only two partial regression coefficients were found to be significant in explaining the variation in total length of comb supports: X_2, the square-root number of the total brood

reared in the comb directly supported (t=2.10, P<0.01, n=150); and X_5, the square-root of the cells in all the combs supported (t=3.21, P<0.05, n=150).

In *D. norwegica*, the multiple regression was also found to be highly significant (F=35.65, P<0.01, df=7,151). The coefficient of multiple determination was 0.625 (adjusted). It is therefore estimated that 62.5% of the variation in comb support length was explained by the seven predictor variables.

Regression equation:

$$\textit{Total length of comb supports (SQRT)} = -44.0 - 2.93 X_1 + 26.3 X_2 - 0.85 X_3 + 1.57 X_4 - 55.1 X_5 + 33.6 X_6 - 7.1 X_7$$

Four of the partial regression coefficients were found to be significant, X_1, the surface area of the comb directly supported (square-root transformed) and X_2, number of brood reared in the comb directly supported (square-root transformed) (t=3.27, 3.71, P<0.01, n=151), and X_4, the total surface area of combs supported (cube-root transformed) and X_5, total brood reared in all the combs supported (cube-root transformed) (t=2.05 P<0.05, t=3.11 P<0.01, n=151).

Question 3. Does the variation in the total length of comb supports result from differences in the length or number of comb supports.

In *D. sylvestris* the multiple regression was found to be highly significant (F=2379.73, P<0.01, df=2,145). The coefficient of multiple determination was 0.97 (adjusted). It is therefore estimated that 97.0% of the variation in total comb support length (square-root transformed) was explained by the number of comb supports (square-root transformed) and the mean length of comb supports.

Regression equation:

$$\textit{Total length of comb supports (SQRT)} = -3.50 + 0.367\ \textit{Mean length of comb supports} + 3.15\ \textit{Total number of comb supports (SQRT)}$$

Both mean comb support length and total number of comb supports were found to be significant in explaining the variation in total length of comb supports (t=24.31, 50.80, P<0.01, n=145, se=0.015, 0.620).

In *D. norwegica* the multiple regression was also found to be highly significant (F=5653.58, P<0.01, df = 2, 156). The coefficient of multiple determination was 0.986

(adjusted). It is therefore estimated that almost 99% of the variation in total comb support length (square-root transformed) was explained by the number of comb supports (square-root transformed) and the mean length of comb supports.

Regression equation:

Total length of comb supports (SQRT) = - 3.28 + 0.246 *Mean length of comb supports* + 3.71 *Total number of comb supports (SQRT)*

Both mean comb support length and total number of comb supports were found to be significant in explaining the variation in total length of comb supports (t=24.67, 87.12, P<0.01, n=156, se=0.01, 0.04).

Question 4. Are comb supports randomly spaced or regularly spaced?

Distance to first nearest neighbour

In *D. sylvestris* the multiple regression was also found to be highly significant (F=4.18, P<0.01, df=7,68). The coefficient of multiple determination, however, was 0.229 (adjusted). It is therefore estimated that only 22.9% of the variation in distance to first nearest neighbour was explained by the 7 predictor variables.

Regression equation:

$$\textit{Mean distance to first nearest neighbour} = 0.42 + 0.225 X_1 - 0.360 X_2 - 0.186 X_3 - 0.0720 X_4 + 0.0756 X_5 + 0.01 X_6 + 0.310 X_7$$

Of the seven partial regression coefficients only the surface area of the comb directly supported was found to be significant in explaining the variation in nearest neighbour distance (t=4.19, P<0.01, n=68).

In *D. norwegica* the multiple regression was also found to be highly significant (F=3.77, P<0.01, df=7, 66). The coefficient of multiple determination was 0.21 (adjusted). It is therefore estimated that only 21% of the variation in distance to first nearest neighbour is explained by the seven predictor variables.

Regression equation:

$$\textit{Mean distance to first nearest neighbour} = -16.0 + 0.277 X_1 - 0.892 X_2 - 0.171 X_3 - 0.254 X_4 + 2.99 X_5 + 5.49 X_6 + 2.27 X_7$$

Of the seven partial regression coefficients only the surface area of the comb directly supported and the total surface area of combs supported were significant (t=3.06, -2.53, P<0.01, n=66).

Nearest neighbour ratio

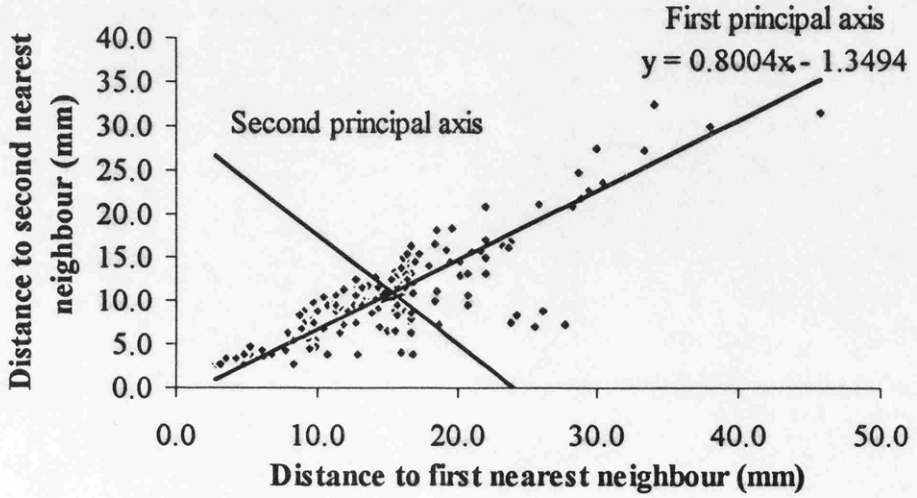
Table 5.2. Comparison of the variance of the first to second nearest neighbour in comb supports of *D. sylvestris* and *D. norwegica* (combs one and two) with an analysis of random scatter. NN1 is the distance from a comb support to its nearest neighbour, NN2 is this distance but to the second nearest neighbour.

	Number of comb supports	Mean NN1/NN2	Degrees of freedom	P<0.05 F-test (2-tail)
D. sylvestris	144	0.723	49, 143	NS
D. norwegica	127	0.693	49, 126	NS
Random scatter	50	0.744	-	-

There was no significant difference between the variance of the ratio of first to second nearest neighbour from that of the analysis of random scatter in both *D. sylvestris* and *D. norwegica* (Table 5.2).

A comparison of the distance from comb supports to the first nearest neighbour with the distance to the second nearest neighbour shows a strong positive linear relationship in both *D. sylvestris* and *D. norwegica* (figures 5.3 and 5.4 respectively).

Figure 5.3. The relationship between distance to second and first nearest neighbours in supports of comb one and two in *D. sylvestris* (pooled from 5 nests). Fitted with a Model II regression line, with 95% confidence zone. (r=0.86, P<0.01, df=142)



As there was no significant difference between nests and between combs, the nearest neighbour ratios of the two combs and five nests in each species were pooled. The one way ANOVA showed no significant difference between the mean ratio of first to second nearest neighbour (arcsine transformed) of the analysis of random scatter for either *D. sylvestris* or *D. norwegica* (F=2.06 at df 2, 318: see Figure 5.5).

Figure 5.4. The relationship between distance to second and first nearest neighbours in supports of comb one and two in *D. norwegica* (pooled from 5 nests). Fitted with a Model II regression line, with 95% confidence zone (r=0.91, P<0.01, d.f.=124)

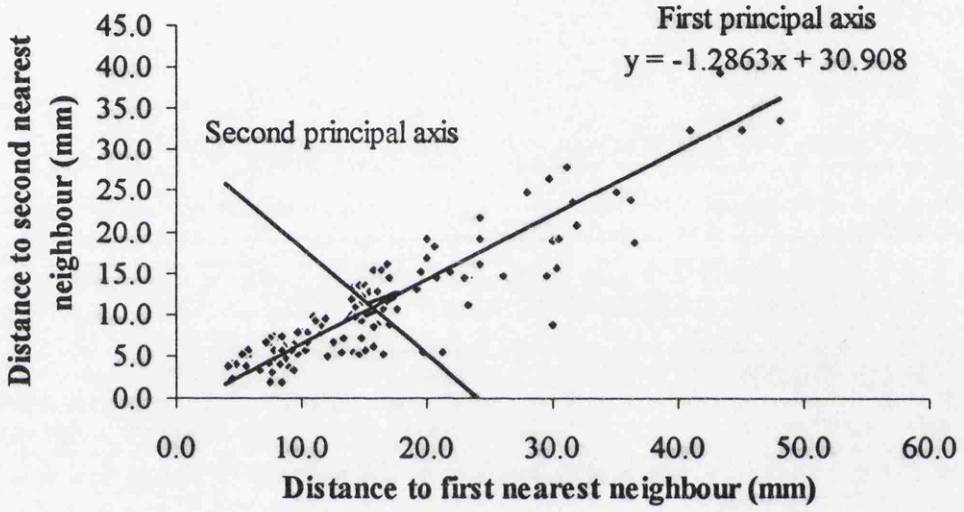
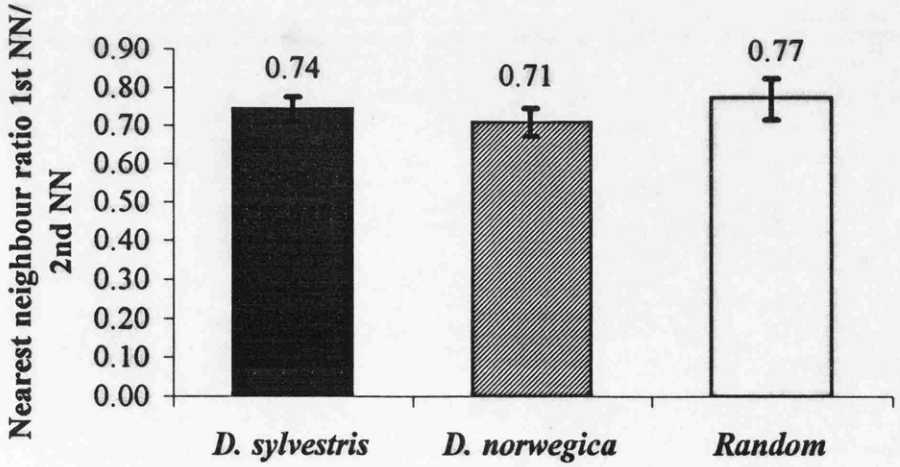


Figure 5.5. The mean ratio of first to second nearest neighbours of comb supports in combs one and two of nests of *D. sylvestris*, *D. norwegica* and an analysis of random scatter with 95% confidence intervals (calculated from the arcsine transformed means and back transformed).



