The tracheal mite, *Acarapis woodi* (Rennie), parasitizes the honey bee, *Apis mellifera* L., an insect used extensively by humans for pollination of domestic crops and for bee products, primarily honey.

This parasite was first discovered in 1921 on the Isle of Wight, a small island situated in the English Channel off the southern coast of England. Controversy as to the impact of *A. woodi* on its honey bee host arose soon after its discovery. This project quantifies the mite-generated mortality of worker honey bees during the flowering period when the bees are active and suggests how mortality of individuals may impact the colony to which these individuals belong. A model of parasitism is derived that demonstrates how swarming may stabilize the populations of host and parasite, how drifting of worker bees between colonies may be directly related to parasite prevalence among the population of colonies and how interruption of swarming may disrupt this system.

It appears that honey bee foraging has little impact on mite transmission, but microclimate probably is important. The mites transfer to new hosts mainly during the night. Tracheal mites
produce both sexes in sufficient numbers to suggest that parthenogenesis is not invoked to take advantage of quickly expanding resources (i.e. young bees) in the spring. Mite fecundity in the Pacific Northwest peaks in June when bee turnover is greatest.

Protein mucopolysaccharide material covers the hemolymph side of infested tracheae and these become darkened with age, suggesting that mite feeding initiates a coagulation-melanization response from the bee.

Within a colony uninfested members are also affected by mite infestation of their sisters, as indicated by studies on individual bee physiology.
Tracheal Mite Parasitism of Honey Bees

by

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TRACHEAL MITE PARASITISM OF HONEY BEES

INTRODUCTION

This introduction presents some basic biology and history of human interest of the host *Apis mellifera* L. as well as discussing the tracheal mite, *Acarapis woodi* (Rennie), from the standpoint of the mite's biology and relationship with other mites. It has been organized into four major sections: 1) Honey bees, 2) Mites, 3) Mathematical ecology and 4) Objectives.

HONEY BEES

The honey bee is a pollen and nectar collecting insect that plays an important role in pollination of plants, including many domestic crops. They are also managed for production of many hive products, principally honey. Their yearly commercial value including both pollination and hive products, totals in the billions of dollars in the US. Recently, mite parasites *Acarapis woodi* (Rennie) and *Varroa jacobsoni* (Oudemans) have appeared in the US and threaten the bee industry. The first of these mites to be introduced, *A. woodi*, is the subject of this work.

History of beekeeping

Human involvement with honey bees is important from a parasite's point of view because this involvement changed bee reproduction, bee density, and parasite transmission efficiency. Early human interest in bees involved honey hunting dated by cave art to a few million years ago (Crane 1983). This type of interaction between humans and honey bees presents humans as predators of the
honey bee, with minor impact on bee reproduction or disease transmission. The earliest evidence of beekeeping comes from drawings made around 2400 B.C. in Egypt. Since that time, a variety of hive types have been used (pottery, log, basket and board). Upright hives existed in European apiaries before 1000 A.D. (Crane 1983). From 1300-1900, bees in Britain were kept in 'skeps' (special wicker baskets) arranged along walls. Often the colonies were destroyed at the time of honey harvest (Crane 1983) because there was no other way to remove honey from a fixed-comb hive. These early beekeeping practices tended to increase density of bees and to increase transmission of parasites, but not interfere with colony reproduction.

In 1851, L. L. Langstroth developed a new management technique, the movable frame (Naile 1976). Colonies could then be managed, checked for disease, prevented from swarming, forced to grow in size and maintained from year to year. Now humans had the ability to manipulate bee density, bee reproduction and, inadvertently, parasite transmission efficiency.

There were no honey bees in the western hemisphere prior to the early 17th century. Colonies of bees brought to the United States by early settlers had to come by boat and must have been either in a swarm state (nonforaging state) or in winter cluster (dormant state). The length of time needed to cross from England to the New World would have been about 3 months (Oertel 1976). The conditions and time required for the crossing must have been quite rigorous for the bees and any weak colonies probably died, leaving healthy colonies (probably mite-free) as the founding sources in the
New World. It is likely that after early imports (between 1622 and 1638), all increase in honey bee colonies in the US was due to natural swarming (Oertel 1976, 1980).

Queen importation to the US, a practice initiated in 1860, presented a possible avenue for parasite introduction (Pellett 1938). Imports were mainly from Italy and were at first essentially unsuccessful (only 1-2% of the queens survived shipment). The few queens that did survive encouraged further attempts. These finally proved successful when Charles Dadant discovered that water was unnecessary for bees in shipment. During the 1870’s he began importing about 250 queens per year from Fiorini, an Italian queen producer (Pellett 1938). Subsequently, queens were imported from other foreign countries including Egypt, Cyprus, the Holy Land, Africa and Hungary (Pellett 1938; Morse et al. 1973). All imports of bees into the United States were halted in 1922 to prevent the spread of tracheal mite parasites, that were discovered in Great Britain in 1921 (Oertel 1976, 1980).

Because shipment of bees provides an excellent avenue for movement of mite parasites (as well as other bee parasites), it may be worthwhile to expand this discussion to include information on package bees and improvements in queen shipment. The US Postal Service approved shipment of queens and their attendants through the mail in 1886 (Pellett 1938). The cage for mailing queens, still in use today, was designed by Frank Benton, and holds 1 queen and 6-8 worker attendants. Designs for cages vary, and many modern versions are made of plastic. A common model in use in the western US consists of a small hollowed-out block of wood open on one side with
a hole bored in each end (width 3cm, depth 2cm, length 8cm, holes 1cm diameter). The open side is covered with a screen. One hole is filled with queen candy (various recipes but is essentially a mixture of confectioner's sugar and water) and the other is used for the introduction of the queen and her attendants. After the bees are caged, the hole is closed. These cages can be mailed all over the world and, because of their small size, may easily be carried through customs without declaration, a practice referred to as 'pocket importation'. This practice may provide a insidious means of honey bee parasite dispersal.

The other common shipping or mailing device, the package, may not be as versatile as the queen cage in terms of smuggling, but may provide better intra-continental movement, because thousands of bees are shipped in one container. The package is a screen cage ca. 15-20 cm deep 45 cm long and 15-20 cm wide, and may accommodate contain 1-5 kg of bees (430 g = ca 4000 bees (Morse 1975). The weight of bees will vary depending on what they have eaten, their genetics, and food supply. Bees for packages are usually removed from a colony in the spring. The process is called 'shaking' because bees are shaken off frames over a funnel that directs them into a cage. The cage is then used to make up the packages. Of course, any parasite associated with these bees is shipped to the new location.

**Honey bee biology**

Honey bees are highly social insects with three types of colony members, the queen, workers, and drones. The queen is the only female reproductive of the colony. She controls colony organization and development through her behavior and pheromones. Her egg laying
ability is phenomenal; she can produce several thousands of eggs in a day during peak egg laying each season and may live several years. A majority of her eggs develop into non-reproductive females and a few into males (drones). When a colony is queenless for some time, a few workers may lay eggs, but these are haploid and develop into males.

Workers perform all the tasks needed for maintenance of the colony, with the exception of egg laying. As a worker ages she changes the tasks that she performs, a phenomenon called polyethism (Lindauer 1952; Winston 1987) The age at which a task is performed by bees in a colony also depends on the general health of the colony and external factors. Tasks of young bees that do not leave the hive include house cleaning, brood care, comb building, guarding, attendance of the queen, and food handling. At about 3 weeks of age, foraging begins. During the nectar and pollen shortages of late summer and fall, food gatherers may become 'robbers' that find weak or abandoned colonies which still have stores of honey and steal these food stores.

Like other Hymenoptera, honey bees have haplo-diploid sex determination, with males being haploid. The only function of drones is mating and, as winter approaches, any drones still within a colony are expelled so that food supplies will not be wasted sustaining them. New queens are produced predominantly in the spring, coinciding with the peak in swarming. Diploid eggs are placed in larger cells, 'queen cups' and fed a diet of royal jelly, that is produced in the worker bees's hypopharyngeal gland. The quantity of royal jelly fed determines the caste into which the larva will develop. Virgin queens require about five to six days after
emergence to complete maturation before they can mate (Winston 1987). They then take several mating flights some distance away from their colony and mate with up to 12 males. Queens can be reared in the colony anytime as long as the old queen is still laying or there are still very young larvae that the workers can rear as queens.

New queens must be produced before swarming can occur to ensure the survival of the parent colony. Several queen cups are constructed at about the same time and eggs are placed in them. Swarming occurs when the larvae in these cells pupate and approach emergence. The old queen usually leaves with the swarm but she must reduce her weight before she is capable of flight. Thus, a few days before the event, the workers force her to cease egg production and to move about over the combs (Winston 1987). When a group of bees (the swarm) issue from a parent colony, they form a cluster on a nearby object, such as a tree branch, and send out 'scout bees' to search for a new nest site. Before the queen can commence egg laying at the new site, comb must be built. The time between production of the first egg and emergence of new workers averages 21 days (Winston 1987). The time between leaving the parent colony and emergence of new workers for the swarm is 4 to 6 weeks (Burgett, personal communication). The parent colony produces a new queen that mates and returns to continue maintenance of this colony. In both the new colony and the parent colony there is an interruption in reproduction. This interruption is longer for the swarm because these bees must find a new site and construct comb, whereas the parent colony will still have emerging brood.
As the spring progresses into summer in the northern hemisphere, colonies of honey bees increase their adult worker bee population with increase in food availability. When both flower availability and colony size peak, the colony stores nectar and pollen in excess of immediate needs. This excess is required during the winter when bees are confined to the hive. These peaks in summer nectar collection are called the 'honey flow'.

When a forager returns from food gathering, she may become confused and enter another hive. This occurrence is referred to as 'drift' when the disoriented bee is accepted by the colony of unrelated bees. Bees on orientation or defecation flights may also drift. The placement of hives in close proximity in an apiary greatly influences the amount of drift that occurs (Jay 1965, 1966, 1968 and 1985). Colonies in hives similar in appearance and placed close together in rows have much higher drift incidence than colonies with hives that differ in appearance, with entrances facing different directions and with random placement. Drift in feral colonies is probably very low or nonexistent (Jay and Warr 1984; Free 1958; Bailey 1958b). Drift may be a primary means of intercolony mite transfer.
**Honey bee immune response**

Hemolymph feeding parasites may initiate a host immune response. Little is known about the physiology of repair and microbial defense in honey bees. A general review of the subject of arthropod immunity may be found in Gupta (1986). Wound responses include a series of reactions related to plugging and repairing of damage; these reactions are also related to micro- and macroparasite defense. A number of systems are involved, including melanization, agglutination, coagulation and encapsulation. Antimicrobial molecules (cecropin, attacins and lysozymes) may also play a role (Gupta 1986).

The melanization processes, which involve a prophenoloxidase cascade, have been studied in the lepidopteran genera *Heliothis*, *Galleria*, *Trichoplusia* and *Bombyx* (Pye 1974; Stoltz and Cook 1983; Sorka and Vinson 1978; Ashida 1981; Ashida et al. 1983), and the dipteran genera *Calliphora* and *Musca* (Thomson and Sin 1970; Yamaura et al. 1980). Melanization has been shown to be inhibited by some parasitoids (Beckage et al. 1987; Stoltz and Cook 1983). Some studies indicate the process of coagulation involving mucopolysaccharides is linked with melanization (Ratcliffe 1975). However, other experiments with trypsin, neuraminidase and hyaluronidase suggest that proteins rather than mucopolysaccharides are involved in encapsulation (Ratner and Vinson 1983). Histochemical studies of *Chironomus* humoral encapsulation also suggest protein involvement (Goetz and Vey 1974). Honey bees are reported to have little coagulation or none (Gilliam and Shimanuki 1970; Gregoire and Goffinet 1979). The honey bee system was compared with the human
system (Gilliam and Shimanuki 1970) which provided information as to how these systems compare, but did not explain the coagulation mechanism in bees.

Arthropod coagulation may involve agglutination (Yeaton 1983; Gupta 1979, 1985, 1986; Komano, Mizuno and Natori 1979), but our knowledge of agglutination is limited. Agglutination in honey bees has been related to invasion by disease organisms (Gilliam and Jeter 1970), and the characterization of agglutinins of a few insects have been made (Suzuki and Natori 1983; Peferoen and de Loof 1982; Stebbins and Hapner 1985). Wounding Cecropia did not increase agglutinin titer (Yeaton 1983), but wounding in Sarcophaga peregrina larvae did result in a titer increase (Komano, Mizuno, and Natori 1980).

Insect encapsulation may involve cellular or noncellular responses (Goetz and Vey 1974, 1986; Rowley and Ratcliffe 1981; Ratner and Vinson 1983). Low blood cell counts and humoral encapsulation appear to be correlated, but none of the insects tested was a hymenopteran (Goetz, Roettgen, and Lingg 1977).
MITES

Mites as a group have diverged broadly from most other chelicerates and may be found in a great variety of habitats. Their small size has allowed invasion of niches that exclude larger organisms. Mites are wingless, but many are phoretic, which allows them to take advantage of the wings of other animals. Their associations with other organisms include many relationships with insects.

Honey bee colonies are rich islands of food and offer ideal habitats for other organisms, including mites, although the housekeeping and grooming habits of honey bees preclude many potential guests. Since many honey bee colonies survive from year to year, those guests that are tolerated or undetected are not required to find new resources as are associates of other insects (bumble bees for example).

