

The relationship between *Varroa jacobsoni*
Oud. (Mesostigmata : Varroidae) an
ectoparasitic mite and its host *Apis mellifera*
L. (Hymenoptera : Apidae), the honeybee.

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In Loving memory of

Mrs. Beatrice Maude Jenkins

‘Mam’

1903-1998

“Live in hope...”.

CHAPTER	TITLE	Page No.
	Contents	i
	Acknowledgements	ii
	Aims & Objectives	iv
	Abstract	vii
1.	Introduction	1
2.	Preferential distribution of <i>Varroa jacobsoni</i> on overwintering honeybee workers and changes in the level of parasitism.	56
3.	Inter-host transfer and survival of <i>Varroa jacobsoni</i> under simulated and natural overwinter conditions.	81
4.	The effect of <i>Varroa jacobsoni</i> infestation on adult worker honeybee emergence weights and their protein, lipid and carbohydrate levels.	119
5.	<i>Varroa jacobsoni</i> as a transmitter of exogenous substances between honeybees.	177
6.	The transmission of Deformed Wing Virus between honeybees by <i>Varroa jacobsoni</i> .	205
	References	227
	Glossary	271
	Appendix (Published papers)	276

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AIMS

The main aim of the project was to investigate the biology of the mite *Varroa jacobsoni* and the host-parasite relationship, between it and its host *Apis mellifera*. Many aspects of the mite's biology are unknown, and the project therefore attempted to focus on problems which have either received little attention, where contradictory conclusions have been drawn in the literature, or where a consideration of more factors simultaneously has never been undertaken. The host-parasite relationship was studied with a view to understanding how effects on individual bees related to the effect on the honeybee colony as a whole.

Although varroa infested colonies frequently die during the winter, very little research has been done previously on the bee-mite relationship during this time. An understanding of the distribution of the mites, within the cluster, on individual bees and aspects of their behaviour to determine whether the parasite displays any selectivity was therefore deemed to be important.

About 50% the bees in a cluster die overwinter even in non-infested colonies. This could have serious consequences on the mite population and it has been argued by some researchers that mites normally die with their host. These workers presented little data to support their reasoning and a series of

experiments were therefore designed to determine overwinter mite mortality and factors affecting their survival.

It is known that varroa feed on the haemolymph of their hosts, and as a consequence, the protein titres of the bees are diminished. However, no previous investigation has been carried out to determine the effects of the mites on the carbohydrate and lipid titres of bees. Additionally, although varroa has been associated with deformity in bees for a long time, no definitive explanation of how the mites cause such deformity has been forthcoming. Thus another of the aims was to attempt to determine whether the drain on the hosts metabolic reserves alone was sufficient to explain the deformities observed.

The possibility of varroa introducing exogenous material into bees was proposed in the 1980's, and although this has been convincingly shown to take place between bee brood, no work has been carried out on inter-adult transmission of exogenous material. Thus an attempt was made to obtain evidence that varroa is a potential vector of exogenous material between adult bees.

If it could be demonstrated that varroa transmitted exogenous material between all stages of the honeybee life-cycle, then varroa could potentially be a

vector of pathogens. This was assessed by investigating the role of varroa as a vector of Deformed Wing Virus (DWV). DWV is lethal pathogen which causes morbidity and mortality in honeybees, but it has never been convincingly demonstrated that varroa can routinely vector this pathogen.

ABSTRACT

Varroa jacobsoni is a highly specialised parasite of *Apis* spp, which ultimately causes colony death. A major contributing factor is their haematophagous habit. It was shown using radioactive tracers that mites consume about 0.67µl of haemolymph every 24 hours. When feeding on overwintering workers, the mites preferentially occupy the left, 3rd and 4th intersegmental tergites. This region is adjacent to the anterior-mesial ventriculus, where nutrients are absorbed.

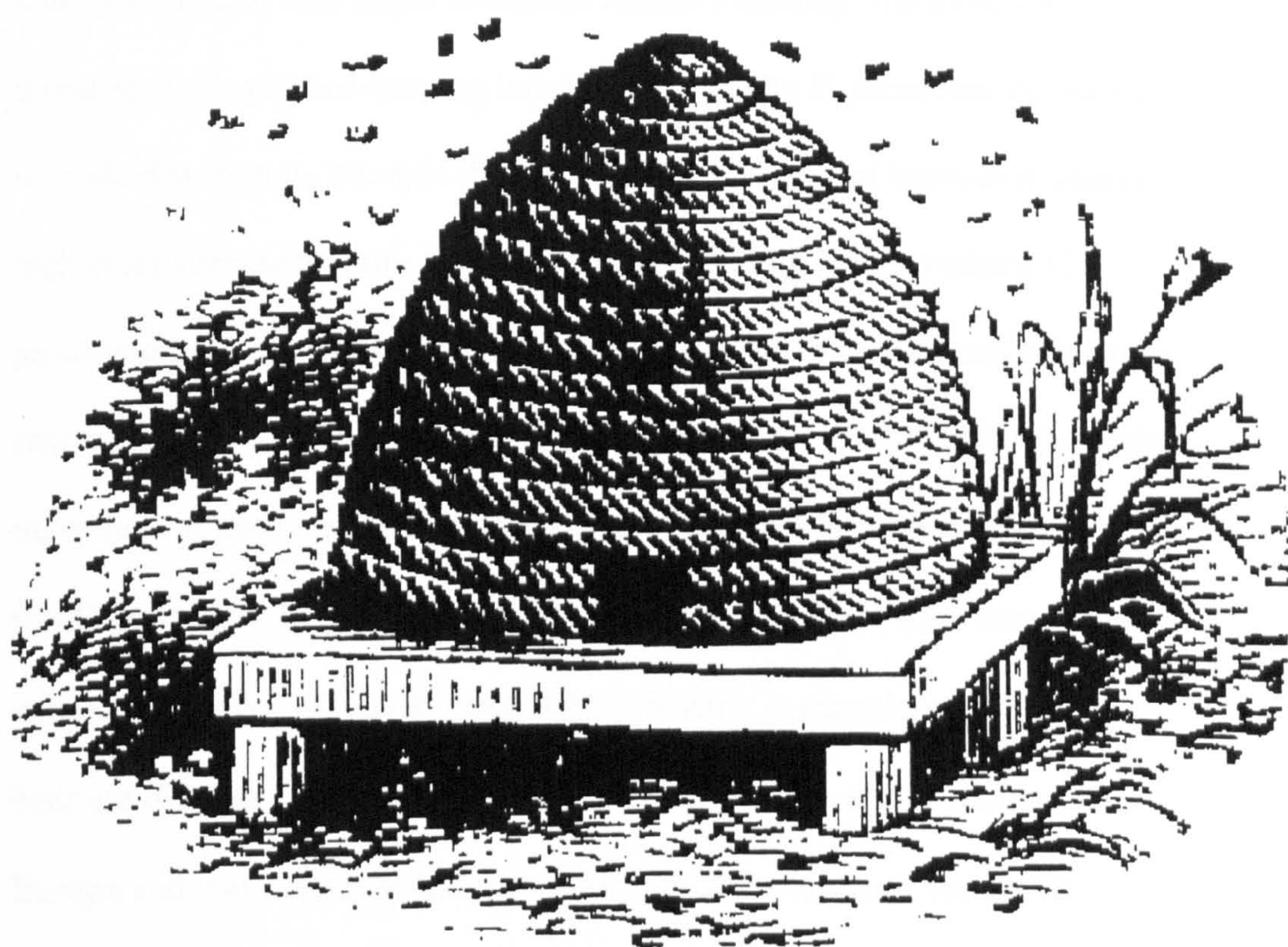
The mites caused significant reductions in the body and haemolymph protein titres of emerging worker bees. Carbohydrate levels were only significantly affected in the abdomen and the mites had no effect on lipid concentrations. Mites also reduced the live weight and water content of emerging worker bees. All the above parameters were negatively correlated with increasing parasitosis. Additionally, worker bees were more likely to emerge deformed with increasing parasitosis.

Using radioactive tracers, it was demonstrated that not only did the mites acquire the tracer from the host, but they also transmitted the tracer into other hosts. Whether the route of transmission was salivarial or via regurgitated gut contents was unclear. It was also demonstrated that varroa acted as a vector for Deformed Wing Virus (DWV).

V.jacobsoni are obligate ectoparasites of honeybees, and can only survive for a few days away from their hosts. Within a winter cluster, mites were found to rapidly transfer off dead / dying hosts onto live hosts. Moreover, mites were shown to regularly transfer between live bees within a cluster. This latter finding coupled with their ability to vector viruses has important implications in disease transmission and thus on colony survival.

If a parasitised honeybee was to die and descend through the cluster the mites could maximise their survival time by feeding on the dead host. This finding has implications in the reinfestation of live bees even if the mites are temporarily removed from the cluster.

INTRODUCTION



Over 40 mite species are presently known to parasitise honey bees (Eickwort, 1988). Six of these parasitise the European honey bee *Apis mellifera* (the European honey bee) (Figure 1.1); namely, *Acarapis woodi* (tracheal mite), *Ac. externus*, *Ac. dorsalis*, *Tropilaelaps clareae*, *Leptus* spp. and *Varroa jacobsoni* (Figure 1.2) (Bailey & Ball, 1991). *V. jacobsoni*, *T. clareae* and *Ac. woodi* are the most damaging species, causing bee death, colony death and thus major economic losses. Presently, the most serious threat to the world bee-keeping industry is posed by *V. jacobsoni* because of its wide distribution, its rapid dispersal between hives and because it causes high colony mortality rates. In many areas (including Britain) where *V. jacobsoni* has become established, colony losses of up to 60% are frequently reported, sometimes rising to 100% (Shabanov *et al.*, 1978). This represents many tens or even hundreds of thousands of colonies in some cases (Griffiths, 1989). In many areas feral *A. mellifera* colonies have been almost entirely wiped out (Kraus & Page, 1995). When it is considered that honey bees are estimated to be worth £7 billion to agriculture and horticulture in Europe and that the honey industry in Britain alone is worth some £12 million, the scale of the *V. jacobsoni* problem is clearly enormous (Walton, 1996). It has been suggested that *T. clareae* poses potentially an even more serious threat than *V. jacobsoni* (Atwal & Goyal, 1971; Burgett *et al.*, 1983). Fortunately it has not yet shown the same propensity for dispersal as *V.*

jacobsoni (Burgett *et al.*, 1983; Burgett & Akkratanakul, 1985) and its known distribution thus far is limited to Indonesia, Philippines, Thailand, Hong Kong (Matheson, 1995) and as of 1995, Nepal (Matheson, 1996).

V. jacobsoni presents the beekeeping industry with a two-fold problem. Firstly, in the wake of *V. jacobsoni* being identified in an area restrictive regulations are normally rapidly enforced which prevent beekeepers from moving bees to profitable areas of forage and from moving or selling equipment which has come into contact with *V. jacobsoni* infested bees (Matheson, 1994). Secondly, *V. jacobsoni* infestation is associated with the decline and, ultimately the demise of bee colonies (Needham, 1988).

1.1 The discovery, classification and spread of *V. jacobsoni*

V. jacobsoni is an obligate ectoparasitic, epizoötic^{*1} mite of honey bees (Eickwort, 1988). According to Dietz & Hermann (1988) it is taxonomically classified as follows

Class	Arachnida
Subclass	Acari
Order	Acarina (Parasitiformes)
Suborder	Mesostigmata
Group	Gamasida

¹ Words marked with an asterix are defined in the glossary

Family	Varroidae (Delfinado & Baker, 1974)
Subfamily	Hypoaspidinae
Genus	<i>Varroa</i> Oudemans, 1904
Species	<i>V. jacobsoni</i> Oudemans, 1904

The only other species in the genus *Varroa*, *Varroa underwoodi* lives on *Apis cerana* (the Asian honey bee) in Nepal, but its biology is hardly known (Delfinado-Baker & Aggarwal, 1987).

V. jacobsoni was first discovered in the brood of *A. cerana* (Figure 1.3) in Java in 1904 and described by Oudemans (1904), who suggested that it may be a parasite. For the next 40-50 years the mite was rarely mentioned in the scientific literature (Buttel-Reepen, [1918]; Zander, [1930]; Örösi-Pal, [1939]). During this time it was suggested that *V. jacobsoni* was not a parasite but survived on bee faeces and discarded brood food (Grobov, 1977; Rademacher & Geiseler, 1986). In 1951 *V. jacobsoni* was '(re)discovered' on *A. cerana* on Singapore Island and erroneously described as a new species and named/renamed *Myrmozercon reidi* (Gunther, 1951).

V. jacobsoni was first observed on *A. mellifera* in 1958 in Japan (Mikawa, 1985; Bailey & Ball, 1991), although it has been suggested that the mite may have been associated with *A. mellifera* since 1915 (Takeuchi & Sakai,

1986) and possibly even earlier as *A. mellifera* was taken to Japan in 1876 (Tanabe & Tamaki, 1986). The movement of *V. jacobsoni* from *A. cerana* to *A. mellifera* probably first occurred in China or East Russia where *A. mellifera* and *A. cerana* became artificially-sympatric* because of bee-keeping practices (Ruttner & Maul, 1983).

Since its discovery, *V. jacobsoni* has spread rapidly throughout most the world owing to migratory beekeeping practices, beekeepers introducing bees into new countries, bee drifting, swarming, robbing, (Crane, 1978; DeJong *et al.*, 1982a; Sakofski & Koeniger, 1986; Bradbear, 1988; Rademacher, 1991) and possibly via flowers, anthophilous (flower-feeding) insects and honey bee predators and nest visitors such as wasps and cuckoo bumble bees of the genus *Psithyrus* (Kevan, *et al.*, 1990). Presently only Australasia and a few isolated islands across the world such as Hawaii are thought to be free of *V. jacobsoni* (Matheson, 1996), although they are unlikely to remain so, if they are now! Indeed, in 1994 (Banks, 1994) varroa was tentatively recorded in the Australian Torres Strait. If correct, this means that every continent is now infested by the mites.

Even though *V. jacobsoni* was discovered at the turn of the century, it was not until the early 1960s that it was recognised as being a highly destructive parasite of *Apis mellifera* (Dietz & Hermann, 1988). However,

by the time this was realised *V. jacobsoni* had been inexorably moved around the world, with the unwitting help of beekeepers. Today it is considered to be the most serious threat to world beekeeping (Shabanov *et al.*, 1978).

1.2 Morphology of *V. jacobsoni*

Adult female *V. jacobsoni* are dorsoventrally flattened with slightly convex idiosomas* which are orange-red in colour. They are wider than long, measuring ~1100µm long by ~1600µm wide (Akratanakul, 1976), and have a mean weight of ~0.34mg (Smirnov, 1978; chapter 3). Adult male *V. jacobsoni* (Figure 1.4) are smaller than the females (being about 820µm long and 800µm wide), have a rounder idiosoma which is white in colour and not heavily sclerotised (Delfinado-Baker, 1984; Steiner, 1988).

Mouthparts of V. jacobsoni

General morphology of the Mesostigmatid mite gnathopodal cavity

The bodies of mites are divided into two main regions, a posterior idiosoma within which the viscera and musculature are located and an anterior gnathosoma* (gnathopodal cavity in Mesostigmata). The gnathopodal cavity is so called because the coxae of the first pair of legs are fused with the gnathosoma. It is essentially a sensory-trophic structure (Evans, 1992) comprised of the subcapitulum, the pedipalps (palps) and

chelicerae. The subcapitulum is comprised of the palpcoxae, labrum, mouth, pharynx and the subcheliceral plate (or shield) (Figure 1.5).

The palpcoxae extend anteriorly and superiorly and come into close contact along their margins to form the floor and ventral walls of the subcapitulum. The floor of the subcapitulum is not fused, but forms a groove called the median capitular groove. Lying above this V-shaped trough, forming a 'roof' to the mouth, is the subcheliceral plate, which extends posteriorly beyond the palpcoxae and back into the idiosoma. Adjacent to the anterior margin of the subcheliceral plate is the labrum. This extends anteriorly beyond the mouth and over-lies the pre-oral cavity. Within the V-shaped trough resides the pharynx, suspended by various supporting structures. The whole of the subcapitulum lies inferiorly within the gnathopodal cavity.

Ventral to the V-shaped median capitular groove and on the outer surface of the Gamasida is the tritosternum. The tritosternum consists of a base and anteriorly directed lacinae (projections). Attached to the inner surface of the tritosternum are two bands of muscle which afford it with limited lateral and vertical movement (Wernz & Krantz, 1976).

Superior to the subcapitulum are the paired chelicerae (Figure 1.6). The chelicerae are morphologically highly variable, trophic (feeding) organs, consisting of three parts (articles). The posterior-most article (basal article)

to which strong retractor muscles are attached, the middle article, which articulates with the anterior margin of the basal article and is dorsally extended in an anterior direction to form a fixed digit, and finally a third article (movable digit), which lies inferiorly to the fixed digit and articulates with the anterior margin of the middle article (Figure 1.5b).

The palps are segmented leg-like sensory structures which extend beyond the anterior margin of the gnathosoma (Milani, 1990). Their coxae form the floor and lateral walls of the subcapitulum.

Gnathopodal cavity of Varroa jacobsoni

The gnathopodal cavity of varroa differs from the general Mesostigmatid plan only in that the fixed digit of the second article of the chelicerae is vestigial. The significance of this modification is discussed below.

Situated antero-superiorly to the brain of varroa are a pair of salivary glands (deRuijter & Kaas, 1983). Two salivary ducts originate from the salivary glands and distally terminate above the labrum (but below the chelicerae) via hollow styli (Gelbe & Madel, 1988; Griffiths, 1988) within the gnathopodal cavity. According to Gelbe & Madel (1988), small drops (presumably saliva) can occasionally be observed forming on the styli.

Feeding behaviour of Varroa jacobsoni

Varroa are stenophagous* and feed on the haemolymph of bees. This they obtain by piercing the inter-segmental membranes of adult bees or the thin cuticle of pupae using their chelicerae, which are 15µm long (Bruce *et al.*, 1988; Bruce *et al.*, 1991b). The exact mechanism of haemolymph acquisition has never been clearly elucidated, but is likely to be by telmophagy* (Lavoipiere, 1965). Strick & Madel (1988) state that the mites use their chelicerae to 'rasp' a wound, first by inserting the tip of the dentate chelicerae into the membrane and then by making 'alternate sawing movements of the chelicerae'. This description requires the mites to have the ability to project the chelicerae forward, which according to Akimov & Yastrebstov (1983) is not brought about by muscular action because varroa do not possess cheliceral protractor muscles. However, by the combined action of hydrostatic pressure maintained by dorsoventral idiosomatic muscles, and individually innervated cheliceral retractor muscles, it is possible for these alternate sawing movements to be undertaken (G.O.Evans, pers.comms; Evans, 1992). According to Strick & Madel (1988a) and Kurscheid (1986), the puncture wounds so formed, measure some 10-15µm wide (max 30µm), whereas the exuding droplet normally measures twice that of the wound in diameter.

To date, no detailed direct observations on live-varroa feeding have been carried out, other than that carried out by Strick & Madel (1988a), who froze feeding mites *in situ* for SEM examination. However, considering the Gamasida are unique in having a tritosternum, which has been shown to have a trophic function in other gamasids (Wernz & Krantz, 1976), then in the absence of evidence to the contrary, it is reasonable to assume that they feed in a similar manner to the other members of the group. Once the fluid food (haemolymph of the bee in this case) is accessed, it flows freely out from the haemocoel of the host (being under pressure). As bee blood does not readily coagulate (Grégoire, 1955; Gilliam & Shimanuki, 1970), fluid escapes easily. The haemolymph then surrounds the chelicerae and other mouthparts which are in close proximity to it. Excess fluid flows posteriorly on the ventral side of the mite to the base of the tritosternum. From here it flows anteriorly and into the the V-shaped groove. Haemolymph reaching the mouth is then ‘sucked’ in by the muscular pharynx of the mite, and later the excess fluid around the tritosternum is also imbibed.

1.3 Adaptations of *V.jacobsoni* for life on *Apis* spp.

The whole life-cycle of *V.jacobsoni* takes place in association with honey bees, including dispersal (Sakofski, 1980; Rademacher, 1991), feeding and reproduction (Saltchenko, 1966). According to Ruttner (1988), *V. jacobsoni*

probably first evolved as a parasite of *A. cerana* sometime after the *A. cerana*-*A. mellifera* species divergence which occurred during the Pleistocene (2 - 0.011 million year ago) epoch. This theory is based on the fact that from the Pleistocene up until the turn of this century, *A. cerana* and *A. mellifera* were allopatric* in their geographical distributions, and varroa was exclusively found on *A. cerana* until this century (Marcangeli *et al.*, 1992b). Therefore it can only be concluded that the mite evolved initially as a parasite of *A. cerana*, sometime after *A. mellifera* and *A. cerana* evolved as two separate species.

Before becoming a true parasite of bees it is possible that, like the other mesostigmatid mites that occupy honey bee hives, that *V. jacobsoni* may have either fed on nest provisions such as pollen or honey, larval food, bee faeces, injured bees, exudates from the brood or were predators of other mites such as the Astigmata (Eickwort, 1993).