5.4. Discussion

The following section will address in turn the questions on the regulation of comb support construction outlined in the introduction.

Question 1. Are there differences in the distribution of supports between combs and between species?

The upper comb in both *D. norwegica* and *D. sylvestris* was found to have a higher density of supports than the lower combs. This finding agrees with the claim of Spradbery (1973) that; *'there are more pillars per unit area in the upper compared to the lower combs'*. There were, however, no significant differences in the density of comb supports between the lower combs in *D. sylvestris*, and no difference between the second and third comb in *D. norwegica*. This does not appear to support the claim of Spradbery (1973), that the quantity of supports constructed is related to the area of comb supported below them.

There was also a significant difference in the density of comb supports between species. *D. norwegica* was found to have a significantly higher density of comb supports on the first comb than *D. sylvestris*. *D. sylvestris*, however, was found to have a significantly higher density of comb supports on comb two and four than *D. norwegica*. This may result from a difference between species in the distribution of mass between combs. This could also be explained, however, by a difference in the behavioural rules for the construction of comb supports between these species.

Question 2. What factors regulate the quantity of comb supports constructed?

The comb morphometric characteristics were significant in explaining a large amount of the observed variation in comb supports length in both *D. sylvestris* and *D. norwegica*. In both *D. sylvestris* and *D. norwegica*, the number of brood reared in the comb directly supported were significant in explaining the variation in total length of comb supports. The number of adults reared in the comb directly supported is likely to give a reliable indication of the maximum mass of the combs during colony development. The construction of comb supports may therefore be regulated by a cue resulting from a change in comb mass. Various cues could result from a change in mass. It could, for example, result in a change in the distance between combs or between the upper comb

and substrate. Alternatively, an increase in the mass of comb suspended could result in a change in the vibration or movement of the comb caused by the worker moving across the comb, as suggested by Downing and Jeanne (1990).

It was found, however, that several other predictor variables were significant in explaining the variation in the total length of supports constructed. In *D. sylvestris*, the total number of cells supported was also significant. In *D. norwegica*, the surface area of the combs both directly and indirectly supports were significant as well as the number of brood reared in the comb directly supported. As several of the predictor variables were significant in explaining the variation in the length of supports between combs, it is difficult to draw any conclusions about the cue used by workers in constructing comb supports. However, the predictor variables explained a large amount of the variation in the total length of comb supports. As all variables result form a change in the amount of comb both directly and indirectly supported, it would appear that workers are using a cue arising directly from a change in comb size.

In *D. sylvestris*, the total surface area suspended and the surface area of the comb directly suspended, did not significantly explain the variation in comb support length. Workers are therefore unlikely to use cues resulting directly from an increase in comb size in the construction of comb supports.

The total brood present in the comb directly suspended and the total combs suspended, did not significantly explain the variation in the total length of comb supports in *D. sylvestris* and *D. norwegica*. The total brood present in the comb suspended directly or indirectly is likely to give a good indication of the current mass of the comb. As there is no evidence that workers remove comb supports when comb use decreases then this would be expected to explain very little of the variation. Similarly the total number of cells supported was not significant in explaining the variation in either species. The number of cells is unlikely to form a direct cue for the construction of comb supports as it would not provide a cue which is readily assessed by workers.

Although there is no quantitative evidence available on the regulation of comb support construction in vespine wasps, some work has been conducted on Polistine wasps. The effect of comb mass on the number of comb supports constructed was investigated in *Polistes fuscatus* by Downing and Jeanne (1990). They investigated this effect experimentally by adding extra weight to combs. When additional load was added centrally, it did not stimulate the construction of additional supports. Similarly adding

weight to the comb off-centre did not stimulate the construction of secondary supports unless the comb tilted and came within 3-4mm from the substrate. They did, however, find that the number of additional comb supports constructed in nests collected from the field was significantly related to the total number of cells in the comb.

Although these findings appear to contradict those in the present study, Downing and Jeanne (1990) did not investigate the relationship between comb size or mass and the number of comb supports constructed. As the number of cells is likely to be highly correlated with comb size, it would be expected that the number of cells would also significantly explain the variation in the number of supports constructed. In the present study as partial regression coefficients were calculated the number of cells was held constant.

Question 3. Does the variation in the total length of comb supports result from differences in the length or number of comb supports?

The variation in total length of the comb supports was explained by both an increase in their number and mean length. In constructing new comb supports, workers can lengthen existing supports, adjoin new supports to existing supports or construct new free-standing supports. Abutting new supports to existing supports can, however, be regarded as another way of extending them. The cues which regulate the decision to lengthen existing supports, or construct new supports, are unknown. It is more energetically costly to extend existing supports rather than construct new supports because of the weight penalty of the end fittings (Hansell 1984). Workers may therefore construct supports to an optimum length above which it is more energetically economical to construct new supports.

The cue for the construction of comb supports appears to arise from an increase in comb size and in *D. norwegica* from comb mass. When workers have decided to construct additional supports they may use cues from existing construction. Supports may be constructed maintaining a minimum distance to immediate neighbours.

Question 4. Are comb supports randomly spaced or regularly spaced?

The distance to the first nearest neighbour was used as a measure of the spacing of the comb supports. If workers use a cue resulting from changes in comb size or mass, then

one or more of the predictor variables should explain a large amount of the variation in distance to the nearest neighbour.

In both *D. sylvestris* and *D. norwegica*, very little of the variation in the distance to the first nearest neighbour was explained by the predictor variables. It is therefore likely that the distance between comb supports is not regulated by cues originating from the size of the comb or the mass of the combs supported. Workers may use cues relating to comb size or mass to regulate the amount of supports constructed. The workers, however, could maintain a minimum distance between new comb supports and surrounding supports. Alternatively, workers may construct comb supports in response to a factor relating to the size of comb supported.

Examining the ratio of the first, to second nearest neighbour tested this hypothesis. The means of the ratio of the first to second nearest neighbour in *D. sylvestris* and *D. norwegica* was compared to that of a random scatter. The analysis of variance indicated that there was no significant difference between the NN1/NN2 ratio in a random scatter and that of both *D. sylvestris* and *D. norwegica*. There was also no difference between the means of the neighbour ratio in *D. sylvestris* and *D. norwegica* and a random scatter. This would suggest that the distance from comb supports to their neighbours is random. Workers therefore do not appear to construct comb supports at a minimum distance from neighbouring supports. It is therefore likely that comb supports are constructed in response to a stimulus resulting from the increase in weight of the combs directly and indirectly supported.

In both *D. sylvestris* and *D. norwegica*, the length and number of comb supports provided a useful measure. Other variations in comb supports may be significant in their function as tension struts. Their thickness and consistency may also affect the strength of comb supports. In addition to the construction of new comb supports workers may thicken existing supports or strengthen them with the addition of silk or oral secretions.

These findings are generally consistent with those of Spradbery (1973) in that there are differences in the amount of suspensoria between combs. However, although surface area explained some of the variation in the total length of suspensoria in *D. norwegica*, it did not significantly explain any of the variation in *D. sylvestris*. In addition, the variation was also explained by the number of brood reared in the combs supported in both species.

In conclusion the stimulus for the construction of comb supports in *D. sylvestris* and *D. norwegica* appears to be a result of an increase in the mass of comb supported. The cue for the construction of comb supports may be a change in the distance between combs, or between a comb, and the substrate. Alternatively, an increase in the weight of the combs supported could result in a change in the vibrations of the comb created by the workers movement or by the movement of the brood. Although the cue to construct comb supports may, however, be a factor related to the increase in the mass of comb suspended. The spacing of suspensoria, however, does not appear to be regulated by comb size or the distance to nearest neighbours.

Chapter 6. The development of colony thermoregulation with respect to envelope construction

6.1. Introduction

The principal function of the envelope is to insulate the nest, and its construction requires a great investment of the time and resources of the colony. The amount of envelope constructed at each developmental stage should therefore reflect the requirement of the colony for thermoregulation and its ability to actively produce heat. This chapter will examine if developmental stages differ with respect to the ability of the colony to regulate nest temperature. This information will be used to explain the differences in the amount of envelope constructed with development. There are several possible factors, such as colony composition, which could determine the ability of the colony to regulate nest temperature. This chapter will examine the effect of several of these factors on thermoregulation.

Information on the mechanisms of nest thermoregulation is fragmented in the literature, and little is known about how these mechanisms function together producing the pattern of thermoregulation observed in colony development. The introduction to this chapter will therefore review available information on the mechanisms of nest thermoregulation and their relative significance.