Parasitic Acari

Parasitic mites are found in all but one suborder of the Acari (the Oribatida) and occur on a wide variety of hosts (Krantz 1978) including honey bees. Known bee parasites are members of the superfamilies Tarsonemoidea and Dermanyssoidea. Honey bee parasites of the genus Acarapis are representatives of the former group, while the superfamily Dermanyssoidea includes the honey bee mite genera Varroa and Euevarroa (family Varroidae) and Tropilaelaps (family Laelapidae), brood parasites of Asiatic and tropical honey bees.

The tarsonemoid family Podapolipidae, diminutive parasites of a variety of insects, is considered the sister group to the family Tarsonemidae (Lindquist 1986). Locustacarus buchneri (Podapolipidae)
is found in tracheae and abdominal air sacs of Bombinae (Husband 1969). Little is known of their biology or their relationship with their bumble bee host.

The tarsonemid subfamily Acarapinae contains the coreid bug associates of the genera Coreiotarsonemus, Asiocortarsonemus and Amcortarsonemus (tribe Coreitarsonemini), along with the genus Acarapis (tribe Acarapini). All of the Coreitarsonemini occur in the metathoracic odiferous glands of coreid bugs, and their cheliceral characters and habitat (all life stages occurring within these glands) suggest that they are parasites (Fain 1970, 1971, 1980a, 1980b; Lindquist 1986).

Not all the mites associated with honey bees are parasites (de Jong et al. 1982). Thirty seven species of mites have been found associated with honey bees in Czechoslovakia and 44 species in Europe and Asia (Haragsim et al. 1978). In most cases the association between the mite and the bee is not understood. Many appear to be phoretic, depending on bees for transportation from flower to flower.

Species related to Acarapis woodi

Four species have been described in the genus Acarapis: A. woodi (Rennie), A. dorsalis Morgenthaler, A. externus Morgenthaler and A. vagans Schneider (Delfinado-Baker and Baker 1982; Schneider 1941; Morgenthaler 1934). These species are similar morphologically and have been confused with each other (Morgenthaler 1964; Foote 1959; Chaneet et al. 1984; Kjer et al. 1989a, b). The species description of A. vagans (Schneider 1941) gives neither measurements nor illustrations and does not designate type specimens, making it difficult to identify. Delfinado-Baker and Baker (1982) consequently
have designated *A. vagans* as a nomen dubium. Species identification in *Acarapis* is often based on their location on the bee; i.e. the dorsoscutellar groove for *A. dorsalis*, venter of the neck for *A. externus*, prothoracic trachea for *A. woodi* and the base of the wings for *vagans* (Schneider 1941; Delfinado-Baker and Baker 1982). We know now that all three defined species of *Acarapis* migrate across the wing base to their respective sites on the bee and may all reproduce in this area (Royce *et al.* 1988, Ibay 1989).

**Biology of *Acarapis woodi***

Detecting tracheal mites is difficult because outward symptoms of both individual workers and colonies are unreliable, and the mites cannot be seen without dissection and microscopic examination. Some researchers recommend cutting sections of the bee thorax to include the main trunks of the prothoracic tracheae and clearing these sections in KOH for later observation under magnification (Shimanuki and Cantwell 1978; Delfinado-Baker 1984). Others call for pinning the bee and cutting or removing bits of its integument to reveal the tracheae (Lorenzen and Gary 1986; Smith, Needham and Page 1987). Both of these techniques are tedious and labor intensive. A serological technique has been proposed but is not yet feasible commercially (Fergala and Ragsdale 1987; Ragsdale and Kjer 1989).

*Acarapis woodi* lives and reproduces in the prothoracic tracheae of adult honey bees. The duration of its life cycle from egg to adult is 11-15 days (Morgenthaler 1931; Bailey 1963; Royce *et al.* 1988). An *in vitro* rearing technique involving the use of exposed bee prepupae as a substrate for the mites has been moderately successful (Giordani 1967). Because honey bee prepupae are more
vulnerable to microorganisms and desiccation outside their cell, mites had to be transferred often to fresh prepupae; this technique is labor intensive and limited in usefulness. The in vitro rearing demonstrated that adult mites can live as long as their adult worker bee host (up to approximately 40 days). Males lived slightly longer than females but the data were not statistically treated. Developmental time was 10-12 days for males and 12-14 days for females under these conditions. These data are comparable to estimates from studies of marked bees in colonies (Morgenthaler 1931; Royce et al. 1988). It is of interest that the temperature (28°C) at which these in vitro life cycle studies were conducted was lower than temperatures expected in bees that are incubating brood (34°C). Giordani (1967) noted difficulty with mite molting and egg oviposition but no data were given regarding fecundity. Females were seen to use a glue to adhere their eggs to the substrate. Developmental times for individual stages were determined to be: 4 days (96 hours) for egg, 4 days (96 hours) for larva and 3 days (72 hours) for nymph. These times were estimated by the appearance of the above mite stages on bees of known ages (marked bees) retrieved from infested colonies (Morgenthaler 1931; Royce et al. 1988).

Female mites probably mate soon after emergence within the trachea and then migrate to a new host bee. New infestations are highest among recently emerged bees (Lee 1963; Morgenthaler 1930, 1931). Male mites wait in the vicinity of a female nymph and mate immediately after her emergence (Sachs 1938). Many tarsonemid males have a modified fourth pair of legs with which they carry the female
nymph until she molts, a behavior not unusual for this group (Lindquist 1986a).

More adult females than males are reported in studies on Acarapis biology and behavior (Otis et al. 1988; Burgett et al. 1989; Morrison 1931) and this discrepancy has led some researchers to suggest that A. woodi is arrhenotokous. Species in which the female disperses phoretically often display a higher proportion of females, as well as a tendency for parthenogenesis (Mitchell 1970). Examples include Iponemus and Pyemotes (Tarsonemidae and Pymotidae) both parasitoids of insects. Their female: male ratios, 10:1 and 20:1 respectively, may optimize dispersal of adult females and efficiency for discovery and utilization of their limited resources (Lindquist 1986a; 1969a, b; Lindquist and Bedard 1961).

Sex ratios determined from general population samples are influenced by behavior and life history; e.g. females migratory but males not, shorter life span for males than females, and males copulating mainly with newly emerged females before dispersal. The primary sex ratio is difficult to determine and the ratios reported vary because sampling methodologies vary. The distinctive male and female characters expressed in the larval stage of most tarsonemids are absent in Acarapis, and cannot be used to determine the primary sex ratio (Lindquist 1988). Studies of Acarapis cytology, and development of in vitro rearing techniques might be helpful in resolving this problem.

Shortly after the discovery of A. woodi, uncertainty arose concerning its pathological effects (Morgenthaler 1929). In areas where beekeeping practices were primitive, the cause of colony losses
could not be determined because colonies could not be examined. High mite infestations may have resulted in colony mortality but death resulting from other causes could not always be ruled out (Morgenthaler 1931). A shortened lifespan of infested honey bees has been reported from Italy on the basis of a study of marked bees held in an incubator (Giordani 1965, 1977). Marked bees placed in colonies in October and recovered in March had fewer infested bees than the hive at the time of introduction (Bailey 1958). This was reported as an indication that infested bees died sooner.

The Langstroth movable frame hive, invented in the United States in 1851 (Naile 1976), was incorporated into British beekeeping at the turn of the century. This radical change in management practices may have resulted in colony stress and possible loss (Bailey 1964). Based on this possibility, Bailey suggested that A. woodi may not have been the primary cause of colony demise seen on the Isle of Wight (Bailey 1958a, 1961, 1964; Bailey and Lee 1959).

The seasonal pattern of population growth and decline of the tracheal mite appears to vary from area to area. Large and frequent fluctuations are reported from individual sites over time. A minimum mite population in March increasing during summer and peaking in autumn has been reported to occur in Switzerland (Morgenthaler 1929, 1930, 1932). On the other hand, low infestation coinciding with midsummer honey flow has been reported from Britain (Morrison et al. 1956; Bailey 1958a). Mite populations of honey bee colonies in areas of poor forage or during years of poor forage in Britain are reported to continue increasing during summer (Bailey 1958a). Population fluctuation from year to year and even from one month to another are
commonly thought to be influenced by external physical factors, but may actually be controlled in large part by intrinsic factors (strength and activity of the colony) (Giordani 1977). Tracheal mite populations have been reported to increase in winter (Otis et al. 1988).

_A. externus_ and _A. dorsalis_, the externally occurring species of _Acarapis_, are reported to decrease in March, late summer and winter in California (Eckert 1961). The highest infestations of _A. dorsalis_ in Oregon were reported from March to June and August and September with the lowest infestation rates in January and July (Ibay 1989). For _A. externus_, mite population was highest in the fall (October and November) and the lowest infestation was recorded in July (Ibay 1989). In New Zealand, reports suggest a decline in population of the external _Acarapis_ species in winter and summer (Clinch 1975). In all of the above studies of these three species of mites, little attention was paid to the host population. Host population fluctuation has never been directly related to the increase or decrease in mite population. Winter studies demonstrate that, in colonies with high infestation levels of _A. woodi_, there are lower bee populations and lower stores at the end of winter (Eischen 1987). Colonies that resulted from 'splits', a practice used to increase colony numbers but that interrupts queen egg laying, had lower infestation levels than the original colony (Eischen, 1988). These two studies, both of which were conducted in Mexico, suggest a negative relationship between mite and bee populations.

Floral bloom (i.e. honey flow) has been correlated with mite populations, but other factors such as temperature and humidity have
not been considered. Variations were common in sampling procedures. Differences such as sample size, location of bees (i.e. off brood comb, from honey comb, from returning foragers), number of colonies per apiary, and frequency of sampling affect reported incidence of mites in an area, and frequency of mites from individual colonies. Differences in colony strength and how data are handled (i.e. pooled data from several colonies, randomness of colony sampling) also may affect population estimates. Many reports give no specific description of sampling methods.

The report that depressed mite population results from foraging during honey flow (Bailey 1958a) was based on percentage of infested bees in a sample, not on actual population estimates and was never followed up experimentally. The statement that bees usually forage at 9 days and perhaps as early as 2-3 days (Bailey 1958a) appears erroneous. The mean age at which bees begin to forage is around 20 days with a range of 9-65 days (Lindauer 1952; Winston 1987; Harrison 1986). This misunderstanding lent support to the idea that transmission would be lower during honey flow. The age at which a bee would have the most migrating mites is about 12-15 days. If these bees were foraging the migrating mites would contact fewer appropriate hosts.

In North America the tracheal mite was first reported from Mexico was in 1980 (Wilson and Nunamaker, 1982) and from the United States in 1984 (Delfinado-Baker 1984), where it aroused controversy similar to that seen in Great Britain concerning its pathology. Using honey production as a measure of pathology, Robinson et al. (1986) could find no difference between infested and uninfested
colonies in Florida. However, honey production may be an unreliable measure of pathology because data for honey production in the USA have not been estimated by a standard method. Furthermore, short-term trends vary widely from year to year and colony to colony (Page et al. 1987). Another Florida study apparently shows that there is little difference between activity of infested foragers compared to uninfested foragers from the same colony (Gary and Page 1989). A colony's ability to maintain productivity, even with large worker loss (Winston and Fergusson 1985) also confuses the issue. Studies measuring productivity over more than one season would probably produce more reliable results.

MATHEMATICAL ECOLOGY

Parasitism as an ecological relationship between the populations of two different species of organisms has some special properties: namely the infection process produces an over-distribution of parasites within the host population, the parasite kills heavily infected hosts, and the parasite has a higher reproductive potential than the host species (Crofton 1971a,b).

On this basis, the technique of truncation of the negative binomial distribution can be understood; that is, hosts with low parasite loads may be expected to have survival rates nearly equal to hosts with no parasites. Parasite-induced host mortality may be demonstrated by extrapolating the negative binomial from the zero and low parasite load classes and comparing this extrapolation with the original distribution. If there is an increase in number of hosts in high parasite load classes of the extrapolated distribution compared with high parasite load classes of the original distribution, then
parasite-induced host mortality has occurred in the high mite load classes (Crofton 1971a). If there is no parasite-induced host mortality, then the truncation of the negative binomial distribution will essentially be the same as the original distribution. This interpretation has been used for Arrenurus pseudotenuicollis and Hydrachna conjecta (Acari: Hydracnellae) water mite parasites of Anopheles crucians (Diptera: Culicidae) and Sigara ornata (Hemiptera: Corixidae) respectively. Laboratory experiments corroborated the benign nature of H. conjecta (Lanciani and Boyett 1980).

The technique, however, may fail if parasitism results in a death rate with a linear pattern. Then the resulting distribution will not differ from a negative binomial distribution and mortality due to the parasite can not be estimated by truncation of the distribution (Lanciani and Boyett 1980). This situation is not biologically likely but cannot be ruled out.

Host-parasite relationships have been modeled for many vertebrate-parasite systems and such models have been found useful in understanding population dynamics of 'two species' interactions (Anderson and May 1979; Briscoe, 1980). Important factors for these models are vertebrate immunity, population growth characteristics and the transmission characteristics of the parasite (thresholds, horizontal, vertical). Because of their importance in biological control, parasitoids and their hosts also have been modeled (Anderson and May 1981; May and Hassell 1988; Munster-Swendsen 1985) in order to better understand population interactions and to formulate more prudent release programs. This type of approach has not been implemented for studies on population dynamics of mite parasites of
honey bees. Honey bees are highly social insects, and models of bee-parasite populations differ from vertebrate-parasite models in some important aspects. For example, the bee’s immune system differs from that of vertebrates, and any model of bee-parasite populations must take this difference into account. Sociality of honey bees and the impact of sociality on reproductive rate also are important considerations in modeling such a system.