The pathological effects of *V. jacobsoni* on *A. cerana* are minor (Moritz & Hänel, 1984), the reason being that the mite population never exceeds about 800 mites per *A. cerana* colony, with the mean number of mites per colony being only 69.8 mites (Rath & Drescher, 1990; Boecking, 1992; Martin, 1997a). Several mechanisms have been shown to contribute to this limited mite population including :

- 1) mites only reproduce within drone brood cells of *A. cerana* which are produced in considerably smaller numbers (~30 cells per day) than worker cells (~388 cells per day) (Rath, 1993);
- 2) *A. cerana* are very efficient at removing *V. jacobsoni*-infested worker cells (Rath, 1991; Boecking, Rath & Drescher, 1990);
- 3) multiple *A. cerana* drone cell infestations frequently result in the death of the host and, because drone cells are seldom cleaned out, the mites die within them (Koeniger *et al.*, 1983; Koeniger, 1987; Rath, 1991; Boecking, 1992);
- 4) *A. cerana* has an effective defensive grooming behaviour which contributes to limiting the mite population on adult bees (Peng *et al.*, 1987).

The relative importance of each of these factors in controlling the mite population has not been resolved, although it is now widely accepted that the case for grooming behaviour as a defensive strategy has been overstated (Boecking, 1992; Boecking *et al.*, 1993).

V. jacobsoni exhibits a number of morphological and behavioural adaptations which enhance its survival on *A. cerana* and *A. mellifera*. The ellipsoid shape of adult female mites (Figure 1.2) enables them to secrete themselves between the abdominal plates of adult bees (chapter 2). The mites are therefore hidden from grooming bees, can easily access host haemolymph, and have a reduced risk of becoming detached from their host when it flies (Bautz &

Coggins, 1992). This shape, as was pointed out by Fain (1969) and later Arlian & Vyszenski-Moher (1987), is a convergent characteristic and is seen in other mites which specialise in parasitising “scaled” hosts, such as reptiles or other arthropods. For example, *Discomegiscus pectinatus* which parasitise millipedes (Trägårdh, 1912), *Omentolaelaps mehelyae* which parasitises snakes (Fain, 1969) and *Geckobia spp.* which parasitise reptiles (Hänel, 1988) are all distinctly wider than long.

V. jacobsoni have strong forward-pointing legs which are laterally flattened (Figure 1.7), enabling them to secure attachment to the adult bees. At the distal ends of the legs are pretarsal pads which are “sucker-like” in appearance (Liu, 1982; Bautz & Coggins, 1992). Adult female *V. jacobsoni* also have strong backward-pointing opisthosomal^{*} setae, which provide the mite with additional anchorage by enmeshing with the cuticular hairs of the host. These setae make dislodging the mites from the bees extremely difficult (Liu, 1982 citing Polyakov *et al.*, 1975).

As is typical of the Gamasida, *V. jacobsoni* have a single pair of latero-median spiracles between the coxae of the third and fourth pair of legs (Strube & Flechtmann, 1985; Bautz & Coggins, 1992). Extending away from these spiracles are movable sclerotised cuticular extensions (300µm long [Richard *et al.*, 1980]) called peritremes (Figure 1.8) which can be articulated, by hydrostatic pressure changes, away from the body. Several functions have been proposed for

the peritremes. Strube & Fletchmann (1985) and Liu (1986) suggested that they facilitate plastron^{*} respiration and / or act as buoyancy-control devices. Ramirez (1987) postulated that they act like snorkels. The purpose accordingly would be to enable the mites to immerse themselves in the semi-liquid brood food (Infantidis, 1988; Boot *et al.*, 1992) and continue gaseous exchange. However, Pugh *et al.* (1992) have proposed an entirely different mechanism of gas exchange in varroa, pointing out that when the mites are immersed in the brood food, the bee larvae lie immediately above them and on top of the food, such that no air-space is available for 'snorkel' gas exchange. Moreover, Pugh *et al.* (1992) have calculated that peritremes themselves could not form a plastron as is the case in some aquatic mite species (Hinton, 1971; Krantz, 1974). Instead the external plastron is formed by an airfilm trapped between the legs and the margins of the dorsal shield of the mite (Pugh *et al.*, 1992). Gas is exchanged between the plastron and the mite at the base of the peritreme (via the outer stigmatic orifice). The function of the peritreme being to facilitate carbon dioxide removal whilst preventing water loss.

By being able to immerse themselves in liquid brood food, mites are less likely to be detected and removed from brood cells by the attending bees (Boecking & Drescher, 1990; Boecking & Drescher, 1991; Rath & Drescher, 1990). *V. jacobsoni* respond to contact with brood food by ceasing all discernible movement (Ramirez, 1987; Rath, 1993). This is a common response

of many acari to submergence (Hinton, 1971; Pugh *et al.*, 1987a; Pugh *et al.*, 1987b). Mites are liberated from the brood food shortly after the cell is capped-over when the developing larva consumes the remaining food.

Another interesting adaptation of *V. jacobsoni* for parasitising honey bees is that if a queen is removed from a colony or dies, the mites react by dismounting off adult bees and enter into brood cells which are about to be capped (DeJong, 1981). DeJong (1981) pointed out that this heightened reproductive activity by the mites, which has been estimated as a three fold increase in reproduction, coincides with the colony's construction of emergency queen cells, which may be the stimulus for the behaviour. This behaviour is probably a defensive strategy which prevents the mites accidentally leaving the colony with the prime swarm or the after-swarms. That this is desirable can be understood when it is borne in mind that the probability of a prime swarm surviving is very low (0% survival [Lee, 1985], 8% survival [Morales, 1986], 24% survival [Seeley, 1978]). The probability of an after swarm surviving is even lower, so it is better to remain with the established colony which has the highest probability of survival.

On *A. cerana* (Figure 1.3), the mites reproduce almost exclusively in drone cells, where they are encountered about ten times more frequently than in worker cells (Koeniger *et al.*, 1983; Martin, 1994a), although they may

occasionally reproduce on worker brood (DeJong, 1988). Several theories have been proposed to explain this preference:

- 1) drone cells have a longer post-capping time (14-15 days) than worker cells (12 days) and therefore a longer period in which the mite offspring can mature (Moritz & Hänel, 1984; Hänel & Koeniger, 1986; Woyke, 1989; Büchler, 1990).
- 2) Drone cells are taller than worker cells and therefore project above the level of surrounding worker cells. This may facilitate recognition by the mites because tall/protruding or worker cells with a larger diameter (worker cell diameter ~ 5.2mm, drone cell diameter ~6.2mm [Frisch, 1974]) are more attractive to *V. jacobsoni* than those of normal size/height (DeJong & Morse, 1988; Message & Gonçalves, 1983; Message & Gonçalves, 1995). This was found to be so even if their depth was artificially made smaller than the depth of normal worker cells (Ruijter, 1986; Ruijter & Calis, 1988), which indicates that it is the height of the cells in relation to other cells that provides the stimulus for selection and not the internal depth of the cell which could also be related to cell height.
- 3) 9-10 day old drone larvae are about 60% heavier than worker larvae of the same age (Jay, 1963). Consequently drone larvae are fed correspondingly more food (Levenets, 1956). As developing larvae depend entirely on nurse bees for food, the nurse bees visit drone brood cells 3-8 times more often than worker cells to meet their different nutritional requirements. This makes drone brood cells more readily accessible to varroa, or more likely to be encountered by them

than worker cells (Boot *et al.*, 1995c). It is interesting to note that towards the end of the drone-production season, the rate of invasion of varroa into drone cells decreases. This may be a behavioural response which enhances their survival, because at the end of the drone rearing period, drones are often tended less by nurse bees and so their mortality rates increase (Otten, 1986; Fuchs, 1990).

4) Drone cells are susceptible to invasion for about three times longer than worker cells. This is because drone brood becomes attractive to adult female *V. jacobsoni* for 40-50 hours before it is capped, whereas worker brood is only attractive for 15-20 hours before capping (Infantidis, 1988; Boot *et al.*, 1992).

According to Boot *et al.* (1995c), because drone cells are 1.7 times larger than worker cells, *V. jacobsoni* are more likely to be brought into close contact with drone cells than worker cells although as worker cells vastly outnumber drone cells it is difficult to appreciate the logic of this reasoning. Consequently the probability that a mite may encounter a suitable drone cell at any given cell visit is higher than that of worker cells (Boot *et al.*, 1992; Boot *et al.*, 1995a)

5) Drone larvae also release volatile chemicals which attract the mites (Le Conte *et al.*, 1980; Issa *et al.*, 1993).

According to Rath (1993), the mean temperature within the brood nests of *A. cerana* and *A. mellifera* are similar ($35.1 \pm 0.2^{\circ}\text{C}$, $33.7 \pm 1.5^{\circ}\text{C}$ respectively). This is probably an important contributing factor in enabling *V. jacobsoni* to move from *A. cerana* to *A. mellifera* because mite reproductive success is thermo-sensitive. Bienefeld *et al.* (1995) showed that well-regulated, uniform temperatures within hives were correlated with higher number of offspring reaching maturity per mother mite. Their experimental methodology did not permit their distinguishing whether this trend was as a result of a slower mite development when temperatures fluctuated, or whether the mites were in some way physiologically damaged. Either way, the outcome for the mites once the cell was opened by the host would be equally unfavourable as their immature stages do not survive outside the cells. LeConte & Arnold (1980) and LeConte *et al.* (1990) showed that the optimal temperature for mite prolificity was $32.5\text{--}33.4^{\circ}\text{C}$. Temperatures above or below this were associated with decreased mite reproduction. High temperatures ($>37.5^{\circ}\text{C}$) and high humidities ($>70\%\text{R.H}$) in the cells also halted oviposition by varroa.

In temperate climates, in which honey bees are unable to produce brood over the winter period, *V. jacobsoni* must tolerate a reproductive break. In the UK, during October to December little brood is normally present in the nest, but as daylength increases brood starts to appear in small irregular quantities in colonies. In February and March brood rearing rates increase rapidly and

continue to do so until peak brood rearing is recorded in late summer (Jefferee, 1956; Avitabile, 1978). Such punctuations in mite reproduction are unlikely to have imposed rigorous demands for additional evolutionary adaptations on the part of *V. jacobsoni* because on their original host *A. cerana*, the mites usually reproduce only within the drone brood cells, which are absent for several months (1-4) during the year (Boecking, 1992; Rath, 1993).

1.4 Life-cycle of *V. jacobsoni*

V. jacobsoni overwinter as adult females on worker bees (Rath, 1993) because little or no brood is available at this time in temperate climes (Jefferee, 1956; Avitabile, 1978). During the winter months, they have a lifespan of 5-8 months (Ritter, 1981; Needham, 1988), but during the summer months, they live for only 2 to 3 months (Grobov, 1977; Ritter, 1981; Ruijter, 1987; Calatayud & Verdú, 1994). When bee brood becomes available, fertilised female mites leave adult bees and enter the cells (Figure 1.9). Drone cells are preferred to worker cells by a factor of 7-12 (Sulmanovic *et al.*, 1982; Fuchs, 1990; Boot *et al.*, 1995c), queen cells being very rarely entered (Grobov, 1977).

The female mites select worker cells containing 5 day old larvae (5th instar) and drone cells containing 5-7 day old larvae (Ramirez & Otis, 1986, *citing* Issa pers. comms.). Having entered the cells, the females immediately immerse themselves in the liquid brood food which lies at the base of the cell

(DeJong, 1984; Infantidis, 1988; Boot *et al.*, 1992). There they wait until the cell is capped by worker bees and the larva eats most or all of the brood food thereby releasing the mite (Infantidis, 1988; Morse & Nowogrodzki, 1990; Martin, 1994a). After leaving the brood food, the mites begin to feed on the haemolymph of the larva. 3-4 hrs after the cell is capped, the bee larva begins to spin a cocoon. When the cocoon is completed (~37 hrs after capping [Jay, 1963]) the larva becomes quiescent and is at this time called a prepupa. Prepupal haemolymph has a high titre of Juvenile Hormone III which is thought to partly be responsible for triggering *V. jacobsoni* oogenesis (Hänel, 1983; Hänel & Koeniger, 1986; Milani & Chisea, 1990). 60-70 hours after the cell is capped, the female mite produces an egg, which is deposited on the antero-dorsal cell wall near the bee pupae and cell aperture (Donzé & Guerin, 1994). Eggs measure approximately 400 x 300 µm. During the time that the egg is inside the mother mite, the embryo develops into a hexapod larvae which remains encapsulated within the egg case, therefore the so-called “eggs” of varroa are not true eggs which develop after being laid but egg-cases containing a larva (Shabanov *et al.*, 1978). Other eggs are laid thereafter at 26-30 hr intervals (Infantidis, 1984; Donzé & Guerin, 1994; Martin, 1994a). The first egg always develops into a male (Rehm & Ritter, 1989; Martin, 1994a), the second and subsequent eggs giving rise to females. The egg/larval stage lasts some 30 hours in total, thereafter the larva changes into an octopod protonymph* which moults, shedding the egg

case (Shabanov *et al.*, 1978; Donzé & Guerin, 1994). The protonymph stage lasts 30-60 hours, after which it moults to the deutonymph* stage. After a further 3 days, the deutonymph moults into the adult morph. The total development time from egg to adult is 134 hours for females and 154 hours for males (Rehm & Ritter, 1989; Martin, 1994a).

Adult female mites lay 5-6 eggs per reproductive cycle, producing about 30 eggs during their lifetime (Ruijter, 1987). Up to 4 eggs can survive to maturity before the bee emerges from its cell (Martin, 1994a), but due to offspring mortality, the mean number of female offspring per reproductive cycle may be as low as 1.45 (Martin, 1994a). Ruijter (1987) found that the mean number of reproductive cycles undertaken by *V. jacobsoni* was 5-6, with 7 being the maximum recorded. Between reproductive periods in the brood cells, the adult female mites attach themselves to bees for periods of 3-15 days (Schulz, 1984) and are thereby distributed within and between colonies.

Following a phoretic period, which seldom lasts more than 20 days, on adult worker bees (Hansen, 1991; Boot *et al.*, 1995a) the mites usually re-invade brood cells again. Many factors can influence whether a mite re-invades or not. Boot *et al.* (1994) have pointed out that re-invasion will be influenced by the availability of suitable brood, which must also be accessible to the mites. *V. jacobsoni* are known to parasitise young bees in preference to older bees (Kuenen & Calderone, 1997). This preference may maximise the chances of a

mite to encounter a suitable cell which to invade, because young bees are known to spend most of their time undertaking tasks which bring them into close proximity with the brood, e.g. brood feeding or cell cleaning.

About 20% of all mites that enter brood cells do not reproduce (reviewed by Martin *et al.*, 1997). Why they should enter cells without reproducing is not known. Al Ghzawi (1993), reported that the longer mites remained on adult bees, the fewer mature offspring they reared. This hypothesis was not confirmed, however, by Boot *et al.* (1995c). It is also interesting to note that when several mites infest a single developing bee, the reproductive success of the mites (i.e. the number of offspring per infesting mother mite) diminishes (Fuchs & Langenbach, 1989; Marcangeli *et al.*, 1992a; Donzé *et al.*, 1996).

The male mite is exclusively found within the brood cells. Males do not survive for more than a few hours after the bees have emerged because their thin cuticles and soft bodies render them vulnerable to dehydration and physical damage (DeJong *et al.*, 1982a). Moreover, because their chelicerae are modified as spermatodactyla* (Alberti & Hänel, 1986; Steiner, 1988), to facilitate podospermy*, it is unlikely that they can prepare a feeding site for themselves and therefore feed independently (Akratanakul, 1976). This is not to imply that adult male *V. jacobsoni* do not feed, simply that they are incapable of preparing a feeding hole in the bee cuticle themselves. Within the confines of the brood cells, males take advantage of the feeding site prepared by the adult female mites and

therefore have no need for trophic (modified for obtaining food) chelicerae (Donzé & Guerin, 1994).

Male *V. jacobsoni* are functionally haploid (7 chromosomes) and female *V. jacobsoni* are diploid (14 chromosomes) (Steiner *et al.*, 1982; Ruijter & Pappas, 1983; Donzé & Guerin, 1994). It has been proposed that males, however, are impaternal*, varroas' reproduction possibly being pseudo-arrhenotokous* (Martin *et al.*, 1997) (also referred to as parahaploidy or paternal genome loss, where diploid male embryos either undergo expulsion of the paternal genome at some early stage of embryogenesis or undergo heterochromatization* of the paternal genome with subsequent retention of the male genome in somatic cells [Wrensch *et al.*, 1993]). Male varroa fertilize their sisters within the brood cells (DeJong *et al.*, 1981; Donzé *et al.*, 1996; Martin *et al.*, 1997). This results in *V. jacobsoni* being genetically highly inbred and consequently being genotypically (Biasiolo, 1992) and phenotypically (Delfinado-Baker & Houck, 1989) remarkably conservative. This is probably advantageous to the mites whose environment is highly stable, in the sense that the mites face the same host/competitor-derived pressures and in that the abiotic parameters within hives such as CO₂ levels and temperature remain remarkably constant (Simpson, 1961; Seeley, 1974).

1.5 Pathology, Parasitic Mite Syndrome, Disease Association

The pathology associated with *V. jacobsoni* infestation of colonies which usually leads to their demise is fairly consistent. The whole range of pathological symptoms was termed Parasitic Mite Syndrome by Shimanuki *et al.* (1994), and includes the following:

Imago-associated pathology

- 1) The worker bee population decreases (Grobov, 1977; Martin 1997a).
- 2) Crawling adult bees are commonly seen in front of the hives (Grobov, 1977; Shimanuki *et al.*, 1994)
- 3) The queen is superceded by younger queens (Shimanuki *et al.*, 1994)
- 4) Heightened rates of colonies swarming or absconding* (Woyke, 1976).
- 5) A reduction in bee lifespan. A 50% reduction in adult bee lifespan was reported by Sadv (1976) if bees were infested within 10 days of emergence. DeJong & DeJong (1983) found that Africanized* bees which had been parasitised as pupae had an average lifespan of 13.6 days- which was about 50% that of uninfested bees. Beetsma *et al.* (1980), however, found that, on average, similarly infested bees had about an 11% reduction in lifespan. Schneider & Drescher (1987) reported that bees infested by 3 or more mites died after 20-25 days post-emergence, whereas only 50% of uninfested bees had died over the same period of time. Kovac & Crailsheim (1988) reported

a maximum shortening of lifespan (40%) of bees in late July, although in their paper they state that “an absolute shortening of lifespan is not inevitable” as they recorded highly parasitised bees (8+) living for longer than 30 days.

6) Emerging bees are frequently deformed. Deformities include bees being smaller in overall size, being lighter in weight than their non-parasitised counterparts, having dented thoraxes, shortened abdomens and deformed wings (Figure 1.10) (Choi & Woo, 1974; Grobov, 1977; Anshakova *et al.*, 1978; DeJong *et al.*, 1982b; DeJong & DeJong, 1983; Schneider & Drescher, 1987; Daly *et al.*, 1988; Kovac & Crailsheim, 1988; Koch & Ritter, 1991; Getchev, 1994).

7) Many parasitised bees (especially those exhibiting morphological damage) are inactive or incapable of normal activity and may be a drain on colony resources (Anshakova *et al.*, 1978; DeJong *et al.*, 1982b; Oku *et al.*, 1983; Choi, 1985; Hara *et al.*, 1986; Schneider & Drescher, 1987; Daly *et al.*, 1988; Koch & Ritter, 1991).

8) The ability of bees to navigate may be disrupted which causes disorientation and also leads to loss of colony members (Sakofski, 1990; Ruano *et al.*, 1991).

9) Varroa-parasitised colonies at the beginning of winter have higher metabolic rates than non-parasitised colonies. During the course of winter, however, the metabolic rates of parasitised colonies decrease, so that by the end of winter their

metabolic rates are about 26% below that of healthy colonies (Byzova *et al.*, 1982). This is thought to be due to the loss of ability of bees which were parasitised during development to contribute to colony thermoregulation. Consequently, the other cohort of bees (those not parasitised during development) have to compensate and expend more energy in thermoregulating. The bees then die prematurely and the colony rapidly loses its thermoregulatory ability (Byzova *et al.*, 1982). That overworked bees display a reduction in life-span is not surprising because high physical activity reduces the life-span of many insects (Sohal, 1976; Ragland & Sohal, 1975; Neukirch, 1982).

11) Emerging bees have also been found to have considerably altered biochemical, cellular and histological parameters (chapter 4)

Brood-associated pathology

1) A spotty brood pattern develops, i.e. empty cells are present within the brood cluster, which normally, would be fully-occupied with developing bees.

2) Symptoms resembling European foulbrood, American foulbrood and sacbrood virus may be present.

3) "Scale" formation has been observed. Scales are dead larvae or dead uncapped pupae, which have desiccated and collapsed to the floor of the cell.

4) Brood death

1.6 Factors influencing the intensity of *V. jacobsoni*-induced pathology

A. mellifera colonies infested with *V. jacobsoni* normally die 3-5 years after the onset of infestation (Shabanov *et al.*, 1978; Weiss, 1984; Rademacher & Geisler, 1986; Korpela *et al.*, 1992). Why they are so susceptible to parasitism by *V. jacobsoni* is unclear. However, a number of factors have been shown to be of significance in influencing the severity of the infestation, namely:

- 1) The sub-species of honey bee infested (Engels *et al.*, 1986; Büchler, 1990; Moritz & Mautz, 1990; Büchler, 1994).
- 2) The abiotic conditions, such as climate, altitude, humidity and ambient temperatures (DeJong *et al.*, 1984; Kovac & Crailsheim, 1988).
- 3) The presence of microorganisms associated with the mites/bees. The mites probably act as vectors of bacterial and viral diseases (Bailey & Ball, 1991; chapter 6).
- 4) The availability of resources (i.e. food) to the bee colony (Kovac & Crailsheim, 1988).
- 5) The defensive response of the bees to *V. jacobsoni* infestation. *A. mellifera* are not as efficient at grooming the mites off and damaging them during the process, as are *A. cerana*. Even though grooming may not be the principal factor in mite population control, the fact that it may be carried out more effectively by *A. cerana* must make at least some contribution in keeping the mite population low (Peng, 1988; Fries *et al.*, 1996).

