A STUDY OF FACTORS AFFECTING QUEEN SURVIVAL, NEST INITIATION, AND NEST DEVELOPMENT IN THE BALDFACED HORNET *Dolichovespula maculata* (L.)(Hymenoptera:Vespidae)

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A STUDY OF FACTORS AFFECTING QUEEN SURVIVAL, NEST INITIATION, AND NEST DEVELOPMENT IN THE BALDFACED HORNET *Dolichovespula maculata* (L.) (Hymenoptera: Vespidae)

Abstract

Mating success and nutrient reserve effects on queen survivial and nest initiation were examined with respect to the reproductive fitness of queens of the baldfaced hornet, *Dolichovespula maculata*. The sperm content of the spermatheca was examined in queens of this hornet species and in queens of 10 other yellowjacket species collected in spring and summer of 1987, 1988 and 1989. Queens of the baldfaced hornet, *D. maculata*, were also analyzed for sperm content prior to nest initiation and during colony development. A regression model was employed to determine sperm utilization. Based on total cell counts and adult census, the sperm use efficiency of this species changed from approximately 90-100 sperm released per egg in the initial stages of colony development, to 2-3 sperm released per egg after 1000 eggs had been laid. Near the end of the season the predicted number of sperm released per egg was 0-1. The number of sperm in *D. maculata* males ranged from 2-2.5 million; a value considerably greater than the mean sperm number (20,223; $\pm$4,669) found in mated spring queens (n=15). Nineteen (18.4%) of 103 yellowjacket queens analyzed in the spring seasons of 1987 and 1988 were uninseminated. The effects of mating success and sperm utilization on colony development are discussed. A significant regression of sperm content on colony size was also found for 8 species of yellowjacket.

Queens of *D. maculata* were collected for studies of nutrient reserves before hibernation in the fall, during nest initiation, and after colonies had

produced more than 2 worker broods. Fresh and dry weights were determined for all queens and a seasonal comparison of the energy reserves of lipids, sugar, and glycogen was performed on the thoracic and abdominal tagmata. Total nitrogen was also quantified to estimate protein changes by season. The results show that lipids accounted for 35% of the weight lost during hibernation, sugars 12%, and glycogen 6%. Total thoracic nitrogen remained constant throughout the year, whereas abdominal nitrogen was the same in the fall and spring queens but increased in the summer queens. The results from this study suggest that most queens which survive the winter and successfully initiate nests have similar energy reserve quantities. The implications of these findings to solitary foraging behavior are discussed.

The eggs of *D. maculata* were examined in the spring and summer to quantify the energetic contributions provided by the queen. Mean egg weight was highest in the spring and decreased with progressive nest development. Both egg weight and energy reserves were variable among and within nests. The nutrient reserves for eggs in embryo nests, in order of importance, were lipid (22%), glucose (9.5%), and glycogen (5%). A 10-day egg incubaton experiment demonstrated a mean loss of 83 ug dry weight; lipids could not completely account for the weight loss, either quantitatively or qualitatively. A study on egg developmental time failed to provide sufficient data; all eggs eventually died although embryonic larval movement was detected in 2 eggs after 16 days. The energetic contributions which a queen provides to the eggs are discussed with respect to foraging behavior and environmental influences.

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CHAPTER 1 - INTRODUCTION

Introduction

Hornets, yellowjackets, and other social wasps have been regarded throughout history as volatile and enigmatic insects. King Menes, the first Pharaoh of Egypt, used the hornet as a symbol of power and strength in Lower Egypt, nearly 5000 years ago. Hornets and other wasps have received some attention in the ancient literature as well. Aristophanes the Greek dramatist, satirized the ancient Athenians in a play entitled *The Wasps*. He likened those who served as jurors in law courts to wasps for their irascible nature and love of money. Many of the early Roman scholars, including Virgil, Ovid, and Pliny the Elder, made references to hornets and wasps in their writings.

The earliest written accounts of social wasp biology and behavior, however, come from the writings of Aristotle in Book IX of *Historia Animalia*. His descriptions were fairly accurate, although the queen was referred to as the king and the workers were considered males. It was only in later centuries that a distinction was made between the sexes. The foundress queen or "leader" received some attention from Aristotle; specifically, the overwintering and nest intiation phases, as well as her interactions with the workers.

Most of the modern accounts of vespine biology and behavior have been written in the last 85 years, beginning with the work of R. DuBuysson in 1905. His monograph described general vespine biology with identification keys from many countries throughout the world. In 1939, C. Duncan published a major account of North American vespine biology, which included a number of anatomical drawings. In the 1970's and 1980's much of the North American yellowjacket

biology and behavior was reported (review in Akre et al. 1981) and it was during these years that an interest was rekindled in the factors affecting annual colony abundance. Numerous biological and environmental factors have been used to explain variation in queen abundance. Most recently, several authors have suggested that the condition or fitness of the queen may affect annual population cycles.

In northern temperate regions of the world, solitary queens of the Vespinae enter hibernation sites in late summer and early autumn. At this time, survival is dependent upon their inherent physiological condition, as well as their choice of hibernation site. Queens become vulnerable to many environmental pressures, resulting in high levels of queen mortality. It has often been suggested the queen mortality is the greatest at this point in the life cycle.

In the springtime, surviving queens emerge from their hibernation sites, usually in late April or early May. The queens feed on floral nectar, honeydew, and insects before they begin construction of their nests. This period of initial nest construction and brood production is an energetically demanding one of the queen. As in the winter, the queen in spring is vulnerable to numerous environmental influences; queen and incipient colony mortality are very high, with some estimates as high as 99% (Spradbery 1973; Edwards 1980).

The present study of queens of the baldfaced hornet, *Dolichovespula maculata* (L.) was conducted to better understand the specific physiological, behavioral, and ecological factors which can affect solitary queen survival, nest initiation, and nest development. Although some general accounts of queen behavior at these points in the life cycle are available, in-depth biological studies

have not been made.

This investigation consists of three major parts. The first part is a study of the effects of mating success and sperm utilization on nest initiation and subsequent colony growth. The second part is a seasonal comparison of the weight and energy reserve changes in queens, with an emphasis on spring queens and the changes that occur at the critical point in the life cycle. Finally, the third part of this investigation involves a study of the energetics concerned with initial egg and brood production.

CHAPTER 2 - Literature Review

Ecological and Economic Importance

Yellowjackets are among the most ubiquitous and important wasps of North America (Akre et al. 1981). Although general aspects of yellowjacket biology and behavior are well-known, the knowledge of their ecology and economic importance is far from complete (Davis 1978; Akre and Davis 1978). Furthermore, the dichotomous nature of the yellowjacket, as a beneficial predator of pest insects and as an economic pest, precludes an accurate estimation of its overall impact.

Yellowjackets prey upon many pest insects. The literature is replete with references to yellowjackets preying upon calliphorid and muscid flies, orthopterans, homopterans, lepidopterous larvae, to mention only a few (Morris 1972; Spradbery 1973; MacDonald et al 1974; Greene et al. 1976; Schmidtmann 1977; Edwards 1980; Akre et al. 1981). However, the inclination of yellowjackets to nest in areas associated with human activity, their strong attraction to human foods, and their tendency towards stinging has earned the yellowjacket its reputation as a nuisance. In addition, yellowjackets have, at times, caused severe economic problems to agricultural, forest and fruit industries. Akre et al. (1981), Akre and Davis (1978), and Akre and MacDonald (1986) have cited numerous cases in which yellowjacket densities were so high that workers in the affected industries refused to work for fear of being stung. Hawthorne (1969) reported that yellowjackets in California cost agricultural operations an estimated $ 200,000 in 1968 from losses that were primarily associated with attacks on fruit pickers and field workers. Davis (1978) also estimated that thousands of dollars are lost

annually by beekeepers throughout the world because of yellowjackets. Stein and Wrensch (1988) surveyed the county extension agents and pest control operators in Ohio and found that most economic disturbances were attributed to yellowjackets associated with homeowners, outdoor businesses and outdoor recreational facilities. Ascerno (1981) reported that between 1976-79, the Insect Information Service at the University of Minnesota received an average of 1,000 telephone calls per year from people with yellowjacket problems.

Yellowjacket Biology

Yellowjacket biology and behavior differ among the various species, but the following description provides a general life cycle pattern based on the reports provided by Duncan (1939), Spradbery (1973), Edwards (1980), and Akre et al. (1981).

In late spring, hibernating yellowjacket queens emerge from their hibernaculae. Typical hibernaculae are located inside decayed logs, under loose bark, and in the ground. Although there is no experimental evidence, several theories suggest that a combination of temperature and photoperiod provide the cues for yellowjacket emergence from the hibernaculum and subsequent nest initiation. After emergence, the queens begin to search for nesting sites in various locations. Species of *Dolichovespula* build their nests on branches of trees, or on artificial structures such as soffets, awnings, etc. The members of this genus make their nest paper from wood that is weathered but still has some structural integrity. Other paper sources such as cardboard, paper cartons, and mulch are often used as well. Species of *Vespula* which includes the *Vespula vulgaris* and

Vespula rufa species groups, build their nests in the ground, although they may place them inside structures, such as wall voids and the attics of houses. The nest paper of the *Vespula* is made from weathered and decayed wood, dead grasses, and the stems of other dead plants. Unlike the *Dolichovespula* which have relatively strong nests, the nests of the *Vespula* species groups tend to be weak and very brittle.

After locating a nesting site, the queen begins to form a nest pedicel by adding well-masticated wood or plant materials to this site (Spradbery 1973; Edwards 1980). After pedicel formation, more paper pulp is added for the manufacture of hexagonal nest cells and a surrounding paper envelope. Jeanne (1977a) found that queens of *D. maculata* (L.) and *D. arenaria* (F.) coat the pedicel of the nest with a rubbery, translucent material that enables the nest to swing from side to side. The flexible pedicel permits the queen to move freely between the comb and nest envelope, as the space between these structures becomes restricted due to nest growth. Most foundress queens build 20-45 cells during the nest initiation period (Spradbery 1973; Akre and MacDonald 1986). However, the number of cells produced by the queens of a given species are variable; the number of cells which are produced by queens of *D. maculata* has not been reported. The foundress queen performs all duties during the period of nest initiation with major efforts devoted to nest construction, nectar foraging, and prey collection.

The queen will lay an egg in each of the cells, after a cell is partially built. Eggs hatch within approximately 5 days (Spradbery 1973; Edwards 1980), giving rise to larvae which pass through 5 instars. The larvae require proteinaceous

foods for their development; the most common being live arthropod prey (Spradbery 1973; Edwards 1980; Akre et al. 1981). However, Edwards (1980) suggested that larvae will occasionally feed on carbohydrates. He provided several anecdotal accounts of workers passing colored sugar water and the juices of cherries to larvae. In colonies with adult workers, differences exist between species with regard to the types of food collected. Yellowjackets of the *Vespula vulgaris* species group tend to be both predators and scavengers (Akre et al. 1981). In contrast, members of the *V. rufa* species group and the *Dolichovespula* are exclusively predaceous (Akre et al. 1981). Prey are collected by an adult and malaxated for some time before being given to the larvae (Spradbery 1973; Edwards 1980; Akre et al. 1981). Larvae chew on the prey, swallowing both digestible and non-digestible parts. The larvae will often discard uningested portions of the prey, if it has not been sufficiently malaxated (Spradbery 1973).

During the larval stage, the hindgut is occluded and the Malpighian tubules are functionless; therefore, the larvae cannot excrete wastes in the usual manner (Spradbery 1973). The larvae void water and excess free sugars and amino acids via labial gland (trophallactic) secretions. The labial glands extend nearly the entire length of the body and are thought to provide a type of water excretion which substitutes for the non-functioning excretory system. Ishay and Ikan (1968a) have questioned whether the sugars produced by the larvae are in fact excess. They found that larvae of *Vespa orientalis* (F.) are able to convert proteins into sugar; thus, sugars in the saliva originated from the excess proteins on which the larvae were fed. Ishay and Ikan (1968b) also demonstrated the

importance of the trophallactic secretions to colony survival. They found that colonies of *V. orientalis* could not survive when the adults were deprived of larval secretions; therefore, colony survival is dependent on the reciprocal feeding relationship that exists between the larvae and adults.

After completing development in the 5th instar, a larva spins a silken cocoon and develops into an exarate pupa. After approximately 10-15 days, it emerges as a worker and assumes the duties previously performed by the queen (Spradbery 1973; Edwards 1980). As the worker population increases, the queen is relegated to the role of egg-layer.

The nest continues to grow by the addition of concentric rings of cells added to the periphery of the comb. Nest construction is a gradual process. Nest paper is removed from the internal envelopes, re-chewed and used to make new cells. Simultaneously, new paper is added to expand the outer envelope.

A colony normally produces workers until a sufficient worker population is present (mid-summer), at which time the workers begin to construct cells for the production of queens and males. The transition from worker to reproductive production is a gradual one (Greene 1984) and the factors which stimulate the building of cells for both queens and males have not been fully examined. However, several studies have been made. Potter (1964) transferred a foundress queen from a nest with large cells to a young nest without large cells; the transfer resulted in the construction of large cells by the workers. Ishay (1975) found that in the absence of the queen, large cells were never initiated at the end of the season. Apparently, the presence of a strong queen influences male and queen cell construction.

As queen and male cells are constructed, the queen begins to produce eggs destined to become reproductives. Males are produced from unfertilized eggs. Queens, like the workers, develop from fertilized eggs. Currently, there are no definitive explanations for why queens are larger than workers. Edwards (1980) reviewed caste determination and suggested that whether an egg becomes a worker or queen larva would depend on cell size and the amount of food which the larva is given. Montagner (1967; cited in Spradbery 1973) found that the transfer of a larva from a large cell to a worker cell would result in the production of a worker. However, when the reverse was done, the worker larva did not become a queen larva.

Sometime in late summer and/or early autumn, the newly emerged queens and males mate. Mated queens then seek hibernation sites. At this time, the cohesive behavior of the colony dissipates as males and new queens leave and the old queen and workers die. The nest then decomposes; a process which is facilitated by various scavenging Coleoptera, Diptera, Lepidoptera, and Orthoptera.

Pre-Wintering and Wintering Biology of Queens

Foraging Behavior

Newly emerged queens exhibit a variety of foraging behaviors. Edwards (1980) reported that new queens feed on nectar and other carbohydrates collected by workers. Similarly, Spradbery (1973) found that young queens feed on larval secretions and regurgitated foods, but would collect food while foraging

outside of the colony. Akre et al. (1982) reported queens of *V. consobrina* (Saussure) to obtain liquid food from workers. In addition, Akre et al. (1982) and Greene et al. (1976) reported that new *Dolichovespula* queens will malaxate prey which has been collected by workers. The feeding and foraging behavior of the pre-wintering queens provides the queens with increased energy reserves before leaving the nest to hibernate. Spradbery (1963) reported that new queens increase their lipid reserves by as much as 40% before the overwintering period.

The Biology of Winter Queens

The queen spends the winter in a hibernaculum and unless disturbed, remains in this site. There are, however, accounts of yellowjacket queens flying on warm winter days (Fox-Wilson 1946; Spradbery 1973). These queens are believed to return to their original hibernation site, although a new site may be located. Occasionally, queens will die by failing to return to a site before the onset of cold temperatures.

Queens of *D. maculata* are typically found inside decomposing logs during the winter (Rau 1929 and 1934; Gibo 1972). Gibo (1972) demonstrated 100% survival of *D. maculata* and *D. arenaria* queens at a temperature of 0°C, at -5°C he found that only 80% of the *D. arenaria* queens survived, whereas none of the *D. maculata* queens died. At temperatures ranging from -5°C to -10°C all of the queens died. Gibo (1972) recorded temperatures within the hibernation sites of both species in Toronto, Canada, and found a gradient of temperatures, with the surface temperatures being the coldest. He concluded that both species were sufficiently isolated from the extreme cold and would rarely be exposed to lethal

temperatures.

Duman and Patterson (1978) and Duman et al. (1984) investigated the survival strategies of *D. maculata* queens and found that ice-nucleating proteins (MW 74,000) were used in conjunction with glycerol as a frost tolerant mechanism. They also reported that queens were tolerant of ice formation in their body fluids at temperatures as low as -14°C. These results conflict with those reported by Gibo (1972); however, Gibo (1972) has provided the only study of the effects of temperature on adult queen mortality for any vespine species.

Studies which have extensively examined the biology of winter queens are lacking. This is due in part to the difficulty of locating natural hibernation sites selected by queens and to a lack of success in finding sufficient queens for analysis.

Archer (1980) has reported that approximately 98% of yellowjacket queens die during the winter, but did not describe specific mortality factors. In addition, his estimates came from a study in which he counted 20,500 queens in the fall (from one area) and was able to locate only three nests in the same area in the spring. Archer (1980) also made calculations from the thesis data of Arnold (1966) and reported the following winter queen mortalities: *V. germanica* (F.), 85%; *V. rufa* (L.), 59%; *D. sylvestris* (Scopoli), 98%. Unfortunately, he did not provide any data, such that, the fate of most winter queens is still largely unknown.

Biology of Spring Queens and Embryo Nests

The Spring Queen and Embryo Nest

Few studies have examined the physiological condition of post-hibernating queens. Spradbery (1973) reported that yellowjacket queens in Britain lose 30% of their dry weight during the hibernation period. Similarly, Matsuura (1969) found that *Vespa* queens in Japan lose 38% of their dry weight during hibernation.

The energy reserves of queens are low after the hibernation period and queens begin feeding shortly after emergence. Spradbery (1973) and Edwards (1980) reviewed several anecdotal accounts of the foraging behavior of spring queens, noting that queens have been observed to collect honeydew, tree sap, floral nectar, and live arthropod prey. These authors also noted that queens will delay nest construction for 2-3 weeks after the hibernation period. During this time, queens not only forage; but simultaneously begin to develop their ovaries. After this initial foraging period, queens begin to construct nests and lay eggs. At this time, a queen forages exclusively for nesting materials until the eggs hatch and the larvae require feeding (Spradbery 1973).

Although many details of nest construction and development have been reported, the biology of the queen, eggs, and larvae have not been studied as well. Several authors (Spradbery 1973; Brian and Brian 1952; Potter 1964) have examined the egg development process in the springtime nests. Most vespine eggs are reported to hatch within 5 days in young colonies, although developmental times are variable and are probably influenced by temperature. Spradbery (1973) found that eggs of *V. crabro* required from 5-18 days to hatch;

however, during the summer, the average developmental time was reported to be 5 days. The time required for complete larval development is variable as well. Potter (1965) reported developmental times of 9-17 days in *V. vulgaris* (L.), with an average developmental time of 14.5 days. The time spent in the pupal stage has also been found to be variable among species with an average developmental time ranging from 9-15 days (Spradbery 1973; Edwards 1980).

Thermoregulation in Embryo Nests

Thermoregulation in embryo nests has been studied by Gibo et al. (1977) and Makino and Yamane (1980). Gibo et al. (1977) found that a nest of *D. arenaria* with the foundress queen and 15 eggs had elevated temperatures of no more than 1°C above ambient, but in a nest with larvae found the nest temperatures increased by 2-3°C. Upon death of the queen, the larvae were still able to maintain the nest temperature 1°C above ambient. In comparison, temperatures higher than ambient were not found within a *D. maculata* nest until the larvae were half-grown. At that time, temperatures were found to be 3-4°C higher than ambient temperature. Makino and Yamane (1980) found that resting queens of *Vespa simillima* Smith were able to increase internal nest temperatures by 3-4°C. Whether a 3-4°C increase in temperature is significant to brood development has not been thoroughly investigated, nor has the possibility of more direct heat transfer to larvae. However, it is doubtful that queens of the Vespinae could produce the same quantities of heat as bumble bee queens. Heinrich (1974) demonstrated that bumble bee queens can maintain brood temperatures as much as 25°C higher than ambient, even in the absence of nest

insulation.

Thermoregulation in the summer nests has received more attention. Ishay and Ruttner (1971) found that nests of *V. orientalis* maintain a constant temperature of 30°C and that the adults are stimulated to produce heat by the presence of pupae. In addition, Ishay (1973) found that larvae of *Vespa crabro* (L.) significantly contributed to heat production. Gibo et al. (1974) reported average summer nest temperatures for *D. maculata* and *D. arenaria* of 28.9°C and 31.0°C, respectively.

Springtime Mortality

Yellowjacket wasps are most commonly observed in the summer during the exponential phase of colony growth. However, many colonies do not reach this developmental stage, since many queens and colonies die before or during nest establishment.

Although yellowjackets suffer heavy losses in the winter, mortality in the springtime is also high. Factors often attributed to mortality include adverse weather, disease, predation, competition for nest sites, nest usurpations and the quality or fitness of the queens (Beirne 1944; Fox-Wilson 1946; Spradbery 1973, Archer 1980, 1981 and 1982; Akre and Reed 1981; MacDonald and Matthews 1981; Roth and Lord 1987).

The embryo nest is believed to be the most vulnerable stage of colony development with estimated mortality rates as high as 99% (Spradbery 1973; Edwards 1980). Unfortunately, few studies have followed an appreciable number of embryo nests throughout the spring season. Brian and Brian (1952) are most

often cited for their study of *D. sylvestris* in which they reported that 1 nest of 12 successfully produced workers (92% mortality). Although they could not account for all of the nest failures, they suggested that the physiological condition of the queen, effective foraging, and efficient regulation of nesting behaviors contributed to colony success. MacDonald and Matthews (1981) have also reported high mortalities of spring and summer nests and found that a small percentage of observed nests produced queens.