Honey bees present a parasite with what may be considered to be two hosts, namely the colony and the individual bee. These two hosts have different growth parameters. Colonies reproduce in a fundamentally Malthusian fashion, whereas the population of individuals within the colony grows linearly.
OBJECTIVES

The tracheal mite of honey bees is a recent introduction to North America. Its impact on the beekeeping industry of the United States has not been fully determined. I undertook this study to examine the interaction of tracheal mites and honey bees in the Pacific Northwest with the hope that it would lead to the establishment of a threshold of pathology by the mites on the bees, and eventually lead to a definition of economic threshold for this parasite.

There have been a number of colony level studies of tracheal mite impact in Europe, Mexico, Florida and midwestern US, but few studies on the impact on individual bees. Therefore, the specific objectives of this project were:

A. to determine if mortality could be measured for individual bees in summer;
B. to explain the mechanisms of stabilization of the host-parasite populations using models of populations of honey bee colonies and of individuals within a colony;
C. to determine if host to host transmission is decreased by bee foraging, using nocturnal and diurnal transmission rates as a nonforaging and foraging period respectively;
D. To determine the tracheal mite sex ratio from bees of different ages and from different times of year and relate these ratios to the primary mite sex ratio, for a better understanding of mite behavior and mode of reproduction;
E. to establish mite feeding rates and amount of fluid uptake and
determine if mechanical damage of mite feeding could affect the
individual bee host;
F. to determine if proteins in hemolymph and tissue of infested bees
were quantitatively or qualitatively different from those of
uninfested bees;
G. to determine if phenoloxidase activity was inhibited by mite
feeding, and if agglutination titers for infested bees increased
compared with uninfested bees;
H. to determine if mite infested bees fed more than uninfested bees;
I. to determine if death rates of infested members were greater than
uninfested members of infested colony and if overall death rate for
an infested colony was greater than a uninfested colony.
These objectives were largely met and the results are presented in
the following manuscripts and drafts.
CHAPTER 2

HONEY BEE MORTALITY DUE TO TRACHEAL MITE PARASITISM

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Parasitology (1989)
ABSTRACT

We demonstrate, by truncating the expected negative binomial distribution, that the tracheal mite parasite, *Acarapis woodi*, causes mortality in the European honey bee, *Apis mellifera*, but that this mortality can be documented only during periods of low mite densities (mites per bee). At high mite densities, this technique no longer reveals mite-induced mortality. We suggest that this paradox results from a reduction in the mortality threshold at high mite densities, concealing mortality from our truncation procedures. Longitudinal comparisons of hive and forager bee cohorts, nevertheless, suggest mortality at all levels of infestation. We propose that persistent parasitism overcomes a colony's ability to compensate for losses, leading to its sudden decline and death.

INTRODUCTION

In 1919, British researchers, while investigating dramatic honey bee (*Apis mellifera* L.) colony losses occurring on the Isle of Wight, discovered a new species of bee parasite, the tracheal mite *Acarapis (Tarsonemus) woodi*, infesting afflicted colonies (Rennie 1921). Possibly because of its recent discovery, they suggested that the mite was the etiological agent responsible for the epizootic.

Ever since that report, however, the pathological effects of the mite have been clouded in controversy. Morgenthaler (1929) discussed uncertainty concerning pathological effects of *A. woodi*, but suggested that high infestations eventually do cause colony death (Morgenthaler 1931). Giordani (1965, 1977) corroborated this conclusion and reported a shortened lifespan in infested honey bees and a loss of infested colonies. Subsequent workers, however, noted
the coincidence of the disease with the introduction of new apicultural management techniques and suggested that these could account for losses experienced on the Isle of Wight (Bailey 1958, 1961, 1964; Bailey & Lee 1959). The techniques were the Langstroth movable frame hive, invented in the United States and being incorporated into British beekeeping at the turn of the century. Therefore, *A. woodi* may not have been the primary cause of colony losses seen on the Isle of Wight.

The mite was subsequently reported from Mexico in 1980 (Wilson & Nunamaker 1982) and in the United States in 1984 where its pathology aroused controversy similar to that seen in the Old World. In Florida, for example, no difference in honey production could be measured between infested and uninfested colonies (Robinson, Cromroy, Thel & Carpenter 1986), while in the mountains of northeastern Mexico, infested colonies were demonstrated to have significantly lower bee populations and fewer stores than uninfested colonies (Eischen 1988).

An important paradox exists in the literature concerning the economic and biological impacts of *A. woodi*. Parasitism is, almost by definition, associated with morbidity and mortality of the host, and one would expect that hemolymph-feeding mites would cause measurable loss to its afflicted host. The objective of this work is to determine whether or not tracheal mites cause mortality within a hive, using Crofton's method of determining thresholds of mortality by truncation of the negative binomial distribution. Because mortality appeared to decrease with increasing parasite burden, we also queried if mortality patterns may not change with increasing
stress. We compared these patterns between hive and forager bees, tasks with different stress levels. We propose that the threshold of mortality decreases with increasing demands on both hive and individual bees, and we estimate the total mortality burden of parasitemia in a hive.

MATERIALS AND METHODS

The colony used for this study was maintained in Deep and Western movable frame hive bodies (42 and 26 liter Langstroth hive with 9 movable frames) and managed to prevent swarming. Samples of worker bees returning from foraging were taken with a sweep net at the hive entrance after it had been temporarily blocked. Hive bees were collected from within the colony on brood comb. These samples were taken weekly from April through September. Bees were frozen and subsequently dissected to determine mite infestation. Infested tracheae were removed and opened, and all of the mites were counted. Female mites were recorded as either reproducing or newly emerged.

The expected negative binomial distribution was based on the reproducing females mites. In foraging bees, adult female mites had either died or migrated in about 10% of the bees; of this last, the number of reproducing females, ranging from 1 to 3, was conservatively estimated from the amount of debris, number of mites remaining in the trachea and the amount of tracheal discoloration.

In brief, the equation for \( P \), the frequency of hosts with \( x \) parasites, is \( P(x) = P(x-1) \frac{(k+x-1/x)}{R} \), where \( P(0) = (1-R)^k \) or \( R = Y/(Y+k) \), \( Y \) being the mean number of parasites and \( k \) the aggregation coefficient of the negative binomial distribution (Pichon, Prod'hon and Rivière 1980).
We first initialized, with the estimate \( k = \frac{y^2}{s^2} - Y \), where \( s^2 \) is the variance, a BASIC program to calculate the maximum likelihood value of \( k \) using the iteration
\[
z = d\left(\frac{A_x}{k_x + x}\right) - N \ln(1 + x/k_x), \text{ until } z = 0,
\]
where \( A_x = A_{x-1} - N_x \), where \( A_0 = N - N_0 \), \( N_x \) is the number of hosts with \( x \) parasites and \( N \) the total number of hosts (Bliss 1953).

The truncation technique involves estimating an overall expected distribution by deriving it from the zero and low frequency classes, where lethal effects are unlikely to be significant (Crofton 1971); truncation of the negative binomial is also an iterative BASIC program using the above formulae. Truncation of the negative binomial distribution is not to be confused with the 'truncated negative binomial', which is its estimation following rejection of the zero class. Programs are available upon request from L.A.R.

Chi-square values were calculated and summed for the first four mite load classes (\( C \) value) and were used as an indication of improved fit of the classes to the negative binomial after truncation (Lanciani & Boyett 1980). Density is defined throughout this paper as mites per bee.

RESULTS

First, we investigated whether or not host mortality, if it occurs, varies with parasite load. Knowing that parasite density in Oregon increases from spring to late summer (Royce pers. obs.), we measured and compared parasite load in worker bees, both foragers and hive bees, for this period. From truncation of the negative binomial, we calculated that, in spring, there were approximately 0.5 to 1.5% fewer bees in the observed host cohort than predicted from
the truncation procedure (Table 2.1). In summer, we noted a
difference of approximately 3% bees (Table 2.2). In late summer,
however, there was essentially no difference between observed host
cohort and expected numbers (Table 2.3). Parasites appear,
paradoxically, to cause more mortality at low densities.

The technique of truncation of the negative binomial
distribution relies on the presence of an exponential effect of the
parasite on the host, manifested as a 'threshold' (Crofton 1971;
Lanciani & Boyett 1980). Were there to be no apparent threshold
(i.e. as when mortality is linearly rather than exponentially
cumulative), then the observed distribution would not differ in
general from a negative binomial distribution. Although it would be
different from the initial, pre-mortality distribution, this last
distribution could not be calculated (Lanciani & Boyett 1980). It
may be that high parasitemia stresses a colony to the point where it
cannot compensate for losses, and mortality begins to afflict even
hosts with low parasite load, in essence lowering the mortality
threshold.

To determine if stress could cause such a threshold to change,
we compared mortality of two colony cohorts with different stress
levels: hive bees and foragers. Hive bees, newly emerged to about 3
weeks of age, are confined to the hive, performing tasks such as
cleaning, caring for brood, and comb building, activities that might
be considered relatively stress free. In contrast, foragers must
suffer the fortunes of weather and natural enemies, and are
considered to be more stressed than hive bees (Sakagami & Fukuda
1968; Wilson 1971).
Upon comparing these castes in the previous data, we could not detect as great a difference between observed and expected distributions for foragers as we did for hive bees (forager sample: mite density = 0.74, \( k = 0.74 \) and \( P \), the probability of fitting the negative binomial, is \( 0.8 > P > 0.5 \); hive bee sample: mite density = 1.1, \( k = 0.68 \) and \( 0.5 > P > 0.2 \)). These data suggest a 4% loss in hive bees, but negligible in foragers. Mite density, here, is based on the number of gravid females that are present in their host from the time of infestation (newly emerging bees) until the host dies; of course, the density of all mites stages and sexes will be higher. This pattern was consistent for foragers collected at any time during the flowering period. Thus, as in the previous analysis, mortality appears to be least where it should intuitively be most.

Hive bees are the precursors of foragers. Knowing that mites invade hive bees rather than foragers (Morgenthaler 1931; Lee 1963), a difference between the distributions of mites in equal sized cohorts of both bee castes would indicate that the above paradox is an artifact of the truncation technique. Consequently, we derived the parameters of the expected negative binomial distribution for both cohorts, and, after adjusting cohort sizes so that they were equal, we compared the distributions of mites (Table 2. 4 and 2. 5). We calculated that, although the adjusted samples are equal in size, the distributions of mites in hive bees and foraging bees are distinct (Fig. 2. 1). The forager population was, conservatively, 36% less than the estimated pre-mortality hive bee population as a consequence of tracheal mite parasitism. Mites appear to cause
mortality in both cohorts but at different thresholds, that of foragers being essentially undetectable by truncation.

**DISCUSSION**

We demonstrate that mortality due to the tracheal mite does occur in a honey bee colony. As mite densities increase, however, the technique of truncation of the negative binomial distribution indicates a decrease in mite-related bee mortality. The simplest explanation for this unusual situation is that, at high mite densities, the presumed mortality threshold is reduced, making the technique of truncation unsuitable. We obviated this difficulty by subdividing our worker bee samples into hive and forager bees. From this, we calculated a pre-mortality distribution and demonstrated that mite-induced mortality does indeed occur in foragers and that it is, as expected, greater than mortality in hive bees.

We examined more than 2,000 bees, dissected 1,170 tracheae and counted 16,705 mites (of which 4,189 were females and of these 1,322 were reproductive females) from a single hive. We are confident that our data and techniques give an accurate picture of this hive and feel that our interpretation is not inconsistent with Crofton's concepts of parasitism on the one hand, and with commercial beekeepers' perception of the reality of mite pathology, on the other. Further studies will determine the general validity of our interpretation.

We base our calculations of the negative binomial distribution on reproductive female mites, the invasive stage, and propose that thresholds vary with stress. Mite reproduction may emphasize this difference. After mite invasion, population levels within a trachea
increase, with the largest population of mites occurring between two to three weeks after infestation (Royce et al. 1988), just before or at about the time a bee becomes a forager. In our study colony 30 to 40 mites were often found in a single infested trachea, counting all mite life stages (Royce pers. observ.) and up to 100 have been reputed. The change in the mortality threshold seen in the forager cohort may be amplified by mite reproduction, in addition to other stresses.

Honey bee colonies possess resiliency in that they can compensate for sudden losses in their workforce by recruiting previously ‘unproductive’ members (Winston and Fergusson 1985). This compensation leaves us to wonder whether or not worker losses due to tracheal mites are ever deleterious to colony output. We suggest that this mechanism may be ineffective against tracheal mites because worker losses are continuous and gradually erode the colony’s reserve workforce. In the early stages of an infestation or during honey flow, losses may be undetectable if measured by colony output, but once a critical stage or density is reached, worker losses would become rapidly evident in terms of output. This explanation may resolve the paradoxical conclusions contained in early reports on the Isle of Wight epizootic.

Our study quantifies, for the first time, substantial Acarapis woodi-induced mortality of honey bees. We demonstrate that worker bee mortality occurs at all levels of infestation and increases with density. If we can eventually estimate the critical mite density which leads to losses in colony output, management of tracheal mites may be feasible.
Fig. 2. 1. Comparison of the expected curves of the negative binomial distribution for the initial pre-mortality distribution (triangle) of hive bees, for the observed distribution (circle) among hive bees, and the adjusted distribution (square) of foragers.
Table 2.1. Observed and expected negative binomial distribution of *A. woodi* in forager and hive bees collected in spring 1988 (April and May), including truncation of this distribution at mite load 2, 3, and 4.