6) The reproductive success of the mites (Harris & Harbo, 1999).

1.7 Honey Bee Biology

Honey bees are eusocial* insects belonging to the genus *Apis*. This genus is taxonomically placed within the tribe Apini*, to which the majority of fossil apids are also assigned. The apini are thought to have evolved by the Upper Oligocene (30 - 27 mya), the genus *Apis* probably evolving rapidly between the Upper Eocene (55 - 30 mya) and the Upper Oligocene, i.e. within about 10 million years, possibly in response to “selective pressures accompanying the development of eusociality in the honey bee line” (Culliney, 1983). Deodikar (1978) and Deodikar *et al.* (1959) proposed that the genus *Apis* evolved in or around India, where its greatest diversity exists today. All bees (including the genus *Apis*) belong to the superfamily Apoidea, which are characterised by relying almost entirely on flowering plants for their nutritional requirements, especially that of proteins derived from pollen. As such, it has been suggested that this group evolved monophyletically in the xeric* interior of the ancient landmass of Gondwana from the Sphecoidea (burrowing wasps) concurrently with the rise of the angiosperms as the dominant flora possibly during the Cretaceous period (130 - 65 mya) or possibly even earlier. (Raven & Axelrod, 1974; Michener, 1979). Being reliant on flowering plant resources, *Apis* spp.

have evolved strategies to ensure that such provisions are available all year round as will be discussed later.

Honey bees form haplodiploid matriarchal colonies consisting normally of 40-80 thousand individuals (Jefferee, 1955; Frisch, 1974). The colonies normally have a single reproductively-competent female termed the queen. The queen is diploid (32 chromosomes), but can lay both diploid and haploid eggs. The haploid eggs develop and give rise to males which are called drones, the diploid eggs develop and give rise to females, either non-reproductive workers or other queens.

Unlike a bumblebee queen, the honey bee queen cannot found a colony on her own. At all times, honey bee queens must be attended-to by worker honey bees to groom, feed, protect and thermoregulate *etc.* on her behalf. Consequently, the colony can only reproduce in such a way that workers are always present whenever a queen is present. In the UK, during late April to July, workers build 15-25 queen cells, in which the old queen lays a single egg. Over the course of 16 days, the eggs are tended to by workers and develop into young queens. About a week before swarming (when the original queen leaves the old colony with worker bees to found a new colony) the worker bees feed the old queen less and she responds by halting egg laying and her ovaries regress. She therefore loses weight and regains her ability to fly again (Allen, 1955, 1960).

About a day after the queen cells are capped over, the old queen leaves the colony accompanied by about 60% of the worker bee population in attendance. Normally it is the younger worker bees that join the queen in the swarm (Figure 1.11). There are two reasons that young workers accompany the queen as opposed to older workers. The first is that the young workers have a longer remaining life-span than the workers that remain behind in the old colony, which imparts to the swarm the best chance of establishing itself (i.e. having enough vigour to build a new nest, rear brood to replace them *etc.*). Secondly, back in the old colony, there will be emerging other young workers to replace the ageing population which remained behind. The primary swarm (composed of the queen and accompanying workers) eventually locates another suitable nesting site, by means of scouts, and there construct a new nest. Back in the mother colony, in which about 40% of the original worker population remain, the first young queen to emerge usually does one of two things: she could either fly out with a proportion of the worker population (and thus producing a secondary or after-swarm) or she could attempt to kill the other developing queens before they emerge. If the young queen opts for the latter behaviour and succeeds, then she becomes the sole inheritor of the old colony. If other queens manage to emerge they fight until only one remains. Thereafter, the surviving queen leaves the colony to mate and then returns to begin her egg-laying role.

Often more than one after-swarm can issue from a colony. These seldom yield viable new colonies, at least in temperate latitudes, because they are not headed by a mated queen, and have generally low worker cohorts (Winston, 1987). Evidence exists, however, which suggests that the degree of afterswarming is regulated and only occurs to the extent that the colony of origin can support. That is, smaller colonies, or colonies from climates which do not favour afterswarming tend to produce fewer afterswarms (Winston, 1987). Moreover, in tropical regions, many afterswarms do survive, and some records exist of afterswarms surviving even in temperate regions. At no time during this “swarming” process are the queens without attendant worker bees.

During autumn, bumblebee workers die, and the queens, which mated shortly after emergence, hibernate in isolation (Heinrich, 1979). This option is not open to honey bee queens which must overwinter in the presence of other worker bees. These overwintering worker bees and their queen form tight clusters which can thermoregulate very effectively and thereby maintain a relatively stable temperature within the hive (Seeley & Heinrich, 1981). The bee cluster is sustained by honey which was collected during the summer and stored within the hive’s combs. At this time, little or no brood is present in the colony, the only work for the worker bees being to attend to the queen, to feed themselves, to defend the colony if necessary and to thermoregulate.

1.8 Aspects of honey bee biology promoting them as hosts for parasites.

Honeybees regulate the abiotic parameters (e.g relative humidity, carbon dioxide concentrations and temperature) of their hives rigorously, irrespective of ambient conditions (Seeley, 1974; Free, 1977; Seeley & Heinrich, 1981; Winston, 1987). Consequently, the hive is a highly stable environment. This can be expected to facilitate parasite transmission between hosts and to reduce parasite mortality due to environmental factors. Additionally, the rate of development of bee brood is remarkably consistent: 21 days for worker brood, 24 days for drone brood and 16 days for queens. Variations do exist in the developmental times between different bee races, but they generally reflect other ambient conditions (food/water availability), or bee genetic stock. Nevertheless within their own region/climate, bee colonies are highly uniform.

Colonies are well-defended, consequently only a few organisms are able to gain access to the hive. By reproducing within the brood nest, the number of potential enemies to the parasites are greatly limited.

Honey bee colonies generally have 40-80 thousand members, present all year round. When brood is present, it is represented by hundreds if not thousands of individuals. These factors minimise the problems normally associated with host location and host availability.

Swarming, absconding, inter-colony drifting, robbing, and the existence of drone congregation areas to facilitate mating, all contribute to making them a

host, through whose population a parasite can easily spread. In addition, beekeepers unwittingly aid transmission by uniting weak colonies, moving stray swarms into their apiaries, and through the practice of migratory beekeeping where many hives from a region are brought into close association.

All these factors have contributed to the rapid world-wide spread of this most serious of all honeybee diseases.

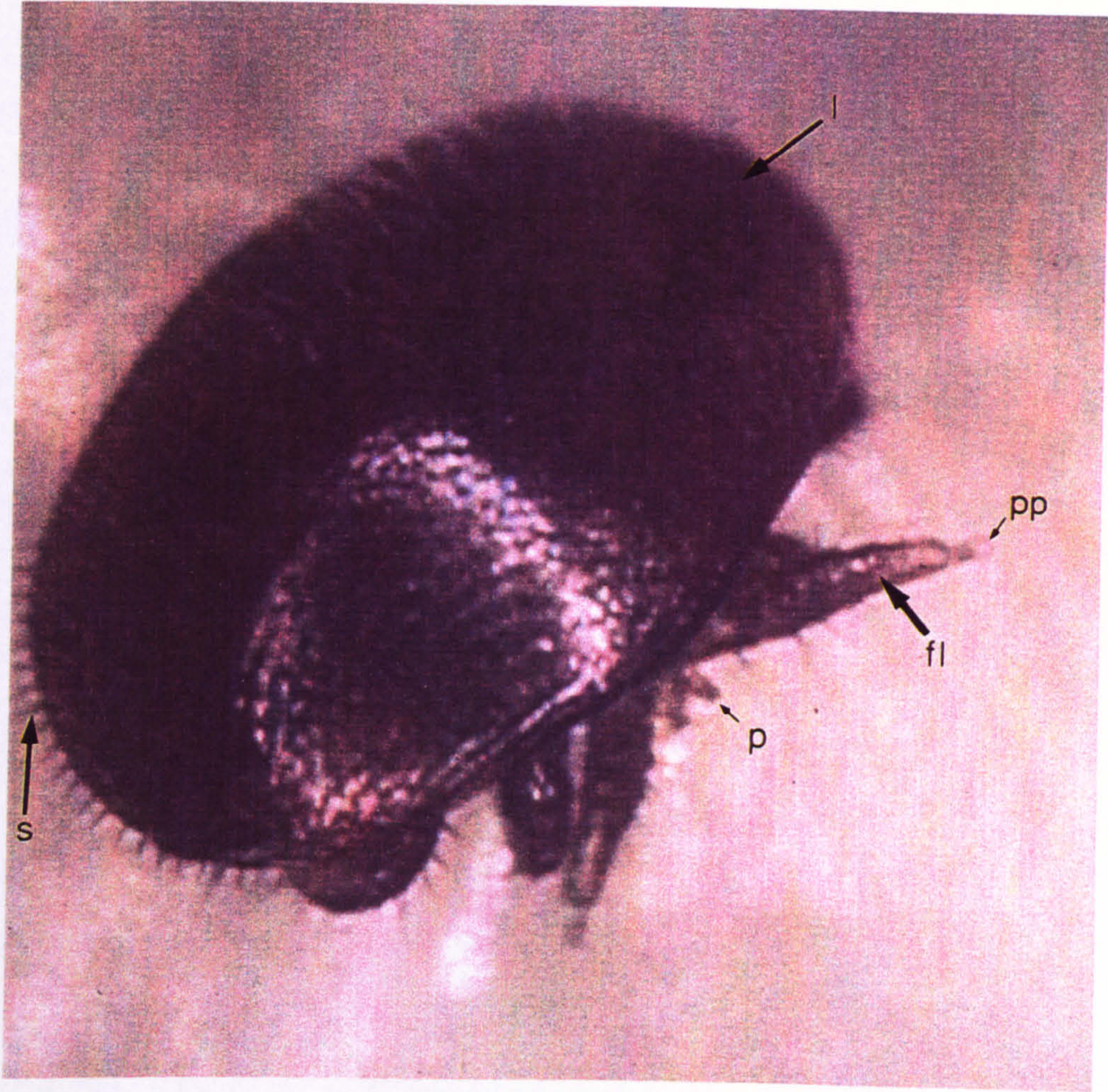
P.T.O

Figure 1.1 The European honeybee, *Apis mellifera*. A foraging worker.
(Scale = x12).



P.T.O

Figure 1.2 Adult female *Varroa jacobsoni*. P = Pedipalps, Fl = front leg, I = idiosoma, S = backward-pointing setae, PP = pretarsal pads. (Scale = x67).



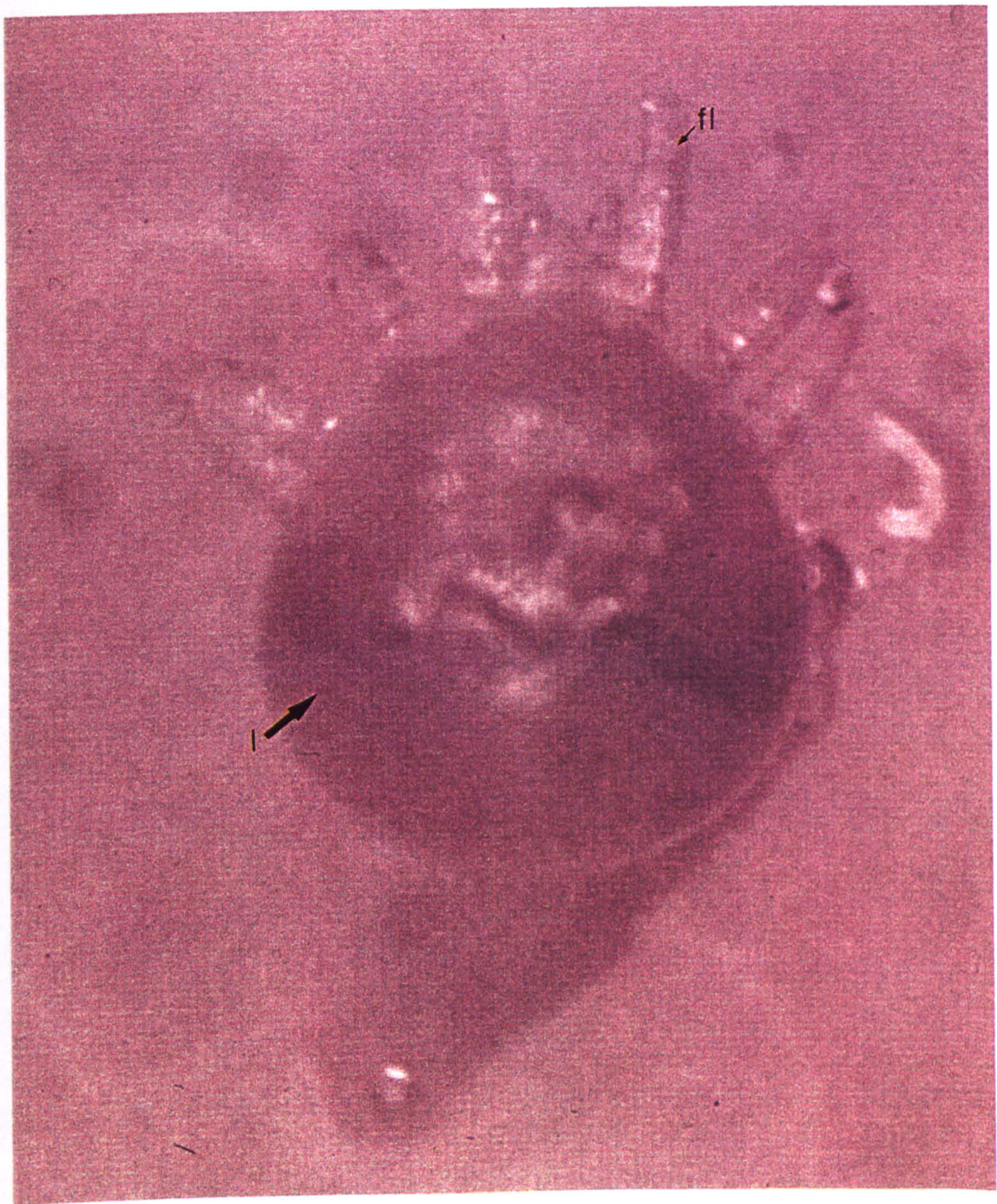
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Figure 1.3 The Asian honeybee, *Apis cerana*. The original host of the parasitic mite *Varroa jacobsoni*. (Scale = x5)



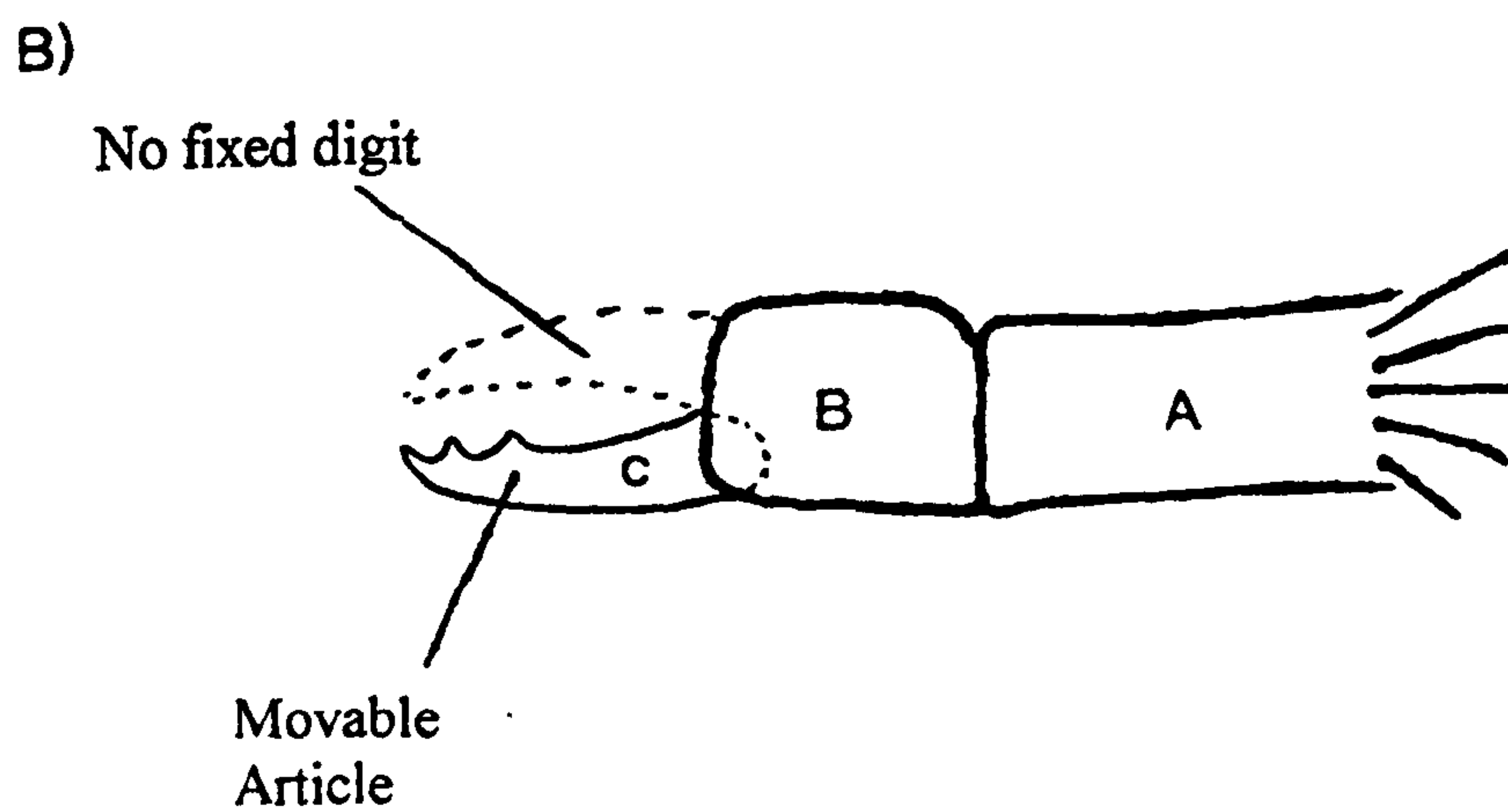
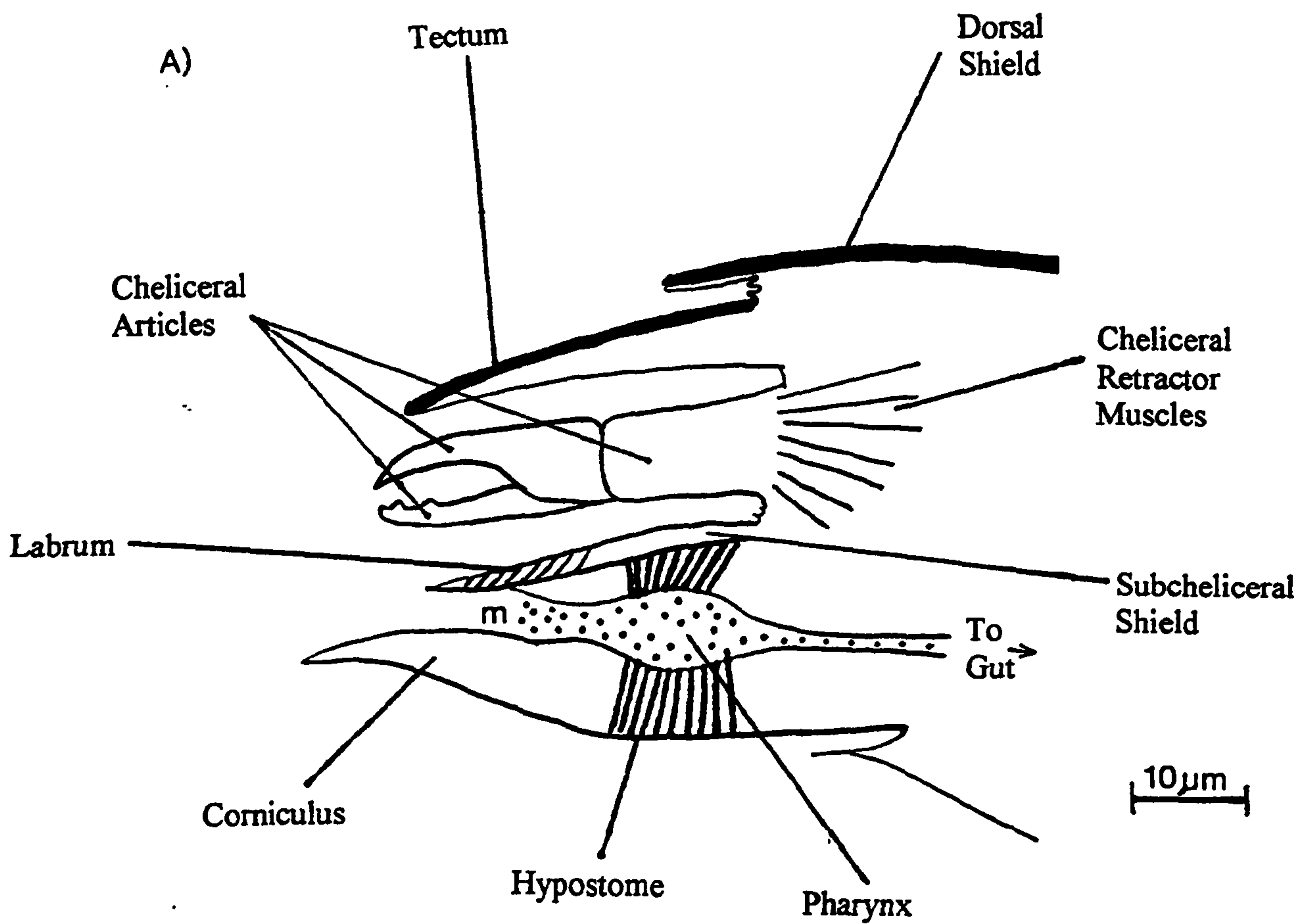
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Figure 1.4 Adult male *Varroa jacobsoni*. Fl = front leg, I = idiosoma.
(Scale = x75).