Thermoregulation requires the active heating or cooling of the nest in response to a change in temperature. The metabolic activity of the colony inside the closed insulating structure of the nest alone, is sufficient to elevate the temperature above ambient. This does not, however, constitute active thermoregulation in which temperature inside the nest should show a degree of independence from fluctuations outside.

Thermoregulation in colonies of Vespinae has long been established in the literature. Sailer (1950) found that colonies of *D. arenaria* were able to maintain a nest temperature of 30°C in a mean ambient temperature of 22°C. Since the discovery of thermoregulation in vespines regulation has been demonstrated in many species for example *V. vulgaris* (Potter 1964; Roland 1969), *V. germanica* (Roland 1969), *Vespa crabro* (Ishay and Rutner 1971), and various other species of the *Vespa* genus (Martin 1990)

Although the ability of the colony to thermoregulate is well documented, its function is poorly understood. Heating the nest is energetically expensive (Gibo 1974) and it is likely that the degree to which the nest temperature is regulated reflects the

requirements of the adults and brood. In order to explain the pattern of thermoregulation in vespines it is therefore necessary to first determine the specific benefits to the adults and brood development. The available information on the relative benefits to the adults and brood will therefore be reviewed. Heinrich (1972) proposed the benefits to egg and larval development were a likely function of nest temperature regulation. *"In social insects such as bumblebees, which must build up their colonies rapidly within a single season, the maintenance of a high nest temperature would permit the more rapid development of poikilothermic eggs, larvae and pupae".* Warming the nest may therefore decrease the developmental time of the brood by raising their metabolic rate (Martin 1990). Himmer (1932) determined that the optimum temperature for brood development in *V. vulgaris* was 29.5-32°C and 29.8-31.8°C in *Vespa crabro.*

Adults may derive some direct advantage from nest heating as they exhibit social thermogenesis outside the nest. Ishay (1972) found that when workers of *V. vulgaris, V. germanica, D. saxonica* and *D. media* are removed from the nest and placed in a box in the absence of brood they form a multi-layered cluster. At the centre of the cluster the temperature was raised to 29-35°C compared to 23-25°C measured in the rest of the box. Wasps that were not in clusters, or were temporarily outside them, did not exhibit thermogenesis. Adult wasps must raise their body temperature prior to many activities such as flight and hunting. Prior to leaving the nest, workers of *V. vulgaris* were observed to have thoracic temperatures of 32-33°C with a nest temperature of 28-30°C. When attacking prey the temperature of the thorax increased to 34-35°C (Heinrich 1984). Social thermoregulation may be more energetically efficient than individual warm up behaviour. Heating the nest may also help to eliminate the time lag in responding to stimuli resulting from the need to warm-up. This may be particularly vital in responding to predators attacking the nest.

Although social thermogenesis may reduce the time required by workers to warm up it is of little benefit to workers once they leave the nest. Heinrich (1984) found that when dead wasps are heated to 30°C above ambient they cool at the rate of 5°C per second. A further reason to suspect that social thermogenesis may not be primarily for the benefit of the adults is that adult vespines are very efficient at regulating their own body temperature (Milani 1982; Heinrich 1984; Schomlz *et al.* 1993; Coelho & Ross 1996).

It is unlikely that the constant expenditure of energy in heating the nest is more efficient than individual thermoregulation.

Adult vespines may also benefit from nest cooling. Gaul (1952) observed that workers of *D. arenaria* abandoned the nest in high ambient temperatures, and flew about 2 metres away returning when the temperature had dropped. Gaul noted that the colony did not seem to suffer, and concluded that nest cooling is for the comfort of the adults not the brood. Workers can also cool the nest through fanning in which forced ventilation is created through movement of the wings. This may also have more benefit to the workers than to the brood. Ishay *et al.* (1973) observed that when hornet workers fan the nest they are frequently dispersed around the outside of the envelope, which is unlikely to have any social function in cooling the nest.

Although thermoregulation may reduce the overall development time of the brood there is very little evidence available as to the specific benefits. Vespines have been observed to warm pupae directly. The relationship between nest thermoregulation and direct incubation of the pupae is unknown. Direct incubation may, however, provide some useful clues as to the mechanism of nest thermoregulation. Ishay & Ruttner (1971), and Ishay (1972, 1973) found that adults of *Vespa crabro*, *V. vulgaris*, *V. germanica*, *D. saxonica* and *D. media* will individually warm pupae, both in their cells and when removed from their cells. When workers were offered brood comb they entered empty cells adjacent to pupae, and heated them by placing their abdomen against the cell cap. The temperature of the pupae was raised by 10 to 12°C above ambient (Ishay 1973).

Direct incubation is a very selective behaviour and workers will preferentially heat pupae close to eclosion. They have not, however, been observed to directly incubate eggs or larvae (Ishay 1972). Direct incubation appears to be stimulated by the release of a pupal pheromone (Ishay 1973; Koeniger 1975). Veith & Koeniger (1978) isolated the pheromone cis-9-pentacosene in pupae of *Vespa crabro*, which is responsible for this behaviour. As workers will warm pupae of other species and genera it is likely that they have a similar pheromone.

The fact that adults directly incubate pupae but have not been observed to directly warm eggs or larvae is evidence that warming of pupae is a priority. Direct incubation appears, however, to be a separate phenomenon to nest warming as it operates only between temperature thresholds. Ishay (1973) found that in various species of *Vespa*, *Vespula* and *Dolichovespula* workers only warmed pupae between 18°C and 27°C.

Below this temperature they clustered together away from the pupae and raised their temperature. This behaviour would suggest that temperature regulation might be more directly important to pupae than to other immature stages.

There is direct evidence that warming pupae improves the success of pupation. Ishay (1972) found that pupae of wasps and hornets incubated at 32°C seem to give rise to perfectly formed adults where as those incubated at 22°C give rise to visibly malformed adults most having underdeveloped or "wet" wings. A critical period therefore seems apparent in older pupae when warming must occur to prevent malformation of emerging imagoes. Ishay (1973a) suggested that the significance of warming in preventing the malformation of imagoes is in the drying of the pupae. Ishay (1973) found that the mean weight of 30 pre-pupae of *Vespa crabro* was 1.21 g compared with a mean weight of 30 newly hatched workers of 0.47g. The reduction of mass is much greater in warmed pupae than unwarmed pupae (32% c.f. 13%). The rate of unsuccessful hatches and the rate of wing malformations in *Vespa crabro*, both decrease with incubation temperature. As direct incubation is important to the development of the pupae, nest thermoregulation is also likely to be beneficial.

Elevating nest temperature may have some benefit to the adults but is more likely to be of importance to the brood. The only specific evidence of the effect of thermoregulation on the brood is its effect on pupae. It would therefore to be reasonable to assume that nest thermoregulation would improve with the number of brood present in the nest and in specifically with the number of pupae. The degree to which nest temperature is elevated will also depend on the ability of the colony to generate heat. The contribution of the various colony members to thermogenesis will therefore be reviewed.

The mechanism of nest heat production in vespine wasps is poorly understood although it appears to result from heat production by both the larvae and adults. The ability of the adults to generate heat is well-documented (Milani 1982; Heinrich 1984). Heat production in vespines is likely to be similar to that in other insects with asynchronous flight. They can apparently mechanically uncouple their flight muscles from their wings (Krammer and Heinrich 1972), and so can raise the temperature of their thorax without visible wing movements. The characteristic abdominal pumping motion often accompanies warm up-activity. During flight and other activities this alternate extension and contraction of abdominal segments ventilates the flight muscles and supplies the elevated 0_2 demand (Krammer and Heinrich 1972). This abdominal

pumping also serves to allow excess heat to be shunted from the thorax to the abdomen. Heinrich (1975) found that adults of *Bombus vosnesenskii* could transfer excess heat produced in the thorax to the abdomen during brood incubation.

Males and newly emerged queens are also capable of thermogenesis and may contribute to nest thermogenesis. Schmolz *et al.* (1993) found that males and new queens of *Vespa crabro* had comparable rates of heat production to that of workers. It is, however, unlikely that new queens would contribute to nest thermoregulation as this would rapidly deplete their fat reserves, which are essential for nesting following diapause. Schmolz *et al.* (1993) suggest that males may also contribute to heating the nest at the end of the season. This again is unlikely as males have less to benefit than workers in terms of fitness from assisting in rearing their siblings.

The eggs and pupae are immobile and are therefore thought to be incapable of raising their temperature significantly to contribute to nest heating (Ishay and Ruttner 1971). The larvae, however, are capable of movement and are able to significantly raise the temperature of the nest. When a comb of *Vespa, Dolichovespula* and *Vespula* species is removed from the nest, the larvae, unwarmed by adults, can raise their temperature by 2-3°C above that of the environment (Ishay 1973; Martin 1990). In nests where the imagoes are removed, larvae are capable of a degree of autonomous thermoregulation (Ishay and Ruttner 1971; Ishay 1972, 1973). Small larvae (instars 1-3) are attached to the cell wall and thus are capable of only limited movement. In addition their small body mass means they are unlikely to contribute significantly to heat production. Older larvae, however, (instars 4 and 5) are capable of moving freely in their cells and are of sufficient mass to generate heat. Ishay (1971) described a rhythmic movement in *Vespa crabro* larvae, which was attributed to thermogenesis. This movement was performed more frequently when the adults were removed from the nest, and was notably different from the characteristic hunger signals and begging movements normally exhibited by larvae.