<table>
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<th>Observed</th>
<th>Expected</th>
<th>Truncation</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>4 3 2</td>
</tr>
<tr>
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<td>483</td>
<td>482.0</td>
<td>483.3</td>
<td>485.6 479.1</td>
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<tr>
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<td>94</td>
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<tr>
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<td>42.3 44.8</td>
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<td>19.9</td>
<td>21.4</td>
<td>23.2 24.2</td>
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<td>10.2</td>
<td>11.8</td>
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<tr>
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<td>8</td>
<td>5.4</td>
<td>6.8</td>
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<tr>
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<td>2</td>
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<td>4.0</td>
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</tr>
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<td>1</td>
<td>1.6</td>
<td>2.4</td>
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<tr>
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<td>1.4</td>
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<td>669.7</td>
<td>677.0 679.8</td>
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<tr>
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Table 2.2. The observed and expected negative binomial distribution of *A. woodi* among forager and hive bees collected in summer 1988 (June and July), including truncation of this distribution at mite load 2, 3, and 4.

<table>
<thead>
<tr>
<th>Mite load</th>
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<th>Expected</th>
<th>Truncation</th>
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Table 2.3. Observed and expected negative binomial distribution of *A. woodi* in foragers and hive bees, late summer 1988 (August and September), including truncation of this distribution at mite load 2, 3, and 4.

<table>
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<td>1.421</td>
<td>1.5</td>
<td>1.5</td>
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<td>0.210</td>
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Table 2.4. The observed and expected negative binomial distribution of *A. woodi* among worker bees collected from the hive in 1988.

Including truncation of this distribution at mite load 2.

<table>
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<tr>
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<td>mean</td>
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<td>1.40</td>
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</tbody>
</table>
Table 2.5. Observed and expected negative binomial distribution of *A. woodi* among worker bees collected as they returned from foraging to their hive in 1988, and the expected distribution of the observed, adjusted to equal the number of bees in the hive bee sample (Table 5).

<table>
<thead>
<tr>
<th>Mite load</th>
<th>Observed</th>
<th>Expected</th>
<th>Adjusted</th>
</tr>
</thead>
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<tr>
<td>0</td>
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<td>1.1</td>
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<tr>
<td>mean</td>
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</tbody>
</table>
CHAPTER 3

MODEL OF TRACHEAL MITE PARASITISM OF HONEY BEES

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Journal of Applied Ecology (submitted)
ABSTRACT

The parasitic tracheal mite, *Acarapis woodi* (Rennie), inhabits the thoracic tracheae of the European honeybee, *Apis mellifera* L. Understanding regulation of this host-parasite relationship presents particular problems because the mite requires two host populations for transmission, namely, a population of colonies, which displays Malthusian growth parameters, and, within a colony, a population of worker bees, which grows linearly. We propose a model in which swarming and colony distribution act as mechanisms to provide oscillatory stability to the parasite population. This model implicates modern techniques of hive management in the sudden historical appearance of the mite on the Isle of Wight.

INTRODUCTION

The tracheal mite parasite, *Acarapis woodi*, lives and reproduces in the prothoracic tracheae of the European honey bee, *Apis mellifera*, where it may have a detrimental impact on the individual host and, ultimately, on the colony (Eischen 1987; Royce and Rossignol 1989). Early this century, the mite was discovered on the Isle of Wight and purported to be the etiological agent of a disease that was seriously affecting apiculture there (Rennie 1921); the mite has since appeared in continental Europe and North America. The duration of its life cycle from egg to egg is 20 days (Bailey 1963; Royce et al. 1988), close to the average bee's life expectancy of 30 days during the peak flowering period (Wilson 1971) in temperate regions. Newly emerged adult female mites probably mate within the trachea (Sachs 1958) and then migrate from the bee of their birth to a new host bee wherein they begin to reproduce.
Because of this relatively long life cycle and the observation that infestations are highest among recently emerged bees (Lee 1963; Gary and Page 1989), it appears that young bees are crucial for a new infestation, older bees having too short a life expectancy for completion of the mite's life cycle.

Conditions required for stable parasitism on a eusocial insect host are difficult to derive from standard host-parasite paradigms because transmission must occur not only between different colonies but also between individuals of a particular colony. On the one hand, the population of host colonies theoretically displays Malthusian growth as does the parasite, while on the other hand, the number of individual hosts within a colony grows linearly because there is but a single reproductive individual, namely, the queen. A parasite must somehow keep up with the growth characteristics of the population of colonies so as not to fall below its own critical density, and yet not overwhelm the population within a colony. Such a parasite must, in essence, cope with two types of hosts, the colony and the individual bee, each with drastically different growth profiles.

To resolve this dilemma, we propose that swarming regulates parasite prevalence within a colony and that colony distribution limits transmission between colonies. Swarming is particularly crucial to stability. Before a swarm becomes a successful colony it must find a nest site, build combs, lay eggs; even the parent colony will not have young bees for several weeks (Winston 1980, 1987). Consequently, the loss of a suitably long lived cohort of hosts might reduce mite population following swarming. A depressed level of
parasites would allow young colonies to grow initially more rapidly than the parasite; when parasite growth approaches threatening levels, swarming occurs again or the annual cycle of temperate colonies results in their becoming broodless for winter. This interaction provides a stable, albeit oscillatory, regulation of parasites. We also explore transmission between colonies and compare the relative importance of different modes of transmission. Based on these assumptions, we are led to suggest that the sudden and dramatic appearance of tracheal mites on the Isle of Wight is linked to coincidental changes in apicultural practices that inhibited swarming and disturbed natural regulatory conditions. Finally, we present preliminary data to support assumptions of the model.

MATERIALS AND METHODS

Two colonies were maintained in western and deep hive bodies (26 and 41 liters respectively) and managed to prevent swarming. Every fortnight from the first of April until 12 October of 1989, the bee population was estimated by the method of Burgett and Burikam (1985). After Oct. 12 cold weather limited examination of every frame within the hives. Brood was seen as late as Nov. 8 when a warm day permitted a detailed examination of colonies.

Colony no. 1 was requeened May 1. In colony no. 2, requeening failed June 5, supersedure was allowed and a new queen started egglaying on June 30. These events provided two contrasting population structures. Worker bees were sampled for mites weekly throughout spring and summer and frozen for later dissection., Tracheae were removed from bees and placed in glass slides where they were opened and all mite stages counted.
RESULTS

Symbols used in these analyses

A  rate of entry of colonies through swarming
X  population of uninfested colonies
Y  population of infested colonies
a  death rate of colonies due to tracheal mites
b  death rate due to natural causes
a  rate of swarming
f  proportion of births from infested colonies which will be infested
B  rate of drifting resulting in new infection
r(c) intrinsic rate of natural increase of bee colony
r(m) intrinsic rate of natural increase of mite
N  total population of colonies
N* population of colonies at equilibrium; N*=A/b in absence of mites
t  time
N(b)_t population of individuals in a colony at time t
N(m)_t population of mites in a colony at time t
x  rate of worker bee production by queen bee
e  base of natural logarithm

Colony to colony transmission

Transmission between colonies probably occurs through drifting, which is the accidental entry of a bee into the wrong hive and is equivalent to horizontal transmission, or through swarming, equivalent to vertical transmission (see Fig. 3. 4a). If we consider colonies as the reproductive unit, then based on Anderson and May
(1979), the equations for the change in populations of infested colonies and populations of uninfested colonies are:

\[
\frac{dX}{dt} = A + a(1-f)Y - BXY - bX \tag{1}
\]

\[
\frac{dY}{dt} = BXY - a(1-f)Y - aY - bY - aY \tag{2}
\]

the sum of which is:

\[
\frac{dN}{dt} = A - bN - aY \tag{3}
\]

The growth characteristic of the population, assuming no immunity, will be:

\[
r(c) - a \tag{4}
\]

there will be a threshold, or critical density, of colonies for successful introduction of mites, given by:

\[
\frac{(a+b-fa)}{B} \tag{5}
\]

for both vertical (through swarming) and horizontal (through drifting) transmission. Now, if \( a > r(c) \), then the host population will be regulated by the parasite at:

\[
N^* = \frac{a+b-fa}{B(a-r(c))} \tag{6}
\]

and, if \( a < r(c) \), then the population of bee colonies will grow exponentially at a rate given by \( r(c) - a \) (May and Hassell, 1988), less than the growth rate would have been without mites.

In the above equations, no density-dependent regulatory effects other than the disease are considered. The rate of vertical transmission in infested colonies, \( fa \), will have a major effect on the critical host density needed to successfully introduce the parasite. In feral conditions, it is likely that drifting is rare (\( a \) small) due to the large distance between colonies (Visscher and Seeley 1982, 1989) and other environmental clues such as hive
similarity; efficiency of vertical transmission, $f$, would then have to be close to unity for the parasite to maintain itself.

**Bee to bee transmission**

Within a colony of honey bees there is a single reproductive female and, although she is tremendously fecund, the population of worker bees within the hive increases linearly:

$$N(b)(t+1) = N(b)(t) + xt$$  \hspace{1cm} (7)

The mite population, however, has many reproductive females and its population increase would be exponential:

$$N(m)(t+1) = N(m)(t) e^{r(m)t}$$  \hspace{1cm} (8)

**Experimental results**

To establish the presence or absence of a relationship between mite and bee populations within a colony, we monitored these populations in two colonies. Colony no. 1 was requeened in April and displays a linearly increasing bee population (Fig. 3. 1), while the mite population in this colony shows an exponential increase.

Colony no. 2 rejected the new queen June 5, and this colony was allowed to then rear its own queen; the fluctuating population in this colony reflects the difficulty of requeening. The worker bee population never exceeded 30,000 individuals and the mite population fluctuated more (Fig. 3. 2) than colony no. 1. Mite population levels appear to be correlated with young bee production; supersedure is accompanied by an absence in young bee production and mite levels decline following such episodes. There was no relationship between mite population changes and honey flow which occurred during June and July in 1988.
DISCUSSION

The tracheal mite-honey bee relationship introduces a difficult problem in parasitology in that the parasite must be capable of maintaining a growth capability equal to host colonies without overwhelming the individuals within a colony. The inherent Malthusian characteristic of mite population growth, however, will lead at some point to a rapid spread within a colony, since the bee population grows linearly. This situation may cause individual pathology and eventual decline of a colony.

We suggest that swarming regulates mite levels within a colony because it inserts a temporal interval during which, as mentioned above, young bees are not available. This mechanism might lead to an oscillatory but stable cycle of parasitism (Fig. 3.3). If we assume such a mechanism to be acting, and our data do suggest that a mite population responds to lapses in reproduction associated with requeening as would occur with swarming, then we can tentatively deduce conclusions concerning the sudden historical appearance of tracheal mites and the role of apicultural practices in this event.

Early human interest in bees would have had little effect on swarming (a) and, since feral hives are widely dispersed (Bailey 1958; Free 1958; Jay and Warr 1988), horizontal transmission (b) was rare although required if f<1, leaving vertical transmission (f) as the most important means of parasite maintenance (Fig. 3.4a). Overall therefore, we may assume that prevalence of mites would have been low both in the population of colonies and in the population of bees within a colony.
When this interaction grew beyond honey hunting, feral colonies were collected and kept close together in beeyards; the previously minor role of horizontal transmission consequently gained importance since drift was facilitated by close proximity and hive similarity (Fig. 3. 4b). In Europe, for example, upright hives existed in apiaries before A.D. 1000 (Crane 1983), and in Britain from 1300-1900 colonies were kept in woven 'skeps', with numerous hives recessed into specially constructed walls. Swarming was not impaired and pathology, a function of $\bar{a}$, was probably low; in any event, colonies were often destroyed at the time of honey harvest (Crane 1983). This new level of interaction would have brought about an increase in prevalence among the population of colonies; prevalence within an infested colony would still have been low because swarming was not interrupted.

In the late 1800, a new management technique was developed; the Langstroth movable frame (Naile 1976). Now colonies could be managed, that is they were checked for disease, kept from swarming and essentially forced to grow in size. They could also be maintained year to year. By preventing swarming, mortality due to parasitism, $\bar{a}$, would have increased because, as the bee population grew within a colony, the prevalence of infestation grew exponentially and may have reached a critical level of pathology not normally reached under previous conditions (Fig. 3. 4c).

The sudden appearance of the Isle of Wight disease may be explained as the interruption of swarming due to management techniques inherent to Langstroth hives in a population where colony prevalence had previously been brought to a peak by close contact.
Bailey (1964) had noted the coincidence between emergence of the disease and new management techniques and suggested that there was a causal relationship; we support this conclusion but propose a different mechanism. In short, the evolution of apiculture towards optimal honey yield eventually broke down two crucial regulatory mechanisms of parasitism, in this case, dispersion and swarming. Management practices are known to reduce mite populations, when colonies are split to increase numbers of productive hives for the beekeeper (Eischen et al. 1988). These splits result in reduction of emerging workers for various time periods and thus would be similar to swarming.

This model might also provide an explanation as to why the mite did not appear in the United States until 1984. Colonies of bees brought to the United States by settlers in the 17th century, had to come by boat and must have been either in a swarm state or in winter cluster. The length of time needed to cross from Britain to the New World would have been about 3 months (Oertel 1976). The conditions and time required for the crossing may have been rigorous enough to destroy the mites (that is the colony recovers from infection) or bees which were infested may not have survived the trip leaving only mite free colonies. After these initial importations all increase in honey bee colonies in the USA was due to natural swarming (Oertel 1976).