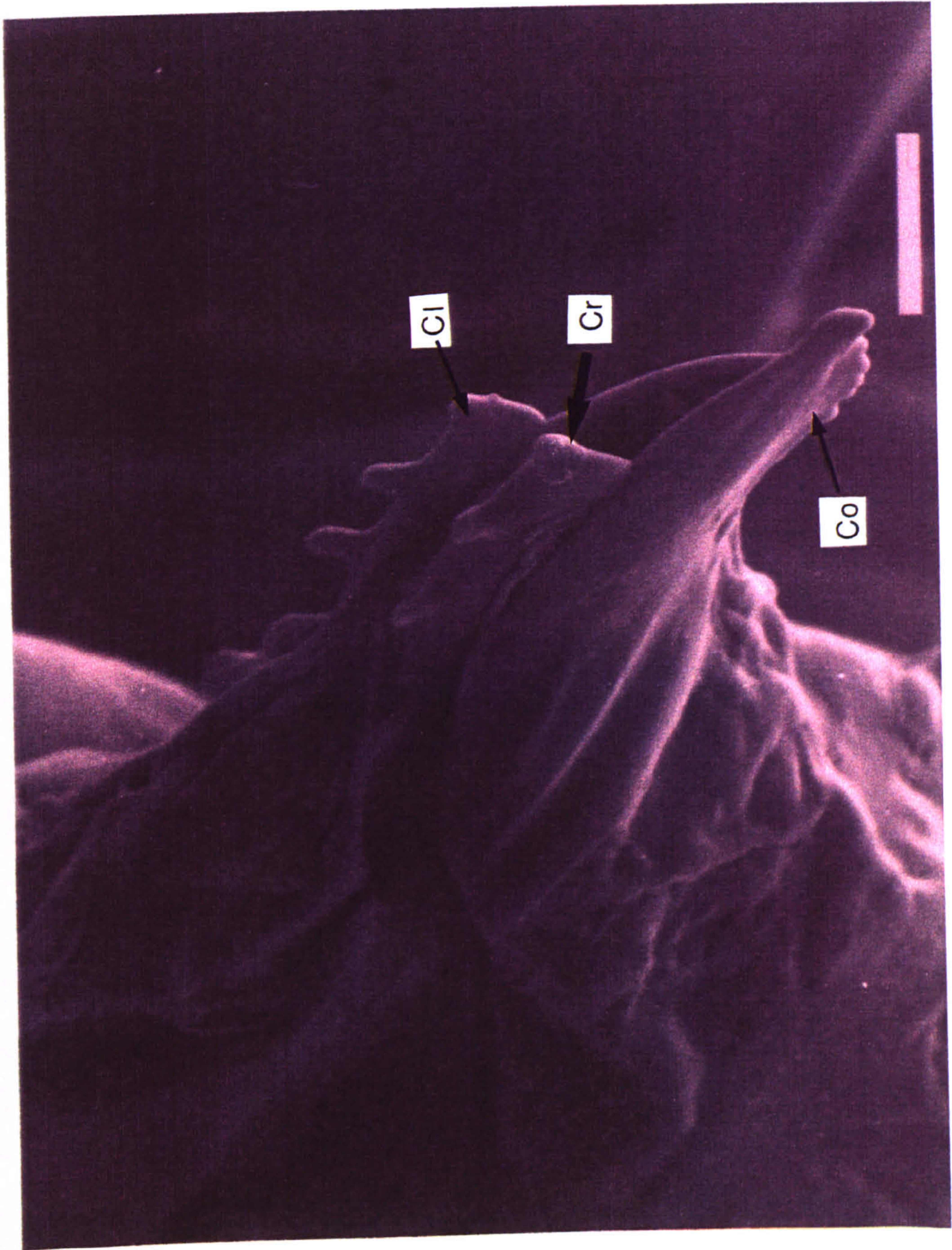
P.T.O

Figure 1.5 A) Cross-sectional diagram of the mouthparts of an adult *female Varroa jacobsoni*. B) Chelicera of an adult female *Varroa jacobsoni*.
m = mouth, A, B & C = cheliceral articles.



P.T.O

Figure 1.6 Scanning Electron Micrograph of the mouthparts of an adult female *Varroa jacobsoni*. Co = corniculus, Cr = right chelicera, Cl = left chelicera. (Bar line = 10 μ m).



P.T.O

Figure 1.7 Scanning Electron Micrograph of an adult female *Varroa jacobsoni*, illustrating the laterally compressed morphology of their legs and pronounced dorso-ventral flattening. M = mouthparts.



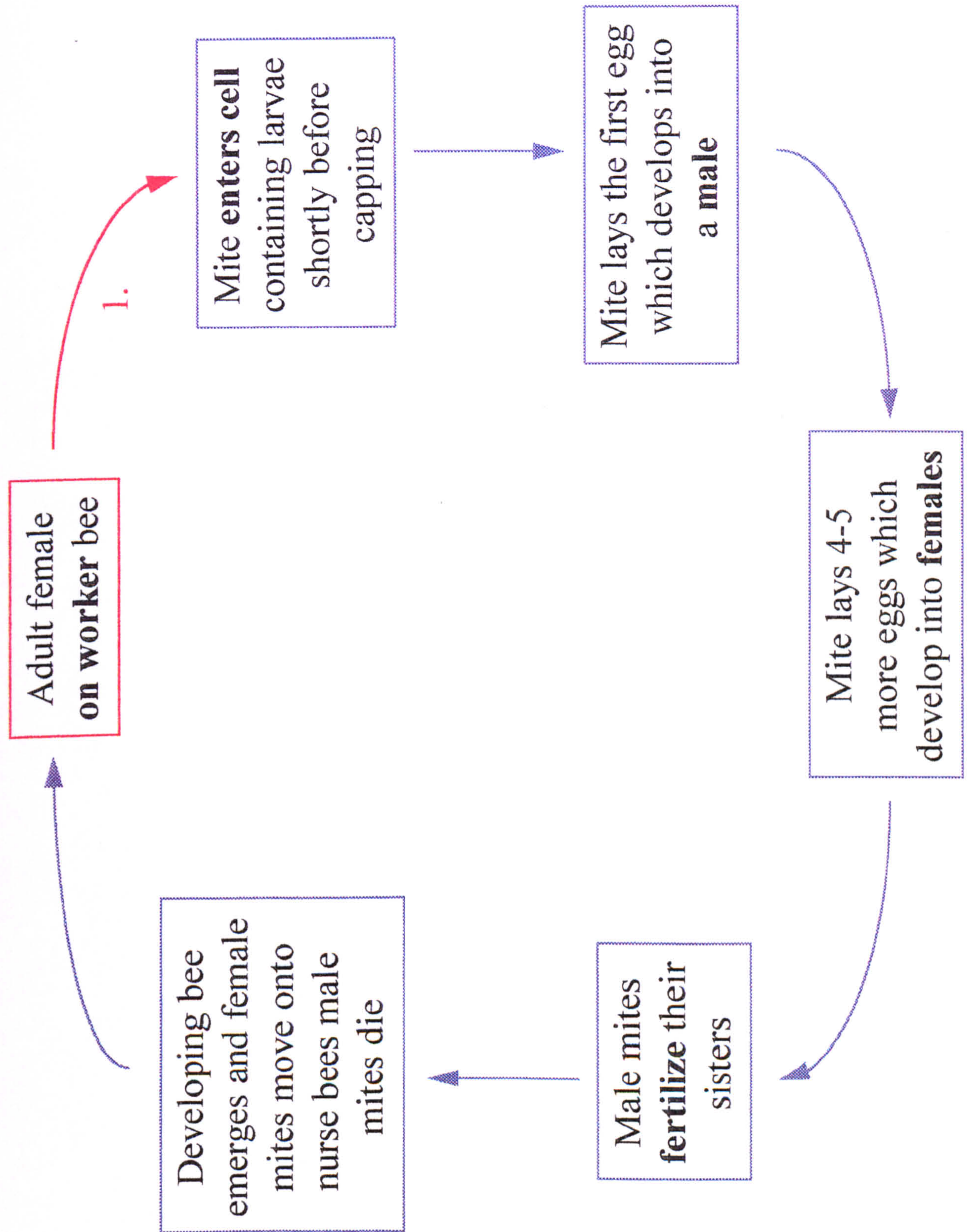
P.T.O

Figure 1.8 Scanning Electron Micrograph of the ventral surface of an adult female *Varroa jacobsoni*. The left legs have been removed to expose the underlying peritremal tube (p). Also note backward-pointing hairs, which enmesh with the hairs of the host. The mite is clearly wider than long, which enables them to secrete themselves between the abdominal plates of adult bees.



P.T.O

Figure 1.9 Life cycle of *Varroa jacobsoni* within an *Apis mellifera* colony.



P.T.O

Figure 1.10 Newly emerged *Apis mellifera* workers. a) = normal condition at emergence, b) & c) = exhibiting morphological deformities – vestigial / crumpled wings and shortened abdomens, following infestation during development with *Varroa jacobsoni*. (Scale = x10)

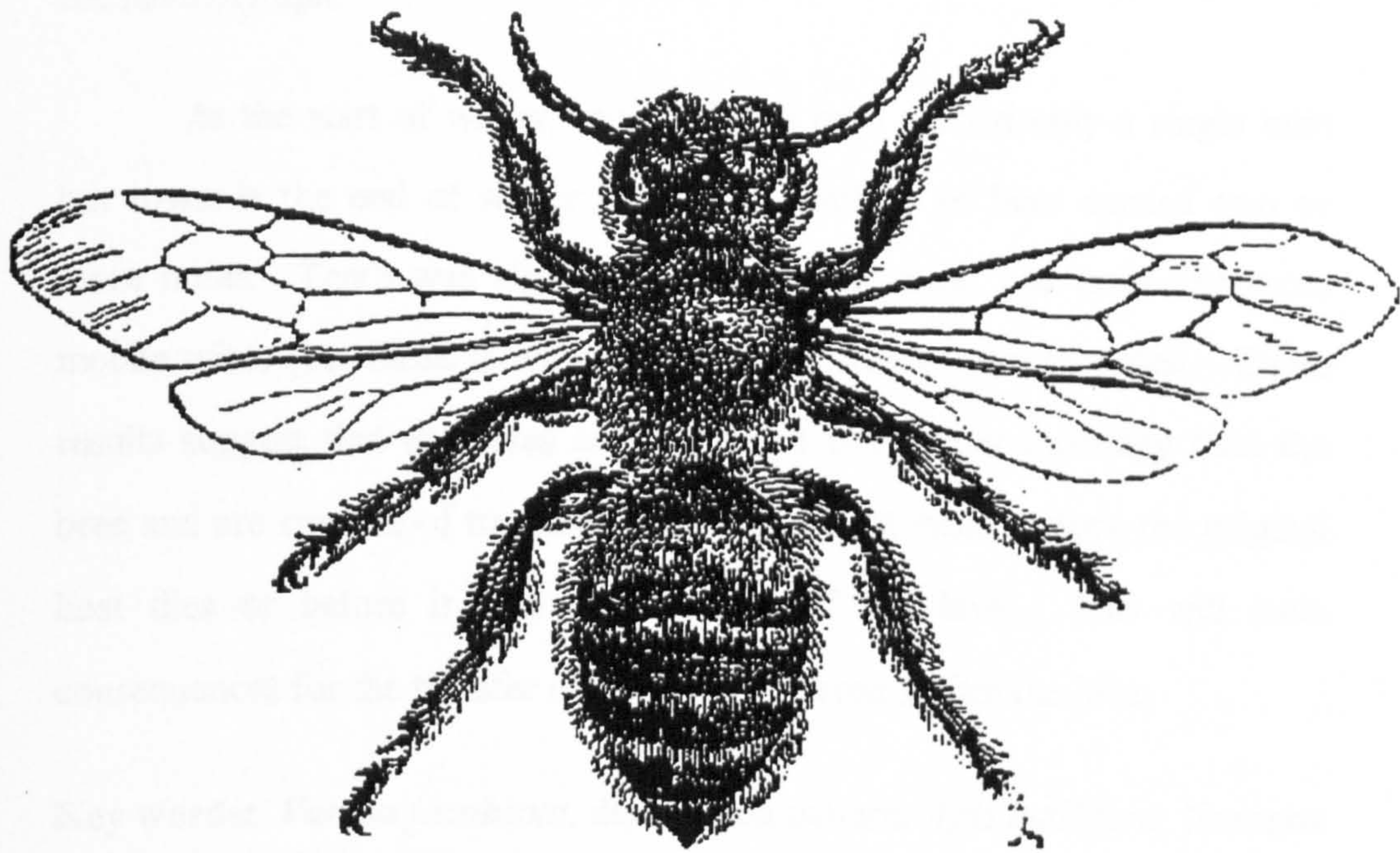


P.T.O

Figure 1.11 An *Apis mellifera* swarm.



PREFERENTIAL DISTRIBUTION OF *VARROA JACOBSONI* ON OVERWINTERING HONEYBEE WORKERS AND CHANGES IN THE LEVEL OF PARASITISM



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SUMMARY

The distribution of *Varroa jacobsoni* on clustered, overwintering workers of *Apis mellifera* was investigated. The majority of mites were found between the 3rd and 4th ventro-lateral tergites of the abdomen with a significant preference for the left side of the host. It is proposed that this position would enable the mites to place their mouthparts in close proximity to the central portion of the bees' ventriculus. This may allow the mites access to nutrients at higher concentrations than would occur elsewhere in the haemolymph.

At the start of winter, most infested bees carried only a single mite but towards the end of winter increasing numbers of bees carried two or more mites. There was also an increase in the mite: bee ratio and more mobile mites (i.e. those moving about on the bees) were recorded. These results suggest that the mites suffer a lower overwinter mortality than the bees and are capable of transferring to a new host either before the original host dies or before it falls to the floor of the hive. This will have consequences for the transfer of diseases by varroa within the hive.

Key words: *Varroa jacobsoni*, distribution pattern, *Apis mellifera*, Nasanov gland secretion, overwintering cluster.

INTRODUCTION

The ectoparasitic mite *Varroa jacobsoni* is an extremely important pest of *Apis mellifera* apiaries in many parts of the world (Morse & Nowogrodski, 1990) and despite stringent control measures it has spread rapidly throughout the UK following its arrival in 1992 (Mobus & deBruyn, 1993) (Figure 2.6). The mites spend much of their time in the brood cells, where they reproduce (De Jong, Morse & Eickwort, 1982a). When out of the brood cells, the adult female mites live on the bodies of the adult bees, where they obtain nutrition and effect dispersal. During winter, when little or no brood is produced, the adult female mites survive by clinging to the bodies of the worker bees (Langhe & Natzkii, 1977; Shabanov, Nedyalkov & Toshkov, 1978).

Although the distribution of the mites on active adult bees has been studied previously the results have been contradictory. Fernández et al. (1993) said that the mites preferred the ventral anterior (2nd and 3rd sternite/tergite) and middle (3rd and 4th sternite/tergite) regions of the abdomen. Ritter (1981) found them between the first sclerites, between the head and thorax and at the petiole. Kraus *et al.* (1986) concluded that the mites preferred the dorsal aspect while Delfinado-Baker et al. (1992) found that they had a strong preference for the lateral inter-tergites (3rd and 4th). De Jong *et al.* (1982a) stated that the mites “are most commonly found on the abdomen, often under the abdominal sclerites or between the thorax and abdomen”. Hoppe & Ritter (1988) stated that mites were most commonly found between the head and thorax of older bees and between the abdominal sternites in nurse bees. LeConte & Arnold (1988) have suggested that these

conflicting observations arise from temperature effects, the mites moving onto the thorax under cool conditions. Other explanations have included the possibilities that the mites are influenced by bee pheromones, especially the Nasanov gland secretions (Hoppe & Ritter, 1988) or that they locate themselves where they are least likely to be detected by bee grooming behaviour (Delfinado-Baker *et al.*, 1992).

As part of the project on the effect of *V.jacobsoni* on overwintering bees the distribution of the mites on clustered *Apis mellifera* was studied- all previous studies have used active bees. The behaviour of the mite at this time of year is of special interest as infested colonies frequently do not survive the winter (Grobov, 1977; De Jong *et al.*, 1982a). It is becoming apparent that this is linked to pathogens (viruses and bacteria etc.) which are transmitted by Varroa (Bailey & Ball, 1991; Gliński & Jarosz, 1992; chapter 6). Factors which enhance the movement of mites between bees will therefore be important in the transmission of disease. Overwintering clustered bees show many differences in physiology and behaviour from active bees. For example, they have reduced metabolic rates and are relatively inactive (Corkins & Gilbert, 1932), they have longer life-spans (Fukuda & Sekiguchi, 1966), they have raised brain cell counts (Rockstein, 1950), their hypopharyngeal glands are hypertrophied (Simpson *et al.*, 1968; Brouwers, 1982), their fat bodies become enlarged (Haydak, 1957), their haemolymph protein and vitellogenin titres rise and their juvenile hormone titres fall (Fluri *et al.*, 1982). Similarly, there are changes in the physiology of the mites which allow them to survive in the absence of brood, such as an increase in life-span (Shabanov *et al.*, 1978) a rise in the amount of guanine

in their malpighian tubules (DeJong *et al.*, 1982a, citing Ionescu-Varo & Suciu, 1978) and changes occur in the electrophoretic mobility values of their proteins towards winter (Barabanova & Galanova, 1993; Barabanova & Piletskaya, 1991). Also investigated was the effect of geraniol, the principal component of Nasanov gland secretion (Pickett *et al.*, 1980), on the distribution of mites in a gradient choice chamber, to determine whether or not the suggestion that mite distribution is influenced by Nasanov gland secretions (Hoppe & Ritter, 1988) could be confirmed.

MATERIALS & METHODS

Distribution of mites on the bees

Between October 1995 and February 1996, groups of 50-100 bees were collected using a pooter (Bailey, 1956) from the top bars of a naturally-infested overwintering colony of *A.mellifera mellifera* X *A.mellifera ligustica*. The bees were placed briefly in a holding container then removed individually using forceps and the position of the mite on the bee recorded. The results were examined using χ^2 analysis.

Effect of geraniol on mite behaviour

Within a concentration gradient chamber, mites were exposed to a filter paper impregnated with 0.001 μ l, 0.01 μ l and 0.1 μ l of geraniol (Sigma, U.K. 98%), at a distance of 10 cm. According to Hoppe & Ritter (1988),

these amounts of geraniol are equivalent to those formed in the Nasanov glands of 1, 10 and 100 bees respectively. After 30 minutes, mites were classified as "attracted" if they were found in the half of the tube close to the geraniol source, or, as "repelled" if they were found in the half of the tube away from the geraniol source (Kennedy, 1978).

RESULTS

Distribution of the mites on the bees

During the period October - December there was usually only a single mite per infested bee but between January - February many more bees were found to carry more than one mite. Increases were also recorded in the numbers of mobile mites, the percentage of infested bees and the mite: bee ratio (Table 2.1). When the mite distributions during the two time periods were analysed, neither the Poisson model ($\chi^2_3 = 11.98$, $p < 0.05$) nor the negative binomial models ($\chi^2_2 = 9.78$, $p < 0.05$) were found to be applicable.

The pooled data for all observed mites, indicated that they showed a preference for the abdomen over the rest of the body, 77.7% being found there (Figure 2.1). On the abdomen, mites had a highly significant preference for the space between the 3rd and 4th true abdominal tergites (Figures 2.2 & 2.3) (χ^2_3 , $p < 0.0005$), 85% of mites on the abdomen being found there. Mites found between the 3rd and 4th abdominal tergites were also observed to show a significant preference for the left side, 66% ($n=441$) of mites occupying that side (χ^2_1 , $p < 0.0005$) (Table 2.2). Also, mites had a highly significant

preference for the inter-tergite spaces as opposed to the inter-sternite spaces, 98.6% and 1.4% being found there respectively (χ^2_1 , $p < 0.0005$).

Mites on the abdomen displayed a clear order of site preference. The most preferred position was between the left 3rd and 4th abdominal tergites (55%), the second most occupied site was between the right 3rd and 4th abdominal tergites (29%), the third most frequently occupied site was between the left 5th and 6th abdominal tergites (6%), and the fourth most frequently occupied site was between the 5th and 6th right abdominal tergites (4.5%) (Table 2.2).

When two mites were found on a bee, the patterns of distribution observed were significantly different to the patterns that would be expected if the mites displayed no site preference (χ^2_{14} , $p < 0.005$). When three mites were recorded on a bee, the combinations of positions occupied varied greatly (Table 2.2).

When bees were dissected it became obvious that the mites were preferentially locating themselves at a site very close to the central ventriculus (Figures 2.4 & 2.5). The midgut is naturally coiled to the left (pers. obs.) but in winter bees it is further displaced sideways and upwards by the grossly swollen rectum (Snodgrass, 1956 and pers. obs. see Figure 2.5). Mites located on the right of the bee would be further away from the midgut and those found between the 4th and 5th abdominal tergites would be even further.