The larvae, however, also contribute indirectly to thermogenesis by providing carbohydrate secretions to the adults. Wheeler (1928) showed that adult hornets feeding their larvae obtain a drop of liquid in return. Brian and Brian (1952) also noted that when the queen of *D. sylvestris* tended the brood, a drop of fluid would pass between them and sometimes she would pinch their prothorax with her mandibles to obtain secretions. Brian and Brian suggested that this is a method of dealing with metabolic waste, and that the concentration of sugars is too low to be of social significance.

Montagner and Courtoise (1963) similarly thought that this behaviour was not of primary importance in colony maintenance.

This secretion was, however, found to contain about 9% sugars and 'considerable' quantities of amino acids (Maschwitz 1966), and Ikan and Ishay (1966) proposed that adults feed larvae on protein sources and in return receive secretions rich in carbohydrates. Ishay and Ikan (1968a) tested this hypothesis feeding larvae and adults of *Vespa crabro* on radiolabelled proteins in solution. The larvae were found to be capable of breaking down the protein, and producing sugars, which appeared in the saliva. No breakdown products of proteins were, however, detected in the bodies of the adults. Ishay and Ikan (1968a) suggested that part of the protein ingested by the larvae was used to assimilate body tissues, while the remainder was degraded to sugars. Ishay and Ikan (1968b) found that larvae also contained amino acids from the breakdown of ingested protein. They found that the saliva of *Vespa orientalis* larvae contained 5.5% carbohydrates and 0-13% protein and free amino acids. The ability of the larvae to break down proteins, results from proteases in larval saliva absent in adult saliva (Ishay & Ikan 1968b).

The volume of secretions produced by larvae increases with age. Ishay and Ikan (1968b) found that fourth instar larvae of *Vespa orientalis* could produce almost 13 times the volume of saliva as first-instar larvae in a 24 hour period. The number of saliva secretions collected from larvae also increases with age. Ishay and Ikan (1968b) found that small larvae (1-2nd instar) of *Vespa orientalis* were paid 53 visits an hour, medium sized larvae (3-4 instar) were paid 74 visits per hour and fifth instar larvae were paid 98 visits per hour. These large larvae are also visited by the queen but only to receive larval secretions rather than to feed the larvae.

Larvae can therefore contribute to heat production directly and indirectly through the supply of carbohydrates to the adults. The number of larvae, particularly old larvae (instars 4&5), in the nest should have an effect on the ability of the colony to elevate its temperature. The role of the larvae in thermoregulation is first apparent in the queen nest. The ability of the queen to elevate nest temperature is limited by the requirement to forage and perform other tasks and when the larvae are young they cannot contribute significantly to thermogenesis directly or indirectly. Gibo *et al.* (1977) measured the temperature of a queen nest of *D. arenaria*. They found that the queen was able to raise the temperature of the nest by up to 1°C above ambient when the brood consisted of

only 15 eggs. On the second week the larvae had increased in size and the nest temperature was maintain at 2-3°C above ambient.

The ability of the colony to regulate its temperature at night is particularly dependent on the contributions of the brood. When workers can no longer forage they must rely on the direct and indirect contributions of the brood. Roland (1969) noted that nest temperature regulation in *V. vulgaris* and *V. germanica* deteriorated when the exterior temperature dropped below 10°C and that thermoregulation could be restored by supplying the wasps with honey. The carbohydrate available in the colony is depleted through the night. Martin (1990) noted that the lowest daily nest temperature in *Vespa simillima*, *Vespa tropica* and *Vespa analis* occurred at first light when energy levels in the colony begin to deplete. Martin found that when the nest entrance is blocked during the night nest temperatures continued to fall until midday when the nest entrance was unlocked and thermoregulation returned. When honey was placed near the entrance during the night the temperature rose by several degrees until the supply was exhausted and temperatures fell.

Nest cooling is also an important part of nest thermoregulation and a variety of nest mechanisms have been described in vespine wasps including fanning, evaporative cooling, cutting holes in the envelope and adults abandoning the nest. The precise mechanisms of cooling and its function are poorly understood. Nest cooling is likely to be of little significance in nest thermoregulation in temperate climates, as the optimum nest temperature in *Vespula* and *Dolichovespula* species is around 30°C and cooling mechanisms have only been observed to operate at temperatures well above the nest optimum.

In this chapter nest thermoregulation was examined only in colonies of *D. sylvestris*. Although it was intended to also examine thermoregulation in nest of *D. norwegica*, insufficient temperature recordings were made for meaningful analysis. This was in part the result of difficulties in locating early developmental stages of *D. norwegica* colonies.

This chapter will first examine the establishment and pattern of thermoregulation in colonies of *D. sylvestris* with colony development. This is achieved by comparing the ability of the colony to raise the temperature of the nest at each developmental stage. The pattern of thermoregulation with colony development is then described with a selective series of temperature recordings taken from nests at various developmental stages.

Secondly, it will examine several factors that may explain differences in the ability of the colonies to regulate nest temperature at different developmental stages. These factors included the number of workers, as they are known to be capable of high levels of heat production. The number of old larvae (instars 4&5) is examined, as they are capable of contributing both directly and indirectly to thermogenesis. As pupae are known to directly stimulate heat production, and may also stimulate nest thermoregulation, the number of worker, male and queen pupae present in the nest will also be examined. Other factors included in the analysis are the number of eggs and young larvae.

6. 2. Methods

Nest temperature was recorded from nests of *D. sylvestris* and *D. norwegica* in the field. These nests were mostly located on domestic premises (see Chapter 2). Temperature probes were used to record the temperature inside and immediately outside the nest. Temperature was recorded using 'Tinytalk Temp' single-channel data loggers (Orion Components, Chichester). The devices used are capable of recording 1800 measurements at predetermined intervals and are small enough to fit into a standard 35mm-film canister. For each temperature recording, the time and date were also logged. The 'Tinytalk' temperature loggers were set up, and the data retrieved via a serial interface to a P.C. To record the temperature inside the nest the temperature sensitive part of the device was remote mounted on a probe, which could be inserted through the envelope. In queen nests the temperature probe was often introduced through the entrance hole to avoid dislodging or damaging the nest.

The temperature probes were allowed to record for 7 days, then the colony was killed and the nest collected as described in Chapter 2. The loggers were set to record temperature at intervals of 6min 24sec. This gave a total recording time of 8 days in case of difficulty in retrieving the logger on the 7th day. Insertion of the probes led to disturbance of the colony and slight damage to the envelope, which was normally repaired within the first two days. This part of the temperature record was therefore not included in the analysis. The total number of temperature recordings taken from each probe was 1126 over the five-day period.

The number of nests at each developmental stage from which nest temperature was recorded is shown in Table 6.1. Although the earlier stages of nests were well

represented, temperature was only recorded from two nests at the CDAB stage and stage CDAC was not represented.

Data on colony statistics was recorded at the same time as data on nest structure and is described in Chapter 2. In determining colony composition, larvae were classified as small or large. Small larvae (instars 1-3) can be distinguished from large larvae as they tend to face outwards in their cells, away from the centre of the comb, whereas older larvae (instars 4 and 5) face inward toward the centre of the comb (Edwards 1980). For the purpose of determining the developmental stages of the nest, the numbers of small and large larvae were pooled.

Table 6.1. The number of nests of *D. sylvestris* from which temperature was recorded at each developmental stage.

Developmental stage	Number of colonies form which temperature was recorded
QN	6
SCN	6
CDL/S	4
CDAA	15
CDAB	2
CDAC	0
Total	33

The relationship between thermoregulation and colony development.

The ability of the colony to thermoregulate was simply taken to be the difference between the mean temperature inside the nest and that outside the nest. The pattern of thermoregulation is then examined statistically by comparing the mean elevation of nest temperature between developmental stages (described in Chapter 2). The pattern of thermoregulation will then be described with a series of selected temperature recordings taken from the nest at various developmental stages.

Factors effecting nest thermoregulation

The ability of the colony to elevate nest temperature above that of the surroundings will be explained in terms of the following seven predictor variables;

1. Number of workers + founder
2. Number of eggs
3. Number of young larvae
4. Number of old larvae
5. Number of worker pupae

6. Number of male pupae
7. Number of queen pupae

Statistical analysis

For ANOVA data were checked for normality with a frequency histogram and homogeneity of variances with the Fmax test (Fowler and Cohen 1996). As the data were normally distributed and homoscadastic, it was not transformed. The difference between developmental stages in ability to thermoregulate was determined by comparing the average difference in temperature with a one-way ANOVA. A Tukey-Krammer test was used to locate differences between means, as there were unequal numbers of nests at each developmental stage (Sokal and Rohlf 1995).

Multiple regression was used to explain nest temperature in terms of predictor variables. The predictor variables were normalised by square-root transformation. Analysis was conducted using the MINITAB (Version 10.1) statistical package. The overall significance of the multiple regression was tested by ANOVA. The significance of partial regression coefficients was determined with T-tests.

The relationship between the number of old larvae (instars 4and 5) and nest temperature elevation was illustrated with a scattergram. The structural relationship was determined by fitting a natural log curve calculated using the least squares method (using the Microsoft Excel package).

6.3. Results

The relationship between thermoregulation and colony development.

The ability of the colony to elevate the nest temperature above ambient at various developmental stages was compared using a one way ANOVA. The ANOVA indicated that there was a significant difference between developmental stages in the ability of the colony to elevate nest temperature ($F=8.27$ at df 4,28: $P<0.01$). Results are illustrated in Figure 6.1.