Mating and Reproductive Biology of Queens

Mating Locations

MacDonald et al. (1974) presented a compilation of the mating behaviors for yellowjacket species which have been observed by various researchers in Europe and North America. Most of these observations were restricted to cage studies, although several accounts from field studies have been published. The initiation of matings on vegetation has been reported for *V. germanica* (Thomas 1960) and *V. crabro* (Rouget 1873; cited in MacDonald et al. 1974). Spradbery (1973) presented 2 accounts of matings that occurred near the nest entrance for *D. sylvestris* and *V. germanica*. Mating in the nest is also a possibility for many species. Schremmer (1962) and Kemper and Dohring (1967) both reported that mating will occur in the nests of *D. saxonica* (F.), *V. germanica*, *V. vulgaris*, and *V. crabro*. Akre et al. (1982) reported that queens of *V. consobrina* mate inside of the nest. Similarly, MacDonald et al. (1974) observed that matings occur inside the nests of both *V. atropilosa* and *V. pensylvanica*. According to most of these authors, mating-site location and mating duration are variable behaviors.

Sibling Mating

Ross (1983) found that queens of *V. maculifrons* (Buysson) readily mated with both sibling and non-sibling males when each of these groups was presented to the exclusion of the other. However, when presented with a choice between non-sibling or sibling males, the queens chose the latter. This finding agrees with the observations of Akre et al. (1982) who suggested that "sibling matings may be the rule, rather than the exception in yellowjackets", with most matings occurring inside the nest. Their conclusions were based on observations of the mating biology of *V. consobrina* and the reports of Spradbery (1973) who found that new queens are rarely seen after leaving the parent nest in the autumn. In contrast, Edwards (1980) reported that yellowjackets mate more frequently with other males outside of the colony. Edwards (1980) believed that matings can occur within a nest, but suggested that a species can only "retain its vigor" by mating with males from other colonies.

Aggregations of male vespines away from nests, which have been likened to leks, have also been observed (Ross 1983; MacDonald et al. 1974; Batra 1980; Yamane et al. 1980). Lekking behavior certainly indicates the likelihood of mating outside of the nest, perhaps, with siblings and non-siblings alike.

Single and Multiple Matings

A considerable volume of literature has been devoted to studies of polyandry, sperm utilization, and kin selection theory in social insects such as ants, honey bees, and polistine wasps (Cole 1983; Page and Metcalf 1982; Ross 1983; Page and Metcalf 1984; Page 1986). Unfortunately, the yellowjackets have

received scant attention. Few studies have been made on the frequency of single and multiple matings with most observations reported from laboratory studies. Thomas (1960) observed that queens of *V. germanica* mate once under field conditions, but found that they would mate multiple times in the laboratory. MacDonald et al. (1974) reported that multiple matings occur in the lab in both *V. atropilosa* and *V. pensylvanica*. In the latter species, the queens were observed to mate with the same males twice. Similarly, Ross (1983) observed queens of *V. maculifrons* mating multiple times in the laboratory. From these studies, it appears mating frequency may be a variable behavior that can be influenced by a number of factors. Several mating strategies may exist and they could be mediated by both genetic and environmental factors.

The Spermatheca and Sperm Utilization

Yellowjackets and other social insects store the sperm received during mating in the spermatheca. The mating process is assumed to provide the queen with sufficient sperm to produce fertilized eggs throughout her reproductive life. However, few studies with social insects have attempted to examine the mated condition and sperm utilization patterns.

Matsuura (1969) reported that approximately 2/3 of *Vespa mandarinia* Smith queens survive the winter without being inseminated. This report conflicts with the findings of MacDonald et al. (1974) who found that queens of *D. arenaria*, *Vespula atropilosa* (Sladen), and *V. pensylvanica* (Saussure) are almost always inseminated.

Harbo (1979) studied the process of sperm utilization using artificially

inseminated queens of the honey bee *Apis mellifera* (L.). He suggested that sperm were released from the spermatheca in a constant volume of spermathecal fluid. As the sperm and fluid are released, the spermathecae fills with fluid, the sperm redistribute, and the number of sperm released per fertilization becomes progressively smaller. Harbo (1979) presented a sperm utilization model for his hypothesis which indicated a logarithmic depletion of sperm.

In the fire ant, *Solenopsis invicta* (Buren), Tschinkel (1987a) found that the age of the queen could be estimated by quantifying the sperm available in the spermatheca. Tschinkel and Porter (1988) reported that queens of *S. invicta* release an average of 2.6 sperm per female egg. Tschinkel (1987b) also demonstrated a relationship between sperm number and ovariole number in ant queens from several different subfamilies.

Population Dynamics

Beirne (1944) and Fox-Wilson (1946) presented evidence that yellowjacket populations fluctuate from year to year. Years of abundance were followed by years of scarcity. Beirne (1944) believed that an "unknown disease" contributed to these fluctuations, whereas Fox-Wilson simply ascribed these fluctuations to an "unknown factor". Archer (1982 and 1985) tested the effects of spring and summer weather on wasp abundance and found no significant weather effects. He did find that: 1) there were large variations in yearly abundance, 2) that there were 2, and possibly, 7 year cycles of abundance, 3) years of abundance tended to occur in pairs, and 4) during years of abundance, spring queens were scarce; and during years of low populations the converse was true.

Archer (1981) and Roth and Lord (1987) both demonstrated a relationship between queen-cell size and the abundance of yellowjacket colonies. In years when yellowjacket colonies were abundant, queen-cell size was small. Conversely, queen-cell size was larger in years when colonies were less abundant. According to Roth and Lord (1987), high springtime densities of large foundress queens results in a large number of colonies. This is followed by increased competition for food resources which results in the production of fewer, smaller queens at the end of the reproductive season. The more fit queens would be favored through competition with weaker queens the following year, resulting in the establishment of fewer colonies. The subsequent low colony densities would reduce worker competition for the available food, resulting in better fed larvae and bigger queens for the following year.

CHAPTER 3

Sperm Utilization Rates Of Baldfaced Hornet Queens, *Dolichovespula Maculata* (L.) And The Regression of Mean Sperm Content On Colony Size Of *Vespula* And *Dolichovespula* Queen Spp. (Hymenoptera: Vespidae)

Introduction

Environmental factors such as weather, predation, and disease have been used to explain the variation observed in annual yellowjacket population densities (Fox-Wilson 1946, Akre and Reed 1981, and Archer 1981 and 1982). More recently, Archer (1985) and Roth and Lord, (1987) have suggested the existence of a biennial cycle regulated by density-dependent factors. Several behavioral and physiological factors have been discussed: competition for nest sites, queen cell-size, and queen quality. However, mating success, has been virtually neglected. This factor has been examined with regard to queen fitness in ants and honey bees, but it has not been investigated as a factor affecting the fitness of yellowjacket queens and its resulting effects on nesting success.

In northern temperate regions of the world, vespid queens mate with males in late summer or early fall before hibernating (Spradbery 1973; Akre et al. 1981). Information on the quantity of sperm received by queens during mating and the frequency of single and multiple matings is lacking. MacDonald et al. (1974) and Ross (1983) have observed multiple matings in the laboratory, but field observations have not been reported.

The contents of the spermathecae of various social Hymenoptera have been examined by several authors with regard to reproductive success. Corbella and Goncalves (1982) have investigated the relationship between queen honey bee, *Apis mellifera* L., weight at emergence, number of ovarioles, and the spermathecal volume. Tschinkel (1987b) investigated several subfamilies of ants and examined ovariole number and spermathecal sperm content. Tschinkel and Porter (1988) discussed the efficiency of sperm use in queens of the fire ant

Solenopsis invicta (Buren) and Tschinkel (1987a) reported that queen longevity and age could be estimated by spem depletion. Harbo and Szabo (1984) examined sperm depletion in honey bees and compared queens that were instrumentally inseminated with those that were naturally inseminated. Moritz (1986) examined sperm competition within bee spermathecae and Page (1986) presented a comprehensive review which discussed the role of the spermatheca as it relates to polyandry and kin selection theory.

In this study, the spermathecae of post-diapausing yellowjacket queens were examined to quantify sperm number, species variability in sperm number, and to determine if a correlation exists between sperm content, successful nest development, and colony size. In addition, sperm depletion and usage rates were examined in the baldfaced hornet, *Dolichovespula maculata* (L.), by collecting queens from colonies in various stages of development.

Materials and Methods

Queens of eleven species of yellowjacket (listed in Table 1) were collected and examined for spermathecal loading over a 3-year period. Queens were caught in the spring seasons of 1987-1989 while they were collecting nectar, water, or wood fibers. After collection, queens were kept alive in glass vials placed on ice and brought back to the laboratory. When possible, queens were dissected immediately to remove the spermatheca for sperm counting. However, they could be frozen at -10^{o}C for up to 2 weeks without any noticeable degradation of the spermathecal contents. After two weeks in the freezer, the spermathecal contents became discolored, changing from white to light brown,

and the sperm could no longer be counted.

Spermathecae were dissected from queens under a modified Hyes' diluent: NaCl (0.9%), $CaCl_2$ (0.02%), and $NaHCO_3$ (0.01%) in distilled water (Ruttner, 1975; Moritz, 1984). After dissection, the spermatheca was placed on the inside wall of a 1.5ml plastic microcentrifuge tube. Twenty microliters of Hyes' diluent were added to the tube and the spermatheca was ground with a microcentrifuge pestle for 1 minute. While grinding, it was important that the spermatheca remain in contact with the tube and pestle, otherwise the sperm were not thoroughly dispersed in the diluent. After grinding, the pestle was rinsed with 80 microliters of distilled water to bring the final volume to 100 microliters. Individual 10 microliter samples were withdrawn and placed on a Bright-Line, Reichert^R hemacytometer for counting. Three replicate counts were made for each extraction, and the results were averaged.

Twelve males of *D. maculata* were also analyzed by dissecting out the testes and seminal vesicles for the determination of sperm number. The same procedure was utilized, but the Hyes' diluent solution was increased in volume to 1ml; calculations were adjusted accordingly.

Estimates of sperm utilization rates were made for queens of *D. maculata* by collecting queens from established nests at various stages of development during the summer season. The nests were obtained at night by plugging the nest entrance with cotton, removing the nest, and then freezing the entire nest. The following day, the nest was dissected and the foundress queen removed for dissection and determination of spermathecal sperm quantities.

Sperm utilization rates were calculated in *D. maculata* queens in 1987 and

1989 by a regression of the sperm counts of individual queens on the total nest cells from which the queens were obtained and on the estimated number of fertilized eggs produced. Vespines reuse cells after adult emergence; therefore, the estimated number of fertilized eggs produced was calculated from cell reuse data provided for *Vespula vulgaris* (L.) by Spradbery (1973). The cells from embryo nests collected in May and early June would have been used only once during this developmental stage. Within the July nests, approximately 10% of the cells were estimated to have been reused. This reuse value was estimated to increase to 30% in August and 70% in September. The total nest cells (including worker, queen, and male cells) collected from nests in July, August, and September, were multiplied by 1.1, 1.3, and 1.7 respectively. Although males are reared in both worker and queen cells (Spradbery 1973, Edwards 1980), the cell reuse estimates are probably low. Thus, the presence of unfertilized eggs for male production in August and September nests should not significantly affect the estimates of fertilized egg production. Also, since the cell reuse estimates were based on data from *V. vulgaris*, different cell reuse estimates were used in the regression model to account for higher levels of fertilized egg production by *D. maculata* queens. These other estimates were obtained by multiplying the total nest cells in July, August, and September by: 1.2, 1.5, 1.9, and 1.5, 1.9, and 2.2, respectively.

An analysis of covariance in the GLM procedure (SAS Institute, 1985) was performed on the 1987 and 1989 models to test whether the data from the two different years could be combined into a single regression. Confidence intervals were calculated with the REG procedure (SAS Institute, 1985) by transforming the

exponential models to logarithmic form. The resulting intervals were then untransformed and graphed accordingly.

The sperm content data collected on eight yellowjacket species (*Vespula maculifrons* (Buysson), *V. squamosa* (Drury), *V. flavopilosa* Jacobson, *V. pensylvanica* (Saussure), *V. vidua* (Saussure), *V. consobrina* (Saussure), *D. maculata*, and *D. arenaria* (F.)) were also examined with respect to possible relationships between mean sperm content and colony size. The nesting biologies of most of the North American yellowjackets have been described (Akre et al., 1981), with values provided for the mean number of total worker and queen cells produced by each colony. Typically, members of the *Vespula vulgaris* species group have larger colonies than either the Dolichovespula or *Vespula rufa* species group (Akre et al. 1981).

RESULTS & DISCUSSION

Sperm Utilization in Queens of the Baldfaced Hornet

The values for mean sperm content in spring yellowjacket queens collected during 1987, 1988, and 1989 are shown in Table 1. The mean quantity of sperm varied from a low of 3,167 in *V. consobrina*, to a high of 176,000 in *V. squamosa*. The mean number of sperm also varied among queens of a given species collected in different years.

The most extensive data examined in this study involved the collection of *D. maculata* nests in an effort to correlate sperm utilization patterns with colony size and colony growth. The May collection period, performed in 2 different years, coincided with nest initiation. At this time, very little sperm, if any, would

have been used. In May 1987, the mean sperm content for the 9 queens sampled was 12,872, ± 2,082 (Table 1 and Fig.1). Nine queens collected from nests in late July 1987 (Fig. 1), had a mean of 11,722 sperm which suggested a slight decrease in sperm number from May. The July value was much lower than in 1988 and 1989, but was not statistically significant (P=0.85). Eight of the 9 immature nests collected during the July period had 1 comb, the other had two combs. The mean cell count of these nine nests was 330 ± 66 and mean worker number was 62 ± 20.

Four of 5 nests collected in August 1987 contained the foundress queen; the mean sperm content of these 4 queens was 2,125 ± 688 (Fig. 1). This illustrates a significant decrease (P=0.001) in sperm number from the preceding months which corresponded well with rapid colony growth that occurred in the preceding period. One of the 5 nests collected in late August had 2 combs, three nests contained three combs, and one nest had four combs. Mean cell and worker number in these five nests was 791 ± 144 and 214 ± 21, respectively. Daughter queens were also present in 2 of the 5 nests. One nest contained 29 queens and the other, only a single queen. Capped cells with queen pupae (n=33) were present in one of these nests, but numerous queen and male larvae were present in both. These data reflected a smaller than average colony population over the 3 year period.

The last group of 1987 queens was taken between September 1 and October 5 (Fig. 1). Thirteen nests were collected during this time period, but only 4 nests (30.7%) contained the foundress queen. An analysis failed to detect any

Table 1. Sperm counts of spring vespid queens.[1]

Species	Year	N	Mean	SEM	Range
V. maculifrons	1987	26	85,471	± 14,374	10,000-255,000
	1988	8	168,750	± 27,142	62,500-292,500
	1989	10	149,475	± 27,370	5,750-280,000
V. flavopilosa	1988	3	90,417	± 38,678	21,250-155,000
V. germanica	1987	9	102,750	± 23,898	40,000-255,000
V. pensylvanica	1989	9	57,500	± 15,413	18,750-158,750
V. squamosa	1987	4	100,438	± 29,322	23,750-154,000
	1988	5	176,000	± 46,194	75,000-318,750
	1989	1	90,000		
V. vidua	1988	2	10,500	± 1,500	9,000-12,000
	1989	3	8,417	± 4,283	1,500-16,250
V. consobrina	1987	2	4,500	± 500	4,000-5,000
	1988	3	3,167	± 600	2,000-4,000
	1989	2	5,000	± 1,250	3,750-6,250
D. arenaria	1988	13	34,750	± 6,126	18,750-78,750
	1989	9	25,250	± 5,353	3,000-57,500
D. arctica	1989	4	23,125	± 2,310	18,750-28,750
D. maculata	1987	9	12,872	± 2,082	5,000-26,250
	1989	6	31,250	± 10,082	5,250-67,500
D. norvegicoides	1988	1	18,750		

[1] *V. pensylvanica* queens came from a Hawaiian population. All other queens came from Montgomery and Giles counties Virginia.

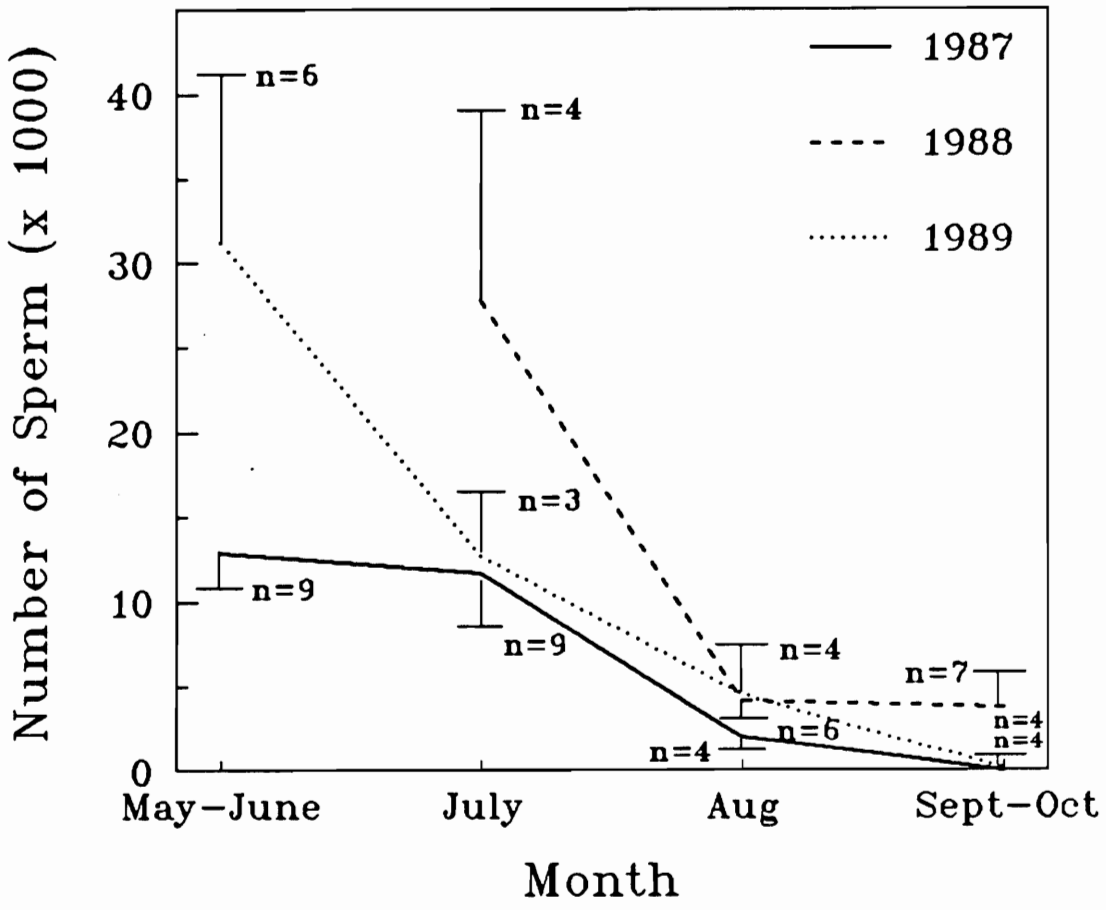

Figure 1. The mean number of sperm in the spermatheca of *D. maculata* queens prior to nest initiation and during colony development. Bars indicate SEM; sample sizes are adjacent to SEM bars.

sperm within the spermathecae of the 4 queens and an examination of the ovaries revealed extensive atrophy. The four nests analyzed within the September 1-October 5 time period had a mean total cell number of 1,523 $\pm$ 132. All nests had 4 combs, with one nest beginning a fifth comb with eight cells.

In 1988, no *D. maculata* queens were analyzed for sperm content prior to nest initiation. Four nests were collected during the last week of July and the mean sperm content of these 4 queens was 27,875 $\pm$ 11,149 (Fig. 1). Mean cell number for these nests was 731 $\pm$ 81. This group of queens had the highest July sperm counts observed for *D. maculata* over the three year period. One queen had 41,000 sperm and another, slightly over 50,000. The other two queens had less than 10,000 sperm. Although the mean cell numbers were higher in July 1988 than in July 1987, none of the July 1988 nests contained queen larvae or queen pupae. Nest development in the 1988 nests showed a more rapid early growth in comparison with those collected in 1987 and 1989; however, there was a reduction in colony growth as the season progressed. This was probably a result of the drought experienced by much of the country in 1988.

Four queens collected from nests in late August 1988 had a mean sperm content of 4,300 (SEM = 2,501; Fig. 1). This was a significant decrease ($P = 0.003$) in sperm number from the July period and followed the same type of trend observed in 1987. The last group of queens were collected from nests in September. Mean sperm content of these queens was 3,071 $\pm$ 1,825 (Fig.1). Two of the queens collected had no detectable sperm in the spermatheca. One nest collected on September 2 contained only 107 cells. This nest had only 3 workers in addition to the foundress queen. The queen of this nest had 250

sperm in the spermatheca, and evidence of an ovarian dysfunction (Fyg 1964). The presence of a well-inseminated foundress queen in late summer nests may not always suggest reproductive competence. Greene et al. (1976) reported observing a *D. arenaria* nest that contained 1,500 males and only 8 queens. The authors described the spermatheca of the foundress queen as "packed with sperm" although no counts were made. Undoubtedly, many factors affect individual queen fecundity.

In May 1989, the mean sperm quantity of 6 *D. maculata* queens analyzed (Fig. 1; Table 1) was significantly greater than the value recorded in 1987, and supports the idea of high insemination variability, not only between individuals, but also between populations in different years. Only 3 of 5 nests sampled in July 1989 contained the foundress queen; the mean sperm number of these 3 queens was 12,750 $\pm$ 3,464 (Fig. 1). The mean cell number of these 3 nests was 380 $\pm$ 191.6. These results compared favorably with those from July 1987.

In August 1989, the mean sperm quantities of 6 queens sampled was 4,694 $\pm$ 1,208 (Fig. 1). For August queens, the numbers of sperm in the spermatheca over the 3 year period were small and showed a significant decrease ($P = 0.003$) from the July groups. The mean number of cells from nests collected in August 1989 was 1,039 $\pm$ 192.1. Based on the size of September nests collected over a 3-year period, these results suggested that August 1989 nests had not attained maximum size. In addition, only 15 of 27 nests (55%) contained the foundress queen. Loss of the foundress queen is therefore common as the colony nears the end of the reproductive season.