Effect of geraniol on mite behaviour

Mites were attracted by the low concentration of geraniol, uninfluenced by the medium concentration, and repelled by the high concentrations (Table 2.3).

DISCUSSION

As *V.jacobsoni* are unable to reproduce in the absence of brood, the increase in the proportion of infested bees and the rise in multiple infestations during the course of winter can only mean that bee mortality was considerably higher than that of the mites. This would agree with the findings of Kovac & Crailsheim (1988) and Korpela *et al.* (1992) who reported that 82-96% of infested bees died during winter, which is far in excess of that of mite mortality (chapter 3). Furthermore, the mites must have been transferring from dead/dying bees before these were separated from the cluster. This was confirmed in later experiments detailed in chapter 3. These results contrast with those of Ritter (1988) who states that the mite population in winter is drastically reduced, and that the mites are mainly removed from the colony by dying host bees, which implies that when an infested bee dies, it will fall to the base of the hive with its mite still attached. Once a mite has fallen to the base of the hive it is extremely unlikely of being capable of returning to the cluster (chapter 3). However, it was found that a dead bee placed in the centre of the cluster could take up to 48 hours to fall to the base of the hive which would allow ample time for any mite to register the death of its host and transfer to another bee. A marked reduction in mite

numbers overwinter could also occur if infested bees, owing to their reduced fitness, suffer higher mortalities outside the hive while on cleansing flights or foraging. Weather conditions during the experimental period however, ensured that the bees remained in a tight winter cluster so it was unlikely that any significant removal of mites occurred this way. Similarly, mite levels were unlikely to have been influenced significantly owing to drifting of infested bees from neighbouring hives - the nearest known of which was 10km away.

The mites showed a clear preference for the left inter-segmental spaces between the 3rd and 4th abdominal sclerites. Similar results have also been described by Delfinado-Baker *et al.* (1992) on *A.cerana* in Thailand and Fernández *et al.* (1993) on *A.mellifera* in Argentina so these results cannot be a strain specific phenomenon. Both Delfinado-Baker *et al.* (1992) and Fernández *et al.* (1993) were working on active bees so the natural orientation of the bees' midgut to the left is probably an important factor in determining mite distribution throughout the year. Contrary to LeConte & Arnold (1988), relatively few mites were found on the thorax of bees. They suggested that the mites preferred the thorax during cold weather because this would be the warmest region. However, according to Esch (1960) there is no difference in thoracic and abdominal temperatures of overwintering bees.

Hoppe & Ritter (1988) state that *V. jacobsoni* are most commonly found between the thorax and abdomen of older bees. They suggested that this is because the mites avoid geraniol released from the Nasanov gland

which, according to them has a strong repellent effect. By contrast, I found that mites were attracted towards low levels of geraniol although, as with Hoppe & Ritter (1988), high levels of geraniol had a repellent effect. This does not preclude the possibility that geraniol may be used by the mites as an indicator of bee age and thereby their suitability as hosts. Indeed, the results would appear to tie in with the observation that mites prefer house bees (<14 days old), which produce low levels of geraniol, to pollen-collecting bees, which produce much higher levels (Boch & Shearer, 1963; Kraus *et al.*, 1986; Kuenen & Calderone, 1997) Finally, when the mites are between the ventral abdominal tergites, with their anterior aspect completely shielded, any effect of the Nasanov secretions, which are low in the winter (Boch & Shearer, 1963), on the mites is probably minimal. The possible shielding effect of the sclerites has previously been suggested by Pätzold & Ritter (1989) with regards to protection against high temperatures. I am also uncertain under what conditions the mites would require (or indeed obtain) protection from high temperatures, but the inter-sclerite spaces between the 3rd and 4th tergites would ensure that the majority of a mite's body would be physically concealed and therefore protected from the attention of bee grooming behaviour (Delfinado-Baker *et al.*, 1992). Moreover, by secreting themselves under the tergites, the mites may be limiting themselves to a more optimal microclimate, perhaps in terms of humidity, to which they are sensitive (chapter 3). This is probably an important factor because *V.jacobsoni* evolved on *A.cerana* which is proficient at detecting and removing the mites (Peng *et al.*, 1987; Fries *et al.*, 1996). However, it does not explain the significant left preference of the mites - which is also found

on *A.cerana* (Delfinado-Baker *et al.*, 1992). Furthermore, clustered overwintering bees are relatively inactive and so the mites - which can remain mobile at much lower temperatures than the bees (chapter 3) - could presumably move with greater safety than they would during the summer.

The distinction that mites preferentially occupy the inter-tergite spaces rather than the inter-sternite spaces has not clearly been made in previous studies. For example, Fernández *et al.* (1993) did not differentiate between mites found under the tergal sclerites and those under the sternal sclerites. A partial explanation for this distribution could come from the fact that in summer the mites preferentially mount bees of late house age (Kraus *et al.*, 1986). This being near the mean age for comb building behaviour in bees (15.2 days old; Winston & Punnett, 1982) and therefore of wax production (max. gland development 5-15 days old; Winston, 1987 citing King, 1933). Wax is produced by the wax mirrors, of which there are four pairs that lie at the anterior of sternites four to seven. The presence of active wax mirrors would increase the thickness of the layer of tissue through which the mites accesses the haemolymph (Snodgrass, 1956). Furthermore, if the mite was positioned adjacent to an actively secreting wax mirror, its legs could become ensnared by the wax. It is therefore possible that the mites avoid the inter-sternal spaces to facilitate feeding and prevent any locomotory compromise.

The most favoured position on the left of the bee is that which would enable a mite to place its mouthparts in closest proximity to the front/central portion of a bee's midgut. The mites have relatively short mouthparts

(Griffiths, 1988) and would be unable to reach this region from any other position. The same location on the right side of the bee would place a mite at a slight distance from the upper portion of the midgut while a position between the 4th and 5th abdominal sclerites, even on the left, would mean that the mite would be feeding considerably below the end of the midgut. In active summer bees the gut is also orientated to the left although as the rectum is not swollen the midgut is not forced as close to the sclerites. Owing to absorption, the region immediately adjacent to the anterior and central midgut probably contains higher concentrations of many nutrients (Jimenez & Gilliam, 1989) some of which must be at a considerably lower level within the general circulation to explain the reluctance of the mites to feed elsewhere. This might be exaggerated in the winter bees in which overall low levels of activity may reduce mixing of haemolymph and/or the rates of transfer through the body. The nature of the nutrient(s) remains conjectural. However, Crailsheim (1988a; 1988b) has demonstrated that both leucine and glucose are 'absorbed within the first two thirds of the midgut'. The carbohydrate content of hymenopteran haemolymph is high (Mullins, 1985) and therefore unlikely to be a limiting factor. The mites have very low protease activities (Tewarson & Engels, 1982), although cathepsin-like proteases have been found in the intestines of female mites (Barabanova & Galanova, 1993, citing Barabanova, 1984), and must therefore rely to a large extent on absorbing amino acids and proteins present in the bees' haemolymph. However, Hymenoptera are also well known for the exceptionally high amino acid concentrations in their haemolymph (Rees, 1977; Mullins, 1985) so unless there are certain ones which are essential to

the mite but at low levels within the general circulation this is also unlikely. Possibly there are certain vitamins and/or cofactors which are absorbed in this region, as has been shown to be the case for amino acids and monosaccharides then rapidly bound to other molecules, taken up by the fat body (chapter 4), metabolised or otherwise made unavailable to the mite necessitating them to feed extremely close to the midgut. The fact that there was an increase in the proportion of bees carrying two mites during winter would suggest that although the left side of the bee is preferred, any mite happening to transfer to an already infested bee will tend to stay with it rather than continue to search for one which is uninfested immediately. This may reflect a relict behaviour from the time the mites parasitised *A.cerana* when, as mentioned above, any mite which exposed itself would be extremely vulnerable to removal by grooming (Fries *et al.*, 1996). Furthermore, this would reduce the energy expenditure and the interruption of feeding. There is evidence which suggests that mites need to feed regularly and so would have to rest and feed frequently.

These observations, assuming they are typical, might explain why hives which are infested but otherwise apparently healthy in autumn often suffer severe losses overwinter (Grobov, 1977; Müller, 1987). Although both mites and bees suffered a natural mortality during the winter months, that of the bees was greater. As the mites were able to transfer to new hosts either immediately before their existing host died or before it fell to the floor of the hive (chapter 3), there was an increasing prevalence of infestation and an increase in the proportion of bees carrying two or more mites. Consequently, not only would the health of more and more bees be reduced

directly by the mites but there would be an ever increasing rate of transmission of the pathogenic diseases for which *V.jacobsoni* has been shown to be responsible, such as Deformed Wing Virus (chapter 6). This could have dire consequences if it occurs when the bees are already ageing and probably weakened by several months of winter. Consequently the colony may be unable to recover when spring returns. These results emphasise the importance of applying rigorous control measures late in the year after the bees have ceased foraging and raising brood. All the mites will then be found on the adult bees and therefore more vulnerable to acaricides, they will be unable to increase in numbers and the more mites destroyed the more the chances of the hive surviving the winter in healthy condition. Furthermore, because no honey will be collected for many months after acaricide application, the risk of residues is reduced considerably.

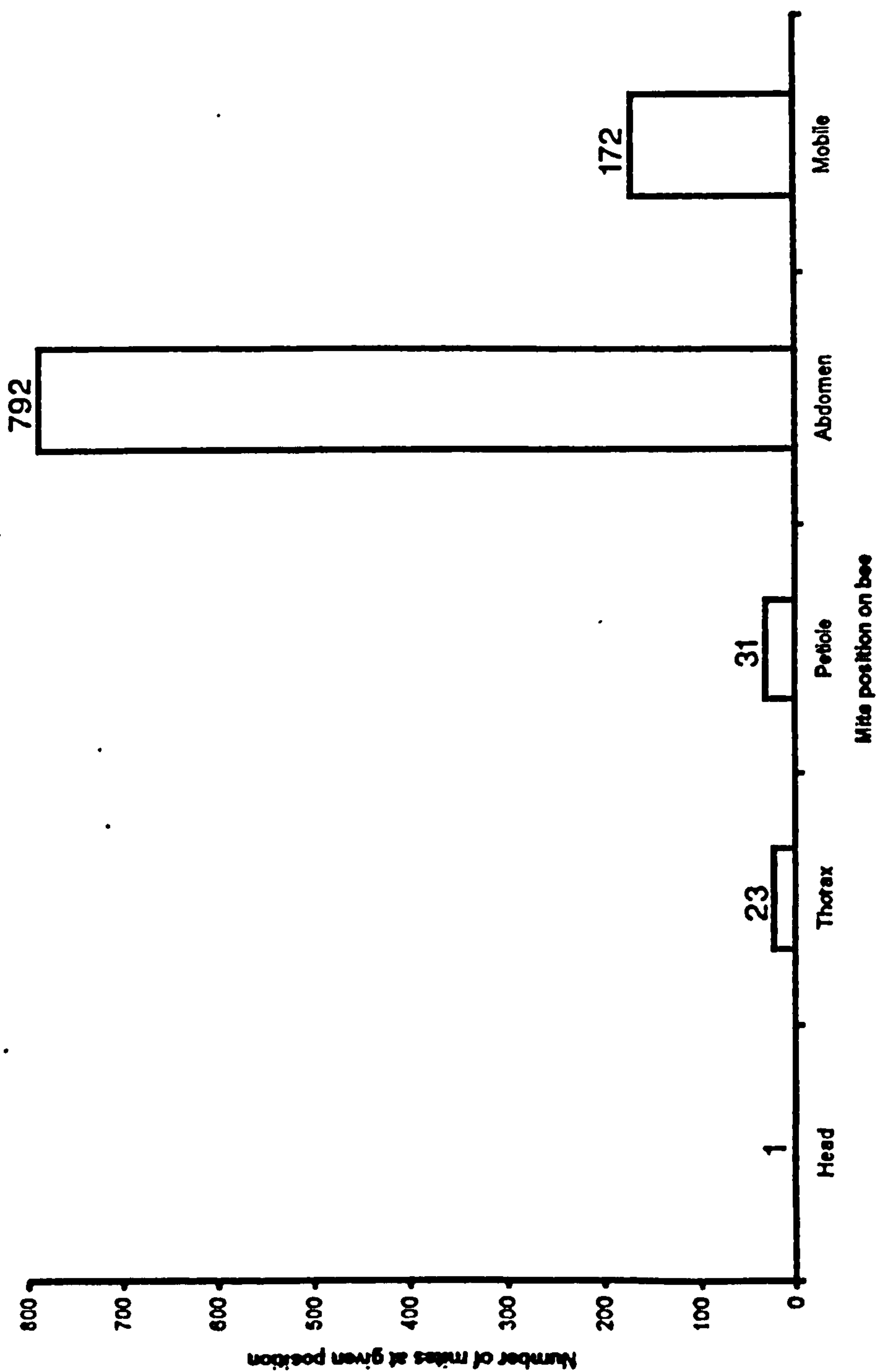


Figure 2.1 Number of *Varroa jacobsoni* ($n=1019$) found at each location on the body of overwintering *Apis mellifera* ($n=3458$). Pooled data gathered between October 1995 & February 1996.

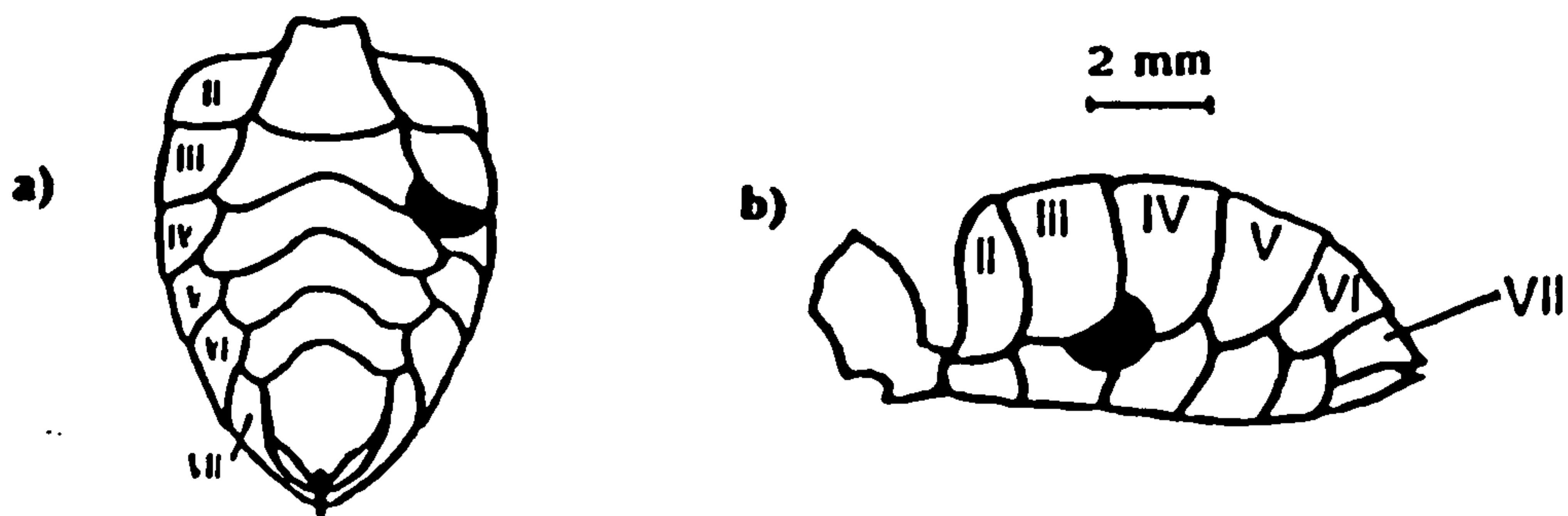


Figure 2.2 Abdomen of *Apis mellifera* a) ventral aspect, b) left lateral aspect; showing the preferred attachment site of *Varroa jacobsoni* on the overwintering host between the left 3rd + 4th tergites. (Redrawn from Snodgrass, 1956).

P.T.O

Figure 2.3 Adult female *Varroa jacobsoni* between the left 3rd (3T) and 4th (4T) tergites of *Apis mellifera*. The black arrow points to the mite. (Scale = x22).



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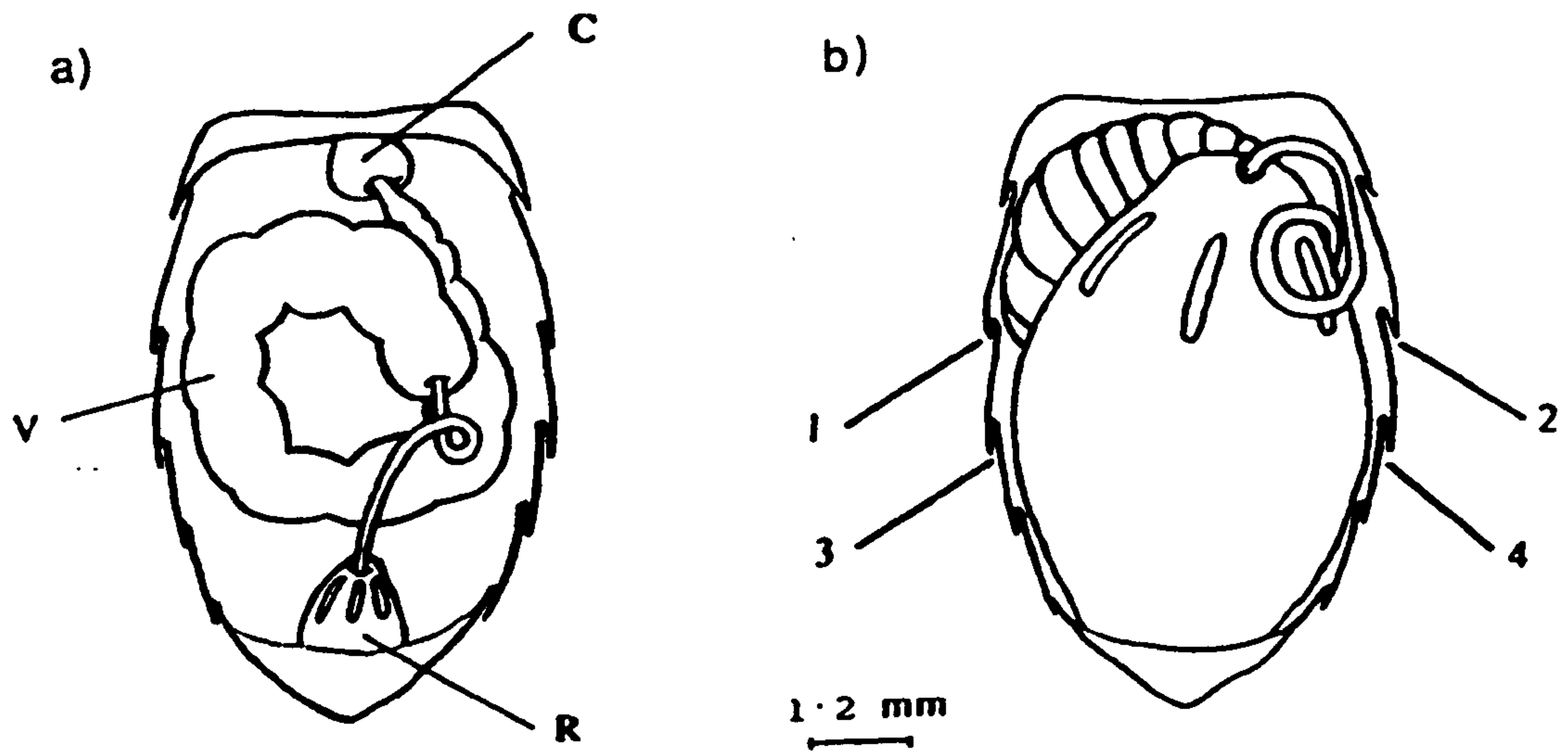


Figure 2.4 Dorsal aspect of dissected *Apis mellifera* gaster showing the positions of the internal organs, **a)** in summer, **b)** in winter. C = crop, V = ventriculus, R = rectum. 1, 2, 3 & 4 = positions occupied by *Varroa jacobsoni* (in decreasing order of preference, respectively) on adult overwintering *A.mellifera*. (Redrawn from Dade, 1962).

P.T.O

Figure 2.5 Dorsal aspect of dissected abdomen of an overwintering worker honeybee (*Apis mellifera*). R = rectum (distended with faeces), V = ventriculus (anteriorly displaced by rectum). (Scale = x14).





Figure 2.6 The movement of *Varroa jacobsoni* in the UK between 1992 and 1996. Data is held by the National Bee Unit. (Figure reproduced by kind permission of S.Martin [Martin, 1997a]).

	October to December	January to February
% uninfested bees	87.1 (1603)	63.2 (1023)
% infested bees	12.9 (237)	36.8 (595)
% parasitised bees with 1 mite	97.5 (231)	73 (434)
%parasitised bees with 2 mites	2.5 (6)	24 (143)
%parasitised bees with 3 mites	0	2.7 (16)
%parasitised bees with 4 mites	0	0.3 (2)
% mobile mites	11.9 (29)	18.43 (143)
% bees with mobile mites	1.57 (29)	7.79 (126)
Bees : Mites	7.57 (1840)	2.09 (1618)

Table 2.1 Prevalence and distribution of *Varroa jacobsoni* within a colony of *Apis mellifera* during early winter (October - December 1995) and late winter (January & February 1996). Number of mites observed in parentheses.