It can be seen from Figure 6.1 that the ability of the colony to elevate nest temperature increases rapidly from the QN stage reaching a peak at the CDL/S stage after which it declines. The Tukey-Krammer test indicated that elevation of nest temperature at the QN stage was significantly lower than at stages CDL/S, CDAA and CDAB. There was, however, no significant difference between stage QN and SCN. Temperature elevation at the SCN stage was significantly lower than at the CDL/S and CDAA stages. No other pairs of means were found to differ significantly (Table 6.2).

Figure 6.1. The relationship between developmental stage of the colony and its ability to elevate nest temperature above ambient. Fitted with SE bars.

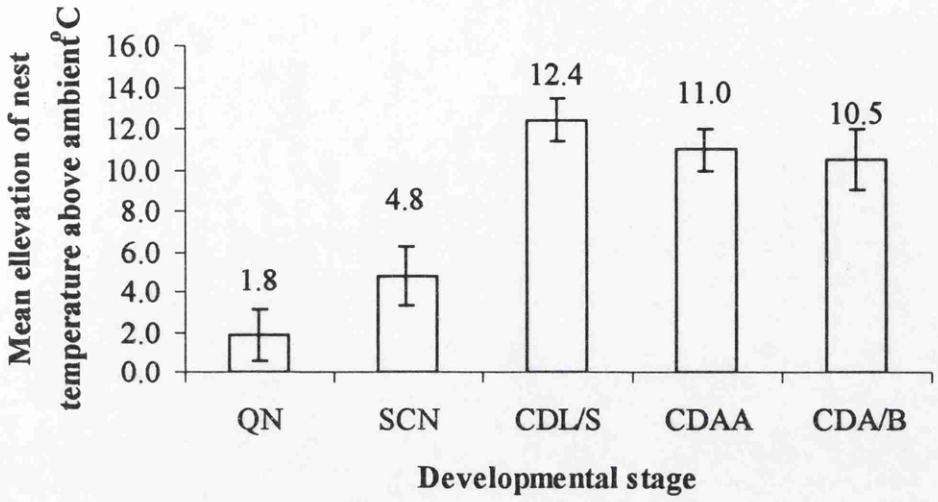


Table 6.2. The results of a Tukey multiple comparison test to locate differences between developmental stages in the ability of *D. sylvestris* colonies to elevate nest temperature above ambient.

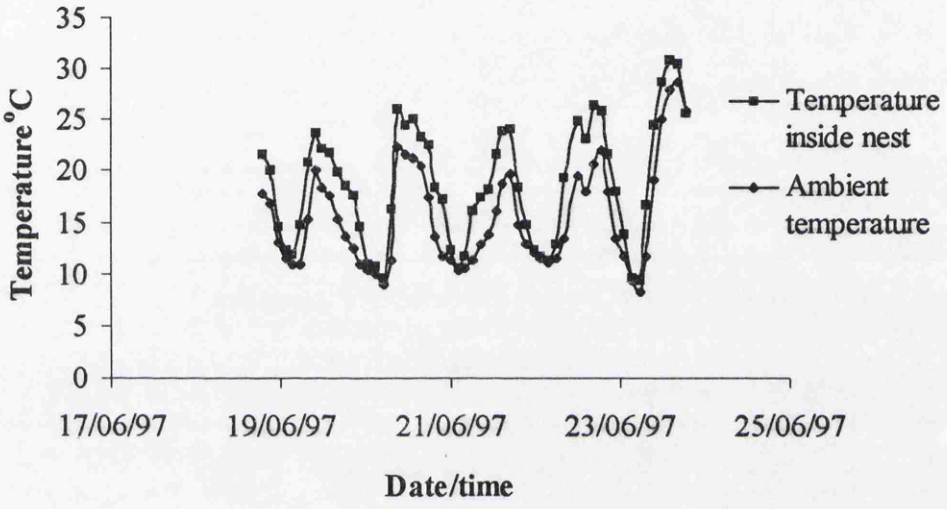
Comparison	Difference between means	$q_{0.05}$, a=5, v=305	*n*	*T*	P<0.05
QN vs SCN	2.91	4.1	6.0	5.84	n.s.
QN vs CDL/S	10.56	4.1	5.0	6.53	Signif.
QN vs CDAA	9.14	4.1	10.5	4.89	Signif.
QN vs CDAB	8.68	4.1	4.0	8.26	Signif.
SCN vs CDL/S	7.64	4.1	5.0	6.53	Signif.
SCN vs CDAA	6.22	4.1	11.0	4.89	Signif.
SCN vs CDAB	5.76	4.1	4.0	8.26	n.s.
CDL/S vs CDAA	1.42	4.1	9.5	5.69	n.s.
CDL/S vs CDAB	1.88	4.1	3.0	8.76	n.s.
CDAA vs CDAB	0.46	4.1	8.5	7.62	n.s.

At the queen nest stage, the colony showed only a moderate ability to elevate nest temperature. Figure 6.2 represents a typical temperature recording from a nest of *D. sylvestris* at the QN stage. During the day the nest temperature was maintained at a mean of 18.6°C in an ambient temperature of 15.3°C. At night the colony showed little ability to elevate nest temperature. The nest contained 7 eggs, 10 small larvae, 9 old larvae and 6 sealed brood

The ability of the colonies to elevate nest temperature improved from the QN stage to the SCN stage with the emergence of the workers. Figure 6.3 shows the temperature recorded from a small cell nest (SCN) of *D. sylvestris*. In this colony nest temperature

was maintained at an average of 22.8C in an ambient of 19.4°C. Although there was not a great increase in the ability of the colony to elevate nest temperature during the day from the QN shown in figure 6.2, the colony shows a greater ability to elevate nest temperature at night. This nest contained 21 workers, 36 eggs and 43 old larvae: no males had yet been reared.

Figure 6.2. Temperature recorded from inside and immediately outside a queen nest (QN) of *D. sylvestris* in the five days prior to collection. Abscissa starts from 00:00 hours on 17/6/97 to 00:00 hours on 25/6/97.



When the colonies began to rear large cell brood at the CDL/S stage, there was a great improvement in temperature regulation, and nest temperature was almost independent of ambient temperature during the day. Figure 6.4 shows temperature recordings from a nest at the CDL/S stage. Nest temperature was maintained at an average of 27.0°C in an average of 16.5°C. The colony was not, however, able to maintain this degree of temperature elevation throughout the night. The nest contained the founder and 29 workers; no reproductives had yet emerged. Forty-five large cell sealed brood were present and a total of 37 old larvae were present in small and large cells.

Figure 6.3. Temperature recorded from inside and immediately outside small cell nest (SCN) of *D. sylvestris* in the five days prior to collection. Abscissa starts from 00:00 hours on 10/7/96 to 00:00 hours on 18/7/96.

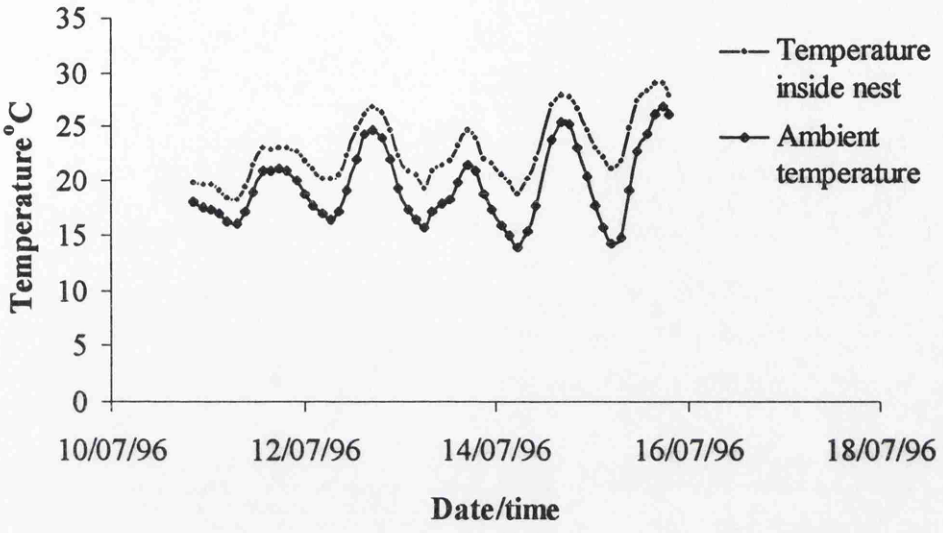


Figure 6.4. Temperature recorded from inside and immediately outside a nest of *D. sylvestris* at the start of the production of the large cell brood (CDL/S) in the five days prior to collection. Abscissa starts from 00:00 hours on 12/7/96 to 00:00 hours on 20/7/96.