During September 1989, 20 *D. maculata* nests were examined; only four

(20%) contained the foundress queen. Three of these queens showed no detectable levels of sperm in the spermatheca; the other queen had approximately 600 sperm. The mean number of cells in 4 nests collected in September 1989 was 1,749 $\pm$ 153.

For comparative purposes, twelve males of *D. maculata* were analyzed for sperm quantities in 1987. The sperm numbers of those males ranged from 2-2.5 million sperm. This quantity is approximately one hundred times the mean number found in the spermatheca of mated queens and supports the hypothesis that males have the potential to mate multiple times. Yellowjacket queens may mate several times in the laboratory (Ross, 1983 and MacDonald et al., 1974), but the frequency of multiple mating in the field remains unknown. Furthermore, the number of sperm transferred per mating has not been determined, although the high degree of variability with respect to sperm numbers between queens would suggest the possibility of multiple matings.

D. maculata sperm use and colony size data obtained during 1987 and 1989 were used to develop a sperm utilization model. An analysis of covariance revealed that the sperm utilization regression models for 1987 and 1989 were not different ($F = 4.69$; $df = 1, 41$; NS). Thus, the 1987 and 1989 data were combined and the results are presented as 2 separate regressions; one to estimate sperm use on the basis of nest cell number, the other, to depict sperm depletion as a function of the estimated number of fertilized eggs produced (Fig. 2). A comparison of the 2 models indicates that the regression of sperm number against estimated egg production proved to be a better indicator of sperm use ($r^2 = 0.65$ vs. $r^2 = 0.54$).

Within both models, the highest variability, which reflects insemination variability, was observed during the early growth period of the colonies, as indicated by the 95% confidence intervals (Fig. 2). Queens collected at nest initiation had probably used very little sperm and therefore, the observed sperm quantities reflect the mated condition. It is also possible that the initial values observed in the lower and upper confidence bands are indicative of single and multiple inseminations, respectively; however, the occurrence or frequency of single and multiple inseminations was not examined.

Due to the high variability in sperm quantities among spring queens, the data were analyzed with the spring queen data omitted. The resulting analysis which only examined queens taken from nests also indicated that an exponential decay model best described the data. Additional analyses of the sperm utilization regression model using the higher estimates for fertilized egg production, caused only a slight decrease in the coefficient of determination. These analyses suggested that changes in cell reuse estimates for total egg production did not have a significant effect on the model.

Given that all queens do not have similar sperm quantities, it follows that initial sperm depletion rates may be different as well. This model predicts that those queens with larger sperm quantities would use sperm at a faster rate than would those having smaller amounts. Further, the rate of sperm use depends on the number of eggs fertilized which are closely coupled to the ability of the workers to construct cells. Thus, differential growth rates in the early phases of colony development, could be a source of variability within the model as well. However, after approximately 1,000 eggs have been laid, which corresponds to a

nest that is a third grown, the rates of sperm utilization among queens appear to be similar and sperm use becomes asymptotic to zero (Fig. 2B).

The estimates of sperm released per individual egg were calculated from the parameters presented in Fig. 2B. Early in the season, during the embryo nest stage, an estimated 90-100 sperm are released per fertilized egg. After 500 eggs have been laid, which corresponds to late July colonies, the number of sperm released decreases to about 13-14 sperm per egg. A queen that begins her nest with initial sperm quantities far below the overall mean of 20,000 may still have a successful nest at this point, with normal numbers of workers. However, resulting low sperm numbers in late July or early August could facilitate nest failure or excessive male production before the normal onset of reproductive production. In mid-to-late August, or when approximately 1,300 -1,500 fertilized eggs have been laid, the sperm use in normally inseminated queens decreases to an average of 1-2 sperm released per fertilized egg. At this point in the season, worker production declines, but does not cease. All of the colonies collected were producing reproductives, although there was considerable variation in the numbers produced among nests. By late September, many of the nests were queenless, and of the nests that had queens, these queens had little sperm in the spermatheca. At this time, it is probable that those queens capable of laying eggs would release an average of no more than 0-1 sperm per attempted egg fertilization.

Harbo (1979) hypothesized that each time a queen honey bee lays an egg, a specific volume of spermatozoa-containing fluid is released from the spermatheca. As the number of sperm is reduced, the spermatheca fills with

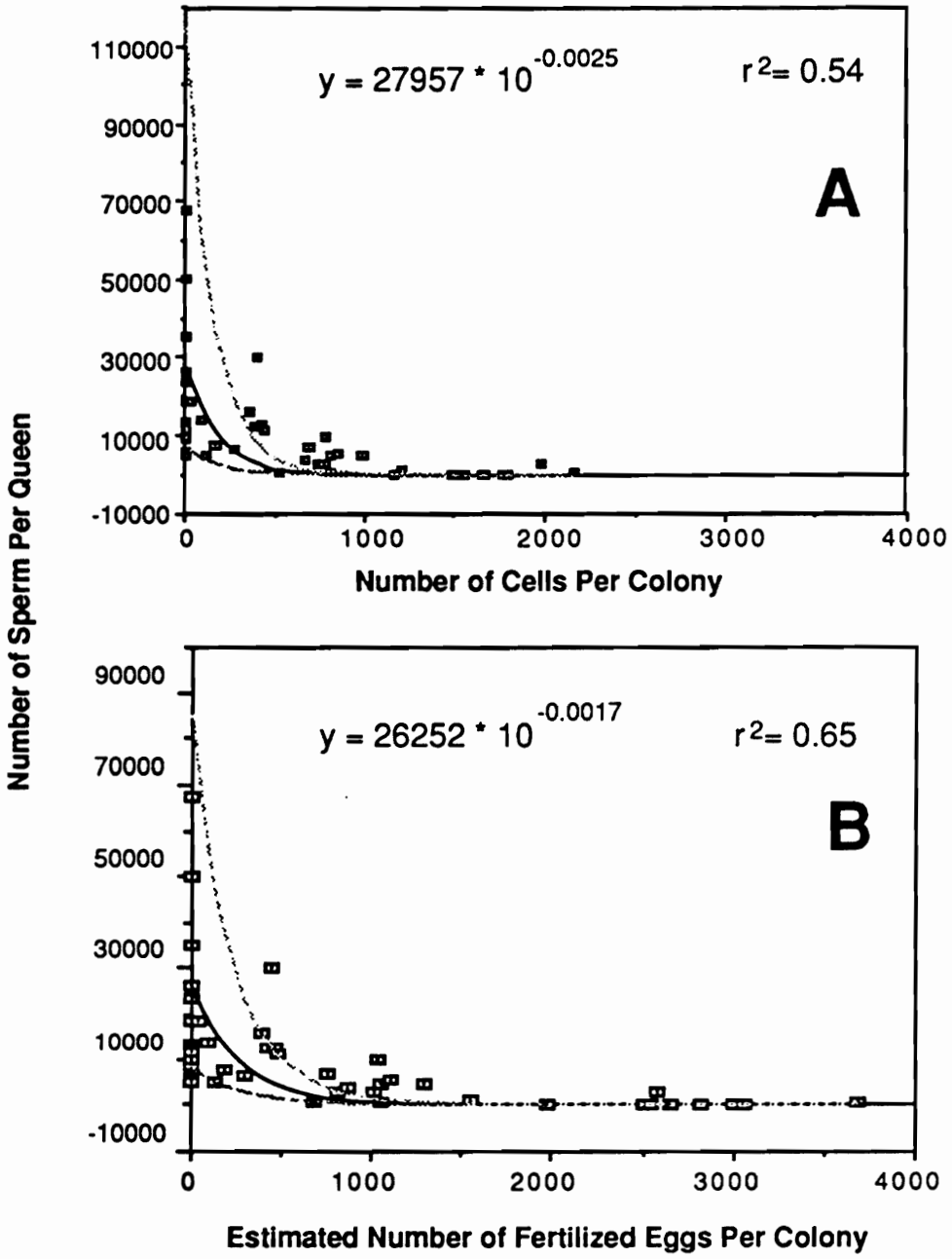

Figure 2. Sperm depletion in *D. maculata* queens as a function of numbers of cells produced by the colony (A) and as a function of the estimated number of fertilized eggs laid (B). Each square represents the spermathecal sperm quantity of one queen and the number of cells (A) and estimated eggs (B) from her nest. The exponential curve is bounded by 95% confidence intervals (dotted lines).

fluid. The same amount of fluid is released with each successive egg; thus, the number of sperm released per fertilized egg becomes progressively smaller. Harbo (1979) cited reports that honey bee queens use 10-100 sperm per egg, but experimentally demonstrated that queens use 20-30 sperm per egg. Although Harbo (1979) presented a detailed treatment of how sperm depletion occurs as a logarithmic decrease, his reported averages reflected a linear response. Similarly, Tschinkel and Porter (1988) discussed the depletion of sperm within fire ant queens as a linear function; reporting that queens of *S. invicta* release an average of 2.6 sperm per egg. If Harbo (1979) is correct, as appears from these data, then these types of estimates do not accurately reflect true sperm use patterns and often underestimate potential sperm use efficiencies, especially at low spermathecal sperm numbers.

One problem which occurs with these estimates, presently, is that the number of sperm which must be released to ensure fertilization is not known. Queens with numbers of sperm significantly below the species mean can successfully establish nests but may not produce workers the entire season. This would account for colonies which go into male production early or which produce a few queens and a large number of males during the reproductive phase.

Since many queenright summer colonies produce males but no queens, some type of queen dysfunction can be suggested; certainly sperm depletion is one possibility. In addition, the sperm utilization regression model assumes that all sperm are viable throughout the year. Although individual queens could have sufficient sperm quantities, the viability of the sperm could be affected with time.

Many of the foundress queens were not found in the mid- to late-season

nests and perhaps these queens died or were destroyed by the workers. Clearly, the absence of the queen would present an opportunity for some workers to develop their ovaries and produce male eggs. Indeed, many of the queenless nests collected during this study contained numerous cells with 6 or more eggs, suggesting worker oviposition. It is also possible that as a queen runs low in sperm, worker aggression increases so that workers can lay their own eggs (Spradbery 1973; Edwards 1980; Greene 1984).

In the spring of all 3 years, many embryo nests observed in this study were abandoned. Although vertebrate predation, and contraspecific and conspecific usurpations are quite common, as well as queen failure (Lord and Roth 1978, Matthews and Matthews 1979, Akre and Reed 1981, Archer 1985), the analyses of spermathecal content in spring queens has also shown that a high percentage of queens may survive the winter without having mated successfully. Nineteen of 103 queens (18.4%) collected in 1987 and 1988 were uninseminated; although none of the 27 queens examined in 1989 were observed to be uninseminated. Ross et al. (1981) observed uninseminated *V. germanica* queens initiating nests in the laboratory, but such nests could not survive. The data from this study indicates that similar conditions may occur in natural situations. Mating problems can thus be considered an important factor contributing to queen failure and nest mortality. Several factors may contribute to this lack of successful mating. Yellowjacket queens mate sometime in late summer or early autumn, and could be restricted by environmental conditions. In addition, yellowjacket queens, like honey bee queens, may only be capable of mating during a relatively short time period in early adulthood (Zmarlicki and Morse 1963). This factor should be

examined in future studies.

The Regression of Queen Sperm Content and Colony Size

Queens of seven yellowjacket species found in Virginia and *V. pensylvanica* queens from Hawaii were also examined during this study to test a regression of mean sperm number on colony size, which was based on the number of cells. The mean yearly sperm contents of each yellowjacket species were combined; the relationship between mean sperm quantity and total nest cell number is presented in Figure 3. The regression was significant ($P=0.01$; $r^2=0.69$) with most of the variability due to the insemination variability within and among queens of *V. maculifrons* and *V. flavopilosa*. The use of colony size data from different geographical locations within the United States undoubtedly affects the accuracy of the regression.

Vespula maculifrons queens were the most common species collected in the spring, during the three year period. The mean sperm content values for *V. maculifrons* collected in 1987 are shown in Table 1; means for this species should be representative for the population in southwestern Virginia. The combined mean sperm quantity of this species over the 3-year period was 115,159 (Fig. 3). MacDonald and Matthews (1981) reported the mean number of worker cells and queen cells per mature colony to be 5,283 and 2,551 respectively (Fig. 3). The position of *V. maculifrons* within the regression model is influenced by the low sperm counts of 1987. The 1987 data were highly variable, whereas values were more similar in 1988-1989 (Table 1). It is also possible that MacDonald and Matthews (1981) reported colony sizes greater than those which occurr in

Virginia. Finally, the sperm use efficiency for *V. maculifrons* may be different from what has been observed with other species (i.e., sperm is used at a lower rate).

The mean sperm content of *V. germanica* (Fab.) queens collected in 1987 was similar to *V. maculifrons* (Table 1). Currently, no published data are available on the mean number of worker and queen cells per colony in North America, thus, the regression of mean sperm quantity on colony size is not presented. However, given the mean sperm quantity and the higher than average worker number for this *V. vulgaris* species group (Akre et al., 1981), it is probable that *V. germanica* would be similar to *V. maculifrons*.

Three *V. flavopilosa* queens were collected in 1989; mean sperm content of these queens are shown in Table 1 and Figure 3. MacDonald et al. (1980) reported mean total worker cells for this species to be 1,871 but queen cell numbers were not given. However, they reported that queen cells accounted for 20% of the nest total; the total cell number was therefore adjusted accordingly. The data presented on *V. flavopilosa* do not follow the same trend as the other species. This may be due to the small sample size (n=3) and/or differences in colony biology. In general, *V. flavopilosa* colonies tend to be the smallest of any of the members of the *V. vulgaris* species group found in eastern North America (Akre et. al. 1981), suggesting that the sperm use estimates are too high, or that the species has a poor sperm use efficiency.

The mean sperm content of 9 *V. pensylvanica* (Saussure) queens collected from Hawaii in 1989 is presented in Table 1. The nesting biology of *V. pensylvanica* in the continental United States was described by MacDonald et al. (1974). They reported the mean number of worker and queen cells for this

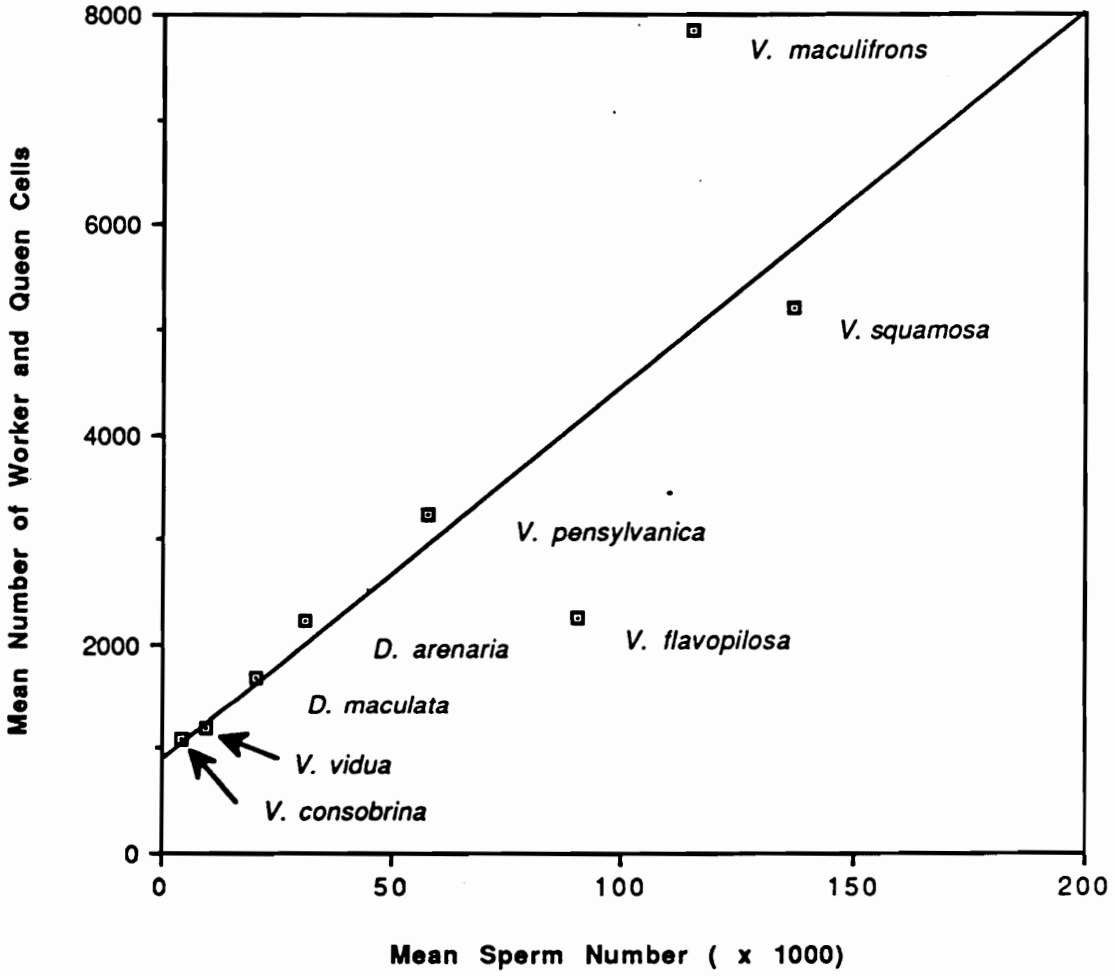

Figure 3. Regression of the number of worker and queen cells produced by mature vespid colonies on the mean spermathecal sperm content of *Dolichovespula* and *Vespula* queens. The regression was significant; $P=0.01$; $Y=875.10 + 38.134x$; $r^2 = 0.69$.

V. vulgaris species group to be 3,248. According to Figure 3, it is probable that Hawaiian *V. pensylvanica* populations have similar nesting biologies and sperm use rates as in North America.

Vespula squamosa queens collected in 1987 and 1988 had similar sperm number estimates as queens of the *V. vulgaris* species group (Table 1). Only one *V. squamosa* queen was collected in 1989, and it contained 90,000 sperm. One *V. squamosa* queen collected in 1988 had 318,750 sperm in the spermatheca, the highest value recorded for any of the species analyzed. The mean sperm number for the 3-year period was 137,175 (Fig. 3). Nests of *V. squamosa* have a mean of 4,200 worker and 1,000 queen cells (MacDonald and Matthews, 1984). Based on these data, and the location of this species on Figure 3, the sperm utilization for this species is more similar to the members of the *V. vulgaris* group, rather than the *V. rufa* species group in which it is often placed (Akre et al. 1981).

Vespula vidua queens collected in 1988 and 1989 had a mean of 9,250 sperm in the spermatheca (Table 1; Fig. 3). MacDonald and Matthews (1976) observed this species to have small nests with a mean worker cell number of 756 and a range of 170-700 queen cells. *Vespula consobrina* queens had the smallest mean number of sperm in the spermatheca for any of the species examined with a mean sperm number for the 3-year period of 4,071 (Fig.3). Nests of *V. consobrina* are also small, with mean numbers of worker and queen cells reported to be 703 and 386, respectively (Akre et al. (1982). Clearly, *V. consobrina* and *V. vidua*, both members of the *V. rufa* species group, have colonies which are much smaller than those in the North American *V. vulgaris* and *Dolichovespula* species groups (Akre et al. 1981). The sperm content data

obtained from *V. consobrina* and *V. vidua* agree with colony size data reported by Akre et al. (1981) and indicate that nest size and reproductive capacity could be limited because of small sperm numbers. In addition, both *V. consobrina* and *V. vidua* could be expected to have similar types of sperm utilization rates.

Dolichovespula arenaria queens had a mean sperm number for the 2 year period of 30,863 (Fig. 3). Greene et al. (1976) reported *D. arenaria* colony sizes with cell numbers for 2 different years; mean cell numbers differed significantly between the two years, with 548 and 2,234 cells per nest in 1974 and 1975. The larger value was chosen as representative of nest size in Virginia (Fig. 3). *Dolichovespula maculata* queens had a mean of 20,223 sperm over a 2 year period (Fig. 3); with mean total cell numbers of 1,680. Queens of two other *Dolichovespula* species were also examined but are not included in Figure 3; sperm count data for *D. norvegicoides* (Sladen) and *D. arctica* Rohwer are presented in Table 1. The latter species is an obligate parasite which has no worker caste (Jeanne 1977b). Knowledge of the nesting biology of *D. norvegicoides* is incomplete (Akre and Bleicher 1985) and, therefore, precludes any estimation of sperm use. Similarly, the behavior and biology of *D. arctica* has been reported, but no estimates of the number of reproductives produced have been made. If the *D. norvegicoides* and *D. arctica* sperm quantities presented in Table 1 are representative for the species, then it might be expected that these are similar in size to those of *D. maculata* and *D. arenaria* and sperm use rates to be similar as well (Fig. 3). However, the use of sperm by *D. arctica* queens would also depend on the vigor and size of the host nest. Since *D. arctica* is a parasite of *D. arenaria*, they may have similar but lower sperm levels.

Conclusion

Tschinkel (1987a) has suggested that the determination of sperm number within social hymenopteran queens would be useful in understanding the demographics of the species, including predictions of queen longevity, age, and age structure of the colony. The results presented contribute additional information to social insect ecology by demonstrating that mating success can affect the ability of vespid queens to establish viable nests. Furthermore, in established nests, mating success can limit the number of fertilized eggs produced by foundress queens, which can ultimately affect nest growth potential. Clearly, mating success is an important factor affecting individual yellowjacket colony fitness.

The sperm utilization and regression models which are presented in Figures 2 and 3 should prove useful in the prediction of potential colony sizes for social insects. Similar mating success studies with other social insects may provide additional insight into social insect ecology.