Position of mite on the bee	% mites at given position for each parasitosis		
	1 mite	2 mites	3 mites
L 3/4	49.02 (326)	34.56 (103)	25 (12)
R 3/4	20.3 (135)	27.85 (83)	22.92 (11)
Mobile	14.14 (94)	19.8 (59)	31.25 (15)
Petiole	4.06 (27)	1.34 (4)	0
L 4/5	4.06 (27)	6.04 (18)	8.34 (4)
R 4/5	3.31 (22)	3.69 (11)	6.25 (3)
Thorax	1.95 (13)	3.02 (9)	2.08 (1)
L 2/3	0.75 (5)	0	0
R 2/3	0.75 (5)	0.34 (1)	0
L 5/6	0.6 (4)	1.34 (4)	0
R 5/6	0.45 (3)	0	0
Head	0.15 (1)	0	0
L 3/4 sternites	0.15 (1)	2.01 (6)	2.08 (1)
R 3/4 sternites	0.15 (1)	0	2.08 (1)
L 4/5 sternites	0.15 (1)	0	0

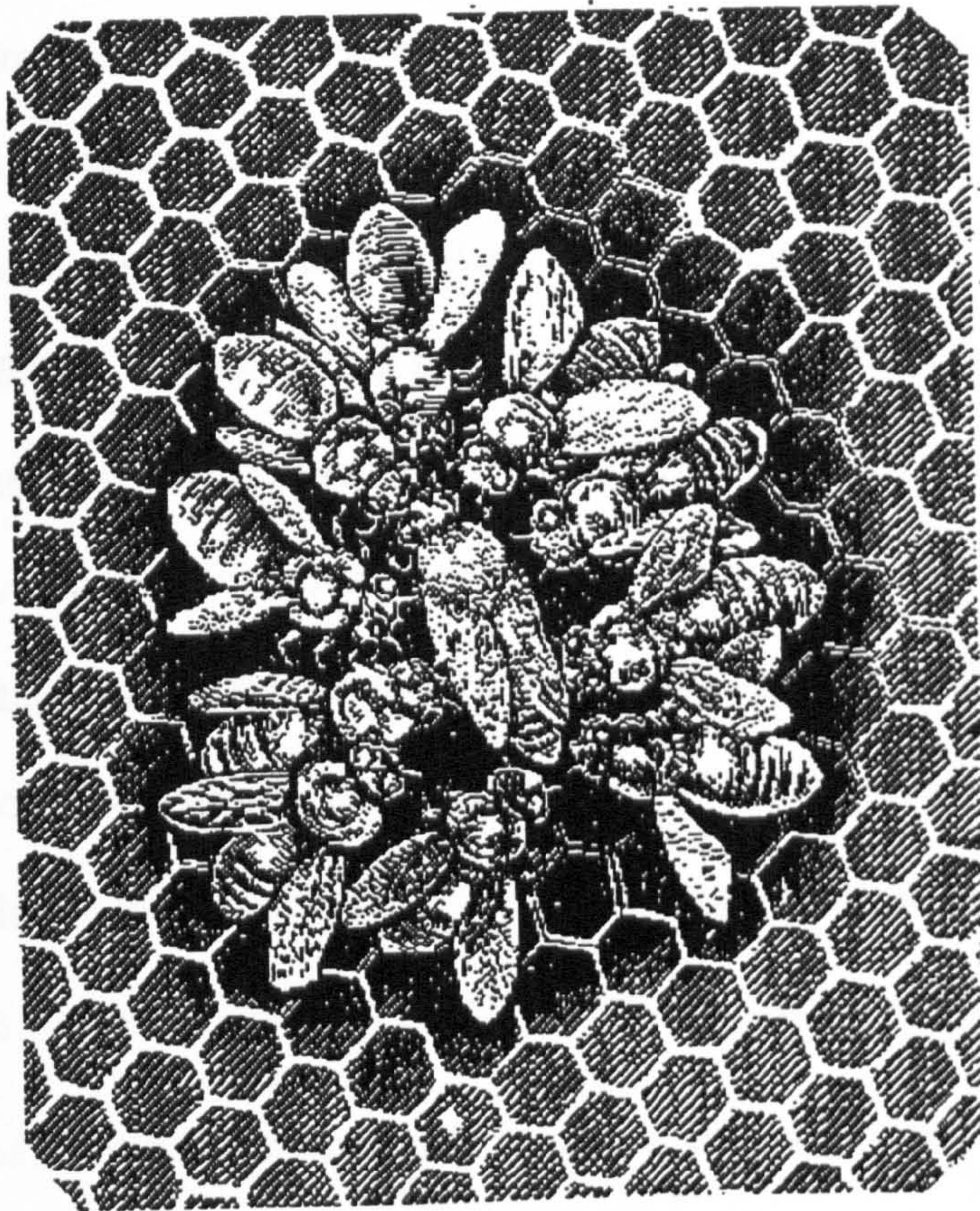
Table 2.2 Pooled data (October 1995 to February 1996) on the distribution (as %) of *Varroa jacobsoni* on *Apis mellifera* when 1, 2 & 3 mites were observed on the host (parasitosis). Number of mites observed in parentheses. L = left, R = right, Number/Number = sclerites between which *V.jacobsoni* were observed.

Volume of geraniol (μ l)	No. mites "attracted" by chemical	No. mites "repelled" by chemical	Significance determined by Chisquare tests
0.001	180	130	$\chi^2_1=8.06$, $p<0.005$ ✓
0.01	63	83	$\chi^2_1=3.18$, $p>0.05$
0.1	72	114	$\chi^2_1=9.48$, $p<0.005$

NS.

Table 2.3 The effect of geraniol on the behaviour of *Varroa jacobsoni*. Significance determined by χ^2 tests. 'attracted' = mites found in the half of the choice chamber nearest the chemical point source, 'repelled' = mites found in the half of the choice chamber furthest away from the chemical point source.

INTER-HOST TRANSFER AND SURVIVAL OF
VARROA JACOBSONI UNDER SIMULATED
AND NATURAL OVERWINTER
CONDITIONS.



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SUMMARY

Varroa jacobsoni readily transferred between living bees and from dead and dying bees onto living bees under both natural and laboratory-simulated winter cluster temperatures. In trials involving 261 bees and 161 mites, 26% of the mites moved from one live host to another within 7 days. When an infested bee was dead or dying, up to 75% of the mites would transfer to a live bee within 24 hours. After host death mites would also transfer from the dead bees to moving objects, such as forceps, which had previously been used for handling bees. However, they would not transfer to other insects, such as adult *Calliphora* spp. This suggests that the stimulus for transfer is a combination of mechanoreception and chemoreception. When their host died, a mite would remain on the dead bee, provided an alternative live bee was not available, for an average of 48 ± 26.5 hours before dismounting. During this time they continued to feed and exhibited behaviour which would probably enhance their chances of transfer to a new host. The distribution of mites within an frozen cluster was found not to be random, the mites showed evidence of selecting previously non-parasitised hosts.

Mites which were removed from their host at its death soon died themselves, all being dead within 48 hours. Isolated mites were unable to climb surfaces angled at more than 35-40°. This suggests that any mite

which falls to the base of the hive would be unable to climb back to the winter cluster.

These results bring into question previous suggestions that *V.jacobsoni* seldom moves between hosts overwinter and that the majority of them would inevitably die with their host. This has important consequences for models of varroa population dynamics and implications for the transmission of pathogens between bees by the mites.

Key words: *Varroa jacobsoni*, honey bees, *Apis mellifera*, winter-cluster, inter-host transfer, survival, bee death, mite feeding, disease transmission.

INTRODUCTION

Many honeybee colonies infested with the ectoparasitic mite *Varroa jacobsoni* collapse during winter (Grobov, 1977; Müller, 1987). The precise cause of this collapse is uncertain, although it is probably linked to the spread of viral and microbial disease by the mites rather than as a direct effect of the mites themselves (Ball, 1994; chapter 6). Factors affecting the survival and movement of mites within hives are therefore of crucial importance in our understanding of mite population dynamics as well as the course of infestation.

Fries, *et al.* (1991) postulated that the winter mortality of varroa may be as high as 50%. Their reasoning is based on three main assumptions:

- 1) that mites do not transfer off dying hosts, remaining with them when the bees fall from the cluster in winter (Müller, 1987),
- 2) that mites do not transfer from dead hosts once these have fallen through the winter cluster onto the hive floor (Müller, 1987) and
- 3) that because 50% or more of bees die overwinter, even in healthy colonies (Avitabile, 1978), and that the mites are randomly distributed within the cluster (Ritter *et al.* 1980), 50% of mites fall down irretrievably to the floor of the hive with the dead bees. The assumptions are tentatively supported by two reports of overwinter mite mortality of 3-40% (Moosbeckhofer, 1991)

and 40% (Korpela *et al.*, 1992) respectively. Furthermore, Fries *et al.* (1991) and Fries (1992) reported that 'many' parasitised dead bees could be found on the floors of their experimental hives overwinter. If it is accepted that on mild winter days, when bees undertake cleansing flights (bees will not defecate within the hive [Snodgrass, 1956]), that a further bee mortality and therefore mite loss also occurs, then it appears justifiable to postulate an overwinter mite mortality of up to 50%.

However, a number of questions remain unanswered. It has been shown (Sakofski, 1980) that mites are capable of transferring from one host to another during summer robbing episodes, and that transfer of mites also occurs during inter-colony drifting of workers and drones (Sakofski & Koeniger, 1986), from drones to queens during mating (DeJong *et al.* (1982a), citing Huttinger *et al.*, 1980) and from adult bees onto brood, when present (Kovac & Crailsheim, 1987; Kovac & Crailsheim, 1988) and *vice-versa* from newly-emerged bees onto older bees (Kuenen & Calderone, 1997). Mites are even known to actively select hosts of a certain age or physiological stage (Kraus *et al.*, 1986; Kuenen & Calderone, 1997). Hence, why could the mites not transfer between hosts during the winter, under cluster conditions, thereby making the first assumption invalid? Secondly, a mite mortality of 3-40% is an extremely wide range. A low mortality rate could as easily be justified as one of 50% using these figures. Indeed, low

(7±1% - 13.6% and 3-10%) overwinter mite mortalities have been recorded by some authors (Grobov, 1977, citing Tatzii *et al.*, 1976; Anshakova *et al.*, 1978; Weiss, 1984; Rademacher & Geisler, 1986; Moosbeckhofer, 1991), who have even gone as far as to suggest that overwinter mite death rates are 'insignificant'. Furthermore, I found that the ratio of parasitised : non-parasitised bees increased from 1:7 to 1:3 during winter and that there was a notable increase in the number of bees exhibiting multiple infestations (chapter 2). This suggests that the mites can and do transfer between hosts either before or sometime after host death. The aim of this study was to explore the readiness and ability of *V.jacobsoni* to transfer between hosts during winter.

MATERIALS AND METHODS

Throughout the chapter, bees are described as dead or dying. Dead bees are defined as those showing no external signs of movement; dying bees showed some weak leg or body movements during the experiments.

Laboratory simulated studies on the relationship between the transfer and survival of *V.jacobsoni* and the death of its honey bee host.

a. At high cluster temperatures

During early winter (November-December 1995), parasitised ($n=134$) and non-parasitised ($n=127$) bees from a single colony were anaesthetised by chilling (1-2 minutes at -22°C) and then individually marked with numbered colour discs (Koniginnen Werden Gezeichnet TM) The mites ($n=167$) already on the bees were individually distinguished by marking with HumbrolTM paint, using an entomological pin. Bees and mites anaesthetised and marked using this method made a rapid and full recovery. Furthermore, in a preliminary investigation, it was observed that 86% ($n=212$) of mites were not chilled to incapacitation during host chilling. The majority of mites remained in their original positions on the hosts, further suggesting that the method was not overly disturbing.

The bees were then placed into an Apidea^{TM*} (a polystyrene queen mating mini-nucleus hive [Exeter Bee Supplies, Devon, UK]), in groups of 16-96 ($n=6$ trials). The bees were supplied with sugar solution and water and the ApideaTM was placed into a Sanyo GallenkampTM incubator at 33°C (the mean brood-rearing central cluster temperature in winter rarely gets cooler than 30°C [Simpson, 1961]) and 55-80% R.H for 7 days. Preliminary experiments indicated that the mites were likely to change host during this

time. After 7 days, the bees were again anaesthetised and individually removed from the Apidea™ and their intensity of infestation (parasitosis^{*1}) recorded. Data was arcsine-transformed prior to analysis.

b. At low cluster temperatures.

Throughout the winter (October 1996 - February 1997), bees ($n=246$) were anaesthetised and individually distinguished by marking as described above and their intensity of infestation (parasitosis*) recorded. Some of the bees ($n=108$) were pierced through the thorax with a pin (at the head/thorax junction). Other bees were left to recover naturally. All bees were then placed into an Apidea™ (supplied with sugar solution and water), in groups of 6-26 ($n=18$ trials), and then placed into a Sanyo Gallenkamp™ incubator at 20°C (commonest non-brood rearing winter cluster temperature [Winston, 1991]) for 24-48 hours. After this time, the bees were individually removed from the Apidea™ and the parasitosis of dead and live bees was recorded and the statistical significance was determined by means of Mann-Whitney U-tests. Many ($n=56$) of the wounded bees exhibited weak limb movement when introduced into the Apidea™, which some ($n=35$) continued to show for 24 hours after introduction but all had died by 48 hours.

¹ Words marked with an asterix are defined in the glossary

Transfer of mites from dying hosts descending through a natural winter cluster.

Throughout the winter (October 1996 - February 1997), parasitised bees ($n=124$) were removed from a colony and killed by piercing their thorax. Both the bees and their attached mites ($n=135$) were marked as described previously. The dying bees were re-introduced into the source colony at the top of the cluster in groups of 6-32, in seven trials. Marking the mites in this way did not cause any noticeable disturbance and they remained attached in their original positions when their host bee was placed on top of the cluster. Following re-introduction, the hive entrance was observed for 30 minutes, thereafter the hive was gently raised at 2-4 hour intervals and any marked bees which had descended through the cluster onto the hive floor or which had been moved outside the hive by house-bees were collected. Also, the varroa floor was examined to recover any marked mites. An indication that the bees were clustering was obtained by confirming that the ambient temperature was below 10°C and noting bee activity at the hive entrance before the hive was raised during experimental manipulations (Free, 1977). On completion of the experiment, the cluster was killed using liquid nitrogen (~25 litres) and the bees were individually examined and colour-marked mites were recovered.

Behaviour of mites after host death under laboratory conditions.

65 parasitised bees were removed from the hive, killed by piercing their thoraces (as described above) and then placed into petri-dishes and maintained at 22°C and 40-50% relative humidity in a Sanyo Gallenkamp™ incubator. The positions of the mites on the host were monitored until they dismounted. On dismounting, the mites were weighed to the nearest 5µg using a Cahn™ 500 microbalance and the statistical significance was determined using Mann-Whitney U-Tests.

10 of the 65 parasitised bees were removed from the experiment after 10 hours and equipment which had previously been used to handle bees was brought into close proximity to the mites. Additionally, mites were exposed to CO₂-killed *Calliphora spp* adults.

Mite survival when isolated from their hosts under winter cluster conditions.

Mites ($n=204$) were removed from overwintering bees and placed individually into Ependorff™ tubes which had been pierced 20 times with a hot pin. The tubes were then suspended from the top bars of the source hive directly into the winter cluster formed by the naturally overwintering bees and mite mortality recorded every 6 hours until all the mites had died.

Mite survival at different relative humidities under laboratory conditions.

20 adult female *V.jacobsoni* were removed by hand from overwintering bees during December 1995 and divided into four groups of five mites each. Each group was subjected to a different humidity regime at a constant temperature of $25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ in a Sanyo Gallenkamp™ incubator. Individual mites were weighed every hour using a Cahn™ 500 microbalance to the nearest 5µg until they died. Mites were defined as being dead if after prodding three times with a pin, no response was noticed.

The apparatus used is shown in Figure 3.1. Each Ependorff™ tube was pierced by 20 pin holes, and contained only one mite. The humidity in each chamber was controlled using either silica gel or dilutions of glycerin in water (Table 3.1), and measured using a Rotronic™ M1 Hygrometer.

Each time the Ependorff™ tube was removed from the flask, a new sheet of paraffilm™ was stretched over the flask aperture to reduce changes in the humidity. Furthermore, each mite was placed into a new flask with a fresh glycerin solution every 8hrs to ensure a constant environment.

Mean weight at death of the mites in each humidity treatment group was calculated as follows:

$$\text{Mean weight at death } (\mu\text{g}) = \frac{\sum([Dwt-1hr + Dwt] \div 2)}{n}$$

where Dwt = Weight of mite at death (μg)

 Dwt-1hr = Weight of mite 1 hour before death (μg)

 n = number of mites in the group

The hive as a barrier to mites re-locating a host after separation.

To investigate how effectively mites separated from their hosts could move about within the hive, a square soft-wood arena and a variable-angle soft wood slope (Figure 3.3) were constructed. Mites were individually placed at the centre of the square arena or onto the slope and their behaviour was constantly observed.

Mite distribution within a winter cluster.

An infested overwintering colony was killed with liquid nitrogen (20 litres at -196°C) (Ritter *et al.*, 1980), instantly killing the whole cluster and fixing it *in situ*. The bees were then individually removed from the combs and were examined to ascertain their parasitosis. The mite distribution on individual bees was then compared to a distribution predicted by the Poisson

model to determine whether it was random or not. A G-test (goodness of fit) was employed to measure the significance of the relationship.

RESULTS

Laboratory simulated studies on the relationship between the transfer and survival of *V.jacobsoni* and the death of its honey bee host.

a. At high cluster temperatures

16% ($n=42$) of bees and 32% ($n=53$) of mites died during the course of the experiments. Of the surviving mites ($n=114$), a mean of 19.7% remained attached to their original host while 25.2% transferred from a live host to a new live host. The movement of the remaining mites (14.5%) remained ambiguous, due to their loss of colour markings. The other surviving mites ($n=37$) were found off the bees, or had transferred from bees which had also subsequently died

b. At low cluster temperatures.

In 18 trials, involving a total of 246 bees and 296 mites, over 75% of those mites which had been on dying hosts ($n=125$) transferred onto new hosts. Consequently, there was a significant difference between the median number of dead bees which remained parasitised (median=1, $Q1=0$, $Q3=2.5$) and the median number of dead bees whose mites had left them (median=5,

Q1=3.5, Q3=8) ($U_{17,17}=185.5$, $p=0.0001$). There was also a significant difference between the median parasitosis at the start of the experiment and at the end of the experiment of the experiment (median=1, Q1=1, Q3=1: and median=2, Q1=1, Q3=3, respectively) ($U_{245,100}=36533$, $p<0.0001$) and live bees were commonly found to have parasitoses of between 3 and 5, and even 7 on one occasion at the end of the experiment.

Transfer of mites from dying hosts descending through a natural winter cluster.

The rate of mite transfer from dying hosts was curvilinear with 50% transferring within 3 hours and over 75% of mites doing so within 24 hours (Figure 3.4). Some mites (~20%) did not succeed in transferring before their host fell through the winter cluster. In these cases, the bee showed some movement and/or abdominal pulsation. Such stimuli may have caused the mites to be 'unaware' of the condition of the host and thus precluded any behavioural response which would have led to their transferring onto new hosts. Examination of the cluster at the end of the experiment yielded seven mites which had been marked with silver Humbrol™ paint. It is assumed that the other mites had lost their paint- under laboratory conditions many mites (~60%) lose their markings after 7 days.

Behaviour of mites after host death under laboratory conditions.

In the absence of live bees, mites spent a mean time of 48 ± 26.5 hours (min= 0.45hrs; max=117.2hrs; $n=43$) on a dead host before dismounting (Figure 3.5). In a separate experiment, 94% of mites ($n=15$) alternated their position on the host during this time between being underneath the tergites and at a prominent position on the host, such as on the uppermost surface. When at a prominent location, mites frequently lifted their first pair of legs in the air and would rapidly transfer onto moving objects (e.g. forceps, fingers) especially if these had been in prior contact with bees ($n=46$ transfers out of 50 attempts). No mites were observed to transfer onto CO₂-killed *Calliphora* spp. adults ($n=22$ attempts).

All mites ($n=55$) eventually dismounted from their dead hosts at which point they had a median weight 15% lower (median=0.29mg, Q1=0.27, Q3=0.31) than those of a control group ($n=46$) removed from live naturally-infested bees (median=0.34mg, Q1=0.33, Q3=0.35) ($U_{42,18}=1525.5$, $p=0.0001$) from the same colony on the same date. Once the weight had fallen to 26% of that of the control group, the mites ceased all discernible movement and died (median=0.25mg, Q1=0.23, Q3=0.28). There was also a significant negative correlation between the weight (mg) of mites at dismounting and the time (hours) spent on the dead host ($r_{s18}=-0.693$, $p<0.002$).

Mite survival when isolated from their hosts.

Over 48% of mites died within 24 hours ($n=98$) when isolated from their hosts. This figure rose to 88% ($n=180$) by 48 hours and 100% after 72hrs, even though the mites were exposed to central cluster conditions.

Mite survival at different relative humidities under laboratory conditions.

During the first six hours, there were significant differences between the mean weight losses of each treatment group, which were 40.2%, 36%, 22.6% and 16.6% for the 0%, 30%, 60% and 100% R.H. respectively ($F_{3,16}=4.63$, $p<0.05$) (Table 3.2). However, the weights of the mites within each treatment group did not change at significantly different rates (0% R.H., $F_{4,20}=0.516$, $p>0.05$; 30% R.H., $F_{4,20}=0.122$, $p>0.05$; 60% R.H., $F_{4,20}=2.74$, $p>0.05$; 100% R.H., $F_{4,20}=0.34$, $p>0.05$); except for mite number 15 (group 3) and mite number 20 (group 4).