Later, queen shipment beginning in 1860 (Pellett 1938) was another possible avenue of mite entry into the United States. However, attempted imports were mainly from Italy, and the tracheal mite range may not have extended into Italy until later. Also,
infested queens like infested colonies may not have survived or may have recovered from an infestation due to the length of transit period. Estimates of the incubation period of infested queens would be informative. Infestivity of 'package bees' could provide similar insight into the history and control of this disease. All import of honey bees into the United States was halted in 1922 by federal legislation (Oertel 1953, 1980).

Models of host-parasite relationships have been developed for many vertebrate-parasite systems and found useful in understanding the population dynamics of these 'two species' interactions (MacDonald, Anderson and May 1979; Briscoe 1980). Apart from parasitoids (Anderson and May 1981; May and Hassell 1988; Munster-Swendsen 1985) few studies have been done on invertebrate host-parasite relationships and apparently none in eusocial insects. This study is a first attempt to implement such an approach to the population dynamics and management of honey bee mites. It suggests that swarming as a stabilizing mechanism of tracheal mite and honey bee populations helps explain the confused issue of tracheal mite pathology. If valid our model has important consequences regarding management of honey bee colonies for human purposes.
Fig. 3.1. Population changes of honey bees (solid line) and tracheal mites (dashed line) in colony no. 1.
Fig. 3. 2. Population changes of honey bees (solid line) and tracheal mites (dashed line) in colony no. 2.
Fig. 3. Illustration of the proposed interaction between tracheal mites (dashed line) and honey bees (solid line) within a colony that is capable of swarming.
Fig. 3.4. Flow charts of population dynamics of tracheal mites and honey bee colonies illustrating possible changes in the relative importance of parameters following human interference;

a) no management, such as harvesting of feral colonies
b) rudimentary management, such as skeps and beeyards
c) modern management, such as Langstroth movable frame hives
CHAPTER 4

EFFECTS OF HOST CROWDING ON THE TRANSMISSION EFFICIENCY
OF THE HONEY BEE TRACHEAL MITE, ACARAPIS WOODI (RENNIE).

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Tel. (503) 737-4392

Apidology (submitted)
The effect of honey bee host (A. mellifera) crowding on tracheal mite (A. woodi) transmission was examined. A confined honey bee colony provided continual host crowding while diurnal and nocturnal periods provided less crowded and more crowded conditions within a free-flight colony out-of-doors. For the free-flight colony the number of mite transfers was greater when all colony members were present (nocturnal) than when some of the colony members were absent because they were foraging (diurnal). Mite transfers were similar for nocturnal periods in the free-flight colony and for periods of continual crowding in the confined colony. The proportion of migrating mites actually entering the tracheae is greatest during daylight hours, regardless of whether or not the colony was confined or in free-flight. This suggests that a microenvironmental cue (perhaps humidity) is an important factor in parasite transmission between hosts.

Transmission efficiency plays an important role in the population increase of a parasite. Bailey (1958) states that Acarapis woodi (Rennie) population growth within an infested Apis mellifera L. colony is seasonal. He suggested that parasite population growth may be inhibited during active foraging periods because most of the older, infectious, bees are outside the hive, hence the crowding of the colony is reduced during the daylight hours. He did not consider nocturnal transmission of mites between adult bee hosts as a factor involved in parasite population growth.
Tracheal mite populations do not experience a population decline in *A. mellifera* colonies under conditions of active foraging during honey flow periods in western Oregon (Royce, unpub). This suggests that foraging activities may not affect transmission as severely as previously believed. Infectious adult bee hosts, i.e., bees with the largest number of newly emerged mite adults, are 12-21 days of age (Royce *et al*., 1988). Bees in this age group do not normally participate in foraging activities (Winston, 1987). Their time is spent within the colony as "hive" bees, feeding nestmates, tending brood, ventilating, grooming nestmates and shaping comb (Seeley, 1985).

Newly mated female tracheal mites leave their host worker's tracheae after mating and migrate to the tips of thoracic hairs around the area of the prothoracic spiracle (Sachs, 1952). Tracheal mites are not found off their adult bee hosts (Harvey, 1921; Morgenthaler, 1931). Therefore, close physical contact between an infected host possessing migratory *A. woodi* females and a potential new host is required for transfer. Once a new host bee is contacted, mites transfer and move into the pelage near the cuticle of the new host. The mites frequently congregate around the hairs surrounding the wing bases, move into the wing axillary region and from there find their way to the prothoracic spiracles (Royce *et al*., 1988).

Crowding of adult bee hosts within the colony may increase the incidence of transmission of mites between bee hosts. Therefore transmission may be enhanced during nocturnal periods when the interior of an infested colony experiences the highest bee density. This study examines the influence of the circadian mediated factor of
nestmate crowding on the transmission of the honey bee tracheal mites between adult honey bee hosts.

MATERIALS AND METHODS

The diurnal rate of transmission of *A. woodi* was determined by introducing cohorts of newly emerged marked worker bees (<24h old) into infested colonies at 7:00 am and removing a subset of the marked bees at 7:00 pm (12h of exposure). Gary *et al* (1989) have shown that newly eclosed honeybee workers <24h of age, are the most susceptible age group for infestation by *A. woodi*. The nocturnal rate was determined in the same manner except the bees were introduced at 7:00pm and removed at 7:00 am the following day.

Transmission rates between worker bees in a colony maintained under confinement and bees from a colony in normal free-flight conditions were compared. The confined colony (80% worker infestation) was maintained in an indoor environmental chamber that dampened external circadian cues, a situation that created more crowding than in normal free-flight out-of-doors. The confined colony was kept in a screen cage (1.8 x 1.8 x 2.4 m) that was in an indoor flight room at a temperature of 27°C, with constant light. The colony was fed sugar syrup and provided with frames of drawn comb containing pollen and brood as needed. The confined colony displayed very little worker bee foraging. The second colony (70% worker infestation) was maintained in free-flight conditions, out-of-doors. Experimentation with the confined colony took place in April and May 1988. Testing under free flight conditions took place in July 25-28, 1989.
Sample bee were examined under 50X for mites in the wing axillaries, in the 'vestibule' or cuticular depression at the prothoracic spiracle, and then dissected and prothoracic tracheae examined. Single classification Anova with two groups of unequal or equal sample sizes were used to analyze the difference between the proportion of mites in the trachea and mites per bee under both test conditions.

RESULTS

For worker bees under conditions of continual crowding (confined) more mites were collected in the prothorascic tracheae in am samples than in pm samples (Table 4.1). This suggests that mites moved into tracheae more quickly during the day. The number of mites infesting callow hosts was essentially the same regardless of time of day for the confined colony (mean value for am period = 1.26 mites/bee, $s^2$=1.13, n=73, with 67% of the sample bees being infested; pm period = 1.31 mites/bee, $s^2$=1.3, n=151, with 50% of the sample bees being infested).

Under free-flight conditions the number of transferring mites was lower and the proportion of mites in the trachea higher for the diurnal sample compared to the nocturnal sample (see Tables 4.2 and 4.3). Thus, the trend of moving into the trachea more quickly during the day was similar to the colony colony held under continual crowding (Table 4.2), but the rate of transmission was greater during the nocturnal period for the free-flight colony (Table 4.3).

DISCUSSION

The total number of mites transferring per bee over a twelve hour period was not affected by this period being diurnal or
nocturnal when the colony of bees was exposed to conditions of continual crowding. In the free flight colony, however, fewer mites transferred during the diurnal 12h period than the nocturnal 12h period. The rate of migration over the external surface of the new host increases during the diurnal period, suggesting that the mites are responding to some factor in their microenvironment (perhaps humidity), making it important for the mites to complete trachea to trachea transfer more quickly during the daylight hours.

Bailey (1958a) reports erroneously that the average age of foragers is 9 days instead of the more accurate average of 21 days (Winston 1987; Harrison 1986) and suggests that foraging would reduce transmission. He ignores the possibility of nocturnal transmission. We suggest that foraging may affect transmission during the day but sufficient time exists at night for the mites to transfer. Infectious bees 15-21 days old are performing tasks that place them throughout the nest and beginning to forage in free flight observation hive (Seeley 1985). Seeley's (1985) observations used health colonies. Worker bees in infested colonies (stressed colonies) may begin to forage at an earlier age (Winston and Fergusson 1985), thus, increasing the advantage of mites transferring at night. The higher proportion of female mites in the trachea after the 12 hours of diurnal exposure compared to 12 hours of nocturnal exposure suggests that environmental factors as well as worker bee behavior is involved in successful mite transfer.
Table 4. 1. Percent of total mites/bee within the trachea from callow bees exposed for 12h in an infested colony under conditions of continual crowding.

<table>
<thead>
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<th>NOCTURNAL</th>
<th>DIURNAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of total mites</td>
<td>19.4%</td>
<td>50.8%</td>
</tr>
<tr>
<td>$s^2$</td>
<td>29.6</td>
<td>43.4</td>
</tr>
<tr>
<td>N</td>
<td>75</td>
<td>50</td>
</tr>
</tbody>
</table>

$F_{0.01(1/123)}=6.85$.  $F=22.36$ significant at 0.01 level

\[^{1}\text{nocturnal = 7 pm to 7 am; diurnal = 7 am to 7 pm.}\]
Table 4.2. Percent of total mites/bee that are within the tracheae from callow bees exposed for 12h in an infested free-flight colony.

<table>
<thead>
<tr>
<th></th>
<th>NOCTURNAL</th>
<th>DIURNAL&lt;sup&gt;1&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>% of total mites</td>
<td>22.8%</td>
<td>58.3%</td>
</tr>
<tr>
<td>s&lt;sup&gt;2&lt;/sup&gt;</td>
<td>46.2</td>
<td>38.2</td>
</tr>
<tr>
<td>N</td>
<td>18</td>
<td>78</td>
</tr>
</tbody>
</table>

F<sub>.01(1/94)</sub> = 7.08. F<sub>=9.94</sub> significant at 0.01 level

<sup>1</sup>nocturnal 7pm to 7am; diurnal 7am to 7pm
Table 4.3. Total mites/bee from callow bees exposed for 12h in an infested free-flight colony.

<table>
<thead>
<tr>
<th>NOCTURNAL</th>
<th>DIURNAL(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>0.77</td>
</tr>
<tr>
<td>S(^2)</td>
<td>0.92</td>
</tr>
<tr>
<td>N</td>
<td>150</td>
</tr>
</tbody>
</table>

\(F_{0.01(1/298)}=6.68\). \(F=55.84\) significant at 1% level

\(^1\) nocturnal 7pm to 7am; diurnal 7pm to 7am
CHAPTER 5

SEX RATIO OF THE TRACHEAL MITE, ACARAPIS WOODI (RENNIE)

(ACARI:TARSONEMIDAE)

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not yet submitted
ABSTRACT

The sex ratio of A. woodi varies with seasons and with host age. Younger hosts have A. woodi sex ratios that favor females, while in older hosts the ratio is more even. Because the proportion of different ages among the host bees varies with the season (i.e. the proportion of young bees is highest in spring and decreases continually through summer, fall and winter) host age appears to be an important factor. Determination of the primary sex ratio will require rearing and cytological experiments.

INTRODUCTION.

The unequal production of sexes in a bisexual population of animals has been of interest to biologists. Fisher (1958) postulated that a sex ratio is in equilibrium when, in the population as a whole, the total efforts spent in producing each sex are equal. Hamilton (1967) pointed out that this may be true for a population, but researchers often deal with local competition between sexes rather than with a population-wide phenomenon. He noted the following exceptional circumstances that invalidate Fisher's principle: 1) sex-linked drive under random mating, 2) sex-linked drive under local competition, 3) non sex-linked control under local competition, 4) inbreeding arrhenotoky, 5) polygyny, 6) thelytoky, and 7) outbreeding. Of these the most likely factor affecting A. woodi populations is inbreeding arrhenotoky, for which Hamilton listed the following criteria:

1) primary sex ratio is spanandrous—that is females greatly preponderate.

2) reproduction is arrhenotokous
3) there is at least one male in every batch of offspring
4) there is gregarious development as a group of siblings from egg to adult
5) adult males eclose first and can mate many times
6) mating takes place immediately after eclosion of adult female
7) males are disinclined or unable to migrate from the batch
8) females can store sperm and one insemination serves to fertilize the whole egg production.

During this study sex ratios of *A. woodi* were recorded from samples of bees of different ages and at different times of the year in order to establish whether *A. woodi* fits the criteria for inbreeding arrhenotoky mode of sexual reproduction. An understanding of reproductive strategy in *A. woodi* is considered essential if a management program is to be established for this mite.

**MATERIALS AND METHODS**

The honey bee colonies used for this study were maintained in deep and western movable frame hive bodies (42 and 26 liter Langstroth hive with 9 movable frames) and managed to prevent swarming. Samples of worker bees returning from foraging were taken with a sweep net at the hive entrance after it had been temporarily blocked. Hive bees were collected from within the colony on brood comb. This provided us with samples of bees which were either three weeks or less of age or older than three weeks of age. These samples were taken weekly from April through September. Bees were frozen and subsequently dissected to determine mite infestation. Infested tracheae were removed and opened, and all of the mites were counted. The sex of *A. woodi* adults was determined by the number of long setae
on the terminal segment (tibia/tarsus) of leg IV, i.e. two for females and one for males. This character was seen visible at 50X magnification.