CHAPTER 4

A Seasonal Comparison of Weight, Energy Reserve, and Nitrogen Changes in Queens of the Baldfaced Hornet *Dolichovespula maculata* (L.) (Hymenoptera: Vespidae)

INTRODUCTION

Most of the ecological research on yellowjackets within the last 40 years has been devoted to nesting biology and population dynamics. More recently, interest has been extended to queen size and queen quality. Both Archer (1981) and Roth and Lord (1987) demonstrated a relationship between queen-cell size and the abundance of yellowjacket colonies (i.e., in years of abundance queen cells are small and vice versa). Roth and Lord (1987) have suggested that size may confer an "ecological advantage" with the greater nesting success advantage possessed by larger queens; however, their studies examined queen cell size only.

Little is known about the behavior of yellowjacket queens during the period following emergence from the pupal cell in late summer or early autumn, until hibernation. Similar information is lacking on hibernation biology and the biology and behavior of queens during the spring nest initiation stage. Most field descriptions of solitary yellowjacket queens tend to be anecdotal with few formal studies having been made (Keyel 1983). Edwards (1980) reported that newly eclosed queens are tended by nurse workers and are fed a diet high in carbohydrates to help accumulate lipid reserves before hibernation. Duncan (1939) and Spradbery (1973) reported that newly eclosed queens obtain regurgitated food from workers, as well as larval secretions, but that queens will forage individually to collect food. Information on the amount of time that queens spend feeding and foraging before mating and hibernation is unavailable.

The foraging habits and dietary requirements of post-hibernating spring queens are also poorly documented. Duncan (1939), Spradbery (1973) and

Edwards (1980) presented accounts of post-hibernating queens foraging on tree sap and floral nectar. Observations of post-hibernating queens foraging on arthropods have been largely restricted to captive settings. Watson (1922) reported that post-hibernating queens will feed on adult calliphorids and Ross et al. (1981) fed house flies, *Musca domestica* L., and crickets, *Acheta* spp. to post-hibernating queens in their nest initiation experiments.

During the solitary phase of colony life, the queen is responsible for all nesting activities (Duncan 1939; Spradbery 1973; Akre et al. 1981). The queen collects wood fibers for nest paper, builds the nest, lays eggs, forages for food, defends the nest, and, perhaps, incubates the brood (Gibo et al. 1977). Since the queen performs so many tasks, her energy demands should increase continuously with colony growth up to first worker emergence. Energy reserve deficiencies, or the inability to obtain food at this point in the life cycle, would be detrimental to both the queen and the survival of her newly founded colony.

To date, most studies on yellowjacket biology have virtually neglected the physiological condition of individual queens and the impact that queen condition could have on nesting success. Spradbery (1963) and Matsuura (1969) quantified the lipid content of vespine queens before and after hibernation, but neither quantified carbohydrates, proteins, or the weight changes that occur thoughout the season.

In this study, changes in weight and nutrient reserves of *Dolichovespula maculata* (L.) queens collected before hibernation, were compared with queens collected after emergence from hibernation, during nest initiation in the spring, and after colonies had produced at least 2 worker broods in the summer. Both

carbohydrate and lipid reserves were quantified and the total nitrogen content of *D. maculata* queens was analyzed to estimate the seasonal changes in total protein. These studies provide a physiological perspective of the weight and nutritive changes that occur in queens during the various seasons and the effect that they may have on nesting success.

MATERIALS & METHODS

Dolichovespula maculata queens were collected during four seasonal periods in Craig, Floyd, Giles, Montgomery, and Pulaski Counties, Virginia between 1987 and 1990. Fall queens were taken from nests in late summer and early autumn, before their overwintering period. Non-nesting spring queens were collected prior to, or during the initial period of nest founding. Nesting spring queens refer to queens captured in their embryo nests which contained eggs, and in some cases, first instars. The non-nesting group of queens were not collected from nests, but were captured while flying, or collecting water, nectar, or wood fibers. While it is possible that some of the queens within this group had initiated nests, their capture during the earlier part of the season would suggest that few had started nests. Summer queens were collected from nests that had produced at least 2 broods of workers.

Queens were kept alive in glass tubes (2.5 x 9.5 mm with a foam rubber plug) placed on ice and brought back to the laboratory. The queens were immediately killed by freezing and placed in a lyophilizer for 4 days. Queen weights were recorded before and after lyophilization. Queens were used for experiments other than those described in this text; therefore, in some cases

there were more fresh weight values recorded than dry weights. After lyophilization, the heads and wings were removed from each queen and the remaining body parts ground before analysis. The heads were removed with scissors by cutting adjacent to the dorsum of the postocciput. The wings were removed near the pleural wing process with scissors and a scalpel.

The thoraces and abdomens were separated by cutting them at the petiole[1]. Thoraces and abdomens were ground separately inside of a 1.5 ml microcentrifuge tube (Fisher Co.) for 1 minute with a Wig-L-Bug^{R} (#3110-31) dental amalgamator. The top 0.5 cm of the microcentrifuge tube was removed with a razor blade to ensure a proper fit in the dental amalgamator. Before placing a tagma into the microcentrifuge tube, 2 stainless steel ball-bearings were placed inside the tube; a 0.4 mm ball bearing on the bottom and a 0.47 mm ball-bearing on top of the first. The individual tagma was then placed above these two bearings, followed by a third ball-bearing (0.67 mm) on top. The tube was then sealed with the original cap. This method provided a homogeneously ground sample which could be subsampled for several types of analyses.

Extraction and Quantitation of Lipids

A 15-20 mg sample was weighed out from each ground queen thorax and abdominal section and placed in a 1.5 ml microcentrifuge tube to which 750 ul of chloroform: methanol (2:1) was added (modified from Van Handel 1965a). The tube was then vortexed and the samples centrifuged at (12,000 rpm) in a Fisher

1 Although the abdomen and gaster are recognized as taxonomically distinct, use of the term "abdomen" is more appropriate for this investigation.

microcentrifuge (#59A) for 5 minutes. The supernatant was transferred with a Pasteur pipette to a tared microcentrifuge tube. The original sample was resuspended and the extraction procedure repeated. After combining the supernatants, the microcentrifuge tubes were placed in a heated sand bath at 35^{o}C for 8-12 hours. The supernatants were evaporated as near to dryness as possible and the resulting lipid within the tube was determined by reweighing the tube. Spikeovers with known weights of corn oil, ranging from 10-15 mg, demonstrated mean lipid recovery of 97 $\pm$ 2%.

Lipid subsamples were taken from the crude extracts of 6 fall and spring queens. The lipids were analyzed by high performance thin layer chromatography (HPTLC) (Judge et al. 1989) to determine the different lipid classes.

Extraction of Carbohydrates

Three samples, weighing from 2 to 4 mg each, were obtained from each thorax and abdomen. Samples were placed into 1.5 ml microcentrifuge tubes to which 1 ml of 66% ethanol saturated with sodium sulfate was added (Van Handel 1965a). The tubes were vortexed and the samples were centrifuged (100 x G) for 10 minutes. The supernatants were transferred to 10 ml glass vials. The pellets were resuspended in 1 ml of 66%. ethanol saturated with sodium sulfate and the tubes were vortexed and centrifuged a second time. The remaining supernatant was added to the first. The ethanolic extracts were then diluted (1:10) by adding and mixing one part of the extract to nine parts of double-distilled water (ddH_2O) inside a 1.5 ml microcentrifuge tube.

The pellets from the previous step were dried by placing the tubes inside a sand bath at 35^{o}C for 1 hour. After drying, 500 ul of a 30% potassium hydroxide solution (KOH) was added to the tubes containing the pellets, along with 50 ul of a saturated sodium sulfate solution. The tubes were then immediately heated to 100^{o}C for 10 minutes. KOH solutions were made fresh before each extraction as a precautionary measure to avoid glycogen loss by contamination with CO_2 (Van Handel 1965b). In addition, the KOH was not added to the pellets until just prior to heating. After heating, the tubes were immediately cooled in an ice bath for 3 minutes. One ml of 66% ethanol saturated with sodium sulfate was added to each of the tubes. The tubes were mixed and centrifuged (12,000 rpm) for 5 minutes. The supernatants were withdrawn with a Pasteur pipette and discarded. The resulting pellets were resuspended in 1 ml of ddH_2O; this solution was diluted (1:10) by adding one part to nine parts ddH_2O.

Quantitation of Carbohydrates

An anthrone reagent was used to quantify both glucose and glycogen (Trevelyan and Harrison 1952; Van Handel 1965a). Fresh reagent was prepared before each analysis by adding 200 mg of anthrone (Sigma Chemical Co.)to 100 ml of diluted sulfuric acid (500 ml H_2SO_4: 200 ml ddH_2O). After mixing the anthrone reagent, undissolved crystals of anthrone were removed with a Pasteur pipette. Glucose or glycogen (oyster glycogen; Sigma Chemical Company) standards (5, 10, 25, 50, and 100 ug/ml) were run with each analysis for the determination of a standard curve.

Five milliliters of the anthrone reagent were pipetted into Pyrex test tubes,

which were then cooled in an ice water bath. A 1 ml volume of each standard, water blank, and sample (sugar or glycogen) was carefully layered on top of the anthrone reagent. When all of the samples had been prepared, the tubes were capped and mixed thoroughly. The test tubes were placed in a boiling water bath for 10 minutes, after which they were removed and cooled in an ice water bath for 3 minutes. The solutions were read with a Perkin-Elmer Lambda 3B Spectrophotometer at 620 nm. Spikeovers with 5 ug glucose and glycogen standards indicated mean recovery rates of 98 $\pm$ 3% and 94 $\pm$3%, respectively.

High performance thin layer chromatography was used to determine the sugars found in the hemolymph of fall and spring queens. One microliter of hemolymph was suspended in 10 ul of 66% ethanol saturated with Na_2SO_4. The sample was mixed and centrifuged at 12,000 rpm for 5 minutes. One ul aliquots of the ethanol extract were spotted directly onto the TLC plates for analysis as described in Fell (1990).

Quantitation of Total Nitrogen

The quantitation of total sample nitrogen was made with a modified micro-Kjeldahl technique (Mullins 1971). Three samples of known weight (400-1200 ug) from each thorax and abdomen were placed onto a tared glass sliver. Glass was used rather than weighing paper because many of the samples were oily. Samples and ammonium sulfate standards (5, 10, 25, 50, and 100 ug/ml ddH_2O) were placed into test tubes (18 x 100 cm), after which 1.5 ml of a oxidizing/hydrolyzing reagent (copper sulfate 313 mg/L, selenious acid 129 mg/L, mixed into 5N sulfuric acid) was added to each tube.

The tubes were placed into an oven at 120-150^{o}C for 8-10 hours, or until the water had evaporated from the digestion mixture. The disappearance of beads of water near the tops of the tubes indicated sufficient evaporation. After the water was driven off, a glass marble was placed on each tube and the temperature was increased to 350^{o}C for 12 hours. The tubes were then removed from the oven and allowed to cool. Four and one-half ml of ddH_2O were added slowly, while rotating the tube in an effort to rinse any accumulated debris from the inside wall of the tube. After the addition of water to all tubes, 4.5 ml of 3.3N NaOH were added to each tube followed by the addition of 3.0 ml of Nessler's reagent (potassium mercuric iodide 7g/L; gum ghatti 1.75g/L). The tubes were vortexed and set aside for 15 minutes to allow for maximum color development. The resulting yellow-orange colored solutions were read at 490 nm on a Perkin-Elmer Lambda 3B Spectrophotometer. Spikeovers with 5 ug/ml of an ammonium sulfate standard demonstrated 93 $\pm$ 3% recovery.

Statistical Analyses

Tests for normality and an analysis of variance (ANOVA) were performed on all class variables using the UNIVARIATE and GLM procedures, repsectively (SAS Institute, 1985). If significant differences were detected (alpha=0.05) among the class variables, then Duncan's New Multiple Range Test was used to locate the differences.

RESULTS

Dolichovespula maculata queens attained their greatest weight during the

late summer and early autumn before the onset of hibernation. The mean fresh and dry weights for queens collected in 1987 are shown in Table 2. The mean dry weight of 15 pigmented queen pupae with adult features was 163.7 mg $\pm$ 3.7 which suggests that newly eclosed queens can increase their dry weight by as much as 79%. The mean weight of autumn queens was significantly higher ($P=0.0001$) than the spring and summer queens, indicating that hibernating queens endure an appreciable weight loss during the hibernation period. Within the spring groups, no weight differences ($P\geq0.05$) were detected among the nesting and non-nesting queens over the 3-year period (Table 2). The mean fresh and dry weights for spring queens (nesting and non-nesting) collected over the 3-year period were 425.1 mg and 136.6 mg, respectively. These data indicate that losses in fresh weight during hibernation may be 30% (179.5 mg), and that dry weight losses may be 53% (157.0 mg). In addition, these data suggest that most queens which successfully emerge from hibernation and initiate nests have similar energy reserve quantities.

The fresh weights recorded for summer queens were the same ($P\geq0.05$) over the 3-year period; however, they were significantly different ($P=0.0001$) from both the autumn and spring groups (Table 2). The mean fresh weight of summer queens was similar over the 3-year period (Table 2), varying from a high of 517.2 mg (1989) to a low of 498.8 mg (1988). The mean dry weight of 8 summer queens collected in 1988 was 177.4 mg and represents a 30% (40.8 mg) dry weight increase between nest initiation in the spring and the summer season.

Seasonal weight changes in queen thoraces and abdomens were analyzed separately (Table 3). The comparison of spring and summer queen thoraces

Table 2. Comparison of mean fresh and dry body weights (mg ± SEM) of *Dolichovespula maculata* queens collected from southwestern Virginia during the autumn, spring, and summer, 1987-1990.

		Fresh Weight		Dry weight	
Group[1]	Year	n	Mean[2] ± SEM	n	Mean[2] ± SEM
Autumn	1987	31	604.6 ± 8.7 a	8	293.6 ±12.5 a
Spring non-nesting	1988	17	422.2 ± 9.0 c	8	129.3 ± 6.4 c
	1989	4	385.5 ± 22.6 c	3	125.7 ±10.3 c
Spring nesting	1988	5	449.1 ± 24.1 c	4	143.3 ±10.5 c
	1989	6	429.2 ± 13.3 c	6	139.4 ± 2.7 c
	1990	8	433.8 ± 8.4 c	8	142.6 ± 2.0 c
Summer	1987	18	498.8 ± 13.0 b	_[3]	—
	1988	15	501.4 ± 10.4 b	_	—
	1989	22	517.2 ± 10.6 b	8	177.4 ±13.5 b

[1] Autumn queens were collected from parent nest before hibernating; non-nesting spring queens were caught in flight; spring nesting queens were collected from embryo nests; summer queens were foundress queens collected from mature nests.

[2] Means within a column followed by the same letter, are not significantly different ($P < 0.05$; Duncan's New Multiple Range test).

[3] Dry weight data were not collected on summer queens 1987-1988.

revealed no significant differences ($P \geq 0.05$) in thoracic dry weight. However, the mean fall thoracic weight (113.5 mg) was significantly different ($P = 0.0001$) from both the spring and summer groups. The average thoracic weight loss that occurred between autumn and spring was 68.7 mg or 39.4%.

Greater dry weight differences were detected among the abdomens of queens. Mean autumn weight was 150.4 mg, which was significantly different ($P = 0.0001$) from both the spring and summer groups (Table 3). Mean weight for all spring queens collected during 1987-89 was 50.1 mg, suggesting a 66.6% loss in dry abdominal weight during the hibernation period.

During the summer, nest development is slow initially, but is followed by a period of accelerated growth (Spradbery 1973; Edwards 1980; Akre et al. 1981). This increase in growth rate reflects an increased oviposition rate of the queen; therefore, overall queen weight should be greater because of increased food intake, vitellogenesis, and ovarian development. Clearly, the summer abdominal weight was significantly different ($P = 0.0001$) from the spring group, showing an increase of 20.1 mg, or 40%.

Mean total thoracic nitrogen in *D. maculata* queens was not significantly different ($P = 0.57$) in different seasons; the mean quantity of thoracic nitrogen ranged from 8.0 mg-8.8 mg (Table 4). Although the nitrogen levels did not change during the year, the percentage of body weight represented by total nitrogen changed from 7.3% in the fall to 12.1% in the spring. The lower value observed in the fall was due to the high levels of both lipid and carbohydrate reserves. Unlike many ant species which undergo flight muscle histolysis during initial brood development (Toom et al. 1976), these analyses failed to detect any

Table 3. Comparison of mean thoracic and abdominal dry weights (mg ± SEM) from *Dolichovespula maculata* queens collected during the autumn, spring, and summer, 1987-1990.

		Thorax		Abdomen	
Group[1]	Year	n	Mean[2] ± SEM	n	Mean[2] ± SEM
Autumn	1987	8	113.5 ± 6.5 a	8	150.4 ± 8.9 a
Spring non-nesting	1988	8	65.5 ± 2.3 b	8	47.4 ± 3.8 c
Spring nesting	1988	5	71.8 ± 1.9 b	4[3]	51.5 ± 2.8 c
	1989	6	70.4 ± 1.1 b	6	52.6 ± 2.2 c
Summer	1989	8	76.6 ± 2.4 b	7[4]	70.1 ± 3.5 c

[1] Autumn queens were collected from parent nest before hibernating; non-nesting spring queens were caught in flight; spring nesting queens were collected from embryo nests; summer queens were foundress queens.

[2] Means within a column followed by the same letter, are not significantly different ($P < 0.05$; Duncan's New Multiple Range test).

[3] Queen with abdominal sugar content of 52% (54 mg sugar; non-normal) was excluded from comparison test.

[4] Queen with abdominal lipid content of 83% (137 mg lipid; non-normal) was excluded from comparison test.

thoracic nitrogen changes in *D. maculata* queens. Successful nest founding involves active foraging by the queen, thus muscle histolysis would not be expected.

Mean abdominal nitrogen ranged from 4.6 mg to 5.7 mg in the spring nesting and non-nesting queens and no significant differences ($P \geq 0.05$) were detected among these queen groups (Table 4). In addition, these 2 groups were not significantly different ($P \geq 0.05$) from the fall 1987 group, indicating that abdominal nitrogen levels remained relatively constant from fall until early summer. However, mean abdominal nitrogen levels increased to their maximum during the summer season and were significantly different ($P = 0.0001$) from both the fall and spring groups. The mean total nitrogen content in the abdomens of summer queens was 6.6 mg, or 9.4% of the abdominal dry weight. The increase in mean nitrogen difference between abdomens of spring and summer queens was 1.5 mg. Given that the mean difference in dry weight between the abdomens of spring and summer queens was 20.1 mg, then nitrogen was approximately 7.3% of the abdominal dry weight difference. This finding suggests that the difference in abdominal dry weight was in part due to increases in protein associated with vitellogenesis and ovarian development.

Although summer queens weighed more than spring queens, an analysis of variance failed to detect monthly fresh weight changes within 55 summer queens over the 3-year period ($P = 0.32$; Table 5). In part, this was probably due to the variability associated with the egg-laying rates of individual queens and the size and vigor of their respective nests. The greatest seasonal weight change in

Table 4. Comparison of mean thoracic and abdominal total nitrogen (mg ± SEM) from *Dolichovespula maculata* queens collected during the autumn, spring, and summer, 1987-1990.

		Thorax		Abdomen	
Group[1]	Year	n	Mean[2] ± SEM	n	Mean[2] ± SEM
Autumn	1987	8	8.2 ± 0.2 a	8	5.3 ± 0.2 a
Spring non-nesting	1988	8	8.3 ± 0.3 a	8	4.6 ± 0.2 a
Spring nesting	1988	5	8.8 ± 0.3 a	5	5.7 ± 0.4 a
	1989	6	8.0 ± 0.3 a	6	5.4 ± 0.2 a
Summer	1989	8	8.4 ± 0.3 a	8	6.6 ± 0.4 b

[1] Autumn queens were collected from parent nest before hibernating; non-nesting spring queens were caught in flight; spring nesting queens were collected from embryo nests; summer queens were foundress queens collected from mature nests.

[2] Means within a column followed by the same letter are not significantly different ($P < 0.05$; Duncan's New Multiple Range test).

Table 5. Comparison of mean *Dolichovespula maculata* queen fresh body weights (mg ± SEM) collected during the summer seasons, 1987-1990.

Month	Year	n	Mean[1] ± SEM
August	1989	11	533.3 ± 13.0
July	1987	9	519.3 ± 20.6
August	1988	6	515.2 ± 11.9
September	1989	7	511.3 ± 20.2
September	1988	5	494.4 ± 27.9
August	1987	4	492.6 ± 17.9
July	1988	4	489.6 ± 8.3
July	1989	4	483.4 ± 26.8
September	1987	5	467.1 ± 20.4

[1] No significant weight differences were detected among any seasonal groups ($P = 0.32$), GLM procedure, SAS Institute, 1985.

D. maculata queens was primarily due to lipid utilization. The mean total lipid weight in the fall queens was 145.2 mg; this quantity accounted for 49.2% of the fall queen dry weight. These results are similar to those reported by Spradbery (1973) who found that lipids accounted for 40% of the pre-wintering dry weight in *Vespula vulgaris* (L.). Within the thorax, mean lipid quantities accounted for approximately 42% of the fall thoracic weight (Table 6). The mean fall thoracic lipid quantities were significantly different ($P=0.0001$) from both the spring and summer groups. Mean thoracic lipid quantities within the spring and summer thoraces ranged from 19.54 mg to 24.4 mg; no differences ($P \geq 0.05$) were detected among the thoracic lipid quantities of spring and summer queens.