Regression analysis showed that mites exposed to a 0%R.H. environment lost mass most rapidly (0.018 mg/hr) and that mites exposed to a 100%R.H. lost mass least rapidly (0.002 mg/hr).

There was a significant difference in the mean survival time (hrs) of each mite group ($F_{3,11}=31.12$, $p<0.001$). Mites exposed to an environment of 100% R.H. lived ~3 times as long as those at 0% and 30% R.H., and ~2 times as long as mites at 60% R.H. (Table 3.2). 50% of mites in groups 1 and 2 (0% & 30% R.H. respectively) died after 6 - 6.5hrs exposure to the

given conditions. 50% of group 3 mites (60% R.H.) and 50% of group 4 mites (100% R.H.) died after 9.5hrs and >23hrs exposure respectively.

There was no significant difference between the mean mite weights at death between groups ($F_{2,9}=1.68$, $p>0.05$) (Table 3.3).

The hive as a barrier to mites re-locating a host after separation.

None of the mites placed into the square arena ($n=99$) were observed to make successfully the transition from the horizontal to the vertical surface. Most of the mites which did attempt this overturned onto their dorsal shields and struggled to correct their orientation.

Using the variable-angle sloping surface, it was found that mites began experiencing difficulty keeping a purchase at an angle of 15° , although they were generally able to recover and continue to walk. Between $25-30^\circ$, mites would overturn and could not recover themselves and at any angle steeper than $35-40^\circ$ they fell off the slope.

Mite distribution within a winter cluster.

The cluster contained 7471 bees, 25% of which were parasitised with between 1 and 3 mites. The distribution of the 2057 mites on individual bees was not random, and differed significantly from that predicted by a Poisson model ($G_{adj3}=31.496$, $p<0.001$). That is to say fewer non-parasitised bees were observed, more singly infested bees were observed and fewer multiply

infested bees were observed than predicted (Figure 3.6). This distribution pattern implies that the mites may have been moving between bees, preferentially selecting non-parasitised hosts.

DISCUSSION

These findings demonstrate that mites transfer between overwintering bees and that many of them are capable of escaping from dead hosts before these fall to the floor of the hive. Previous studies have suggested that movement of varroa between hosts takes place seldomly or not at all owing to its cost in energetic terms and that there is a risk of being separated from the host (Ritter *et al*, 1980). It has also been suggested by Ritter *et al*. (1980) that, in the winter, low temperature would restrict the mites' mobility. However, the normal winter cluster temperature is 20-35°C (Winston, 1991) and the laboratory experiments suggest that this would not compromise mite movement. Although the temperature at the periphery of the cluster may be much lower than 20°C, bees do not remain at the same location all the time, but continually exchange places between its periphery and centre (Esch, 1960). Temperature is therefore unlikely to be an important factor limiting mite movement. It is interesting to note that Rosenkranz (1985) reported that temperature had little or no influence on the distribution of varroa within the brood nest. So temperature (provided it is not at the extremes of toleration),

may not be as important as some workers had previously thought in influencing the distribution of varroa.

Another reason for objecting to the idea that mites routinely transfer between hosts within a winter cluster could be that it would expose them to bee grooming behaviour (Peng *et al.*, 1987). Considerable attention has been paid to the defensive mechanisms of bees against varroa, and grooming behaviour has been identified as having some importance (Fries *et al.*, 1996; chapter 2). Nevertheless, it is known that mites move between adult bees during spring and summer, such as during robbing episodes (Sakofski, 1980) and off drifting bees (Sakofski & Koeniger, 1986). Therefore, as bees are relatively inactive within the winter cluster (Esch, 1960), and the mites can benefit from transferring (see below), I can see no logic in precluding inter-host transfer at this time.

The ability to detect bee death and to transfer onto a live host before the dead bee falls to the floor of the hive is of obvious benefit to the mites - especially as they are unlikely to be capable of returning to the winter cluster unaided. However, if no alternative hosts are available, or the bee falls before the mite has time to transfer, then it remains on the dead bee. This, as DeGuzman *et al.* (1993) have also shown, prolongs mite survival compared to mites kept in isolation. This is not only owing to the dead bee providing a

more suitable microclimate but also because the mites are able to continue feeding for many hours after host death (chapter 5). In addition to providing nutrients, feeding also replaces lost water (metabolic/trans-cuticular) which is an important factor determining mite survival (Bruce *et al.*, 1997).

At comparatively high humidities (>60%R.H.), mites isolated from their hosts survived for over ten hours. This is lower than the survival times of mites obtained by others in related experiments. DeGuzman *et al.* (1993) found that mites lived for 27hrs away from their hosts, and Bruce *et al.* (1997) found that mites lived for 10hrs at 30°C and 42hrs at 20°C. One criticism of the experimental design of Bruce *et al.* (1997), was that they recorded the time it took for a mite to loose 50% of its mass, which would take longer than to loose a 'lethal' amount of water which I found to be in the region of 40% (see later). A possible reason for these discrepancies in survival times could be due to the additional 'stress' that the mites would have endured in my experiment because of the frequent hourly weighing undertaken. My data is, however, similar to that of Sadov (cited by Grobov, 1977), who found that mites could only survive for 24-30hrs on dried bees, dried faeces, or dried mammalian blood. No value was given for the %R.H. associated with these experiments, but it was reported that at 'lower' humidities, mite survival was correspondingly reduced. Once mites have lost enough water to reduce their body weight to about 0.21mg (i.e. 40% weight

loss) then they are unlikely to recover, because even before their weight reaches this low level, they become immobile and would be incapable of responding to a suitable host if one became available.

Perhaps crucially, the dead bees provide a platform from which the mites have a good chance of transferring onto other live bees. Mites on dead bees alternate between 'questing' (i.e. when they sit with their front legs raised and stretched out, probably to expose their tarsal sense organs which are at the distal ends of the first pair of legs [Milani & Nanelli, 1980]) on prominent locations on the upper surfaces, and secreting themselves on the lower surfaces, usually between the sclerites, where they continue to feed (chapter 5). In agreement with the observations of Grobov (1977), questing mites would immediately transfer themselves onto live bees or to moving inanimate objects which had previously been used for handling bees.

However, I found that they would not transfer to another species of insect, namely adult *Calliphora* spp. ($n=23$), suggesting that the stimulus initiating transfer involves both mechanoreceptors and chemoreceptors. It is interesting to note however, that varroa have been found as phoretics on wasps (*Vespula* spp) (Gerig & Lips, 1988), perhaps as social insects they have certain 'odours' in common with *Apis* spp. Within the hive, certain bees specialize in the removal of dead bees (Visscher, 1983). Consequently, any

mite which is unable to immediately transfer upon host death (under laboratory conditions) greatly enhances their chances of encountering another live bee if they remain with the dead host for as long as possible. It is not until around 48 hours after host death that the mites dismount and move away in active search of a new host. This is probably because by this time, owing to decomposition and dehydration, dead bees no longer prove to be a suitable food source or to provide a suitable microclimate. Although isolated mites soon die and the chances of a mite locating a suitable host by itself are slight, because the mite has only a few hours left to live anyway, this strategy is probably marginally less risky than remaining with the now inedible dead bee which shows no sign of being cleared away.

The results indicate that mites also transfer between live bees. This is less easy to explain than the movement from dying bees, but may be linked to the mites' preference for feeding between the left 3rd/4th inter-tergite space (chapter 2). Other sites may be occupied, in a clear order of preference, so it is not essential for survival, but it may provide some selective advantage associated with accessing nutrients. The consequence of this site preference is that many more singly-infested bees are found within a colony than would be the case if mite distribution was random. Mite transfer between live bees may reflect a continual search by the mites for uninfested bees so that they can access the preferred feeding site (chapter 2). There may also be a

difference between the suitability of individual bees as hosts which further contributes to mite movement.

The non-random (non-poisson) distribution of the mites within the cluster, could be explained in a number of ways (such as a non-normal distribution of the mites before winter) although on the basis of the other experiments which have shown that mites move between hosts, the most plausible reason is that of inter-host transfer to access preferred feeding sites. The other explanations for this distribution include; firstly, as mentioned, a non-poisson distribution of the mites existed before the cluster had formed. This would be difficult to verify without destructively sampling a large proportion of the bees and mites at the start of winter, which in itself could adversely affect the behaviour and survival of both bees and mites, thus causing complications for the second sample which would be taken later as winter progressed.

Studies have suggested that the distribution of mites on adult bees is influenced by olfactory cues (Kuenen & Calderone, 1997) and that bees older than nurse bees show no difference in their infestation rates (Steiner, 1993). Because no brood had been present in the colony for over a month, it is reasonable to assume that the physiology of the bees over this time was that of older bees as the stimulus for nurse physiology or olfactory cues would be greatly reduced. Furthermore, the only study which suggests that mites are

randomly distributed within the winter cluster is that of Ritter *et al.* (1980), whose method I believe may yield reliable data on mite distribution between frames within the cluster but their methodology could not provide information on the mite distribution between individual bees. The reason for this is that they only investigated the density of mites in individual beeways (i.e. the gaps between the frames/cells where the bees are found). They did not examine individual bees and relate the distribution of mites to bees.

Secondly, it is possible that multiply parasitised bees died more frequently than their singly infested counterparts, yielding a non-poisson distribution. The effect of varroa infestation on the longevity of bees is unclear (Martin, 1994b). Indeed it is not until a developmental parasitosis of four or more mites per bee is recorded (Schatton-Gadelmayer & Engels, 1988), that a clear reduction in life-span when compared to non-parasitised bees occurs. However, even when a parasitosis of 8 mites or more per developing bee is recorded, some develop without any apparent effect on lifespan (Kovac & Crailsheim, 1988). There is no published data on the influence of phoretic varroa on the lifespan of overwintering bees. However, I would suggest that provided they do not carry pathogens, that their effect on lifespan would be limited, until a high parasitosis was reached. Even if infested bees died more rapidly, the mites would still have to transfer between the overwintering hosts to bring about the distribution observed.

Consequently the main conclusions of this work would remain unchanged, namely, that inter-host transfer of mites between clustered overwintering bees takes place; and that the observation of Grobov (1977) that “one could even notice parasites passing from one living bee to another”, and of Koivulehto (1976) who stated that varroa readily changed hosts both inside and outside the hive (no supporting data or references provided) is not the exception, but more likely the rule.

In conclusion, the portrayal of *V.jacobsoni* as a passive, helpless, phoretic mite, inseparable from its host during winter, which dies when its host dies may be misleading and requires further investigation. Models of varroa population dynamics which predict a mite mortality of 50% overwinter (based solely on the assumption that mites do not transfer between hosts and not on natural mite lifespan/mortality rates (e.g. Calatayud & Verdú, 1994) will need reevaluation. More importantly, the movement of mites between hosts has implications for the transmission of viral and microbial pathogens (chapter 6). Once overwintering bees start to die of old age and disease, more and more mites, potentially carrying disease agents, will become concentrated on the remaining bees. This would help explain the collapse of varroa-infested colonies towards the end of winter.

In conclusion, I would recommend correct pre-winter colony management to reduce mite infestation levels, and to ensure that hives are

well-prepared before going into winter in terms of both location/shelter and ample food supplies. Finally, the overwinter mite mortality rate should be monitored to enable beekeepers to take early action in the spring where necessary to control the mites.

P.T.O

Figure 3.1 Worker honeybees (*Apis mellifera*) marked with numbered colour discs (Koniginnen Werden Gezeichnet™).



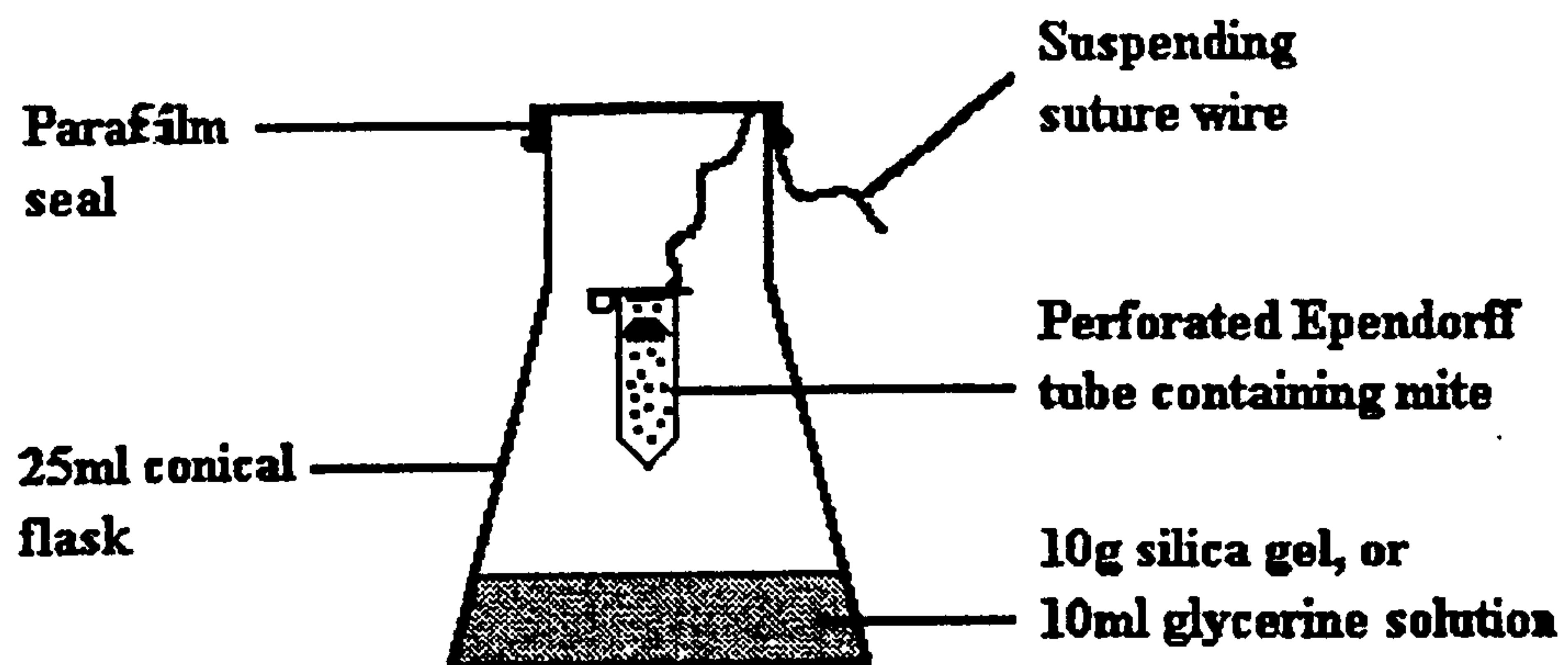
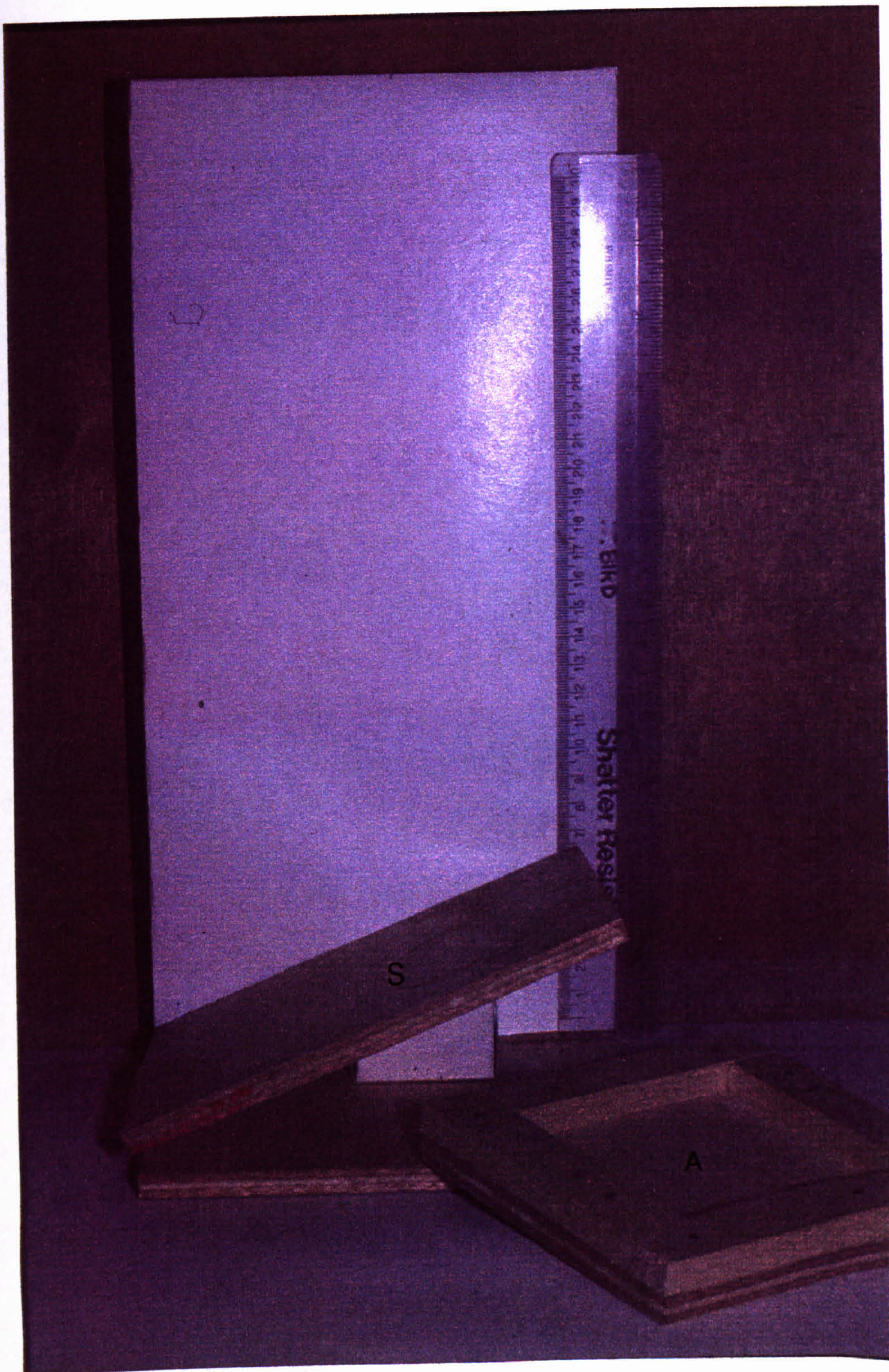


Figure 3.2 Apparatus used to expose adult female *Varroa jacobsoni* to 0%, 35.3%, 57.6% and 100% Relative Humidity respectively over 24 hrs.

P.T.O

Figure 3.3 Soft-wood arena (A) and variable-angle slope (S) on which *Varroa jacobsoni* were placed to investigate their potential mobility within a hive when not attached to a honeybee (*Apis mellifera*).