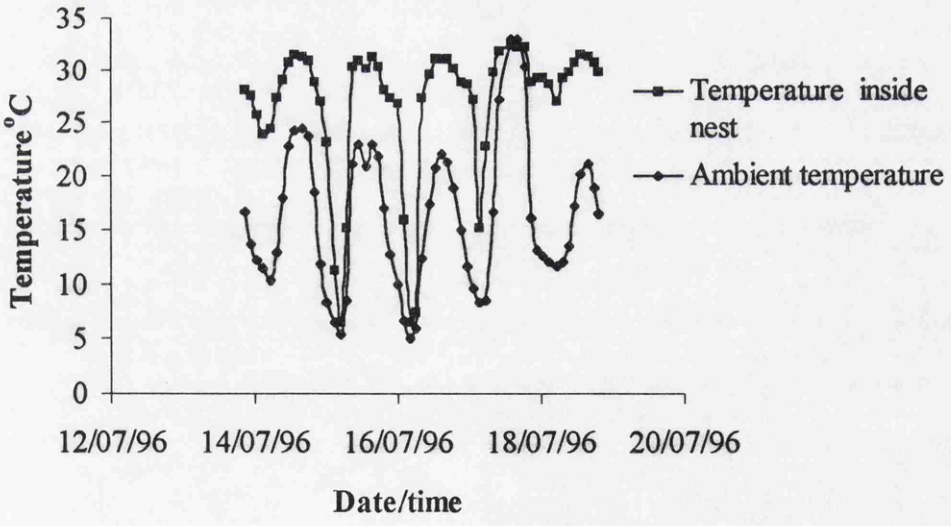
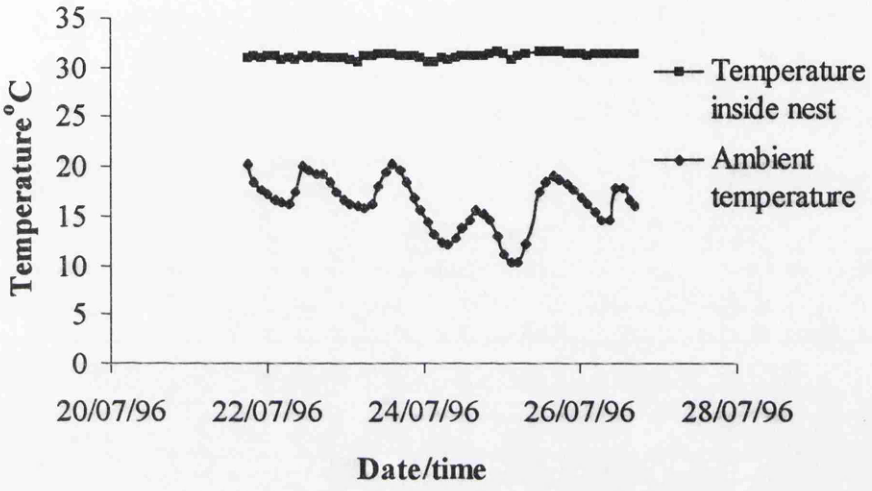


Figure 6.5 illustrates temperature recordings from a nest of *D. sylvestris* at the CDAA stage and it can be seen that the temperature inside the nest was almost independent of the ambient temperature. Nest temperature was maintained at an average of 31.1°C in an ambient temperature of 16.3°C. The ability of the colony to regulate its temperature was especially noticeable at night when nest temperature remained near optimum. At

the time of collection the adults consisted of the founder 53 workers, 19 males and 27 new queens. The brood contained 84 old larvae and 216 sealed brood of which 106 were new queens and 22 were males.

Figure 6.5. Temperature recorded from inside and immediately outside a mature nest of *D. sylvestris* at the peak of emergence of large cell adults (CDAA) in the five days prior to collection. Abscissa starts from 00:00 hours on 20/7/96 to 28/7/96.



There was no significant difference between stages CDAA and CDAB in the Tukey multiple comparison test, and there was very little difference apparent in the temperature recordings. Temperature recordings for stage CDAB are therefore not presented.

Factors effecting nest thermoregulation

The ANOVA indicated that the 7-predictor variables were highly significant in explaining the variation in nest temperature elevation ($F=5.13$, df 7,25: $P<0.01$). The coefficient of multiple determination was 0.475 indicating that 47.5% of the variation in nest temperature elevation was explained by variation in the predictor variables.

Regression equation:

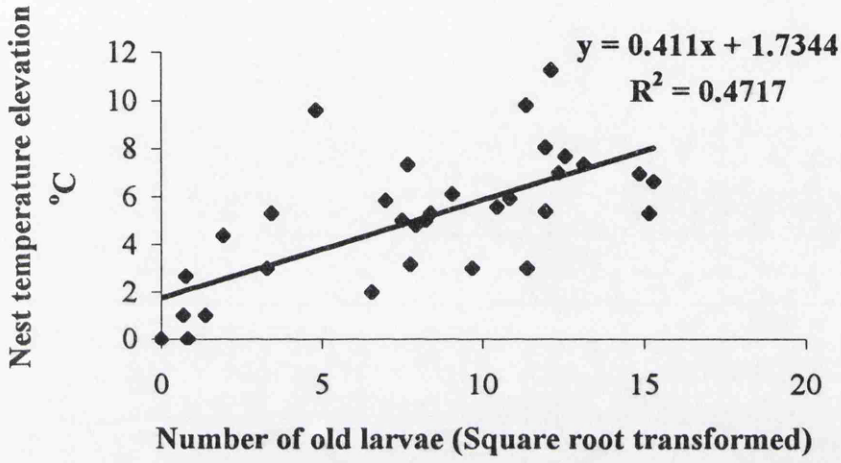
Temperature elevation *= 3.39 – 0.131(number of workers and founder) – 1.21(Eggs) – 0.170(Young larvae) + 0.170(Old larvae) + 0.515(Worker pupae) + 0.087(Male pupae) – 0.055(Queen pupae)*

Of the partial regression coefficients only the number of old larvae (square root transformed) was significant in explaining the variation in nest temperature elevation ($t=3.01$ $P<0.01$).

The total number of old larvae was plotted against nest temperature elevation to illustrate the structural relationship, and fitted with a least-squares regression line

(Figure 6.6). There was a highly significant positive relationship between the number of old larvae in the nest and nest temperature elevation ($F=27.68$ df 1,31: $P<0.01$). From the coefficient of determination it can be seen that the number of old larvae (square root transformed) explains 47% of the variation in nest temperature elevation.

Figure 6.6. The relationship between the number of old larvae (instars 5&6) and elevation of nest temperature above ambient. Fitted with a least squares regression line.



6.4. Discussion

The ability of the colony to elevate its temperature above that of the environment was apparent from the earliest developmental stage, the queen nest (QN). This ability appeared to increase with the emergence of the workers, although this was not significantly different. The ability of the colony to elevate its temperature above that of the environment increased rapidly between the small nest stage (SCN) and the start of the rearing of the large cell brood (CDL/S). It can be seen from the temperature records (Figures 6.2. to 6.5.) that the increasing ability of the colony to elevate its temperature from the QN to the emergence of the reproductives is especially evident at night. Throughout the production of the large cell brood, the colony retained a similar ability to raise its temperature above ambient. The senescent stage of the colony (CDAC) in which production of large cell brood had greatly reduced was not represented, and it is therefore difficult to determine at which point the colony looses its ability to regulate its temperature.

Various factors could effect the ability of the colony to thermoregulate. The variables examined were all correlated were all correlated with each other and for example both the number of workers and larvae increase with colony development. Several of the seven factors therefore increased significantly with nest temperature elevation. The multiple regression, however, examines the relationship between each variable in turn and that of temperature elevation while keeping the other six variables statistically constant. Several variables were examined in this chapter including the number of workers, eggs, young larvae, old larvae, worker pupae, male pupae and queen pupae. Of these only the number of old larvae (instars 4 and 5) were found to be significant in explaining the variation in thermoregulation.

Evidence in the literature suggests that the eggs and young larvae (instars 1 to 3) are too small to contribute directly to thermogenesis, and that small larvae produce relatively little carbohydrates in the form of saliva, which can be utilised by the adults in thermogenesis (Ishay and Ikan 1968b). The results presented in this chapter therefore confirm that the eggs and small larvae do not play a significant role in regulating nest temperature.

It was established in the introduction that the workers are capable of significant levels of heat production, and could therefore be responsible for a large part of nest thermoregulation. From the findings in this chapter, however, the number of workers present in the nest does not appear to be critical to the ability of the colony to raise its temperature. This does not mean that the workers are not responsible for a considerable part of thermogenesis in the nest. The number of workers is, however, a limiting factor, and it is possible that thermogenesis may be achieved with relatively few adults. There may be two reasons why the number of workers may not be limiting in the ability of the colony to elevate its temperature. Firstly, although workers have been observed to directly warm the brood, they may be only partly responsible for nest thermogenesis. Secondly, if workers are responsible for a large proportion of nest thermogenesis, their ability to heat the nest may be limited by the availability of carbohydrates.

If carbohydrate is limiting in colony development, the contribution of the adults to nest thermoregulation may be highly dependent on the provision of saliva from late instar larvae for carbohydrate. There is evidence that vespine colonies can thermoregulate with relatively few adults. Martin (1990) found that a mature colony of *Vespa tropica* was able to maintain a constant nest temperature when it contained only 17 workers. Ishay (1973) found that when the adults of a nest of *Vespa crabro* were removed from

the nest, the larvae were able to elevate the temperature of the nest by approximately 4°C above ambient as opposed to around 7°C when the adults were returned to the nest. Pupae do not appear to be capable of significant levels of thermogenesis. They are, however, capable of stimulating direct warming through the production of a pheromone (Ishay 1972, 1973a; Koeniger 1975) and may also stimulate nest thermogenesis. The numbers and types of pupae present in the nest were not, however, significant in explaining the variation in nest temperature elevation. It is likely then that pupae do not stimulate nest thermoregulation through the production of pheromones, and direct brood incubation appears to be a separate phenomenon.

The number of old larvae present in the colony was found to be a limiting factor in the ability of the colony to thermoregulate. This may be a result of their direct contribution to thermogenesis (Ishay 1972; Martin 1990), or because they are capable of supplying the adults with considerable quantities of carbohydrates for thermogenesis (Maschwitz 1966, Ishay and Ikan 1968a,b). It is not, however, possible from these results to determine the relative importance of the direct and indirect contributions of the larvae to thermoregulation.