Greater mean lipid differences were detected within the queen abdomens. The mean lipid quantities within the abdomen of fall queens accounted for 64.4% of the dry weight (Table 6). This value was significantly higher ($P=0.0001$) than the other groups. By spring, the mean lipid quantities within the abdomens ranged from 18.7 mg to 25.9 mg, representing 45% of the abdominal weight of spring queens. The most abundant lipids found in both fall and spring queens were triacylglycerides (35-50%), free fatty acids (25-40%), and cholesterol (20-40%). No significant differences ($P \geq 0.05$) in abdominal lipid were detected within the spring groups (Table 6). Similarly, the abdominal lipid quantities of summer queens were not significantly different ($P \geq 0.05$) from the spring groups. However, the lipid analyses revealed an atypical abdomen of which 83% of the total abdominal weight was lipid; this extreme value was excluded from the comparison tests. The unusually high lipid content of the respective queen, as well as the high abdominal nitrogen levels observed among the summer queens,

Table 6. Comparison of mean thoracic and abdominal total lipid (mg ± SEM) from *Dolichovespula maculata* queens collected during the autumn, spring, and summer, 1987-1990.

		Thorax		Abdomen	
Group[1]	Year	n	Mean[2] ± SEM	n	Mean[2] ± SEM
Autumn	1987	8	48.4 ± 4.9 a	8	96.8 ± 8.3 a
Spring non-nesting	1988	8	21.7 ± 1.6 b	8	21.3 ± 1.3 b
Spring nesting	1988	5	24.4 ± 1.2 b	5	25.9 ± 2.8 b
	1989	6	19.5 ± 3.9 b	6	18.7 ± 1.2 b
Summer	1989	8	19.6 ± 1.4 b	7[3]	26.1 ± 2.1 b

[1] Autumn queens were collected from parent nest before hibernating; non-nesting spring queens were caught in flight; spring nesting queens were collected from embryo nests; summer queens were foundress queens collected from mature nests.

[2] Means within a column followed by the same letter are not significantly different ($P < 0.05$; Duncan's New Multiple Range test).

[3] Queen with 83% (137 mg lipid; non-normal) abdominal lipid content excluded from comparison test.

probably reflects extensive feeding and/or a large number of eggs in the abdomen ready to be laid.

Sugars were the second most abundant energy reserve found in queens before the hibernation period. The mean thoracic sugar quantity from queens collected in the autumn of 1987 was 12.3 mg or 11% of the total thoracic dry weight (Table 7). This value was significantly higher ($P=0.0001$) than those recorded in the other seasons. After the autumn period, the sugar quantities ranged from 1.3 mg-5.0 mg and never accounted for more than 2% of the total thoracic weight. In addition, no significant differences ($P \geq 0.05$) in sugar quantities were found among the thoraces of spring and summer queens.

High performance thin layer chromatography (Fell 1990) revealed trehalose to be the predominant sugar in the hemolymph of 6 *D. maculata* queens. The mean quantity of trehalose was 29.25 ug/ul $\pm$ 4.47, ranging from 15.6 ug/ul to 42.4 ug/ul of hemolymph. Although trehalose was the most abundant sugar within the hemolymph, traces of glucose were apparent, but at levels too small to be quantified.

Mean sugar levels within the abdomen followed the same trend as those in the thorax (Table 7). Mean sugar quantities (20.4 mg) were highest ($P=0.0001$) in the fall abdomen, representing 13.5% of the total fall abdominal weight. The high levels of sugar observed in the fall thorax and abdomen were probably used for glycogen and/or polyol synthesis. After the hibernation period, the abdominal sugar quantities ranged from 3.1 mg-5.9 mg, never rising to levels higher than 6%. As in the thorax, the mean sugar quantities were not significantly different ($P \geq 0.05$) among the abdomens of spring and summer queens. However, one

Table 7. Comparison of mean thoracic and abdominal total sugar (mg ± SEM; glucose equivalents) from *Dolichovespula maculata* queens collected during the autumn, spring, and summer, 1987-1990.

		Thorax		Abdomen	
Group[1]	Year	n	Mean[2] ± SEM	n	Mean[2] ± SEM
Autumn	1987	8	12.3 ± 0.3 a	8	20.4 ± 1.5 a
Spring non-nesting	1988	8	1.3 ± 0.3 b	8	3.1 ± 1.1 b
Spring nesting	1988	5	2.6 ± 0.6 b	4[3]	3.9 ± 1.0 b
	1989	6	5.0 ± 2.0 b	6	5.1 ± 1.1 b
Summer	1989	8	4.7 ± 1.3 b	8	5.9 ± 1.7 b

[1] Autumn queens were collected from parent nest before hibernating; non-nesting spring queens were caught in flight; spring nesting queens were collected from embryo nests; summer queens were foundress queens collected from mature nests.

[2] Means within a column followed by the same letter, are not significantly different ($P < 0.05$; Duncan's New Multiple Range test).

[3] Queen with abdominal sugar content of 52% (54 mg) was excluded from comparison test.

queen collected from a spring embryo nest in 1988, had an abdominal sugar content of 52%. Although this value is over one-half the abdominal weight, the sugar quantity within the thorax was at normal levels. The high sugar content was probably due to extensive nectar or honeydew feeding and would most likely have been associated with the crop or midgut.

Glycogen was also a relatively abundant energy reserve in fall queens (Table 8). Similar to the lipid and sugar reserves, both fall thoracic ($P=0.047$) and abdominal ($P=0.0004$) glycogen levels were significantly different from the spring and summer groups. The mean glycogen quantity within the fall thorax was 4.2% (4.7 mg) of the fall thoracic weight. Within the fall abdomen, the mean glycogen quantity was slightly higher at 9.7% (14.6 mg) of the total abdominal weight. In both the spring and summer queens, the glycogen quantities of the thoracic and abdominal tagmata ranged from 0.2 mg to 1.1 mg. These quantities reflected trace amounts and never exceeded 1% of the thoracic or abdominal weight. In addition, no significant differences ($P \geq 0.05$) were found among the spring and summer groups (Table 8). Low levels of glycogen in the spring and summer queens indicate that glycogen is primarily an energy reserve used during the hibernation period and is probably associated with the formation of glycerol at low temperatures (Alford 1969; Storey and Storey 1983; Duman and Patterson 1978).

DISCUSSION

The results presented here, show that lipids accounted for 35% of the weight lost during hibernation, sugars 12%, and glycogen 6%. Spradbery (1963) reported that vespine queens in Britain undergo a 30% lipid dry weight loss during

Table 8. Comparison of mean thoracic and abdominal total glycogen (mg ± SEM) from *Dolichovespula maculata* queens collected during the autumn, spring, and summer, 1987-1990.

		Thorax		Abdomen	
Group[1]	Year	n	Mean[2] ± SEM	n	Mean[2] ± SEM
Autumn	1987	8	4.7 ± 2.4 a	8	14.6 ± 4.1 a
Spring non-nesting	1988	8	0.4 ± 0.1 b	8	0.8 ± 0.2 b
Spring nesting	1988	5	0.2 ± 0.1 b	5	0.3 ± 0.1 b
	1989	6	0.8 ± 0.1 b	6	1.1 ± 0.4 b
Summer	1989	8	0.3 ± 0.1 b	8	0.6 ± 0.5 b

[1] Autumn queens were collected from parent nest before hibernating; non-nesting spring queens were caught in flight; spring nesting queens were collected from embryo nests; summer queens were foundress queens collected from mature nests

[2] Means within a column followed by the same letter, are not significantly different ($P < 0.05$; Duncan's New Multiple Range test).

the hibernation period. Similarly, Matsuura (1969) reported that Japanese *Vespa* queens undergo a 38% lipid fresh weight loss during hibernation.

The difference in energy reserves observed between autumn and spring queens suggest that nutrition prior to diapause is an important factor contributing to overwintering success. Unfortunately, little is known about the foraging behavior of individual queens after emergence from the pupal cell and before hibernation. The data show that *D. maculata* queens may increase their dry weight by as much as 79% during this period. Queens therefore, need an ample supply of food from which to build their energy reserves before winter. Duncan (1939) and Spradbery (1973) reported that newly emerged queens solicit food from larvae and workers, and may forage away from the nest. However, it seems unlikely that individual queens would spend a significant amount of time foraging for insects or nectar before hibernation. Foraging away from the nest not only requires considerable time and effort, but also increases the queen's vulnerability to predation. It seems more reasonable to expect queens to obtain as much food as possible within the nest, building reserves without active foraging. Once reserves reach a specific level, queens could then leave the nest, mate, and either return to the nest to continue feeding, or seek a hibernation site.

Given the above scenario, queens emerging from their pupal cells when larvae and worker numbers are high should have a better chance of building sufficient energy reserves than would queens from less vigorous nests. Declining autumn nests have low numbers of larvae and workers, and queens which emerge in such nests may not be able to feed sufficiently to build energy reserves. This would increase a queen's need to forage away from the nest and

thus affect her probability of survival. Insufficient storage of lipid and carbohydrate reserves could well account for a large percentage of the mortality of hibernating queens.

Similar high demands on the energy reserves of queens can be found in the spring. Nest founding queens must seek out suitable nesting sites, collect wood pulp, construct the nest and distribute energy reserves for brood production, all when environmental conditions are usually the harshest. Extremes in precipitation or cold temperatures would also limit foraging and could decrease the availability of floral nectar and insect prey. Thus, the energy reserves of queens which survived the winter could also affect survival and successful nest initiation.

Active foraging by spring queens could offset some of the problems from low energy reserves. In southwestern Virginia, both *Vespula* and *Dolichovespula* queen spp. have been observed collecting nectar from blueberry (*Vaccinium* spp.) and Russian olive (*Eleagnus angustifolia* (L.)). Less commonly, *D. maculata* queens were observed to feed on honeydew, a behavior noted by several authors (Duncan 1939, Spradbery 1973, and Fell and Morse 1977). Further evidence of foraging is provided by the queen which had an abdominal sugar content of 52%. The high concentration of sugar is indicative of nectar foraging, or honeydew collection.

Roth and Lord (1987) suggested that queen cell-size may be important in determining individual queen fitness, and suggested that bigger queens may have an advantage over smaller queens. This study has focused on the energy reserves of foundress queens, with particular attention given to queens prior to,

and during nest initiation. The results suggest that queens which survive the hibernation period begin the nest initiation process with similar weights and energetic reserves. These results do not indicate that bigger queens have an "advantage" (Roth and Lord 1987) over smaller queens, but do suggest the importance of energy reserves. However, size could play an important role during the nest initiation stage should attempted nest usurpations occur.

It would be extremely difficult to examine all of the possible causes of queen mortality and embryo nest failure. Factors such as rainfall, microbial infections, vertebrate predation, and conspecific and contraspecifc usurpations have been used to explain queen and embryo nest mortality (Beirne 1944; Fox-Wilson 1946; Spradbery 1973; Edwards 1980). Although environmental and behavioral factors are undoubtedly important causes of mortality; nutritional factors have been overlooked. The results presented on *D. maculata* suggest that the time of year the queen ecloses from the pupal cell and the time spent foraging both before and after hibernation may be important factors mediating queen survival. Clearly, more research focusing on the solitary phase of the queen's existence is needed to better understand vespid ecology.

CHAPTER 5

The Embryo Nest of the Baldfaced Hornet *Dolichovespula maculata* (L.): Egg Weight Variation, Energy Reserves, and Internal Nest Temperatures (Hymenoptera: Vespidae)

INTRODUCTION

Embryo nests are built each spring in northern temperate regions of the world by solitary queens of the Vespinae. Details of nest construction have been described for many species, with reviews provided by Duncan (1939); Spradbery 1973; Edwards (1980) and Akre et al. (1981).

The embryo nest has been reported to be the most vulnerable stage of yellowjacket colony development. Spradbery (1973) and Edwards (1980) have suggested that embryo nest mortality might be as high as 99%, although Brian and Brian (1952) reported a 92% mortality rate for twelve nests which they studied. Many of the nests which survive the initial founding stage fail to reach maturity or produce reproductives. MacDonald and Matthews (1981) studied 36 nests of *Vespula maculifrons* (Buysson) and reported that only 2 of these nests produced reproductives at the end of the summer.

Despite the amount of literature devoted to yellowjacket population ecology (Beirne 1944; Fox-Wilson 1946; Spradbery 1973; Edwards 1980; Archer 1981, 1982; Roth and Lord 1987) embryo nest biology and ecology have received little attention (Matthews et al. 1982). Spradbery (1973) studied egg developmental rates in several vespine genera and Edwards (1980) reviewed vespine egg embryology. Ross et al. (1981) and Matthews et al. (1982) have examined the sequence of events involved in nest initiation and development in the laboratory. However, studies of the energetics involved in initial egg and subsequent brood production are absent.

The chemical analyses of insect eggs has previously relied upon the pooling of samples to ensure sufficient quantities for analyses. Recently, high

performance thin-layer chromatography (HPTLC) techniques have been developed by Fell (1990) and Judge et al. (1989) for the quantitative and qualitative analyses of sugars and lipids from insect hemolymph samples as small as 0.5 ul. These techniques have now been utilized for the analysis of eggs produced by queens of the baldfaced hornet *Dolichovespula maculata* (L.).

This investigation examined the dry weight, total nitrogen, and energy reserves of *D. maculata* eggs collected during nest initiation and throughout the summer in an effort to understand the energetic investment or contribution of the queen to egg production. In addition, *D. maculata* embryo nests were allowed to develop in an incubator to identify egg developmental rates and the utilization patterns of lipids and carbohydrates. In conjunction with this study, the internal temperatures of a *D. maculata* embryo nest were recorded to identify potential brood incubation trends. Gibo et al. (1977) and Makino and Yamane (1980) examined thermoregulation in embryo nests and have speculated on its significance to brood development. However, heat production by the queen and the effects of this heat on brood development are not well-understood.

Materials & Methods

Dolichovespula maculata nests used in this study were collected in Craig, Floyd, Giles, Montgomery, and Pulaski Counties, Virginia between 1988 and 1990. Nests collected in May and early June which contained the foundress queen, eggs, and in some cases first instars, are referred to as embryo nests; all other nests are referred to as summer nests. Embryo nests were collected from buildings and trees after foundress queen removal. Nests were removed from

trees by cutting the supporting branch within 5 cm of the point of nest attachment. Nests attached to buildings were removed by scraping the paper envelope(s) and nest pedicel with a penknife. The nests, including paper envelopes, were placed in paraffin-coated paper cups (Sweetheart® 18 oz.) before placement in an insulated container. Summer nests were collected at night after vacuuming the workers from the nest (Akre et al. 1973) and plugging the nest entrance with cotton. After returning to the laboratory, the nests were killed by freezing. The paper envelope(s) were removed from each nest before placing the entire structure in a cooler of dry ice for a minimum of 15 minutes. It was found that placing the nest combs in dry ice prevented rapid thawing and leakage of egg contents before the lyophilization process. The combs containing eggs and brood were then lyophilized for 3 days. To prevent egg damage, the eggs were not removed from the cells until after lyophilization.

After lyophilization, the cells containing eggs were removed . The walls of each cell were removed with fine scissors and discarded. The egg was then removed from its point of attachment to the cell base by cutting the base with a scalpel. After removing the eggs from a nest, the individual eggs were placed in 1.5 ml microcentrifuge tubes (Fisher®), and lyophilized a second time for 24 hours. This lyophilization was performed to remove any excess moisture which may have been absorbed by the egg. After removal from the lyophilizer, the tubes were sealed with the attached cap and frozen until use. Eggs were weighed immediately before all experiments on a Mettler® (#AE163) balance.

Egg Incubation Study

Embryo nests used in the incubation study were collected within a five-mile radius in Montgomery Co., VA., on May 18, 1990; between the hours of 0900-1400. These nests were collected and treated as outlined above but kept alive. Seven of the 8 nests contained eggs only; 1 nest had 12 eggs and 3, first instars. Three of the intact nests were placed in an incubator for a study of egg development; these nests were removed after 18 days. Five of the nests were used for a study of developmental changes in egg weight and lipid content. These nests were cut into halves. One half was scored as the initial group and these were killed by freezing; the other half was placed in an incubator for a 10 day period (Percival Manufacturing Co., Boone, Iowa; #E-30B). An incubator light regime of 14L: 10D was used. The light was provided by two 25-watt incandescent bulbs and six 20-watt fluorescent bulbs. The incubator temperatures were maintained at 20°C$\pm$0.5 and 14.5°C$\pm$0.5, day and night, respectively. Temperatures were based on environmental conditions in the Montgomery Co. VA area during the period when the embryo nests of *D. maculata* are normally found. The embryo nest stage generally extends from May 10 through the first two weeks of June. Temperature data from Blacksburg, VA. (May 10-June9, 1987-1989) were used in conjunction with a modified Fortran program (F.W. Ravlin; unpubl.) to generate the simulated temperature regime. Humidity in the incubator was not controlled but ranged from 60-75% during the day to 75-90% during the night.

Nest halves which were used for the egg weight and lipid analyses were removed from the incubator after 10 days. The youngest eggs at the time of nest

collection were positioned in the cells at the outermost edge of the nest; therefore, these were the eggs used in the experiments. Only healthy eggs were used and these were identified by their white color and robust appearance. During the incubation period, all eggs within each of the nests were also examined with a dissecting microscope (40x) twice daily for eclosion and/or larval movement.

Many of the eggs in the center of the nest produced fungal mycelia after 7-12 days. In an effort to determine the type of fungus associated with the nests, twelve of these eggs were aseptically placed onto a potato-dextrose agar amended with chloromycetin and streptomycin (200 ug/ml each). The plates were then incubated at a temperature of 25°C for 1 week.

Extraction of Sugars

Anthrone analysis of total sugars: Lyophilized eggs were placed into 1.5 ml microcentrifuge tubes to which 120 ul of 66% ethanol saturated with sodium sulfate (Na_2SO_4) was added (modified from Van Handel 1965a). The egg was then ground with a microcentrifuge pestle for 1 minute. The contents of the microcentrifuge tube were thoroughly mixed for 1 minute before centrifugation at 12,000 rpm (Fisher Microcentrifuge) for 5 minutes. Samples were diluted (1:10) by mixing 1 part of the ethanolic extract in 9 parts of double-distilled water (ddH_2O).

HPTLC Analysis: Eggs were treated as above except 10 ul of 66% ethanol (saturated with Na_2SO_4) was added rather than 120 ul. The samples were ground and centrifuged and the supernatants spotted directly onto the TLC plate for analysis without further dilution.

Extraction of Glycogen

Pellets from the anthrone extraction were dried by placing the tubes inside a sand bath at 35^{o}C for 1 hour. After removing the tubes from the sand bath, 500 ul of a 30% potassium hydroxide (KOH) solution was added to the pellets along with 50 ul of a saturated Na_2SO_4 solution (in water). The tubes were immediately placed in a Multi-Blok Heater (Lab-Line Instruments Inc.) and heated to 100^{o}C for 10 minutes. KOH solutions were made fresh before each extraction as a precautionary measure to avoid glycogen loss by contamination with carbon dioxide (Van Handel 1965b). In addition, the KOH was not added to the pellets until just prior to heating. After heating the tubes for 10 minutes, they were immediately chilled by placement on ice for 3 minutes. One ml of 66% ethanol saturated with Na_2SO_4 was added to each of the tubes. The tubes were vortex mixed and centrifuged at 12,000 rpm for 5 minutes. The supernatants were withdrawn with a Pasteur pipette and discarded. The pellets were dried in a 35^{o}C sand bath for 1 hour, then dissolved in 1 ml of ddH_2O. A final dilution (1:5) was made by transferring 1 part of the glycogen solution to 4 parts of ddH_2O inside of a microcentrifuge tube.

Anthrone Quantitation of Carbohydrates

An anthrone analysis was used to quantify both total and glycogen (Trevelyan and Harrison 1952; Van Handel 1965a). The anthrone reagent was prepared before each analysis by adding 200 mg of anthrone to 100 ml of diluted sulfuric acid (500 ml H_2SO_4: 200 ml ddH_2O). After mixing the reagent,

undissolved crystals of anthrone were removed with a Pasteur pipette. Glucose (Sigma Chemical Co.) and glycogen (oyster glycogen; Sigma Chemical Co.) standards were used (5, 10, 25, 50, and 100 ug/ml) for each of the respective analyses. Glucose standards were made in a 6.6% ethanol solution; glycogen standards in ddH_2O.

In earlier experiments involving carbohydrate extraction from homogeneously ground abdomens of *D. maculata* queens, 5 ug of glucose and glycogen were added to three subsamples (2-4 mg each) from each abdomen to examine extraction and analysis efficiency. Three subsamples (2-4 mg each) from the same abdomen were carried through normal analysis procedures. A total of 8 queen abdomens were used for the confirmation analyses.

Five ml portions of the anthrone reagent were pipetted into Pyrex test tubes which were cooled in an ice water bath. The standards, blanks, and 1.0 ml glucose or glycogen samples were slowly pipetted on top of the anthrone reagent. When all of the samples had been prepared, the tubes were capped and mixed thoroughly. The test tubes were placed in a boiling water bath for 10 minutes, after which they were removed and cooled in an ice water bath for 3 minutes. The solutions were read with a Perkin-Elmer Lambda 3B Spectrophotometer at 620 nm. Spikeovers with 5 ug glucose and glycogen standards indicated recovery rates of 98% $\pm$ 3% and 94% $\pm$ 3%, respectively.

HPTLC Analyses of Sugars

Merck pre-coated silica gel 60 plates for HPTLC (10 x 10 cm) were treated and activated as described by Fell (1990). Mixed 1 ul standards, consisting of

glucose, fructose, trehalose, and sucrose (0.25, 0.5, 1, 2, 4 and 6 ug/ul) and 1 ul sample solutions were applied to the treated plates with a Camag^R Nanomat 1 applicator and Drummond^R microcaps; samples were run in duplicate.