RESULTS

Sex ratios for mites from samples of hive bees ranged from 3:1 to 7:1 through spring, summer and fall. Numbers of females and males from foraging bees were virtually equal (1:1) from early spring to late fall. Sex ratios of mites from samples of winter bees were also near 1:1 (Tables 5.1 and 5.2). Thus, while young hive bees usually had a sex ratio that favored female mites, the older foraging and winter bees had a non-skewed ratio of about 1:1 (Tables 5.1 and 5.2).

DISCUSSION

The more young bees in a sample, the higher the female:male ratio. The mite infest bees which are newly emerged (Lee 19; Gary and Page 1989) and begin reproduction within the first two days (Royce et al. 1988). This phenomena will result in young bees having a bias toward female mites. Sampling methodology certainly affects the observed sex ratio, with the result that the primary sex ratio is uncertain.

Certain of Hamilton's criteria for inbreeding arrhenotoky are met in A. woodi. For example, there appears to be one male in most batches of offspring, there is gregarious development dictated by the restrictions of the trachea, adult males eclose first but it is not clear at this time if they mate more than once, mating takes place immediately after female eclosion, and males are disinclined to migrate from the batch. We do not know, however, if reproduction is
arrhenotokous, if females can store sperm, or if one insemination serves to fertilize all of the female eggs. There have been reports that females are more numerous than males, but the ratio reported is only 3:1 (Otis 1988; Burgett et al. 1989; Morrison 1931). It is questionable if such a ratio fits Hamilton's definition of 'great preponderance of females'.

Sampling methodology and mite migratory behavior may have a profound effect on reported sex ratios (Lindquist 1986). In fact, a variety of ratios may be generated from our data, depending on the age of bees in a given sample and/or on the season during which the sample was taken. Clearly the use of sex ratio data as a basis for invoking arrhenotoky in A. woodi is debatable and the use of cytological and rearing studies will be required to understand mode of reproduction for A. woodi.
Table 5.1. Observed sex ratios of *A. woodi* from samples of honey bees taken from within and in front of hive 2 during 1988, in Corvallis, Oregon. Ratios are expressed in terms of number of females/male (a total of 2125 bees sampled and 7405 adult mites counted).

<table>
<thead>
<tr>
<th>SEX RATIO</th>
<th>hive bees</th>
<th></th>
<th></th>
<th>foragers</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPRING</td>
<td>SUMMER</td>
<td>FALL</td>
<td>WINTER</td>
<td>SPRING</td>
<td>SUMMER</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>2.7</td>
<td>2.4</td>
<td>1.4</td>
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<tr>
<td></td>
<td>3.3</td>
<td>2.1</td>
<td>2.6</td>
<td>0.6</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

| total number of adult mites counted, female/male |
| SPRING | SUMMER | FALL | WINTER | SPRING | SUMMER | FALL |
| 6/1    | 584/206| 261/110| 42/30 | 219/153| 608/640| 252/245|
| 108/33 | 701/342| 373/142| 15/23 | 246/312| 312/572| 129/130|
| 289/151| -----  | -----  | ---    | -----  | -----  | 76/94  |

| total number of worker bees sampled/number infested |
| SPRING | SUMMER | FALL | WINTER | SPRING | SUMMER | FALL |
| 55/5   | 232/114| 90/58| 20/10  | 207/57 | 423/181| 80/52 |
| 161/30 | 110/81 | 80/50| 10/3   | 291/76 | 132/79 | 30/22 |
| 164/63 | -----  | ---  | ---    | -----  | -----  | 30/16 |
Table 5.2. Observed sex ratios of *A. woodi* from samples of honey bees taken from within hive 1 during 1988, in Corvallis, Oregon. Ratios are expressed in terms of number of females/male (a total of 941 bees sampled and 1909 adult mites counted).

<table>
<thead>
<tr>
<th>SEX RATIOS</th>
<th>SPRING</th>
<th>SUMMER</th>
<th>FALL</th>
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<tbody>
<tr>
<td></td>
<td>5.4</td>
<td>7.3</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>4.2</td>
<td>1.9</td>
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</tr>
<tr>
<td></td>
<td>0.8</td>
<td>---</td>
<td>3.5</td>
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</table>

**total number of adult mites counted female/male**

<table>
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<tr>
<th>SPRING</th>
<th>SUMMER</th>
<th>FALL</th>
<th>WINTER</th>
</tr>
</thead>
<tbody>
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<td>42/8</td>
<td>47/6</td>
<td>92/60</td>
<td>229/262</td>
</tr>
<tr>
<td>5/1</td>
<td>151/32</td>
<td>242/126</td>
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</tr>
<tr>
<td>23/29</td>
<td>----</td>
<td>431/123</td>
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</tr>
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</table>

**total number of worker bees sampled/number infested**

<table>
<thead>
<tr>
<th>SPRING</th>
<th>SUMMER</th>
<th>FALL</th>
<th>WINTER</th>
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<tr>
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<td>40/10</td>
<td>20/18</td>
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<tr>
<td>58/4</td>
<td>120/36</td>
<td>20/15</td>
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</tr>
<tr>
<td>223/12</td>
<td>----</td>
<td>40/31</td>
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</table>
CHAPTER 6

RESPONSES BY THE HONEY BEE, *A. MELLIFERA* (HYMENOPTERA:APIDAE) TO FEEDING INJURY OF THE TRACHEAL MITE *A. WOODI* (RENNIE) (ACARI:TARSONEMIDAE) WITH NOTES ON HONEY BEE HEMOLYMPH UPTAKE

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ABSTRACT

Feeding by tracheal mites causes the appearance of amyloid material around the hemolymph side of the trachea. Mites feed often, and may provide a means for pathogenic microorganisms to gain entry into the bee's hemocoel.

INTRODUCTION

Feeding by *A. woodi* provides nutrients for their reproduction and growth and may also evoke an immune response from the host bee. Irritation and injury caused by the feeding wounds may induce physiological responses in the host. Abnormal increase in bacterial counts and in the number of bacterial species present in the hemocoel of infested bees (Fekl 1956) suggests the introduction of contaminated foreign material into the body cavity through breaching of the tracheal wall by their mite parasites. Mites may inject salivary secretions toxic to the host. Furthermore, the volume of hemolymph consumed by the mite occupants of an infested trachea may in itself be a significant injury factor. In short, the secondary effects of feeding may be as or more important than the injury itself. An understanding of these effects however is dependent on an understanding of the primary injury itself. Accordingly, the major objectives of this study were 1) to determine the volume of hemolymph uptake by *A. woodi*, and 2) its effect on the host; 3) to clarify host response to mite feeding.

MATERIALS AND METHODS

Feeding by *A. woodi* was monitored through observation of the appearance of dye in the mites ventriculus following the dye injection into the hemolymph of the bee host. Congo Red, the dye
used by Őrös-Pál (1934) in his feeding studies, was chosen for these experiments because it remains in solution when injected into the hemolymph. The dye was dissolved in Hayes saline and filtered twice before use. Twenty bees were individually anesthetized with CO₂ and then injection with 5 μl of a 3% Congo Red-Hayes saline solution through the dorsal neck membrane using a calibrated microcapillary tube drawn to a fine point. Injected bees were kept in a group with 20 uninjected bees in a small cages held in an incubator at 32°C, and provided with sugar water (1:1 solution by volume). Injected bees were dissected after 6, 12, 22 and 43 hours, and stained mites were recorded. Observations were made on the condition of the tracheal wall emphasized by the dye.

Volume of the mite ventriculus was determined by multiplying its depth by the area. Depth of the gut was measured using units of the fine focus on a Zeiss phase contrast photo microscope. The units of fine focus were calibrated with Vitro Dynamics Inc. 0.05mm microcell, i.e. the number of units/0.05mm depth = units/mm. Area was estimated by drawing mites with dye in the ventriculus at 400X on Keuffel and Esser Co. paper, 10 x 10 to 1/2 inch (100 squares/0.5 inch²) using a camera lucida and counting the squares covered by the stained gut.

By noting the color of known dilutions of dye in 0.1mm microcells (3% serially diluted to 0.38% and 0.3% to 0.038%), we hoped to estimate the concentration of dye in the mite ventriculus compared to that in the bee hemolymph and thereby estimate the amount of fluid ingested.
RESULTS

Congo Red first became discernible in the gut of mites six hours after injection into the bee hemolymph. Dye may have been present before that time but in quantities too low to be observed. At six hours, all mites in the trachea other than newly emerged males and females had acquired discernible quantities of dye in the ventriculus, indicating that feeding had taken place. Dye concentration in single mites varied visually from 0.3% to 3%. Gut volume estimates for 5 female mites was 0.11 ul. (std error=0.02). This volume is not an estimate of hemolymph uptake because we do not at this time have information on how hemolymph is digested by A. woodi. We also feel a more accurate method of calibrating the fine focus divisions is needed that the use of glass microcell may overestimate the divisions because of the difficulty of discerning the inside boundaries of the cell.

We demonstrated a reduction of Congo Red concentration in bee hemolymph over a 40 hour post-injection period by comparing the color of honey bee hemolymph in 0.1mm microcell to known concentrations of dye in 0.1mm microcells. This reduction appears to be the result of elimination processes of bee metabolism. Fine particles of dye were passed through the Malpighian tubules to the midgut, and finally eliminated from the rectum. Dye concentration in the hemolymph immediately following injection was estimated to be 0.3 %. At 6 hours it was 0.2 %, at 14 hours it ranged from 0.1 to 0.075 %, at 24 hours 0.05 %, and at 40 hours 0.075 to 0.038 %. Inconsistency in the time required for elimination of dye by bees, and due to the
difficulty in detecting subtle differences in dye concentration in the mite gut, we were unable to accurately determine volume of fluid uptake by mites in bee tracheae. While the initial estimate of .3% should give an estimate of the total volume of bee blood we feel this estimate would give an inaccurately high vol. because dye is essentially particles in the blood and are probably caught or in essence filtered as the blood is pumped through the bee. So while the ratio of .3/3 suggests a blood volume 10 times the 5 ul injected or 50ul, this estimates is probably much too high and it is impossible to estimate by how much. Kirkurt and Gilbert vol 3

Accumulations of a gelatinous material were found on the hemolymph side of sections of tracheae infested by mites. This material was stained by Congo Red (Fig. 6. 1) which indicates that it is an amyloid or an extracellular deposit of protein-mucopolysaccharide substance (Humanson, 1979). Darkening or melanization of the tracheae follows its appearance.

DISCUSSION

Sachs (1958) observed that *Acarapis woodi* (Rennie) apparently will feed through a wax membrane of unknown thickness and suck up water, bee hemolymph and even vaseline. Mites that were offered water or vaseline diets lived only a short time, but those that were fed hemolymph did produce eggs. These experiments corroborated the in vivo findings that *A. woodi* feeds on hemolymph (Örösi-Pál 1934). Furthermore, their parasitic mode of life is clearly indicated by the morphological characteristics of the mouthparts (Sachs 1951) and by the fact that all life stages are found within tracheae (Lindquist 1986).
We suggest that mites feed often, because all mites, except those newly eclosed, have taken up dyed hemolymph 6 hours post-injection. The amount of hemolymph removed from an individual bee as the result of mite feeding could not be accurately measured. But, considering the small size of *A. woodi* relative to their host bee (1/200,000, a rough estimate calculated from volume of mite/volume of bee) the amount ingested by mites is probably negligible. A heavy mite infestation may result in consumption of moderate amounts of host hemolymph, but host distress is more likely mediated by feeding injury and, possibly, by the consequent breaching of the tracheal wall barrier by pathogenic microorganisms.
Fig. 6. 1. Infested (IT) trachea with protein mucopolysaccharide (PM) on its hemolymph side, beside an uninfested trachea (UIT)
CHAPTER 7

WINTER FEEDING BY TRACHEAL MITES IN WORKER HONEY BEES.

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not yet submitted
ABSTRACT

Mite feeding continues in winter within host bee's trachea, while mites in wing axillaries of winter bees do not feed.

INTRODUCTION

Bee behavior and physiology change with the onset of winter. Foraging ceases because flowering ends and temperatures become too cool for flight. Bees in a 'winter cluster' are older on average because they survive longer than their summer counterparts. In early winter no eggs are laid and there are no bee larvae and no newly emerging bees for several weeks depending on the climate. Without developing larvae and fresh pollen and nectar, hive odor probably changes during winter. All the bees in the colony form a compact group over comb containing honey, the winter cluster, bees circulate from the center to edge of the cluster and temperature varies from 24°C to 7°C (Owens 1971). As resources become depleted at one site the cluster moves over the comb.

At some point near mid-winter, brood rearing commences, allowing colonies to reach swarming strength earlier in spring or summer than would be otherwise possible (Seeley 1985). Winter brood rearing is accomplished without fresh forage and temperature of 34°C are maintained where brood exists despite low ambient temperatures.

The significance of bee behavioral and physiological differences to the mite populations have never been determined. Mites are reported to lay eggs continually during winter though apparently at lower rates than during summer months (Otis et al. 1988; Royce et al. 1988). Changes have been noted in sex ratios (Royce et al. 1988) with males being more prominent and more females
being seen in wing axillaries during winter (Royce et al. 1988). The objectives of this study are to observe winter mite feeding and compare feeding of mites within tracheae and mites that are found at the bases of the wings of worker bees during winter.