The findings presented in this chapter concerning the importance of the late instar larvae in thermoregulation are consistent with the evidence in the literature. The ability of the queen to raise the temperature of the embryo nest improves greatly when there are significant numbers of larvae present. Gibo *et al.* (1977) noted that the founder of a nest of *D. arenaria* was unable to raise the temperature of the nest by more than 1°C until there were a significant number of larvae present in the nest, when it was heated to 2-3°C above ambient. The effect of the brood is also noticeable at the end of the colony lifecycle. Martin (1992) found that deterioration in the ability of a colony of *V. simillima* to thermoregulate coincided with a decline in the number of brood present. Workers were found to be present in the colony until 1-2 months after the loss of thermoregulation.

If the late instar larvae principally effect nest thermoregulation through their supply of carbohydrates, then this would suggest that carbohydrate is limiting in thermogenesis. There is evidence in the literature to support that carbohydrates are both limiting in thermogenesis and in nest growth. Roland (1969) noted that when colonies of *V. vulgaris* and *V. germanica* are offered carbohydrate in the form of honey, the nest temperature did not decline at night. Martin (1990) similarly noted that supplying

honey to colonies of *V. simillima* raised the temperature of the nest by several degrees, where as supplying the colony with a protein rich source did not. As carbohydrates seem to be limiting in nest thermoregulation, they may also limit the growth of the colony. Martin (1990) found that supplying a colony of *Vespa simillima* with carbohydrate had a greater effect on final nest size than did transferring a similar colony to a cabinet heated to the 30°C. It therefore seems likely that larvae have their greatest influence on thermogenesis through the supply of saliva.

The amount of energy contributed by the larvae to heating the nest was estimated by Gibo *et al.* (1977). They observed that before the nest contained larvae, queen nests of *D. arenaria* and *D. maculata* were only capable of elevating their nest temperature by 1°C. It was determined that in elevating nest temperature by 1°C, the *D. arenaria* queen must expend energy at the rate of 123 cal/hr and the *D. maculata* at a rate of 165cal/hr. When the nests contained larvae, however, the temperature was elevated to 4°C above ambient. The energy required maintaining this temperature elevation was 423 cal/hr in *D. arenaria*, and 480cal/ hr in *D. maculata*. Gibo *et al.* therefore estimated that the brood must be responsible for producing 300 and 315 cal/hr of this energy respectively. The extra energy expenditure could be expended directly by the larvae in heating the nest, or could be supplied to the queen in the form of saliva secretions.

Gibo *et al.* calculated that in order to heat the nest at a rate of 400cal/hr, assuming that the queen can respire 100 cal/hr, the larvae must supply the queen with approximately 67mg of secretions per hour therefore respiring the equivalent of 201mg per hour themselves. Firstly this would rapidly deplete the mass of the larvae, and further Ishay and Ikan (1968b) found that a larva of *Vespa crabro* could produce a maximum of only 0.0051mg of saliva in a 12 hour period. Alternatively if the larvae respired body fat Gibo *et al.* estimated that they would loose only 33mg of fat per hour to maintain the same temperature, and that if the larvae were capable of respiring half their mass they would be able to heat the nest for 5 hours.

It would seem likely then that much of the production in queen nests result from the direct respiration of body fat by larvae. In older nests, however, the larvae may be able to supply the demand of adults for carbohydrates, and hence have a less direct role in heating. As larval saliva consists mainly of water, the larvae would loose a lot of their body mass during extended periods when foraging is prevented. This may explain the early morning peak in foraging activity for water noted in *V. vulgaris* (Potter 1964).

Larvae may increase their body weight above that required for normal development in order to act as a reservoir for carbohydrates. Harris (1995) found that worker larvae of *V. vulgaris* could successfully pupate after starvation for up to 16 days, despite a reduction in body weight of up to 56%. Larvae then may therefore be able to respire a large proportion of their body weight in heating the nest, and successfully pupate. The storage of energy as fat may be respired directly by larvae, or may be supplied to the adults for thermogenesis. Brian and Brian (1952) assessed the effect that removing these larval secretions had on the larvae by dividing larvae into two groups; one of which was desalivated regularly, the other was not at all. After two days the desalivated larvae appeared and behaved normally while the other larvae appeared distended and salivated copiously when their mouths were touched with food. It is likely therefore that removal of this salivary secretion is a normal part of larval development

There is evidence, based on the ability of the adults to produce heat, that thermogenesis by larvae is important in mature nests. Schmolz *et al.* (1993) measured the heat production by workers of the hornet *Vespa crabro*. At ambient temperatures of 20°C, workers were observed to produce heat at a maximum rate of 0.028W. They calculated the colony must heat the nest at a rate of 1.3W in order to maintain the nest at 25.6°C in an ambient temperature of 16°C. If the brood did not contribute directly to thermogenesis, in order to maintain this level of thermogenesis the nest would therefore require a minimum of 46.4 workers engaged in thermogenesis at any one time. Martin (1990), however, observed that a colony of *Vespa tropica* was able to elevate its nest temperature by over 10°C above ambient when only 17 workers remained in the nest. A similar level of heat production has been noted in workers of other species. *V. germanica* workers are capable of producing heat at the rate 0.04W to 0.0626W and *V. maculifrons* at a rate of 0.048W (Milani 1982; Coelho and Ross 1996). Higher levels of heat production may be necessary to maintain nest temperature in other species. Gibo *et al.* (1974) measured that nests of *D. arenaria* and *D. maculata* required heat production rates of 3.93W and 3.26W respectively to maintain a nest temperature of around 28°C at an ambient temperature of 5°C.

It can be seen from Chapter 2 that envelope thickness increases significantly up to stage CDAA. Temperature regulation, however, reaches its peak somewhat earlier in colony development at stage CDLS. However, it was shown in chapter 2 that the relative thickness of the envelope is unaffected by nest size. From a consideration of the surface

area to volume ratio and the amount of energy per unit biomass required to heat the nest (Gibo *et al.* 1974), the ability of the colony to heat the nest will improve with size. The only direct evidence of an effect of nest thermoregulation on the brood is the effect on the success rate of pupae. As the quality of the males and newly emerged queens will have a direct effect on the reproductive success of the colony, it is logical that the amount of insulation constructed should reach its maximum at the peak of production of the reproductives.

Potter (1964) provided evidence of the effects of temperature on the proportion of forage trips for pulp. Although this would seem to suggest that nest temperature has an effect on the rate of nest construction and in particular envelope construction, there is little evidence to support this (Chapter 6). More experimental work therefore is required to determine the effects of temperature on the rate of envelope construction. This can be achieved by maintaining a captive vespine colony in a heated nest box. By manipulating the temperature at which the nest is maintained the effect of on envelope manufacture can be examined. The nest box and entrance trap presented in Chapter 3 were developed for this purpose.

The function of thermoregulation in colony development remains a key question. The effect of incubation temperature on survival of pupae is the only solid evidence of its benefits. As the temperature of the nest is raised when only eggs and small larvae are present in the nest, thermogenesis must have more general benefits to brood development.

In this chapter the pattern of thermoregulation was examined by measuring nest temperature from a large number of colonies in the field. An alternative approach is to monitor the progress of temperature regulation in individual colonies throughout their development and experimentally manipulating the environment and forage of the colony (Martin 1990). As the envelope obscures the colony, this method does not allow temperature regulation to be related directly to colony composition. This was, however, achieved by the approach adopted for this chapter.

Although the ability of adults and late instar larvae to produce heat is well documented, it is not clear as to what extent each contributes. The role of each of the colony members and of external factors in thermoregulation could be explored with a mathematical model. A model of colony thermoregulation could be developed to predict the ability of the colony to raise its temperature at each developmental stage based on the number of each type of colony member present and their ability to raise the

temperature of the nest. The mechanism of thermogenesis could be determined by comparing the prediction of thermoregulatory ability from the model with that observed in colonies at similar developmental stages in the field.

Chapter 7. General discussion

Vespine workers appear to have a simple behavioural rule regulating the amount of comb and envelope constructed. This resulted from workers allocating a constant proportion of material to the manufacture of comb and envelope. Spradbery (1973) and Edwards (1980) claimed that small vespine nests have proportionally thicker envelopes than large nests. This claim was not, however, supported by the findings presented in this thesis. A linear relationship was found between envelope thickness and nest diameter.

There are advantages to the colony in employing simple behavioural regulation at the level of the individual. The use of more sophisticated cues in regulating behaviour may allow the amount of envelope constructed to more closely match the demands of the colony, and the characteristics of the nest site. In deciding whether to construct comb or envelope, for example workers would have to evaluate cues and survey the comb and envelope to decide which construct. If, however, workers simply allocated a fixed proportion of their time in the construction of comb and envelope, they would not have to survey both the comb and envelope.

The ability of the colony to thermoregulate should increase through colony development reaching a peak at the maximum colony biomass. The amount of energy required to heat the nest per unit biomass will decrease with colony size and the surface area to volume ratio will decrease with nest size. The ability of the colony to thermoregulate should therefore increase with colony size. The findings presented in this thesis show that this ability of colonies of *D. sylvestris* to thermoregulate reached a peak at the start of the production of the reproductives. The only specific evidence of the benefits of thermoregulation is on the success of pupation (Ishay 1972, 1973). The quality of the reproductives emerging would directly effect the reproductive success of the colony. It is therefore logical that the ability of the colony to thermoregulate should reach a peak during the production of the reproductives. This was confirmed by the finding presented in this project. However, as temperature regulation is apparent from the earliest developmental stage when the only brood present in the nest are eggs and small larvae, thermoregulation must have more general benefits to brood development.