After sample application, the plates were dried with a hair dryer. The plates were developed with acetonitrile:ddH_2O (85:15) three times and dried between each development. The plates were allowed to cool after the final drying. The plates were dipped into a ceric sulfate/H_2SO_4 solution (Fell 1990) and then charred by placing them in an oven at 110°C for 15 minutes. The dipping reagent was made by diluting 1 part of 0.1N ceric sulfate in 2N H_2SO_4 (Ricca Chemical Corp.) Quantitative measurements were made by absorbance scanning, using a CAMAG TLC scanner II, in conjunction with a Spectra Physics #4270 integrator. The plates were scanned using the parameters described by Fell (1990).

Extraction, and Analysis of Lipids

Lyophilized eggs were individually placed inside 1.5 ml microcentrifuge tubes to which 400 ul of chloroform: methanol (2:1) was added. The eggs were ground with a microcentrifuge pestle for 1 minute; 400 ul of ddH_2O was then added. The tubes were mixed for 1 minute and then centrifuged at 12,000 rpm for five minutes. The aqueous layer was extracted with 400 ul of 2:1 chloroform/methanol; the chloroform layer (subnatant) was saved. The aqueous layer was washed 2 more times and the resulting chloroform layers were combined. The combined chloroform extract was then dried under a stream of dry N_2. The lipid was removed from this tube by 3 chloroform washings (100 ul/wash) and transferred to a 400 ul microcentrifuge tube. The final solution was

evaporated to dryness under nitrogen.

Merck pre-coated 5633 silica gel 60 plates for HPTLC (10 x 10 cm) were treated and activated as described by Judge et al. (1989). Mixed lipid standards, consisting of mono-, di-, and tristearin, and cholesterol palmitate (0.5, 1, 2, 4, 6, 8, and 10 ug/ul) and 1 ul sample solutions were applied to the plate with a Camag^{R} Nanomat 1 applicator and Drummond^{R} microcaps; samples were run in triplicate. Sample solutions were prepared by adding 20 ul of chloroform to the sample tubes and vigorously mixed immediately before spotting.

The HPTLC plates were developed in a linear separation chamber (CAMAG). The first development utilized benzene:ether:ethanol:acetic acid solution (60:40:2:0.2; v/v), followed by a second development with a hexane:heptane:ether:acetic acid solution (63:18:18:1; v/v) (Judge et al. 1989). After each development, the plates were dried with a hair dryer and then allowed to cool before proceeding.

The developed plates were visualized with 1% TNS (6-*p*-toludino-napthalenesulfonic acid) in methanol. Quantitative measurements were made by fluorescence scanning, using a CAMAG TLC scanner II,in conjunction with a Spectra Physics #4270 integrator. The plates were scanned using the parameters described by Judge et al. (1989). Spikeovers with 2.0 ug/ul of the mixed lipid standard added to the eggs before the extraction process demonstrated recovery rates of 94% $\pm$ 4%.

Quantitation of Total Nitrogen

The quantitation of total nitrogen involved a modified micro-Kjeldahl

technique (Mullins 1971). Lyophilized eggs and ammonium sulfate (NH_4SO_4) standards (5, 10, 25, 50, and 100 ug/ml) were placed into test tubes (18 cm x 100 cm), after which 1.5 ml of a oxidizing/digesting mixture copper sulfate ($CuSO_4$), 313 mg/L, selenious acid (H_2SeO_3) 129 mg/L, mixed into 5N H_2SO_4 was added to each tube.

The tubes were placed into an oven at 120-150^{o}C for 8-10 hours, or until the water was evaporated from the digestion mixture. This was apparent by beads of water which collected near the tops of the tubes; followed by complete evaporation of the beads with continued heating at the same temperature setting. After the water was driven off, a glass marble was placed on each tube and the temperature was increased to 350^{o}C for 12 hours. The tubes were then removed from the oven and allowed to cool. Four and one-half ml of ddH_2O were slowly added while rotating the tube to rinse any accumulated debris from the inside wall of the tube. After the addition of water to all tubes, 4.5 ml of 3.3N sodium hydroxide (NaOH) were added to each tube, followed by the addition of 3.0 ml of Nessler's reagent (potassium mercuric iodide 7g/L; gum ghatti 1.75g/L). The tubes were mixed and set aside for 15 minutes to allow for maximum color development. The resulting yellow-orange colored solutions were read on a Perkin-Elmer Lambda 3B Spectrophotometer at 490 nm. Spikeovers with 5 ug/ml of an ammonium sulfate standard demonstrated 93 $\pm$3% recovery.

Nest Temperature Study

A *D. maculata* embryo nest which contained the foundress queen and 8 eggs was located on May 26, 1990. An incision was made with a scalpel into the

nest envelope and a thermistor probe (Model # 101; Campbell Scientific Co.) was inserted immediately above the nest comb (0.5 cm) and adjacent to the nest pedicel. A second thermistor probe was placed 7 cm away from the nest. Data were continuously recorded from May 31-June 6 with a CR21 Micrologger (Campbell Scientific Co.) and downloaded to a cassette recorder. The resulting data were transferred to an IBM-PC for analysis using a Cassette Interface (#C20; Campbell Scientific Co.). By June 2, 1990, the nest contained 8 eggs and 3 first instars. Data collection was discontinued on June 7, three days after the foundress queen was lost.

Statistical Analyses

Seasonal Egg Analyses: Tests for normality, variance homogeneity (Cochran and Cox, 1957), and an analysis of variance (ANOVA) were performed on all class variables, using the UNIVARIATE, DISCRIM, and GLM procedures (SAS Institute, 1985), respectively. If significant differences were detected (alpha=0.05) among the class variables, then Scheffe's Test was used to find the differences.

Egg Incubation Experiment: A *t* test procedure (alpha =0.5) (SAS Institute, 1985 was used to compare mean weights between the control and incubated eggs. A multivariate analysis (MANOVA procedure; SAS Institute, 1985) was used to quantitatively and qualitatively compare lipid differences between the 2 treatment groups, with the 5 lipid classes as the dependent variable.

Results

Egg Weight, Energy Reserve, and Total Nitrogen Comparisons

Table 9 shows the seasonal changes in mean dry weight of *D. maculata* eggs. The mean dry weight of embryo nest eggs was significantly higher ($P = 0.0001$) than eggs collected in June, July, and August. The difference in mean dry weight between the 2 groups was 26%. Among the summer eggs, mean dry weight was not significantly different ($P \geq 0.05$). These results show that the first eggs produced by a queen are larger and indicate greater investment per egg earlier in the season. In an attempt to determine which egg components were associated with seasonal egg weight changes, the total nitrogen, sugar, glycogen, and lipid reserves of the individual eggs were compared (Table 9). However, the DISCRIM procedure revealed that variances among the energy reserve classes (sugar, glycogen, lipid, and nitrogen) within the embryo nest eggs and summer nest eggs were non-homogeneous. Therefore, energy reserve and nitrogen comparisons were made among summer nest eggs only; embryo nest eggs were not included in the comparisons although the means are presented in Table 9.

The HPTLC analyses revealed that glucose was the only detectable sugar in all of the eggs examined throughout the season. The mean sugar quantity per egg (combined for all seasons), determined with the HPTLC and anthrone analyses were 19.2 ug $\pm$ 2.8 (standard error of the mean) and 19.0 ug $\pm$ 4.9, respectively. A variance ratio test revealed that the HPTLC and anthrone sugar quantitation techniques were not different ($F = 2.23$; $df = 1,43$; NS). Thus, the HPTLC and anthrone sugar data were combined with the mean sugar quantities

Table 9. Comparison of mean (ug ± SEM) dry egg weight, energy reserves, and total nitrogen of *Dolichovespula maculata* eggs collected from nests in Southwestern Virginia, 1987-1989. The percent of each component is shown directly below the mean.

	Dry egg weight		Sugar[3]		Glycogen		Lipid		Nitrogen	
Group[1]	n	Mean[2] ± SEM	n	Mean[2] ± SEM	n	Mean[2] ± SEM	n	Mean[2] ± SEM	n	Mean[2] ± SEM
Embryo	87	266.4 ± 5.2 a	24	25.4 ± 3.4 @	18	12.2 ± 1.3 @	16	60.7 ± 11.4 @	33	22.6 ± 0.7 @
				(9.5%)		(4.6%)		(22.8%)		(8.5%)
June	10	214.0 ± 9.8 b	2	17.9 ± 10.3 a	3	7.3 ± 0.2 a	4	28.9 ± 10.4 a	--[4]	---
				(8.3%)		(3.4%)		(13.5%)		
July	15	212.0 ± 8.3 b	6	9.2 ± 4.3 a	4	14.1 ± 2.8 a	4	18.8 ± 4.7 a	4	17.9 ± 0.3 a
				(4.3%)		(6.7%)		(8.9%)		(8.4%)
August	25	179.6 ± 5.1 b	11	9.2 ± 1.9 a	9	11.5 ± 2.3 a	6	13.0 ± 4.2 a	7	15.2 ± 0.3 b
				(5.1%)		(6.4%)		(7.2%)		(8.5%)

1 Embryo refers to the eggs obtained from the initial stage of nest development which contained the foundress queen and no more than 15 eggs; all other eggs came from summer nests which had the foundress queen and workers.
2 Means within a column followed by the same letter are not significantly different ($P < 0.05$; Scheffe's test).
3 Sugar is expressed in glucose equivalents.
4 June eggs were not analyzed for nitrogen.
@ Due to unequal variances, this group could not be analyzed with the other groups (Cochran and Cox, 1957).

presented in Table 9. The mean sugar quantity was highest in the embryo nest eggs, accounting for approximately 9.5% of dry egg weight (Table 9). Mean sugar quantities among the summer nest eggs were not different ($P=0.42$); percent sugar ranged from 4.3 to 8.4% of the egg dry weight (Table 9).

Glycogen was the least abundant of the energy reserves within the embryo nest eggs and accounted for less than 5% of the total dry weight (Table 9). Within the summer egg groups, glycogen quantities were not significantly different ($P=0.38$), ranging from 3.4 to 6.6% of the total egg dry weight (Table 9).

Lipids were the most abundant energy reserve found in embryo nest eggs and summer nest eggs (Table 9). The HPTLC analyses revealed monoacylglycerol, diacylglycerol, triacylglycerol and esters of cholesterol to be the only detectable lipids. Lipid quantities were highest in the embryo nest eggs and accounted for 23% of the mean dry egg weight. Within the summer groups, no significant differences were detected ($P=0.24$); mean percent egg lipid ranged from 7.2 to 13.5% (Table 9).

Mean total nitrogen was also highest in the embryo nest eggs, representing 8.5% of the total dry weight (Table 9). Among the summer eggs, mean total nitrogen in July eggs was significantly higher ($P=0.0004$) than the August eggs, which suggests that egg protein quantities decrease with decreasing egg weight. However, percent nitrogen values were not only the same for both July and August eggs (8.4%), but also similar to the eggs from embryo nests.

An analysis of variance (ANOVA) was used to test for weight differences and examine variability within both embryo nest egg and summer nest egg

groups. The ANOVA revealed significant weight differences (P=0.001) among the embryo nest eggs. However, Scheffe's test did not detect differences in mean egg weights (which ranged from 231 ug to 310 ug) in spite of a 25% difference between the highest and lowest mean weights (Table 10). Similarly, ANOVA revealed significant weight differences (P=0.003) among the summer egg groups, with considerable overlap among the groups as indicated by Scheffe's test (Table 10). These results indicate that an appreciable amount of egg weight variability exists, not only within eggs from individual nests, but among nests as well.

Some of the variability that was observed within and among the different egg groups could have been due to egg age. The experiments described above were conducted without regard to the age of the egg analyzed. Eggs positioned toward center of the embryo nest are older and, as a result of metabolic activity, might weigh less than younger eggs which are positioned near the nest edge. Furthermore, the analyses presented in Table 9 show that lipids exhibit the largest percent decrease when the embryo nest eggs are compared to the eggs of summer nests. As a result of these findings, an incubation study was conducted in an effort to quantify and compare changes in the dry weight and lipid content of eggs after a known period of developmental time. Since the eggs in the outermost edge of the embryo nest are the youngest, and thus have the longest developmental time, they were used for the study.

Table 10. Comparison of mean dry egg weights (ug ± SEM) from *Dolichovespula maculata* nests collected in southwestern Virginia, 1987-1989.

Date[1]	n	Mean[2]	SEM
Embryo Nest Eggs			
May 24, 1989	9	310 **a**	± 12.8
June 2, 1988	9	290 **a**	± 10.0
May 21, 1988	9	289 **a**	± 21.5
May 30, 1989	7	279 **a**	± 15.0
June 1, 1988	4	270 **a**	± 28.6
May 24, 1989	10	268 **a**	± 16.5
May 24, 1989	9	254 **a**	± 13.7
May 26, 1989	5	246 **a**	± 9.8
May 24, 1988	15	243 **a**	± 11.4
May 30, 1989	10	231 **a**	± 11.3
Summer Nest Eggs			
June 23, 1988	10	214 **a**	± 9.8
July 26, 1989	15	212 **ab**	± 8.4
August 31, 1988	12	181 **ab**	± 6.5
August 14, 1989	13	179 **b**	± 8.1

[1] Nests collected during May and the first week of June contained the foundress queen and no more than 15 eggs; all other nests were mature.

[2] Means with a column followed by the same letter, are not significantly different ($P < 0.05$; Scheffe's test). The embryo and summer nest eggs were tested as separate groups.

Egg Weight Comparison and Lipid Analyses

The results of the egg incubation study are shown in Table 11. A *t*- test revealed the initial group to be significantly higher (P=0.0001) in dry weight than eggs that were incubated for 10 days. The mean dry weight loss over the 10-day period was 83 ug, or 29%. The MANOVA revealed no significant quantitative differences (F=1.22; df=5,15; P=0.35; Wilkes' Criterion) in lipids between the 2 treatments (Table 11). In addition, the MANOVA did not detect significant qualitative differences among the 5 lipid classes: monoacylglycerol (P=0.22), 1,2-diacylglycerol (P=0.32), 1,3-diacylglycerol (P=0.69), triacylglycerol (P=0.59), and esters of cholesterol (P=0.77) (Table 11). These results indicate that the individual lipid classes were the same between the two groups and that there was no significant mobilization or catabolism of lipid reserves. However, differences may have been masked by high variability between samples.

Egg Development

The egg incubation study failed to produce any discernible developmental data. Only 1 larva was observed to fully eclose from its egg after five days in the incubator; this egg came from a nest which initially contained 3 larvae. Within most of the eggs, the developing larval characters could be seen through the chorion. In fact, larval movement was observed in 2 of the eggs after 16 days of incubation; however, these 2 eggs also failed to hatch. The eggs followed a similar course of development before dying: larval characters would appear on the developing embryo, the chorion would distort, then shrivel, and the embryo/egg would lose its white color.

Table 11. Dry weight and total lipid changes in *Dolichovespula maculata* embryo nest eggs[1].

Dry Weight[3]	Control[2] n	Mean ± SEM	Incubated n	Mean ± SEM
	10	282.0 ± 7.6a	9	199.0 ± 8.7b

Lipids[4]	n	ug ± SEM	n	ug ± SEM
Monoacylglycerol	11	30.6 ± 7.7	10	18.2 ± 6.0
1,2 - Diacylglycerol	11	7.2 ± 2.2	10	4.5 ± 1.4
1,3 - Diacylglycerol	11	18.5 ± 5.0	10	15.4 ± 6.1
Triacylglycerol	11	30.2 ± 3.6	10	27.6 ± 3.2
Esters of Cholesterol	11	9.7 ± 2.3	10	8.8 ± 2.0
Mean total		96.2 ± 10.4 ug		74.5 ug ± 12.6

1 Embryo refers to the initial stage of nest development which contains the foundress queen and no more than 15 eggs.

2 Embryo nests were cut into halves; control eggs were killed by freezing; incubated eggs were placed in an incubator for 10 days.

3 Means in a row followed by the same letter are not significantly different ($P < 0.05$; t-test procedure, SAS Institute 1985).

4 Mean lipids were not significantly different for each class, by treatment ($P < 0.05$, MANOVA procedure; SAS Institute, 1985).

Many of the eggs which were located in the center of the nest were observed to have fungal mycelia after 7-12 days. Fungi representing five genera including, *Pullularia* (=*Aureobasidium*), *Hormodendrum*, *Pennicillium*, and *Aspergillus* spp. (all Fungi Imperfecti) were isolated from the 12 eggs examined. These fungi are assumed to be external saprophytes, but they may be pathogenic under certain environmental conditions (R.J. Stipes; pers. comm.).

Nest Temperature Analysis

Figure 4 illustrates the ambient vs. internal nest temperature differences that occurred within one *D. maculata* nest between May 31 and June 4, 1990. Figure 5 shows this comparison of temperature more clearly over a 24hr period (May 31-June 1). The internal nest temperatures followed the same daily pattern. Typically, between the hours of 2300 and 0600 the nest temperature was closely coupled to ambient temperature. However, at approximately 0630 hours the internal nest temperature began to increase toward a point 4.0°C higher than ambient. This increase in temperature was probably associated with queen activity such as flight muscle warm-up before foraging. Following the initial nest temperature increase, ambient temperature increased steadily but the nest temperature remained 1°C to 2.5°C higher. As darkness approached, (period between 1900 and 2200) the internal nest temperature increased briefly, reaching a level 3-4° higher than ambient, before dropping back to ambient levels.

On June 4, the queen was lost and internal nest temperatures rapidly became similar to ambient temperatures (Fig. 6). No differences between ambient and internal nest temperatures were noted in the following 3-day period. These findings suggest that the queen was largely responsible for the observed

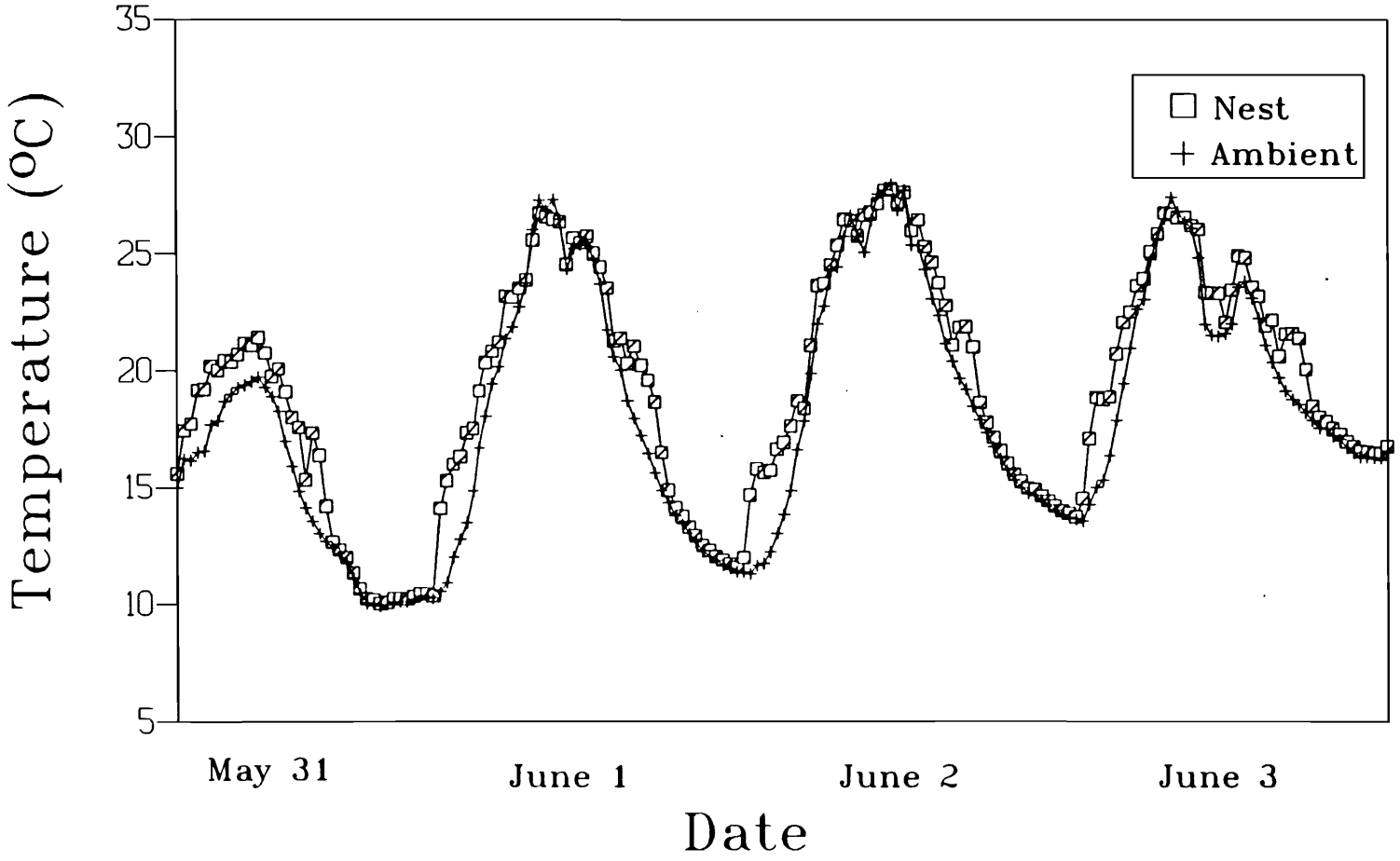

Figure 4. Ambient and internal temperatures of a *D. maculata* embryo nest which was monitored continuously (presented in 30 minute intervals) between May 31 and June 4, 1990 in southwestern Virginia. The nest contained the foundress queen, 8 eggs, and 3, first instars.