MATERIALS AND METHODS

Five ul of 3% Congo Red dye was injected into bees collected from winter cluster during December 31, and January 8, 1989. Injections were made into the dorsal neck membrane of the honey bee with a capillary tube drawn to a fine point. The colony from which worker bees were taken had 90-100% of its individuals infested with A. woodi. No brood was seen during the short interval of worker bee collection and we assumed that winter brood production for this colony had not begun. Bees into which dye had been injected were held in small cages in an incubator at 32°C and fed sugar syrup (1:1 solution by volume). Twenty uninjected bees were also placed in these cages, because it improved survival of the injected bees.

A sample of injected bees were examined 22 hours after injection and the remaining injected bees were examined at 43 hours postinjection. Mites from the wing axillaries and tracheae of same bee were checked for dye-positive ventriculi. On January 12, this colony was found to be so depopulated that the experiment had to be terminated.

RESULTS

Few mites in wing axillaries had fed at 22 hours and about half the mites at this site had fed after 43 hours. In tracheae, however, all mites had fed by 22 hours, except those individuals that were newly emerged (Table 7.1). Females mites were observed laying eggs
in wing axillaries, suggesting that there are too few bees or suitable tracheae and too many mites.

DISCUSSION

The observations that mite eggs are present and that there is an increase in the number of infested bees within a colony during the winter (Royce et al. 1988; Otis et al. 1989) suggest that mites continue to mate, lay eggs and require feeding. Newly emerged female mites can find only older bees as hosts, but the mite life cycle can be completed in these bees because the bees survive much longer than their summer counterparts.

Overcrowding of mites within a colony of bees could produce a situation of too few tracheae and female mites may resort to laying eggs in the wing axillaries.
Table 7.1. Feeding by mites in trachea and wing axillaries in December 1988, + mites that had fed, - mites that had not fed.

<table>
<thead>
<tr>
<th></th>
<th>new females</th>
<th></th>
<th></th>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>wing axillaries</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>trachea</td>
<td>32</td>
<td>5</td>
<td>13</td>
<td>0</td>
<td>23</td>
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<table>
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<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>4</td>
<td>0</td>
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<tr>
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<td>10</td>
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CHAPTER 8

PROTEIN LEVELS IN HEMOLYMPH AND HYPOPHARYNGEAL GLANDS OF WORKER HONEY BEES INFESTED WITH A. WOODI (RENNIE) COMPARED WITH UNINFESTED BEES.

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not yet submitted
ABSTRACT

Protein levels in hemolymph and hypopharyngeal glands were lower for infested worker bees and infested colonies than uninfested workers or colonies during fall and winter of 1988. These results could not be demonstrated in spring and summer because colonies had lower mite levels and food was in abundance.

INTRODUCTION

Parasitoids have been known to change protein composition of their host's hemolymph (Beckage et al. 1987). Blood feeding mammal parasites such as the rabbit flea use hormone changes in the blood of their hosts as cues for their biological processes (Rothchild and Ford 1964). We therefore felt that because A. woodi feeds on bee hemolymph this was a good tissue in which to look for mite initiated physiological changes in the bee. Hemolymph protein determinations were made initially to enable electrophoresis of hemolymph. Depression in hemolymph protein levels correlated with mite infestation were dramatic, leading us to examine possible differences in hypopharyngeal gland proteins. These glands are the brood food glands and are well developed in bees that are feeding brood. Because bees change physiologically for winter and are thought to use the hypopharyngeal gland for brood food production during winter, we expected the most dramatic difference to occur in winter.

MATERIALS AND METHODS

Maintenance of honey bee colonies

The colonies for this study were maintained in deep (46 1 with 9 frames) and western (21 1 with 9 frames) hive bodies and managed to prevent swarming.
Marking bees

Emerging brood was removed from a colony unrelated to the study colony and placed in an incubator. Workers were collected from this brood daily so that their age within a 24 hour period was known. These bees were marked and placed in the test colonies to allow experiments with bees of known age. Acrylic paint was used to mark the bees on the head, thorax or abdomen. Previous studies (Royce et al., 1988) have shown that the paint on the bees has no effect on the mites.

Bleeding bees

For protein analysis 2 ul of blood were bled from the neck of a worker bee with a microcappillary tube (microcap 5 ul) and immediately mixed with 4°C 0.2 M sucrose and 1% EDTA (10:1 hemolymph:sucrose).

Hypopharyngeal gland protein

Hypopharyngeal glands were dissected from winter bees in Hayes saline. Glands were ground, centrifuged to remove cell debris and tested for total protein using the Bradford method (Bradford 19). Optical density was read at 595 nm. A standard curve using bovine serum albumin was used to compute mg protein per pair of glands. Infested workers were compared to uninfested workers in the same colony and to workers from an uninfested colony.

Electrophoresis

Polyacrylamide electrophoresis SDS gels were run with 9% running gels, 10 x 18 cm and 17 x 19 cm, 5% stacking gels and a constant voltage power source. Gels were stained with Comassie Brilliant Blue.
RESULTS

Samples of hemolymph taken from bees 0 to 4 weeks old from infested and uninfested colonies were compared during October 1987 (Fig. 8. 1). Individuals from infested colonies had lower protein levels. The electrophoresis gels showed some differences in quantity of certain bands (Fig. 8. 2) even though difficulty in obtaining cell free samples of serum made comparisons difficult.

Protein levels in the hypopharyngeal glands were lower for infested bees than uninfested bees during January and February 1988 (Table 8. 1). Also, overall protein levels in hypopharyngeal glands from heavily infested colonies were lower than those of slightly infested colonies and uninfested colonies. However, during spring (May 1988, Table 8. 2), no differences could be found between infested and uninfested bees or colonies. It should be noted that the infestation level dropped and was 30% for the infested test colony during the month of May.

During winter at high mite infestation levels, tracheal mites appear to lower protein levels of honey bee hemolymph and hypopharyngeal glands. However, this effect could not be demonstrated in the spring when mite infestation rates had dropped.

DISCUSSION

Lower protein levels were noted in the hemolymph of infested bees during October. Fall is a time when honey bees are changing physiologically for winter by eating more pollen to build up fat body and maintain active hypopharyngeal glands (Winston 1987). Lower hemolymph protein levels in infested bees in October suggest that these bees would be less able to rear winter brood or to live long
enough to be active when spring arrives. Attempts to repeat this experiment in the fall of 1988 failed because field conditions could not be replicated.

Honey bee cells appear to rupture even at low centrifugation, resulting in lack of control over the number of cells present in samples of hemolymph for protein determination or electrophoresis.

Hypopharyngeal gland total protein was less in glands removed from workers of a heavily infested colony compared to moderately infested and uninfested colonies. Infested workers from the heavily infested colony had lower values than did uninfested workers. Differences at both the individual and colony level (Table 8.1) suggest that the mites had an effect on individual bees and this effect was eventually reflected in the uninfested members of the colony.

If maintenance of adequate protein levels are indeed a problem for mite infested bees, then feeding protein supplements in fall and into winter may increase the bee's ability to rear winter brood and help assure colony survival.
Fig. 8.1. Comparison of total proteins in hemolymph of aged infested and uninfested worker bees for October 1988.
Fig. 8. 2. Polyacrylamide SDS gel showing protein bands from infested workers, lane c, f, h with 50, 30, and 15 ug protein/lane respectively and uninfested workers, lane b, e, g with 50, 30, and 15 ug protein/lane and molecular weight standards a, d, i (250,000-40,000)
Table 8. 1. Comparison of total protein levels in the hypopharyngeal glands in ug/pair of glands of infested and uninfested workers from colonies with mite prevalence of 90 and 8 %, hive 1. and 2 respectively, during winter 1988. All samples consisted of 10 workers, the numbers in () are standard errors.

| month | HIVE 1  |  |  | HIVE 2  |  |
|-------|---------|  |  |---------|  |
|       | infested| uninfested | infested| uninfested |
| Jan.  | 21.9    | 54.1        | 75.8    | 80.8     |
|       | (4.6)   | (12.8)      | (15.2)  | (10.5)   |
| Feb.  | 32.8    | ----        | 53.8    | 63.1     |
|       | (4.5)   |             | (15.5)  | (14.1)   |
Table 8.2. Comparison of hypopharyngeal gland total protein in ug/pair of glands of infested and uninfested worker bees from an infested hive with an infestation level of 30% (hive 2) and a uninfested colony during May 1988. All samples consisted of 10 workers, the numbers in () are the standard errors.

<table>
<thead>
<tr>
<th>AGE OF BEES IN DAYS</th>
<th>INFESTED</th>
<th>UNINFESTED</th>
<th>UNINFESTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>121.4</td>
<td>101.0</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>(13.6)</td>
<td>(5.5)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>143.4</td>
<td>157.3</td>
<td>139.4</td>
</tr>
<tr>
<td></td>
<td>(16.6)</td>
<td>(13.9)</td>
<td>(10.6)</td>
</tr>
<tr>
<td>20</td>
<td>118.8</td>
<td>111.5</td>
<td>119.3</td>
</tr>
<tr>
<td></td>
<td>(13.0)</td>
<td>(17.9)</td>
<td>(10.0)</td>
</tr>
</tbody>
</table>
CHAPTER 9

HEMIAGGLUTINATION IN AN A. WOODI INFESTED COLONY COMPARED WITH THAT IN AN UNINFESTED COLONY

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ABSTRACT

More individuals from an infested colony had positive tests for hemmiagglutination than individuals from uninfested colonies. A positive test for hemmiagglutination and individual mite load showed no correlation. Because agglutination could not be correlated with mite load we suggest mite infestation predispose the entire colony to secondary infection by pathogenic microorganisms and that microorganism infection of individual bees resulted in positive agglutination tests.

INTRODUCTION

Wounding and molting appear to affect the titer of insect agglutinins (Komano et al. 1980). Increases in honey bee agglutinins were noted after injection of pathogenic bacteria (Gilliam and Jeter 1970). Tracheal mites may cause both wounds and an increase in hemolymph bacterial counts (Fekl 1956) and may inject toxins with their saliva; we, therefore, proposed to determine whether the agglutinin titer was higher in worker bees from an infested colony than in those from an uninfested colony.

MATERIALS AND METHODS

Hemolymph collection

Bees were collected from the colony just before bleeding in January and February 1989. The oral opening and anus of individual bees were sealed with melted bees wax, the legs were severed and the bee placed in a 0.5 ml microfuge tube from which the end had been cut. These tubes were placed in a 1.5 ml microfuge tube. The bee was held by the smaller tube while her blood was collected into the larger tube. The tubes were spun in a Phillips Drucker combination
table top centrifuge in a 1°C room at RCF = 139.9 x g for five minutes to collect hemolymph.

Agglutination assay

Agglutination was detected at 22°C, in equal volume of hemolymph and 1.3% solution of red blood cells (RBC) in Dulbecco's's phosphate buffered saline (DPBS; 1.5 mM KH₂PO₄, 8 mM Na₂PO₄, 0.9 mM CaCl₂, 2.7 mM KCl, 0.5 mM MgCl₂, 135 mM NaCl; pH 7.2 containing 1 mM phenylthiourea) (Stebbins and Hapner 1985).

Red blood cells obtained from the OSU Veterinary Diagnostic Laboratory from dog, horse, cow, sheep, llama and cat were tested for agglutination with bee hemolymph. Bee hemolymph was found to agglutinate dog RBC and these were used in the following assays. The tests did not conclusively eliminate the other species and further tests would be required to tell which species and blood types were most active with bee hemolymph.

Erythrocytes were washed four times by centrifugation at 4°C in DPBS, RFC = 139.9 x g, prior to use. They were prepared by incubating 0.5 ml cells for 1.5 hours at 34°C with 3 mg neuraminidase (type 5, Sigma) in 10 ml DPBS at pH 5.7. The asialo-erythrocytes were washed 4 times with 4°C DPBS pH 7.2 and used in the assay.

Hemolymph was carefully removed from the microfuge tube so that only the serum was used in the test. Serum was frozen on dry ice in wells of microtiter plates (one bee per well) until hemolymph from the entire sample was collected (usually 36 bees). The hemolymph was then thawed at 4°C and the RBC added; after RBC addition, the hemolymph containing the RBC was incubated at 22°C for 1 hour.
Controls (equal volumes of buffer saline and 1.3% RBC) not containing bee hemolymph were included. Tests were considered negative if the RBC settled to the bottom of the well to form a distinct dot, and positive if the RBC did not settle but instead formed a diffuse mat (Fig. 9.1). Bees from infested and uninfested hives were compared. Each sample of bees contained infested and uninfested workers and these were recorded so that hemagglutination and mite infestation could be related.

RESULTS

There were more positive agglutination tests for workers from the colony infested with tracheal mites than for workers from clean colonies. However, there was no correlation between positive tests and mite infestations, possibly because the assay was not sensitive enough to determine small differences in agglutinin titer. Possible contamination from the honey stomach cannot be ruled out, even though we made every attempt to seal the oral opening of the bee. Trials 4 and 5 were not consistent with trials 1, 2, and 3 (Table 9.1), and the infested colony died in March.