The primary function of the envelope is in nest insulation. Envelope construction should therefore be regulated such that the amount constructed matches the needs of the colony for thermoregulation. One way in which envelope construction could be regulated so

that the amount constructed matches the requirement of the colony is to use temperature as a cue in deciding to produce comb or envelope. Potter (1964) found evidence that the rate of foraging for pulp in *V. vulgaris* is regulated by temperature at the nest site. Although this evidence suggests that envelope construction is regulated by temperature, Potter did not determine what proportion of the pulp returned to the nest was used in the construction of comb or envelope. In addition he found that the proportion of trips made for pulp in *V. vulgaris* increased with temperature and reached a peak at 28°C which is close to the nest optimum. In a temperate species of Vespine, the requirement for insulation would be expected to decrease up to the optimum.

There are, however, other requirements on the time of the workers. Environmental factors are known to have an effect on the activity of the colony. Wasps are known for example to forage more for water following a rainstorm Potter (1964). The increase in collection of water at this time is probably because less energy is required to collect it when it is abundant than at other times (Edwards 1980). The proportion of time available for pulp collection may therefore be related to the effect of environmental factors on foraging for carbohydrate, water and carrion.

If workers do not use the thickness of the envelope as a cue for its construction the amount of envelope constructed is not stimulated by the previous construction. The placement of pulp, however, is effected by previous construction. In order to construct the large continuous sheets of envelope, workers must have a greater tendency to extend an existing sheet of envelope than to initiate a new one.

Potter (1964) presented further evidence on the effect of temperature at the nest site on envelope construction. When the envelope of a colony of *V. vulgaris* was removed and the nest was maintained at 32°C in the dark, envelope was reconstructed very slowly. This is consistent with the hypothesis that temperature acts as a cue that regulates the rate of envelope construction. The slow reconstruction of envelope would, however, also be consistent with the allocation of a constant proportion of material to comb and envelope. If workers spent a constant proportion of time on the construction of comb and envelope, then removal of the envelope would not stimulate accelerated reconstruction. Potter's observations may also provide evidence that wasps do not construct envelope to a threshold thickness, as this would result in the rapid reconstruction of the envelope.

If workers were constructing envelope to a threshold thickness they would have to survey the thickness of the envelope before the addition of pulp. As envelope thickness cannot be measured directly, apart from at the nest entrance, this would be a time consuming process. Nest site restrictions were found to have no effect on the total amount of envelope constructed in *D. sylvestris*. If workers were measuring envelope thickness, restrictions at the nest site, where construction of part of the envelope is prevented, would reduce the total amount of envelope constructed.

The examination of envelope structure in a large number of nests of *D. sylvestris* and *D. norwegica* collected from the field gave a lot of valuable information on construction behaviour. It is, however, important to obtain more information on the regulation of envelope construction behaviour. An experimental approach would provide more detailed information on construction behaviour, and in particular on the influence of environmental factors on envelope construction.

The nest box and entrance trap presented in **Chapter 3** was designed to study the effects of environmental factors such as nest temperature on the rate of comb and envelope construction. The nest box was found to be effective in maintaining a colony of *D. sylvestris* at a range of temperatures determined by the experimenter. The entrance trap was similarly effective in separating incoming and outgoing wasps and could be used to sample foragers returning to the nest. The equipment presented could be used to verify the finding of Potter (1964) on the effects of temperature on the rate of foraging for pulp. It could further be used to determine if nest temperature has a different effect on the rate of comb and envelope construction.

Comb and envelope paper were found to be constructed to different specifications. There is therefore a difference in the behavioural regulation of the manufacture of comb and envelope material. The difference in construction behaviour between comb and envelope material results from a difference in the structural requirements. Comb material must support its own weight and that of the brood where as envelope functions to weatherproof the nest, and must principally support its own weight in tension. Although comb material is potentially subject to higher tensile and compressive forces than envelope in the nest, it is additionally strengthened by the accumulation of the silk lining and meconia resulting from pupation.

Differences in the mechanical properties of comb and envelope paper were found to result from both fibre processing and paper manufacture. Comb paper was found to be

thinner, consisting of shorter fibres than that of envelope in *D sylvestris*, *D. norwegica* and *V. vulgaris*. The difference in fibre length between comb and envelope appears to result from workers spending more time masticating pulp for the manufacture of comb paper than envelope paper. This may be due to the selective use of material removed from the envelope and re-processed for comb production (Akre *et al.* 1976; Makino 1980). Recycling of paper would lead to fibres being masticated twice resulting in the reduced fibre length observed in *Dolichovespula* comb.

It was not clear from this study whether differences in comb and envelope paper resulted from a difference in the fibre selection behaviour. If workers select fibre from different sources for comb and envelope, then the decision to manufacture comb or envelope must be made before leaving the nest. If the preference for constructing comb or envelope is determined by age, the preference for types of fibre source may also change.

One way in which differences between fibre sources can be determined is through chemical analysis. McGovern *et al.* (1988) analysed the chemical content of comb and envelope paper in various species of *Dolichovespula*. They found that comb and envelope were very similar in chemical composition, which suggested that they were collected from similar fibre sources. Comb paper, however, had a higher nitrogen content, which they suggested was the result of the use of more saliva in its manufacture. The characteristics used by wasps in the selection of pulp sources is, however, unknown. Vespine workers do not seem to make a destination between hard and soft woods and it would appear that the degree to which the wood is weathered is more important in fibre selection (McGovern *et al.* 1988).

The results presented in this thesis show that *D. sylvestris* and *D. norwegica* have many similarities in fibre selection and processing, and in the manufacture of paper. *Dolichovespula* species tend to select fibre from sound or slightly weathered wood (Weyrauch 1935, Arnold 1966). This results in a relatively strong paper consisting of long fibres. The nest paper of *V. vulgaris* differed from that of the *Dolichovespula* species in several characteristics (i.e. having a thicker, weaker envelope and consisting of short chunks of fibre bundles). This results from the selection of fibres from rotten sources in *V. vulgaris* (Akre and Davies 1978).

It was found that the ability of the colony to thermoregulate was limited only by the number of old larvae (instars 4 and 5) in the nest. It is unclear, however, to what extent this results from the direct contribution of the larvae through movement in their cells

(Ishay 1971), and the indirect contribution through the supply of carbohydrate rich saliva (Maschwitz 1986; Ishay and Ikan 1966, 1968a). Nest thermoregulation was found to reach a peak at the CLD/S stage when the production of the reproductives starts. The peak thickness of the envelope appears at the CDAA stage, when the maximum number of large cell sealed brood is present in the nest. The only specific evidence of the effects of thermoregulation on the brood is on the success rate of pupation. As the nest is heated from the time when only eggs and small brood are present in the nest, thermoregulation must also have more general benefits to brood development.

Envelope construction is regulated such that it remains a constant proportion of nest thickness through development. However, as the nest increases in size, the surface area to volume ratio and the amount of energy per unit biomass required in heating the nest decreases. Therefore the colony should improve until it reaches its maximum biomass. The regulation of envelope construction such that a constant proportion of material is allocated to its construction therefore allows thermoregulation to reach a maximum when the reproductive pupae are being produced.

Spradbery (1973) claimed that the number of comb supports constructed are related to the area of comb supported which results in a higher density of pillars in the upper combs than the lower combs. In chapter 5 it was found that there was a significant difference in the density of supports constructed between combs. One way in which comb support construction can be regulated is to construct supports at a minimum distance from neighbouring supports. The spacing between comb supports was, however, found to be random. Workers may, however, use a cue originating from the size or mass of combs supported in the construction of supports. Downing and Jeanne (1990) found evidence that the thickness of the petiole in *Polistes fuscatus* was positively related to comb mass. They did not, however, find any significant effects of comb mass on the number of secondary comb supports constructed. In both *D. sylvestris* and *D. norwegica* the number of brood reared in the combs directly and indirectly supported were significant in explaining the variation in the total length of comb supports constructed. As the meconia produced during pupation contributes significantly to the mass of the comb, the number of brood reared in a comb is reliable indicator of mass. The cue for comb support construction therefore appears to result from a change in comb size or mass. In *D. norwegica* the comb surface area was also significant. It is unlikely that workers use a direct measurement of nest size, as this would require extensive surveying of all the

combs supported before adding a pulp load to the supports. Workers cannot directly measure the mass of combs supported. Downing and Jeanne (1990) suggested a mechanism whereby comb mass could serve as a cue for the construction of secondary supports in *Polistes fuscatus*. They suggested that workers may indirectly detect the mass of combs supported through the frequency of vibration of the comb as they walk on it.

A further cue for comb support construction, which could be used by workers, is the distance between combs. An uneven increase in the mass of combs supported would result in the combs tilting. Downing and Jeanne (1990) found that when the brood comb of *Polistes fuscatus* was weighted unevenly, workers were stimulated to construct secondary supports only if the comb tilted and came within a threshold distance of the substrate. A change in the distance between combs could therefore form a cue for the workers to construct suspensoria. This would cause several problems, firstly that the combs would have to move before the supports are put in place and secondly this does not provide a cue for the initial comb supports.

The results presented in this thesis have shown that wasps have very simple behavioural rules in nest construction. In the introduction to the thesis it was shown how complex, organised construction behaviour at the level of the colony, could result from simple behavioural rules at the level of the individual (Downing and Jeanne 1989,1990; Karsai and Penzés 1993, 1998; Franks and Deneubourg 1997). More complex behavioural rules at the level of the individual would have some benefits in terms of the efficiency of allocation of materials. Workers for example appear to have a fixed behavioural rule for the allocation of material to comb and envelope. A more sophisticated rule may involve cues arising from temperature, nest site restrictions and developmental stage. The use of additional cues would allow envelope construction to more closely match the needs of the colony. It would, however, have penalties in that workers would have to spend more of their time in surveying the nest and in making decisions.

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