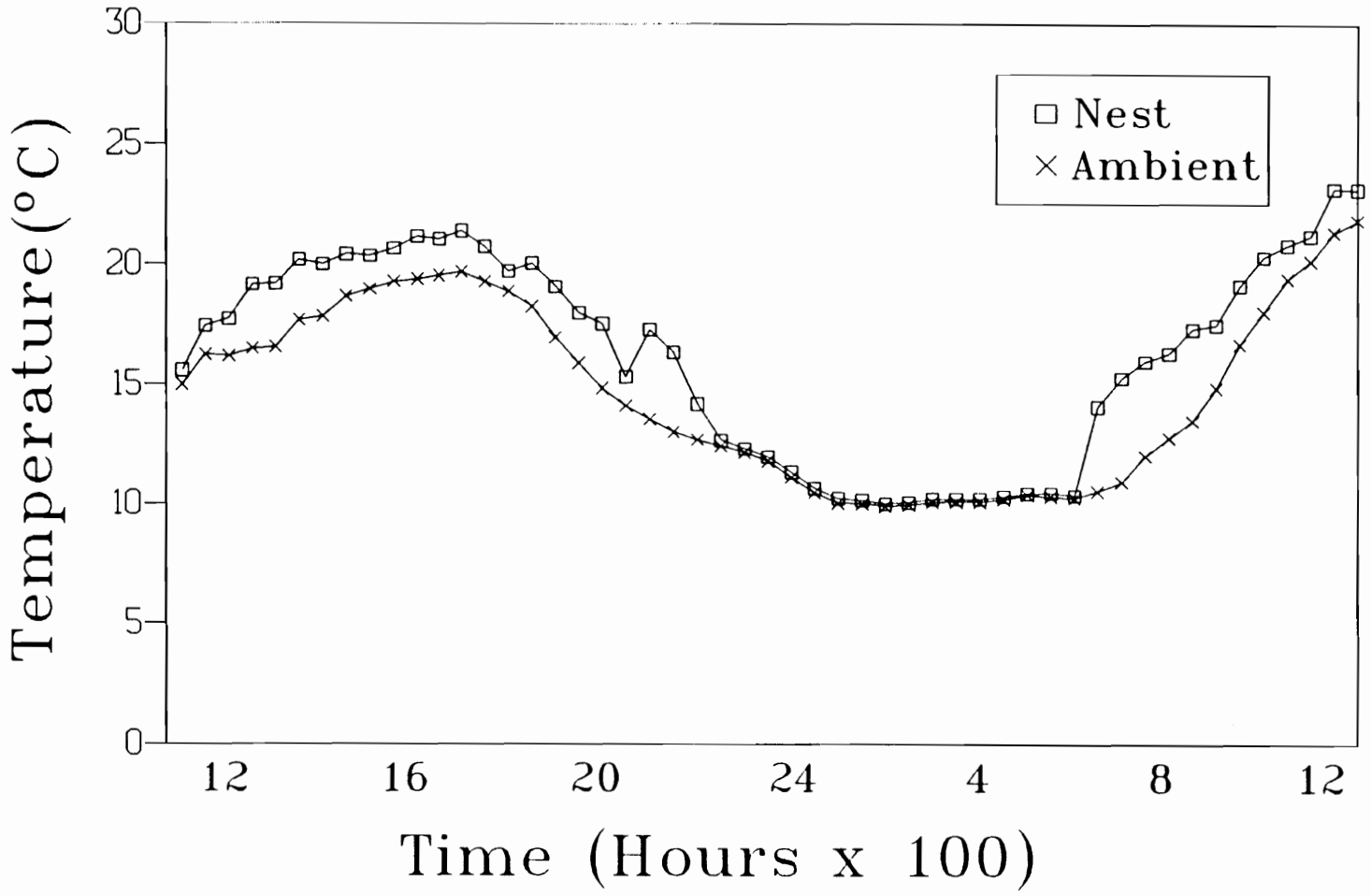

Figure 5. Ambient and internal temperatures of a *D. maculata* embryo nest which was monitored continuously (presented in 30 minute intervals) for a 24 hour period between May 31 and June 1, 1990 in southwestern Virginia. The nest contained the foundress queen, 8 eggs, and 3, first instars.

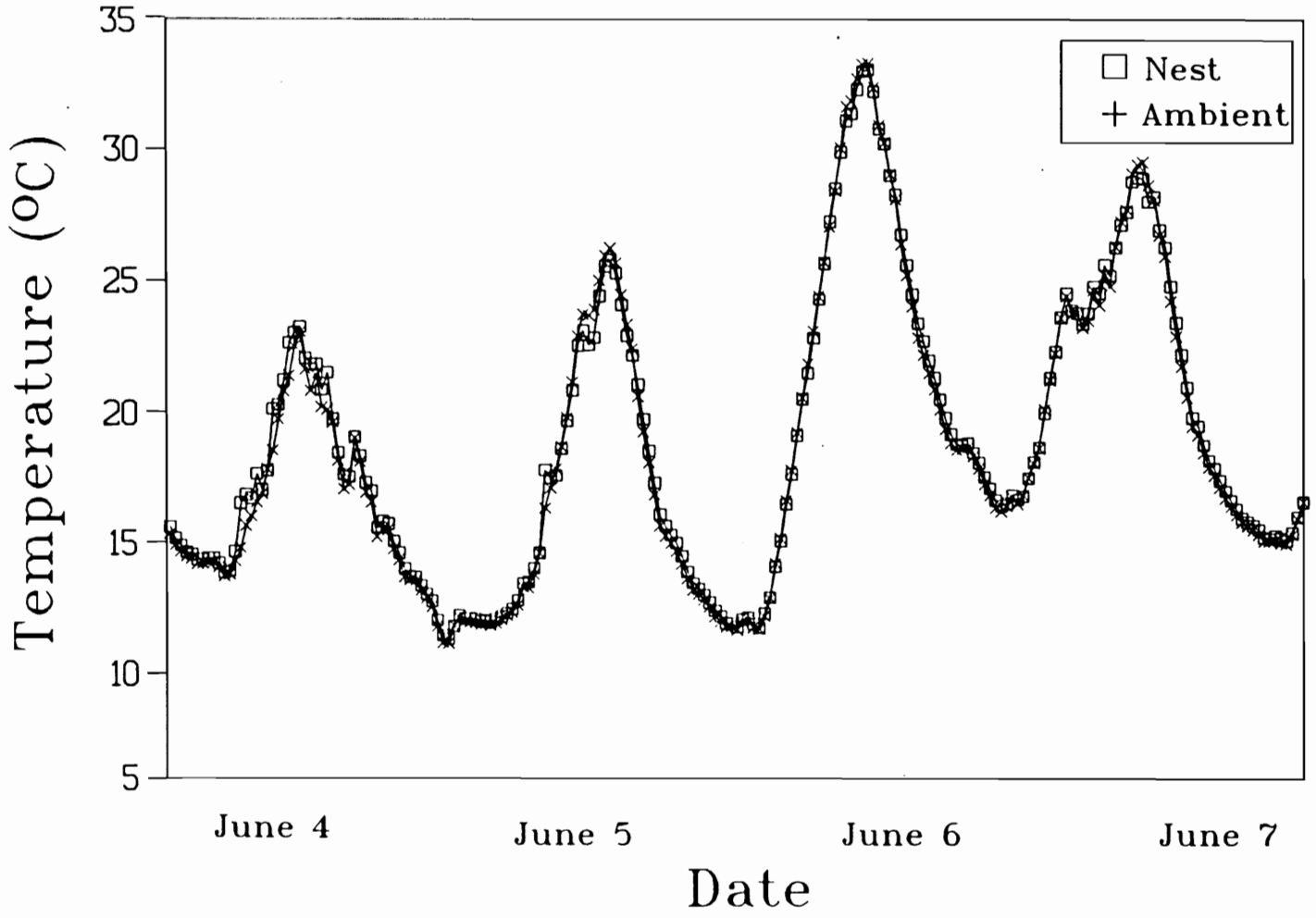

Figure 6. Ambient and internal temperatures of a *D. maculata* embryo nest which was monitored continuously (presented in 30 minute intervals) between June 4 and June 7, 1990 in southwestern Virginia. The nest contained 8 eggs, and 3, first instars.

temperature increases within the nest and that first instar contribution towards heat production is negligible.

Despite the internal nest temperature fluctuations observed in Figures 4 and 5, the average nest temperature over the 4-day period was 1.1°C higher than ambient. The average nest and ambient temperature were 19.3 and 18.2°C, respectively. If the evening and early morning temperatures are excluded from the calculation of averages, the average temperature difference is 1.9°C. In comparison with the incubation study, the average temperature difference between the nest (19.3°C) and the incubator setting (17.7°C) was 1.6°C. This difference could have had a substantial effect on developmental times, not only preventing development, but also, increasing mortality.

Discussion

Egg Development and Nest Temperature- The results of the egg developmental study were inconclusive, yet showed the ability of eggs to remain alive without the foundress queen for relatively long periods. Spradbery (1973) reported that on the average, eggs of *Vespa crabro* (L.) took 5 days to hatch, but could take as long as 18 days. Spradbery (1973) also suggested that as a colony became larger and the temperatures higher, the egg incubation period became shorter.

Although one egg was observed to completely eclose, it is probable that more embryonic larvae were alive than were noted. Embryonic larval movement tended to be sporadic and there may have been periods of greater activity which may not have coincided with periods of observation. The gradual shrinking,

discoloration, and eventual death of the eggs could have been partially the result of suboptimal developmental temperatures. Schnetter (1934) investigated the effects of temperature on honey bee eggs and found that 50% of the eggs failed to develop and malformations were common when incubated at temperatures of 29-30°C, compared to the optimal 35°C. It is also likely that the relative humidity within the incubator may have been below the optimal range. Doull (1976) reported that honey bee eggs were able to hatch successfully at normal brood temperatures and humidities ranging from 80 to 100%; however, at 50% humidity, no eggs hatched. Doull (1976) found that the eggs failed to eclose because the hatching fluid could not dissolve the chorion. Unfortunately, embryo nest humidity data are not available for any vespid species. Undoubtedly, other factors could also have contributed to egg mortality, including handling of nests twice a day.

Whether the queen's contribution to nest temperature is important to brood development cannot be established from the temperature data collected from the one nest in this study. Gibo et al. (1977) reported that *D. arenaria* (3 nests) were able to heat the embryo nests during the nest initiation period, whereas *D. maculata* (1 nest) did not heat the embryo nest until it was eleven days old and had well-developed larvae. The data presented in Figure 4-6 indicates that *D. maculata* queens can produce heat within the nest during the initial phases of development; however, the importance of this heat needs to be more carefully examined in future studies. Is the heat necessary for initial egg and larval development, or is it simply a metabolic by-product of queen activity?

Energy Reserve Utilization- The results of the 10-day egg incubation study demonstrated a significant change in dry weight. Although lipids were found to be the primary energy reserve in the embryo and summer nest eggs, total lipids were not significantly different during the 10-day study. However, a decreasing trend was apparent in all 5 lipid classes and variability between eggs may have prevented detection of differences. Other reserves must have been utilized during this period but were not quantified. It is likely that glucose and glycogen were used, and perhaps, yolk proteins. Sander et al. (1985) reported that lipids are the primary energy reserve used during egg development in most insects; but that carbohydrates are often the major energy source during the early phases of development. Irie et al. (1979) reported that glycerol and a polysaccharide, consisting of glucose, mannose and sorbose, were the primary energy reserves used during embryonic development within eggs of a cricket, *Teleogryllus* spp. Proteins are not thought to be a common energy source because of nitrogenous waste buildup (Babcock and Rutschky, 1961). However, at suboptimal temperatures and during the latter stages of embryogenesis, proteins can be used (Yamashita and Irie, 1980; Bownes and Hames, 1977; Sander et al. 1985).

Egg Weight Variability- The results of this investigation have demonstrated that egg weight of *D. maculata* is not only highly variable, but also decreases progressively with colony development. In the honey bee *Apis mellifera* (L.), Taber and Roberts (1963) have suggested that egg weight and egg size are controlled by genetic factors, although environmental factors contribute to the total variability. The authors observed a 30% difference in mean (live) egg weight

between hybrid high- and low-weight lines. In addition, they demonstrated that hybrid crosses of queens known for their production of high- and low-weight - eggs, produced eggs of intermediate weight.

Roberts and Taber (1965) also demonstrated that there was no correlation between the egg-laying rate and the size of the eggs produced. However, the studies were conducted in small nucleus colonies that prevented maximum oviposition. The maximum egg production rates they recorded were 150-160 eggs per day, a figure which is approximately 10% of that which would occur in a normal-sized colony (Moeller 1958). The results of Roberts and Taber (1965) do not agree with the earlier findings of Hejtmanek (1961) and Jordan (1961). Both of these authors demonstrated a decrease in egg size as the rate of egg laying increased, and suggested both seasonal and colony size affects.

Dolichovespula maculata and other vespids do not have the same nesting biology as honey bees. The egg-laying rate of most *D. maculata* foundress queens is probably 1-2 eggs per day during the nest initiation period. *D. maculata* foundress queens have been observed to produce 9-16 eggs in the first 1-1/2 to 2 weeks of nest development (Stein; unpubl.). Although information on mid-summer oviposition rates are not available on *D. maculata*, Spradbery (1973) has reported that *V. vulgaris* (L.) and *V. germanica* (F.) queens can lay as many 200-300 eggs per day during periods of maximum oviposition. Nests of D. *maculata* are considerably smaller than those of *V. germanica* and *V. vulgaris*; therefore,the *D. maculata* oviposition rate in mid-summer is probably less than half of what Spradbery (1973) reported. Nevertheless, the oviposition rate of queens in the summer, would be considerably higher than that which would occur

in the spring.

These data suggest that the larger embryo nest eggs found within the spring embryo nest are a function of the queen's oviposition rate. Like the honey bee, some of the variability in weight may be due to genetic factors, although other factors such as queen foraging and the available reserves within the fat body may exert their influence as well. However, egg size and energy reserve variation could have ecological implications as well. As shown in Table 9, larger eggs have proportionately more energy reserves than the smaller summer eggs. These extra reserves could be important for egg (or 1st instar) survival during the prolonged periods of unfavorable weather often found in early spring. In contrast, eggs which are produced in the summer develop under more optimal humidities and temperatures. Weather is seldom unfavorable for extended periods, nest temperature can be maintained at high levels (ca. 30°C), and workers can forage on a routine basis. Food is seldom limiting and larvae are regularly fed, so that extra reserves in first larval instars would be unnecessary.

The success of the nest initiation process by queens of the baldfaced hornet is undoubtedly affected by many factors. The literature is replete with references alluding to nest failure due to microbial infections, vertebrate predation, queen competition for nesting sites and/or nest usurpations (Beirne 1944; Fox-Wilson 1946; Spradbery 1973; Edwards 1980; Archer 1980, 1981; Akre et al. 1981). The inability of a queen to forage successfully because of adverse conditions is one of many possible explanations. Furthermore, the foraging behavior of a queen and her available reserves could affect and possibly limit, the energetic contributions and care which she can provide her offspring. Thus,

energy reserve variability and/or deficiencies could affect the initial rate of embryo nest growth, and perhaps nest mortality. These results suggest that the nutritional status of the queen can ultimately influence the survivability of her first brood. Additional studies with other vespines are needed to more clearly define the energetics involved in initial brood production and their relative importance with respect to other envirionmental influences.

CHAPTER 6 - CONCLUSION

The factors which have been most frequently reported to affect queen and embryo nest mortality in the spring have included adverse weather, disease, predation, competition for nest sites, and nest usurpations (Beirne 1944; Fox-Wilson 1946; Spradbery 1973; Edwards 1980; Akre and Reed 1981). Several authors (Archer 1973, 1980, 1981, 1982; Roth and Lord 1987) have extended the factors to include various attributes of queen fitness. While the mortality rates of wintering and spring queens and embryo nests are reported to be high (Spradbery 1973; Archer 1980), there is very little supporting data and studies of solitary queens are lacking. This lack of information was the basis for this dissertation; the results of which have provided new information on the biology of the solitary vespine queen when she is most vulnerable to environmental influences and mortality is reported to be the greatest.

In mid to late summmer, the solitary queen ecloses from her pupal cell. What a queen does from this point in her life cycle until hibernation is not well-known, although several anecdotal accounts are available. Edwards (1980) reported that upon eclosion, new queens are tended by nurse workers for some time before leaving the nest. Duncan (1939) and Spradbery (1973) found that new queens feed on larval trophallactic secretions, regurgitated food from workers, and perhaps, forage away from the nest. The amount of time that new queens spend in the nest feeding and the physiological changes that they undergo before winter have not been clearly established.

The study of pre-hibernating queens has revealed that on average, *D. maculata* queens have the capacity to increase their total dry body weight by as much as 79% before entering hibernation. The analyses of these queens has shown that lipids accounted for (49%) of the fall queen weight, followed by the carbohydrates, sugar (13%) and glycogen (7%). Previous studies of other energy reserves in fall queens have not been made, with the exception of Spradbery (1963) who found that lipid reserves accounted for 40% of the pre-wintering weight of vespine queens.

In addition to feeding and increasing energy reserves, yellowjacket queens mate sometime before entering hibernation. Although mating is not requisite for successful overwintering as shown in this study and by (Matsuura 1969), queens must mate in order to produce fertilized eggs in the spring. The location of matings, the amount of sperm transferred per mating, as well as other aspects of yellowjacket mating biology have not been well-studied.

In mid to late autumn, queens seek hibernation sites that favor survival. These may include any secluded area that protects the queen from the elements and predators. Hibernaculae may include decayed logs, under the bark of trees, and in the ground (Rau 1929; Spradbery 1973; Akre et al. 1981). Queens spend the entire winter inside their hibernaculae and emerge sometime in spring. At present, there have been no major investigations devoted to hibernating queens. Clearly, hibernation biology is an area in which studies need to be undertaken.

The studies of fall and spring queens indicated that queens undergo an appreciable weight loss (53%) during the hibernation period. Furthermore, the analyses of these queens indicated that lipids accounted for 35% of the weight

lost during hibernation, sugars 12%, and glycogen 6%. Spradbery (1963) reported that vespine queens in Britain undergo a 30% loss in lipid during hibernation. Similarly, Matsuura (1969) reported that *Vespa* queens in Japan undergo a 38% loss in lipid fresh weight during hibernation.

Although lipids have been reported to be a primary energy reserve used in hibernating vespine queens, lipid use accounted for only 66% of the dry weight loss in hibernating *D. maculata* queens. While these results agree with the findings of Spradbery (1963) and Matsurra (1969), carbohydrate reserves have not been previously quantified. The results obtained from the analyses of *D. maculata* queens indicate that carbohydrates are important energy reserves for hibernating queens. Both sugar and glycogen accounted for 23% and 11%, respectively, of the total weight loss during hibernation.

The comparison of spring queens revealed no significant differences in weight or energy reserves between non-nesting and nesting queens over a 3-year period. This finding suggests that spring queens do not undergo a significant weight increase during the nest initiation period; however, the maintenance of a constant weight suggests the continuous need for feeding/foraging after emergence from hibernation. During this period, queens feed on nectar and honeydew as well as seek nesting sites. After choosing a nesting location, the queens devote most of their time and energy to nesting activities. These activities include the collection of wood fibers, water, nectar, and prey, in addition to allocating nutrients and energy reserves to their first brood. The total dry weight invested in the first 15 eggs within an embryo nest is only 4-5 mg, or 2-3% of the dry weight of the queen. This initial energy contribution appears to be small;

however, in view of the energy invested in nest construction and brood-rearing activities, it is possible that low energy reserves could be a limiting factor in the production of viable eggs.

An examination of the initial eggs produced by the queen indicated that the energy reserves, in order of importance, were lipid (22%), sugar (9.5%), and glycogen (5%). Egg reserves were found to be variable, with the variability attributed to the reserves that are available to a queen. However, the foraging behavior of the queen could influence both the quantity and quality of reserves that a queen could direct into her eggs.

This study of embryo nest eggs also revealed that egg weight was highest in the spring and decreased with progressive nest development. This decrease in egg weight was attributed to the oviposition rate of the queen; although, it is also likely that extra reserves in embryo nest eggs could be important for egg (or first instar) survival during periods of unfavorable weather in the spring. Furthermore, energy reserve variability and/or deficiencies within embryo nest eggs could prove to affect egg viability and ultimately, incipient colony survival. In comparison, summer eggs were found to weigh less and contain less energy reserves. Within these eggs, extra reserves would be unnecessary for egg development because larvae are regularly tended by workers and are seldom exposed to adverse conditions.

In conjunction with the nest development study, the egg incubation experiment was conducted to examine egg developmental rates and energy reserve utilization. Many of the eggs died and several developed fungal infections during the course of development. While many factors can affect egg survival, the

incubator temperatures and humidities may have been below the optimal range. As partial evidence, a mean difference in temperature was found between a *D. maculata* embryo nest and the incubator of 1.6°C; this temperature difference could have affected egg development. An embryo nest temperature analysis also revealed that nest temperatures were 1.1°C higher than ambient over a 4-day period. Gibo et al. (1977) reported nest temperatures of 3-4°C higher than ambient in his studies with *D. maculata* but only after the larvae were half-grown. Although the results of this nest temperature study differ slightly from those of Gibo et al. (1977), slight temperature increases were found when the queen was present. These temperature increases, whether a metabolic by-procuct or a routine incubation activity, could be important to the development and survival of the first brood. In addition, the maintenance of nest temperatures slightly higher than ambient, requires the expenditure of energy. Therefore, queens with inadequate energy reserves and/or restricted foraging abilites due to unfavorable weather would be at a disadvantage. Clearly, the energetic contribution of queens and the effects of temperature on egg and embryo nest developmental rates/mortality needs to be more thoroughly examined in future studies.

Although queens that survive the winter are similar in weight and energy reserves, not all queens are mated. A large number of queens (18.4%) which were examined in the spring seasons of 1987 and 1988 were found to be uninseminated. However, in 1989, all of the queens were found to be in the mated condition. Similar observations on mating success have been reported by Matsuura (1969) who noted that as many as 2/3 of *Vespa mandarinia* queens survive the winter without having mated. Although MacDonald et al. (1974) have

reported that spring queens in North America are almost always inseminated, the results of the mating success study indicated that unmated queens can survive the winter; although, the frequency of the unmated condition may vary from year to year. The failure of queens to mate before overwintering is a significant cause of reproductive failure.