Bees may lose their ability to produce required precursors for immune reactions as they use up their limited resources during winter, this may explain the lack of agglutination seen in the February trials. Positive response from uninfested members of an infested colony suggests that high mite densities affect the colony overall. Hemocyte rupture during centrifugation was again a problem in these experiments.
DISCUSSION

We suggest that agglutinins are indirectly affected by mites, because uninfested and infested bees in the same colony had positive agglutination assays. The mites may weaken the entire honey bee colony, infested and uninfested members alike, predisposing individual members to secondary infections. Uninfested bees may have to work harder to make up the difference in work that their infested sisters cannot accomplish. In this way, even bees that are not infested in a colony come under more stress and are weakened. Higher numbers and more species of bacteria have been reported in infested bees (Fekl 1956); therefore, a colony with high mite infestation might also have high bacterial infestation. The lower number of positive agglutinin tests seen in the month before the death of the colony suggests that bees continually challenged by parasites may eventually become unable to respond.

A more sensitive agglutinin test is needed to determine differences between different mite loads. If the agglutinin could be purified and an antibody developed to it, sensitivity would be enhanced.
Table 9.1. Percent positive tests of agglutination for an infested and uninfested colony of honey bees, number of bees in test is in ()

<table>
<thead>
<tr>
<th>Trial</th>
<th>Uninfested</th>
<th>Infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.1 (48)</td>
<td>77.0 (22)</td>
</tr>
<tr>
<td>2</td>
<td>---</td>
<td>56.0 (16)</td>
</tr>
<tr>
<td>3</td>
<td>6.2 (32)</td>
<td>56.0 (32)</td>
</tr>
<tr>
<td>4</td>
<td>23.3 (30)</td>
<td>3.1 (32)</td>
</tr>
<tr>
<td>5</td>
<td>0 (35)</td>
<td>2.9 (35)</td>
</tr>
<tr>
<td>total</td>
<td>6.9 (145)</td>
<td>38.0 (137)</td>
</tr>
</tbody>
</table>
Fig. 9. 1. Hemiagglutination assay, example of positive A.2.4., example of negative B.1.1.
CHAPTER 10

COMPARISON OF FEEDING RATE OF A. WOODI-INFESTED WORKER HONEY BEES
WITH UNINFESTED INDIVIDUALS

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ABSTRACT

No differences could be detected between infested and uninfested bees either in food consumption or hemolymph protein levels when samples of these bees were subjected to similar conditions of low stress.

INTRODUCTION

Honey bee colonies in temperate climates must accumulate enough stores to sustain themselves through winter. A colony whose members use up winter stores too rapidly will starve before good weather in the spring allows foraging on early flowering plants. Infested bees are reported to be more active and to use food stores more quickly than uninfested bees (Scott-Dupree personal communication). In fact, infested colonies wintered at high elevation in Mexico were found to have fewer stores remaining after winter than did uninfested colonies (Eischen 1987). These reports led us to propose that infested bees deprived of protein (pollen) would have lower hemolymph protein levels than uninfested bees similarly deprived, and that infested bees would consume more pollen than uninfested bees.

MATERIALS AND METHODS

Brood was removed from colonies and held in an incubator. Newly emerged bees were marked with acrylic paint on the abdomen, thorax or head. Marked bees (>24hrs old) were placed in test colonies. One hundred 7 day old marked bees were removed from test colonies and caged on a 13.5 cm x 20.5 cm frame of comb. These cages were held in an incubator at 32°C. Two controls, consisting of bees from uninfested colonies, were maintained: one with pollen pats and sugar water and one with only sugar water. Two test groups of bees from an
80-100% infested colony were maintained in the same manner. Sugar water was supplied *ad libido* and pollen consumption was measured daily by weighing the small pollen pat given the test and control groups at the beginning of the test period. Two pats isolated from bees also were weighed. This experiment was repeated a second time using 3 day old bees.

Hemolymph samples were taken from the first trial and analyzed for protein levels using the Bradford method.

**RESULTS**

In this experiment no differences in hemolymph protein levels or in pollen consumption were apparent between infested and uninfested bees (Figs. 10. 1. and 10. 2, and Table 10. 1). Bees held in the above manner and given only sugar water did have lower hemolymph protein than bees given pollen.

**DISCUSSION**

Bees deprived of pollen had lower levels of hemolymph protein than bees that were allowed to feed on pollen, but there were no measurable differences between *A. woodi-*infested bees and uninfested bees. Rates of feeding by mite-infested bees were not different from rates of feeding by uninfested bees. Differences may not have been detected because bees held in this manner were under no physiological stress (i.e. they did not have to care for brood). Therefore, the infestation made little overall difference to them. Results suggest that the low protein levels seen in fall bees from hive 1 are the result of pollen deprivation, because this colony had recently been moved from a quarantine situation (flight room) to a free flight situation at a time when flowers were becoming scarce. Workers from
this colony were unfamiliar with the floral sources present at the
time of conversion of the colony from quarantine to free flight.
Without some familiarity with floral sources, these bees apparently
were unable to gather adequate stores rapidly enough to recover from
the stress of being in a flight room before our testing was carried
out.
Table 10.1. Hemolymph protein levels for infested and uninfested bees held in cages without pollen (IWOP and CWOP) and for infested and uninfested bees held in cages and fed pollen (IWP and CWP)

<table>
<thead>
<tr>
<th>Experimental time in weeks</th>
<th>Age of bees in days</th>
<th>CWP</th>
<th>IWP</th>
<th>CWOP</th>
<th>IWOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
<td>8.26</td>
<td>8.26</td>
<td>8.26</td>
<td>8.26</td>
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<tr>
<td>1</td>
<td>14</td>
<td>20.28</td>
<td>14.10</td>
<td>8.60</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.90</td>
<td>15.60</td>
<td>11.60</td>
<td>6.74</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>12.50</td>
<td>12.86</td>
<td>3.74</td>
<td>6.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.74</td>
<td>11.18</td>
<td>6.12</td>
<td>4.44</td>
</tr>
</tbody>
</table>
Fig. 10. 1. Consumption of pollen by bees caged at 7 days of age (solid line uninfested; dashed line infested).
Fig. 10.2. Consumption of pollen by bees caged at 3 days of age (solid line uninfested; dashed line infested).
CHAPTER 11

COMPARISONS OF WINTER MORTALITY BETWEEN AN 80% A. WOODI-INFESTED COLONY AND TWO MINIMALLY INFESTED COLONIES

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ABSTRACT

Infested colonies had higher death rates. More bees caught in outer cage of trap suggests cold tolerance of infested bees is lower than uninfested bees.

INTRODUCTION

Tracheal mites have been reported to reduce the life span of infested worker bees of Europe and Britain (Bailey 1958; Giordani 1965). No work has reported the effect on uninfested members in colonies with moderate to heavy tracheal mite infestations. This experiment was intended to confirm the shortened life span for infested bees and determine whether or not death rate of uninfested bees in infested colonies was greater than for bees in uninfested colonies of the Pacific Northwest.

MATERIAL AND METHODS

Brood was placed in an incubator and 700 to 1200 emerging bees (>24 hours old) were marked daily in September 1989 and placed in four test colonies. Two colonies were infested at 80% and 40% levels and two which served as controls were 6 and 9% infested. 'Norm Gary dead bee traps' were placed on the colonies at the time marked bees were introduced. These traps were designed by Gary (1960) and form a series of two screen cages on the hive entrance. The first fits the hive entrance and is open directly above the entrance allowing arrival of foragers and departure of bees without loads. A departing bee with a load, such as a dead bee, cannot fly up and out this opening because the weight of the load demands a more gradual take off. They crawl into the second cage 4 inches wide and 12 inches high carrying their burden. They attempt to exit through the top of
this cage which has a screen mesh large enough for one bee to get through, but not large enough for the burden. The burden is consequently dropped as the bee exits. This second cage can be lifted off the first one and the dead bees removed (Fig. 11. 3). Dead bees were collected daily from these traps and the total number of marked and infested individuals were recorded. The number and species of Acarapis found on these bees also was noted. Large numbers of live bees were collected in traps in February, and these also were counted. The remaining marked bees were recovered from within the colonies in March.

RESULTS

The 80% infested colony died before results could be obtained. However, there were nearly twice as many dead bees recovered from the trap of the remaining infested hive as from traps placed on the two control hives (Table 11. 2). Recovery of marked bees was considered to be too low to reach any conclusions. Twenty two percent of the bees recovered in the trap on the infested colony in February were live individuals that had been unable to find the colony entrance before becoming chilled. Live bees collected in the traps on the two control hives at the same time amounted to only 9 and 3% of the total individuals.
DISCUSSION

We were unable to recover enough marked bees to draw conclusions on length of a worker bee's life. A major obstacle to data collection in this type of study is that an estimated 90% of non-surviving colony members die outside the colony (Gary 1960, 1989). However, more dead bees were recovered from the infested test colony overall than from the control colonies. Changes in the bee population of these colonies could not be quantified in winter and comparisons between dead bees recovered as a function of the total population of a colony was not possible. For example 400 bees dying in a colony with 40,000 individuals is not as severe a loss as 400 bees dying in a colony with 4,000 individuals. The bee populations of the infested hive was dwindling during the winter months at what appeared to be a more rapid rate than the control colonies and consequently it perished in March. Thus, dead bees recovered from the infested colony probably represented a higher percent of the total colony population than dead bees recovered from the control colony. It appears that infested and uninfested individuals from infested colonies are dying sooner than individuals from uninfested colonies.

Apparently some bees returning to their colony on which a dead bee trap was placed landed on the upright cage and crawled through the top screen and into the cage. From the cage many were unable to find the hive entrance and had to remain outside the colony. The increase in dead bees recovered from traps during the months of January and February was probably due to more 'flight days' during these months. Days suitable for bee flight in winter are likely to
be very close to minimal flight temperatures. Because bees will not defecate inside the hive, days warm enough for flight attract many colony members outside the colony for defecation flights. Winter days usually cool very quickly in the evenings and bees that had tried to returned to the hive through the cage of the trap may have become chilled before they were able to get back into the hive. More live bees recovered from the trap on the infested colony than from the traps on the control colonies suggests that infested bees may be more susceptible to chilling.
Table 11.1. Dead bees recovered from dead bee traps for two minimally infested colonies and one heavily infested colony during November 1988 through February 1989.

<table>
<thead>
<tr>
<th>month</th>
<th>%infested</th>
<th>control colony 1</th>
<th>control colony 2</th>
<th>infested colony</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>total dead</td>
<td>total live</td>
<td>total marked</td>
</tr>
<tr>
<td>NOV</td>
<td>6</td>
<td>246</td>
<td>----</td>
<td>9</td>
</tr>
<tr>
<td>DEC</td>
<td>2</td>
<td>233</td>
<td>----</td>
<td>13</td>
</tr>
<tr>
<td>JAN</td>
<td>9</td>
<td>560</td>
<td>----</td>
<td>5</td>
</tr>
<tr>
<td>FEB</td>
<td>4</td>
<td>766</td>
<td>69</td>
<td>2</td>
</tr>
<tr>
<td>NOV</td>
<td>2</td>
<td>320</td>
<td>----</td>
<td>18</td>
</tr>
<tr>
<td>DEC</td>
<td>7</td>
<td>177</td>
<td>----</td>
<td>21</td>
</tr>
<tr>
<td>JAN</td>
<td>8</td>
<td>457</td>
<td>----</td>
<td>3</td>
</tr>
<tr>
<td>FEB</td>
<td>1</td>
<td>602</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>NOV</td>
<td>68</td>
<td>576</td>
<td>----</td>
<td>4</td>
</tr>
<tr>
<td>DEC</td>
<td>58</td>
<td>372</td>
<td>----</td>
<td>7</td>
</tr>
<tr>
<td>JAN</td>
<td>60</td>
<td>1077</td>
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<tr>
<td>FEB</td>
<td>57</td>
<td>1059</td>
<td>232</td>
<td>7</td>
</tr>
</tbody>
</table>
Fig. 11.1. Norm Gary dead bee trap
SUMMARY

Tracheal mite parasitism of a honey bee colony results in a steady premature death of its worker honey bees. This leads to eventual change in colony social structure, e.g. increased death rate of foragers causes increasingly younger bees to be recruited from hive duties into foraging. The continual premature loss of workers leads to a diminution in the colony’s accumulation of ‘resting bees’ and a decrease in its ability to rebound from stress.

Human interruption of the natural cycle of honey bee colonies probably interferes with the mechanism by which tracheal mites and bees are able to maintain stability. Honey bees normally produce new colonies by swarming but with the invention of the movable frame hive in 1850, this process may now be prevented. Swarming reduces the number of bees in a colony, therefore, it is to man’s advantage to prevent swarming because a colony produces more honey when the bee population is high. Man has gained control over the bee population, but in the process has upset the balance between the bees and their tracheal mite parasite.

The female-biased tracheal mite sex ratio is probably a sampling ratio rather than a representation of a primary sex ratio. Death of older bees must remove some of the sample units (bees) that would have had males mites in their tracheae, while migration of females mites would augment the female count. Sampling bees only from within the colony may result in recovery of large numbers of young bees, which have only female mites.

Tracheal mite transmission studies suggest that mites move more quickly into the tracheae during the day than at night. We suspect
that daytime colony humidity is lower than nighttime humidity. If mites are sensitive to low humidity they may need to find a trachea more quickly during daytime migration than at night.

Tracheal mites feeding in honey bee tracheae cause an amyloid material to accumulate on the hemolymph side of the infested tracheae, possibly a physiological response of the bee host to mite feeding. Mite feeding seems to take place mostly within the trachea and not during migration.

Physiological changes in infested honey bees as compared to uninfested individuals of the same colony were difficult to determine, however, differences between infested and uninfested colonies were apparent.


Morgenthaler, O. 1931. An acarine disease experimental apiary in the Bernese Lake-District and some of the results obtained there. Bee World 12: 8-10.