Although mated queens establish viable nests, not all nests reach full maturity. Summer nests may become all male before the normal onset of reproductive production, despite the presence of a foundress queen. Early male production within a population of vespines may be caused by dysfunctions of the queens, genetic factors, or sperm depletion. Several authors (Spradbery 1973; Greene et al. 1976) have questioned the depletion of sperm in foundress queens as a mechanism that initiates early male production. However, the study of sperm utilization in queens of *D. maculata* has suggested that queens can deplete their sperm supplies prematurely, if the initial sperm numbers are low. Furthermore, the sperm utilization regression model indicates that the decline in sperm numbers follows an exponential decay. Sperm utilization has been previously recognized as an efficient process in which exceedingly small quantities of sperm are released per female egg (Harbo 1979; Tschinkel 1987 a & b, Tschinkel and Porter 1988). The sperm utilization regression model presented in this study provides an alternate view of sperm use efficiency in which large numbers of sperm are released per egg fertilization during early colony development, followed by decreasing numbers per fertilization event as the colony develops. In terms of fitness, this suggests that queens with low sperm numbers would deplete their sperm supplies earlier in the season than would a queen with average or higher

than average sperm numbers. Therefore, the mating success of fall queens is as important to the fitness of the queen, as is the storage of sufficent energy reserves.

Although specific causes of queen mortality were not examined within this investigation, the results and analyses suggest many questions with regard to overwintering survival and queen fitness. For example, how much time do newly-eclosed queens spend feeding before they seek out hibernation sites? Is the vigor and size of the summer nest important to the sufficient storage of energy reserves by the queen? Can a significant amount of overwintering mortality be attributed to insufficient storage of energy reserves? Is there a relationship between the time of year a queen ecloses and successful mating? What are some of the factors involved in successful mating? What are the optimal conditions that favor nest initiation and survival?

Successful queen survival and the nest initiation process are undoubtedly affected by many factors. This investigation has provided new insight into several aspects of *D. maculata* queen fitness. Additional studies with queens of other vespine species should reveal that physiological and behavioral factors are more important to the understanding of individual queen fitness and incipient colony mortality than are currently recognized.

CHAPTER 7 - LITERATURE CITED

Akre, R.D., W.B. Hill and J.F. MacDonald. 1973. Artificial housing for yellowjacket colonies. J. Econ. Entomol. 66: 803-805.

Akre, R.D. and H.G. Davis. 1978. Biology and pest status of venomous wasps. Ann. Rev. Entomol. 23: 19-42.

Akre, R.D., A. Greene, J.F. MacDonald, P.J. Landolt, and H.G. Davis. 1981. The yellowjackets of America north of Mexico. USDA Handbook No. 552. 102pp.

Akre, R.D., and H.C. Reed. 1981. Population cycles of yellowjackets (Hymenopteras: Vespinae) in the Pacific Northwest. Environ. Entomol. 10: 267-274.

Akre, R.D., H.C. Reed, and P.J. Landolt. 1982. Nesting biology and behavior of the blackjacket, *Vespula consobrina* (Hymenoptera: Vespidae). J. Kan. Entomol. Soc. 55: 373-405.

Akre, R.D. and D. P. Bleicher. 1985. Nests of *Dolichovespula norwegica* and *D. norvegicoides* in North America (Hymenoptera: Vespidae). Entomol. News 96: 29-35.

Akre, R.D. and J.F. MacDonald. 1986. Biology, economic importance and control of yellowjackets. *In* Economic impact and control of social insects, ed. S.B. Vinson. New York: Praeger Publishers.

Alford, D.V. 1969. A study of the hibernation of bumble bees (Hymenoptera: Apidae) in southern England. J. Anim. Ecol. 38: 149-170.

Archer, M.E. 1973. The population ecology of *Vespula vulgaris* (L.), Vespidae. Hymenoptera, and the computer simulation of colonial seasonal development. IUSSI Proc. VII Int. Congr. pp. 2-10.

Archer, M.E. 1980. Population dynamics. *In* Social wasps, their biology and control. Rentokil Limited, England. 398p.

Archer, M.E. 1981. Successful and unsuccessful development of colonies of *Vespula vulgaris* (L.) (Hymenoptera: Vespidae). Ecol. Entomol. 6: 1-10.

Archer, M.E. 1982. A population dynamics hypothesis for *Vespula* in England, U.K. *In* The biology of social insects (Ed. by M.D. Breed, C.D. Michener and H.E. Evans), pp.76-79, Westview Press, Colorado.

Archer, M.E. 1985. Population dynamics of the social wasps *Vespula vulgaris* and *Vespula germanica* in England. J. Anim. Ecol. 54: 473-485.

Arnold, T.S. 1966. Biology of social wasps. Comparative ecology of the British species of social wasps belonging to the family Vespidae. MSc. thesis, Univ. Lond.

Ascerno, M.E. 1981. Diagnostic clinics: more than a public service. Bull. Entomol. Soc. Amer. 27: 97-101.

Babcock, K.L. and C.W. Rutchschky. 1961. Lipids in insect eggs: A review with new evidence from the milkweed bug, *Oncopeltus fasciatus* (Hemiptera, Lygaeidae). Ann. Entomol. Soc. Amer. 54: 156-164.

Batra, S.W.T. 1980. Sexual behavior and pheromones of the European hornet, *Vespa crabro germana* (Hymenoptera: Vespidae) J. Kan. Entomol. Soc. 53: 461-469.

Beirne, B.P. 1944. The cause of the occasional abundance or scarcity of wasps (*Vespula* spp.). Entomol. Mon. Mag. 80: 121-124.

Bownes, M. and B.D. Hames. 1977. Accumulation and degradation of three major yolk proteins in *Drosophila melanogaster*. J. Exp. Zool. 200: 149-156.

Brian, M.V. and A.D. Brian. 1952. The wasps *Vespula sylvestris* Scopoli: feeding, foraging, and colony development. trans. R. Entomol. Soc. Lond. 103: 1-26.

Cochran, W.G. and G.M. Cox. 1957. Experimental Designs. John Wiley and Sons, Inc.

Cole, B.J. 1983. Multiple mating and the evolution of social behavior in the Hymenoptera. Behav. Ecol. Sociobiol. 12: 191-201.

Corbella, E. and L.S. Goncalves. 1982. Relationship between weight at emergence, number of ovarioles and spermathecal volume of africanized honey bee queens (*Apis mellifera* L.). Rev. Brasil. Genet. 5: 835-840.

Davis, H.G. 1978. Yellowjacket wasps in urban environments. *In* Perspectives in Urban Entomology, ed. C.S. Koehler, G.W. Frankie. New York: Academic Press, Inc.

Doull, K.M. 1976. The effects of different humidities on the hatching of the eggs of honeybees. Apidologie. 7: 61-66.

Duman, J.G. and J.L Patterson. 1978. The role of ice nucleators in the frost tolerance of overwintering queens of the bald faced hornet. Comp. Biochem. Physiol. 59: 69-72.

Duman, J.G., J.P. Morris, and F.J. Castellino. 1984. Purification and composition of an ice nucleating protein from queens of the hornet, *Vespula maculata*. J. Comp. Physiol. 154: 79-83.

Duncan, C. D. 1939. A contribution to the biology of North American vespine wasps. Stanford Univ. Publ. Biol. Sci. 8: 1-272.

Edwards, R.E. 1980. Social wasps. Their biology and control. The Rentokil Library, East Grinstead, England. 398 pp.

Fell, R.D. 1990. The qualitative and quantitative analysis of insect hemolymph sugars by high performance thin-layer chromatography. Comp. Biochem. Physiol. 95A: 539-544.

Fell, R.D. and R.A. Morse. 1977. Honeydew. Amer. Bee J. 117: 52-54.

Fox-Wilson, G. 1946. Factors affecting populations of social wasps, *Vespula* species, in England (Hymenoptera: Vespidae). Proc. R. Entomol. Soc. Lond. (A), 21: 17-27.

Fyg, W. 1964. Anomalies and diseases of the queen honey bee. Ann. Rev. Entomol. 9: 207-224.

Gibo, D.L. 1972. Hibernation sites and temperature tolerance of two species of *Vespula* and one species of *Polistes* (Hymenoptera: Vespidae). J. N.Y. Entomol. Soc. 80: 105-108.

Gibo, D.L., H.E. Dew, and A.S. Hajduk. 1974. Thermoregulation in colonies of *Vespula arenaria* and *Vespula maculata* (Hymenoptera: Vespidae). II. The relation between colony biomass and calorie production. Can. Entomol. 106: 873-879.

Gibo, D.L., A. Temporale, J.P. Lamarre, B.M. Souter, and H.E. Dew. 1977. Thermoregulation in colonies of *Vespula arenaria* and *Vespula maculata* (Hymenoptera: Vespidae) III. Heat Production in queen nests. Can. Entomol. 109: 615-620.

Greene, A. 1984. Production schedules of vespine wasps: an empirical test of the bang-bang optimization model. J. Kan. Entomol. Soc. 57: 545-568.

Greene, A., R.D. Akre, and P.J. Landolt. 1976. The aerial yellowjacket, *Dolichovespula arenaria* (Fab.): nesting biology, reproductive production, and behavior (Hymenoptera: Vespidae). Meland. 26: 1-34.

Harbo, J. R. 1979. The rate of depletion of spermatazoa in the queen honey bee spermatheca. J. Apic. Res. 18: 204-207.

Harbo, J. R. and T. I. Szabo. 1984. A comparison of instrumentally inseminated and naturally mated queens. J. Apic. Res. 23: 31-36.

Hawthorne, R.M. 1969. Estimated damage and crop loss caused by insect/mite pests-1968. Calif. Dept. Agric., Bur. Entomol. 11 pp.

Heinrich, B. 1974. Thermoregulation in bumble bees. I. Brood incubation by *Bombus vosnesenskii* queens. J. Comp. Physiol. 88: 129-140.

Hejtmanek, J. 1961. The variability in honey bee eggs. (In Czech.) Ved. Prace Vyzkumn. Ustavu Vcelar. CSAV V 1960, 2: 169-196.

Irie, K., K. Suzuki, and K. Miya. 1979. Change of carbohydrate content during ovarian development in Emma Field Cricket, *Teleogryllus emma*. Appl. Entomol. Zool. 14: 278-284.

Ishay, J. 1973. Thermoregulation by social wasps: behavior and pheromones. Trans. N.Y. Acad. Sci. 35: 447-462.

Ishay, J. 1975. Caste determination by social wasps: cell size and building behavior. Anim. Behav. 23: 425-431.

Ishay, J. and R. Ikan. 1968a. Gluconeogenesis in the oriental hornet *Vespa orientalis* F. Ecol.. 49: 169-171.

Ishay, J. and R. Ikan. 1968b. Food exchange between adults and larvae in *Vespa orientalis* F. Anim. Behav. 16: 298-303.

Ishay, J. and F. Ruttner. 1971. Thermoregulation in hornissennest. Z. verg. Physiol. 72: 423-434.

Jeanne, R.L. 1977a. A specialization in nest petiole construction by queens of *Vespula* spp. (Hymenoptera: Vespidae). J. N.Y. Entomol. Soc. 85: 127-129.

Jeanne, R. L. 1977b. Behavior of the obligate social parasite *Vespula arctica* (Hymenoptera: Vespidae). J. Kans. Entomol. Soc. 50: 541-557.

Jordan, R. 1961. Relations between egg size and the rate of egg-laying. (In German.) Bienenvater 82: 132-134.

Judge, D.N., D.E. Mullins, and J.L. Eaton. 1989. Microquantity analysis of insect hemolymph by high performance thin layer chromatography. J. Plan. Chromat. 2: 442-446.

Kemper H. and E. Dohring. 1967. Die sozialen faltenwepsen mitteleuropas. Paul Parey, Berlin.

Keyel, R.E. 1983. Some aspects of niche relationships among yellowjackets (Hymenoptera: Vespidae) of the northeastern United States. Ph. D. Dissertation thesis, Cornell University, Ithaca, New York. 161 pp.

Lord, W.D. and R.R. Roth. 1978. Is there intraspecific competition in *Vespula maculifrons* (Hymenoptera: Vespidae)? J. N.Y. Entomol. Soc. 86: 304-305.

MacDonald, J.F., R.D. Akre, and W.B. Hill. 1974. Comparative biology and behavior of *Vespula atropilosa* and *V. pensylvanica* (Hymenoptera: Vespidae). Meland. 18: 1-66.

MacDonald, J. F. and R. W. Matthews. 1976. Nest structure and colony composition of *Vespula vidua* and *V. consobrina* (Hymenoptera: Vespidae). Ann. Entomol. Soc. Am. 69: 471-475.

MacDonald, J. F., R. W. Matthews, and R. S. Jacobson. 1980. Nesting Biology of the yellowjacket, *Vespula flavopilosa* (Hymenoptera: Vespidae). J. Kan. Entomol. Soc. 53: 448-458.

MacDonald, J. F. and R. W. Matthews. 1981. Nesting biology of the Eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). J. Kan. Entomol. Soc. 54: 433-457.

MacDonald, J. F. and R. W. Matthews. 1984. Nesting biology of the Southern Yellowjacket, *Vespula squamosa* (Hymenoptera: Vespidae): Social parasitism and independent founding. J. Kan. Entomol. Soc. 57: 134-151.

Makino, S. and Yamane Sk. 1980. Heat production by the foundress of *Vespa simillima*, with descriptions of its embryo nest (Hymenoptera: Vespidae). Insecta Matsum. 19: 89-101.

Matsuura, M 1969. Behavior of post-hibernating female hornets , *Vespa*, in the pre-nesting stage, with special reference to intra and interspecific dominance relationships. Jap. J. Ecol. 19: 196-203.

Matthews, R.W. and J.R. Matthews. 1979. War of the yellowjacket queens. Nat. Hist. 88: 56-65.

Matthews, R.W., K.G. Ross, and R.A. Morse. 1982. Comparative development of queen nests of four species of yellowjackets (Hymenoptera: Vespidae) reared under identical conditions. Ann. Entomol. Soc. Amer. 75: 123-129.

Moeller, F.E.. 1958. Relation between egg-laying capacity of queen bees and population and honey production of their colonies. A. Bee J. 98: 401-402.

Montagner, H. 1967. Contribution a l etude du determinisme des castes chez les guepes du genre *Vespa*. Thesis, Univ. Nancy.

Moritz, R.F.A. 1984. The effect of different diluents on insemination success in the honey bee using mixed semen. J. Apic. Res. 23: 164-167.

Moritz, R.F.A. 1986. Intracolonial worker relationship and sperm competition in the honey bee (*Apis mellifera* L.). Experientia. 42: 445-448.

Morris, R.F. 1972. Predation by wasps, birds, and mammals on *Hyphantria cunea*. Can. Entomol. 104: 1581-1591.

Mullins, D.E. 1971. An investigation into the nitrogen balance of an insect, *Periplaneta americana* (L.) with specific refernce to urate storage and mobilization, the urate storage complex, and nitrogen excretory products. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, 231 pp.

Page, R. E. 1986. Sperm utilization in social insects. Ann. Rev. Entomol. 31: 297-320.

Page, R.E., and R.A. Metcalf. 1982. Multiple mating, sperm utilization, and social evolution. Am. Nat. 119: 263-281.

Page, R.E., and R.A. Metcalf. 1984. A population investment sex ratio for the honey bee (*Apis mellifera* L.). Am. Nat. 124: 680-702.

Potter, N.B. 1964. A study of the biology of the common wasp, *Vespula vulgaris* L., with special reference to the foraging behavior. Ph. D. Thesis, Bristol Univ.

Rau, P. 1929. The nesting habits of the bald-faced hornet, *Vespa maculata*. Ann. Entomol. Soc. Amer. 22: 659-675.

Rau, P. 1934. A note on hibernating queens of the wasp, *Vespa maculata*. Bull. Brooklyn. Entomol. Soc. 29: 170-171.

Roberts, W.C. and S. Taber. 1965. Egg-weight variance in honey bees. Ann. Entomol. Soc. Amer. 58: 303-306.

Ross, K. G. 1983. Laboratory studies of the mating biology of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). J. Kan. Entomol. Soc. 56: 523-537.

Ross, K. G., R. W. Matthews, and R. A. Morse. 1981. Laboratory culture of four species of yellowjackets, *Vespula* spp. foundress nest initiation. Ann. Entomol. Soc. Amer. 74: 247-254.

Roth, R. R. and W. D. Lord. 1987. Cycles of queen size and abundance in a population of *Vespula maculifrons* (Hymenoptera: Vespidae). Environ. Entomol. 16: 649-652.

Rouget, A. 1873. Sur les coleopteres parasites des vespides. Mem Acad. Dijon (Ser 3) 1: 161-288.

Ruttner, S. K. 1975. The instrumental insemination of the queen bee. Bucharest: Apimondia Publishing House.

Sander, K., H.O. Gutzeit, and H.Jackle. 1985. Insect embryogenesis: morphology, physiology, genetical and molecular aspects. *In* Comprehensive insect physiology biochemistry and pharmacology. Vol. 1. Embryogenesis and reproduction. (Ed. by G.A. Kerkut and L.I. Gilbert) Pergamon Press.

SAS Institute. 1985. SAS user's guide: statistics, version 5 edition. Cary. N. C.

Schmidtmann, E.T. 1977. Muscid fly predation by *Vespula germanica* (Hymenoptera: Vespidae). Environ. Entomol. 6: 107-108.

Schnetter, M. 1934. Physiologische untersuchungen uber das differenzierungszentrum in der embryonlentwicklung der honibiene. Wilh. Roux's Archiv. 131: 285-323.

Schremmer, F. 1961. Bemerkenswerte wechselbeziehungen zwischen orchideenbluten und insekten. Natur. u Volk. 91: 52-61.

Spradbery, J.P. 1963. A study of British vespine wasps (Hymenoptera: Vespidae) with reference to the problems of caste differentiation. Ph. D. thesis, Univ. Lond.

Spradbery, J. P. 1973. Wasps: An account of the biology and natural history of social and solitary wasps. Univ. Wash. Press, Seattle. 408pp.

Stein, K.J. and D.L. Wrensch. 1988. The pest status of yellowjackets in Ohio (Hymenoptera: Vespidae). Gr. Lakes. Entomol. 21: 83-89.

Storey, K.B. and J.M. Storey. 1983. Biochemistry of Freeze tolerance in terrestrial insects. Trends Biochem. Sci. 8: 242-245.

Taber, S. and W.C. Roberts. 1963. Egg weight variability and its inheritance in the honey bee. Ann. Entomol. Soc. Amer. 56: 473-476.

Thomas, C.R. 1960. The European wasp (*Vespula germanica* Fab.) in New Zealand. Inf. Ser. Dep. Scient. ind. Res. N.Z. 27: 1-73.

Toom, P.M., C.P. Johnson, and E.W. Cupp. 1976. Utilization of Body Reserves During Preoviposition Activity by *Solenopsis invicta*. Ann. Entomol. Soc. Amer. 69: 145-149.

Trevelyan, W.E. and J.S. Harrison. 1952. Studies on yeast metabolism 1. Fractination and microdetermination of cell carbohydrates. Biochem. J. 50: 298-302.

Tschinkel, W.R. 1987a. Fire ant queen longevity and age: Estimation by sperm depletion. Ann. Entomol. Soc. Am. 80: 263-266.

Tschinkel, W.R. 1987b. Relationship between ovariole number and spermathecal sperm count in ant queens: A new allometry. Ann. Entomol. Soc. Am. 80: 208-211.

Tschinkel, W.R. and S.D. Porter. 1988. Efficiency of sperm use in queens of the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 81: 777-781.

Van Handel, E. 1965a. Microseparation of glycogen, sugars, and lipids. Anal. Biochem. 11: 266.271.

Van Handel, E. 1965b. Estimation of glycogen in small amounts of tissue. Anal. Biochem. 11: 256-265.

Watson, E.B. 1922. The food habits of wasps. Bull. Chamber. Hort. 1: 26-31.

Yamane, Sk., R.E. Wagner, and S. Yamane. 1980. A tentative revision of the subgenus *Paravespula* of eastern Asia (Hymenoptera: Vespidae). Insecta Matsum. 19: 1-46.

Yamashita, O. and K. Irie. 1980. Larval hatching from vitellogenin-deficient eggs developed in male hosts of the silkworm. Insect Biochem. 9: 547-553.

Zmarlicki, C. and R. Morse. 1963. The mating of aged virgin queen honey bees. J. Apic. Res. 2: 62-63.

Vita

Kenneth J. Stein was born on November 10, 1957 in Lorain, Ohio to Raymond F. and Lucille K. Stein. He exhibited an interest in insects at a very early age. In both elementary and high school his hobbies were always related to one of the sciences, although he maintained an avid interest in insects. He graduated from Brookside High School in 1975, and entered Lorain County Community College in Ohio. After completing one full year, Ken decided to join the United States Navy to "see the world" and earn money for his return to college. On August 9, 1976, Ken began his naval service, during which time he worked as an aviation ordnanceman aboard 2 different aircraft carriers. He was honorably discharged from active duty on June 13, 1980 and 10 days later returned to Lorain County Community College as a full-time student. He completed one year of classes before transferring to the University of Toledo in September of 1981. While at the University of Toledo, Ken joined the U.S. Naval Reserves. In his final year at the University of Toledo, he renewed his interests in entomology before receiving his Bachelor's degree in Biology on June 23, 1983. Ken then decided to pursue a Master of Science in Entomology at The Ohio State University. He completed a project on several aspects of yellowjacket ecology, and was awarded his Master of Science degree in December of 1986. In the spring of 1987, Ken came to VPI&SU to pursue a Ph.D. in Entomology. In December of 1987, he was commissioned as a Medical Entomologist in the United States Naval Reserve and currently serves as a Lieutenant.