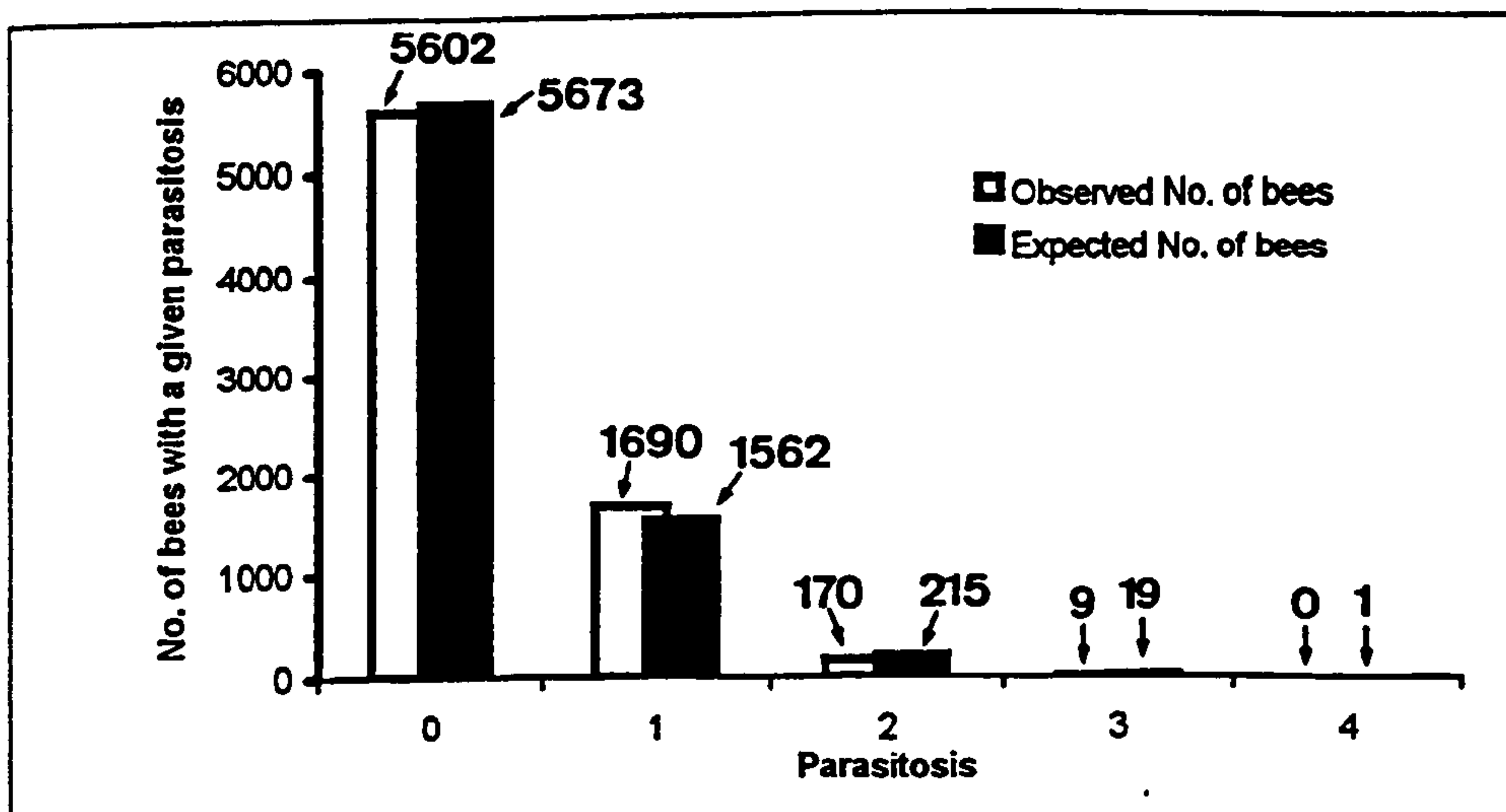
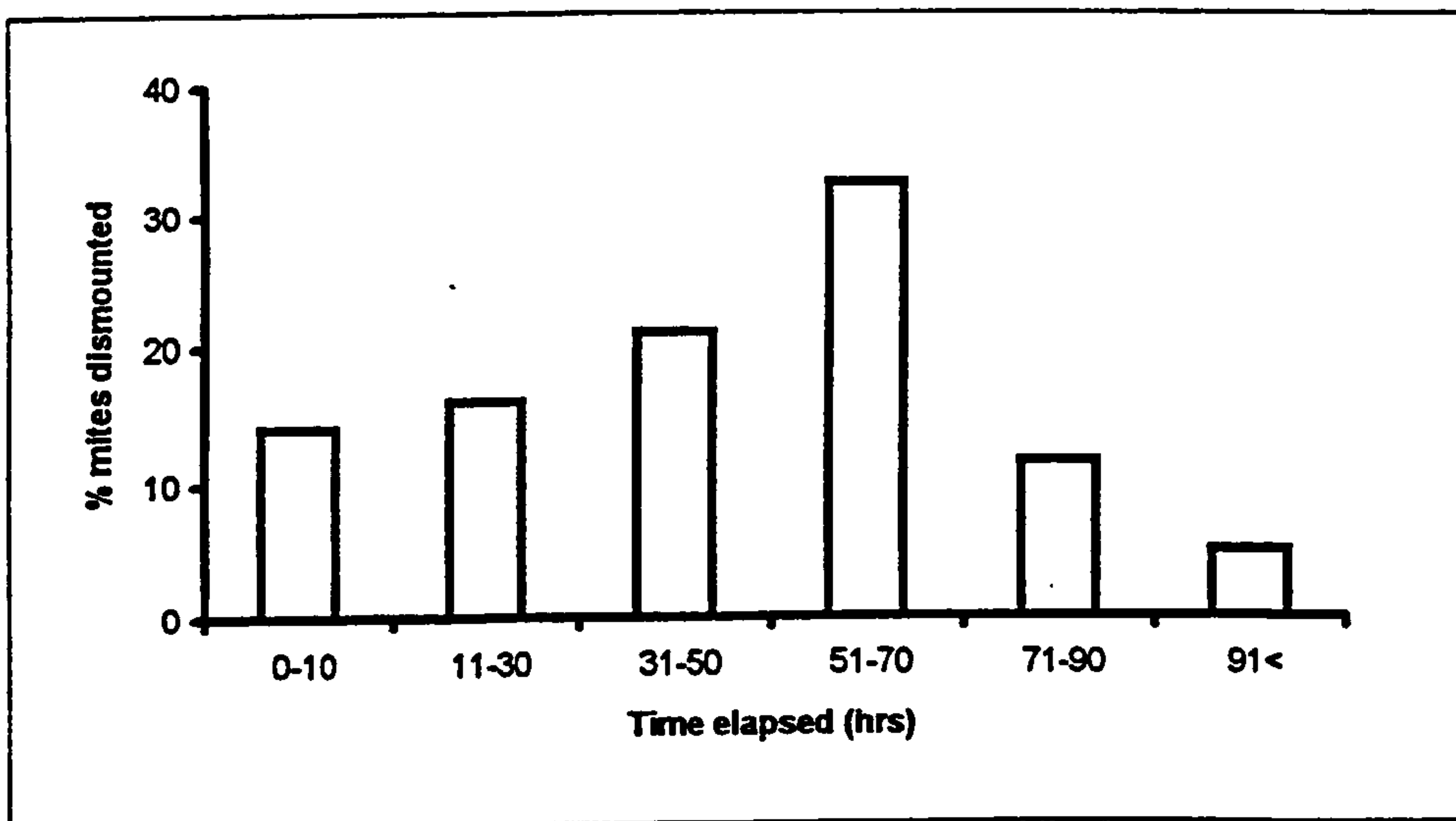
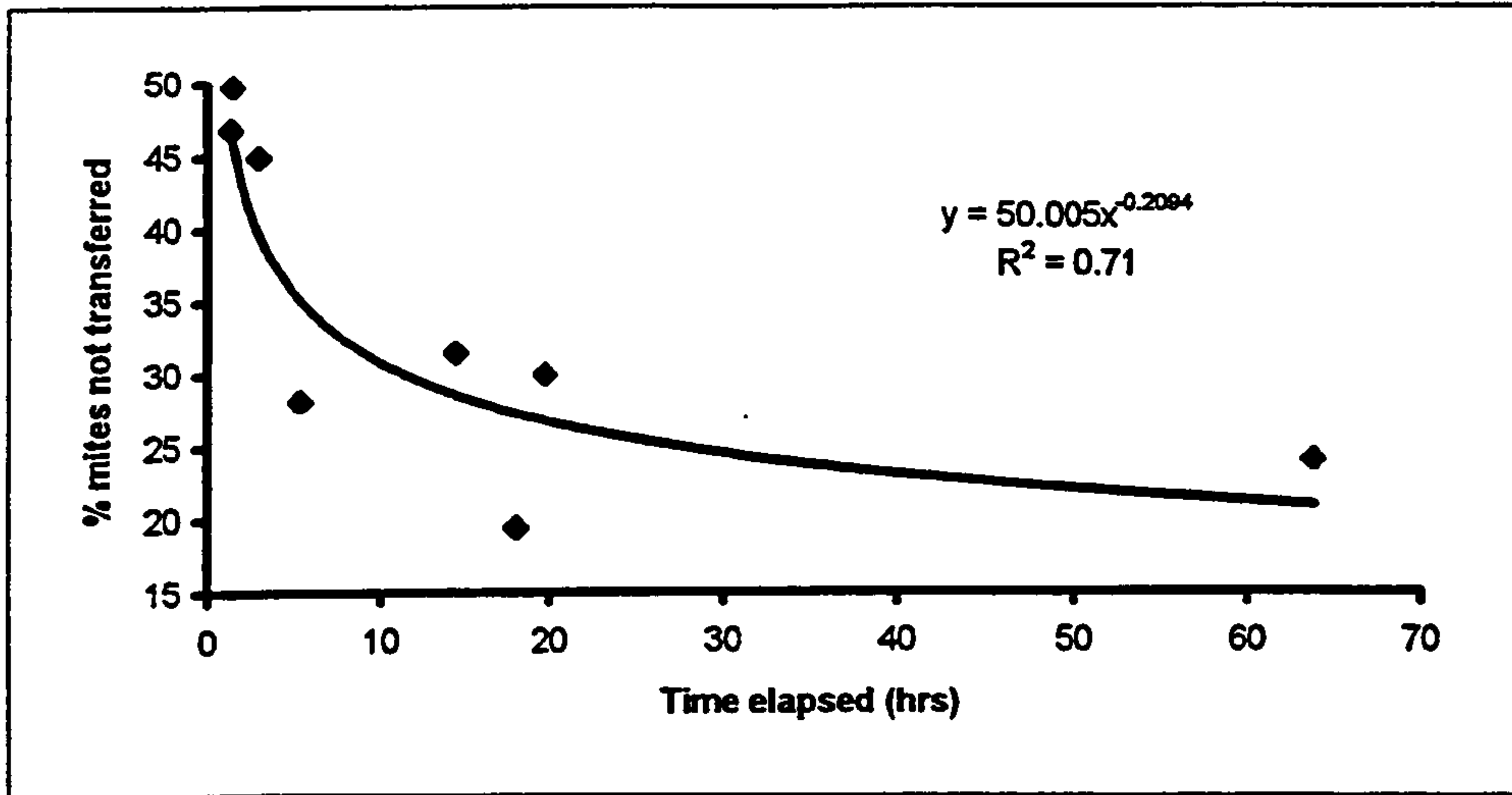


P.T.O

Figure 3.4 Rate of transfer of 135 *Varroa jacobsoni* from 124 naturally-infested dying *Apis mellifera* descending through a winter cluster.

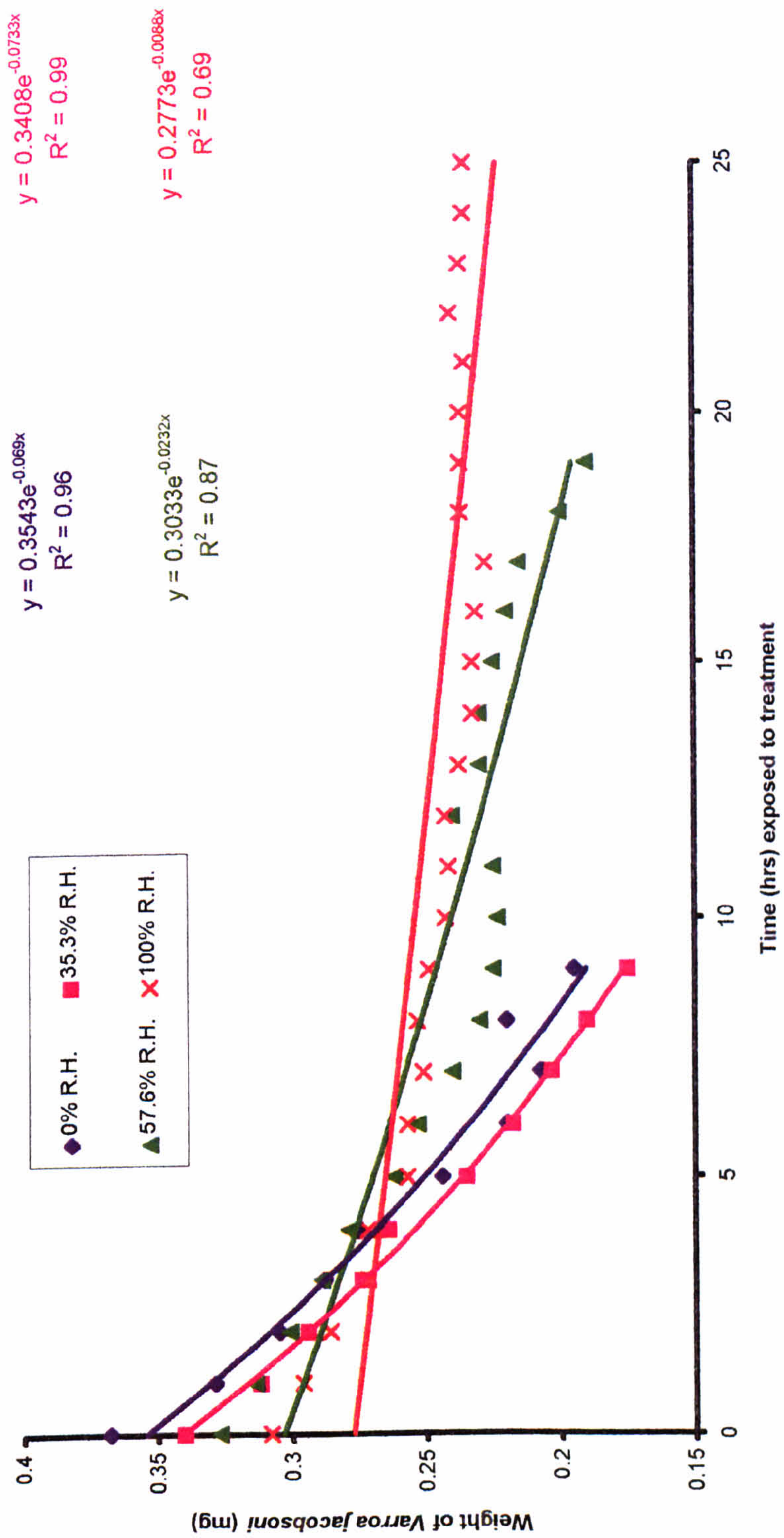
Figure 3.5 Time taken for 55 *Varroa jacobsoni* to dismount from their dead, singly infested hosts (*Apis mellifera*) under laboratory conditions.

Figure 3.6 Observed and expected (as determined by the poisson model) parasitosis of an overwintering *Apis mellifera* colony naturally-infested with *Varroa jacobsoni*.



P.T.O

Figure 3.7 Changes in the weights of adult female *Varroa jacobsoni* ($n = 20$) when exposed to 0%, 35.3%, 57.6% and 100% Relative Humidity respectively over 24 hours.



Group	Weight of glycerine (mg)	Weight of water (mg)	Theoretical %R.H. at 25°C	Actual %R.H. at 32°C
1	Silica gel	0	0	0
2	33	67	30	35.3
3	70	30	60	57.6
4	0	100	100	100

Table 3.1 Weights of glycerine (mg) and water (mg) required at 25°C to prepare solutions above which are generated fixed %Relative Humidities (Archenhold *et al.*, 1978). Actual %R.H. generated at 32°C were determined using a hygrometer.

Group	%R.H.	Mite weight (mg±sd) at start	Mean survival time (hrs±sd)	Mean weight loss over 6hrs±sd
1	0	0.368±0.013	6.7±1.095 a	0.148±0.0217 e
2	35.3	0.34±0.01	6.7±1.304 a,b	0.1225±0.008 f
3	57.6	0.327±0.018	10.9±4.39 b,c	0.074±0.0185 g
4	100	0.308±0.031	22.8±4.38 d	0.051±0.0139 h

Table 3.2 Mean survival times (hrs) and mean weight losses (mg) of *Varroa jacobsoni* held in fixed humidity environments. Mean survival times and mean weight losses over 6hrs followed by the same letter are not significantly different from one another, those followed by different letters are significantly different from one another ($p < 0.05$, Tukey test).

Group	%R.H.	Mean mite weight at death (mg±sd)	% Mortality after 24 hours
1	0	0.205±0.01	100
2	35.3	0.205±0.02	100
3	57.6	0.204±0.01	100
4	100	0.2	20

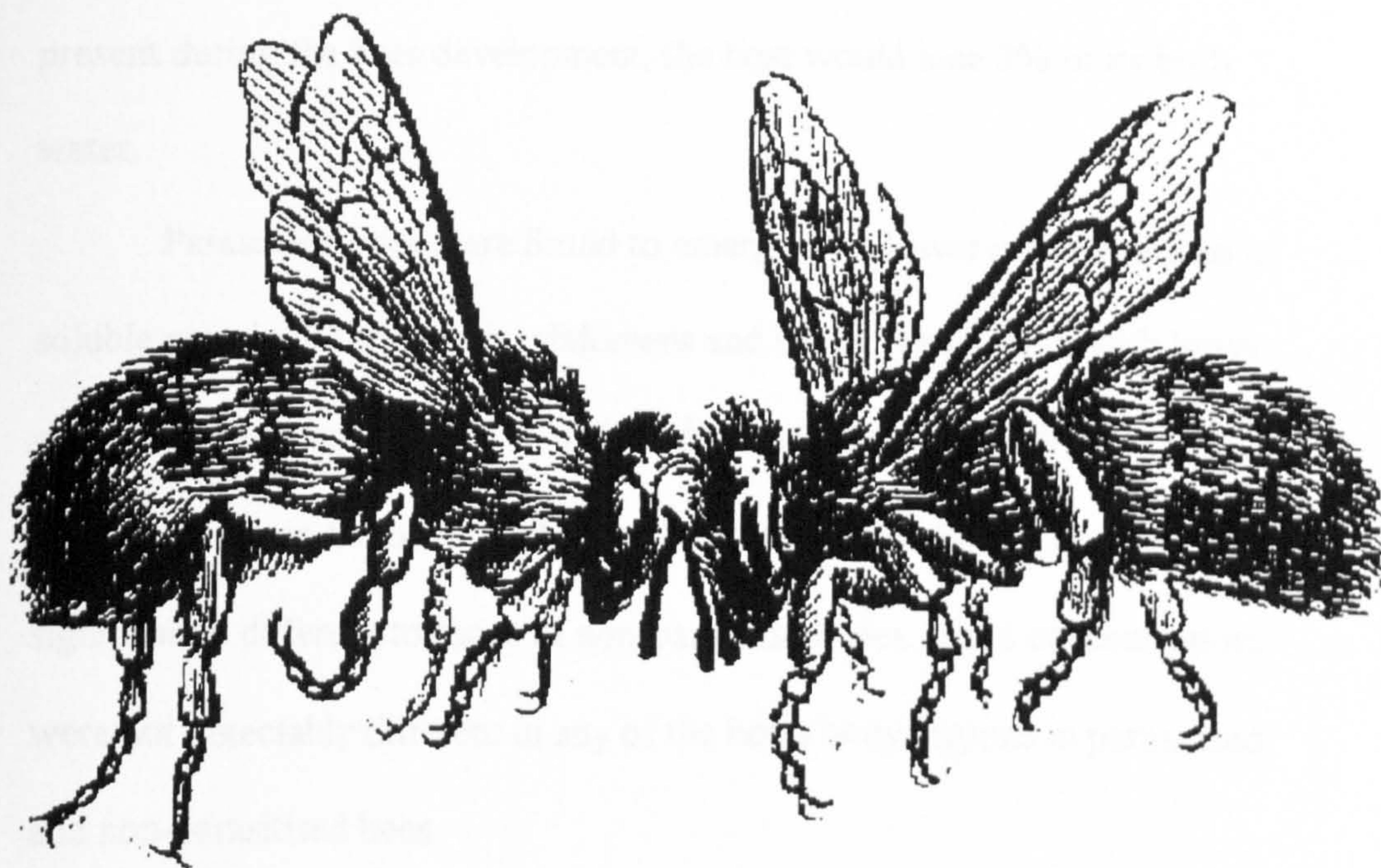
Table 3.3 Mean weights of *Varroa jacobsoni* at death after exposure to 0%, 35.3%, 57.6% and 100% Relative Humidity Environments. (n=5 for each group). sd = standard deviation.

Authority	% R.H.	Temp. (°C)	Survival time (hrs)
Bruce, Needham & Potts, 1997.	0	20	16±1.8
	60	"	42±5.1 Time taken
	97	"	123±22 to lose
	0	30	5±0.4 50% body
	60	"	10±0.8 mass
	97	"	29±3.1
DARG booklet	Hive	Hive	3-5 days
	Room	Room	A 'few hours'
DeGuzman <i>et al.</i> , 1993		26	27±0.97
Engels, 1992 (cited by DeGuzman <i>et al.</i> [1993] pers. comms.)	High	20-25	5-10days
Grobov, 1977	10 to 20	35	24
	50	35	3 days
	85	28	9 days

Table 3.4 Literature concerning the effect of humidity on the survival of *Varroa jacobsoni* maintained off their host.

SUMMARY

THE EFFECT OF *VARROA JACOBSONI*
INFESTATION ON ADULT WORKER
HONEYBEE EMERGENCE WEIGHTS AND
THEIR PROTEIN, LIPID AND
CARBOHYDRATE LEVELS.



SUMMARY

Honeybees infested with *Varroa jacobsoni* during development are profoundly different from their non-infested sisters at emergence. Parasitised bees exhibited a 3-30% wet weight loss, which was negatively correlated with increasing numbers of mites. The whole dry body weights and water contents of parasitised bees were also negatively correlated with increasing parasitosis. It was estimated that for every parasitising adult female mite present during the bees development, the host would lose 3% of its body water.

Parasitised bees were found to emerge with lower concentrations of soluble protein in their heads, abdomens and haemolymph, and, with lower carbohydrate concentrations in their abdomens. The carbohydrate concentrations in the heads and thoraces of parasitised bees were not significantly different to those of non-parasitised bees. Lipid concentrations were not detectably different in any of the hosts body regions in parasitised and non-parasitised bees.

8.5% of the emerging bees ($n=543$) exhibited morphological deformities. Morphological deformity was positively correlated with increasing parasitosis. Deformed bees were, however, found in all categories

of parasitosis, suggesting that other factors such as secondary infections may also have a role in the onset of bee deformity.

Key words: *Apis mellifera*, *Varroa jacobsoni*, metabolic reserves, proteins, carbohydrates, lipids, haemolymph, water, morphological damage, weight loss.

INTRODUCTION

Apis mellifera colonies normally die within three to five years of becoming infested with *Varroa jacobsoni* (Nedealkov, 1977; Shabanov *et al.*, 1978). Why this should be so has been the subject of much research over the last 20 years. It has become evident that colony collapse results from many interrelated factors which act detrimentally either on individual bees or cumulatively on the whole colony. These factors include the sub-species/strain of honeybee infested (Moritz & Hänel, 1984; Engels *et al.*, 1986; Buchler, 1990; Moritz & Mautz, 1990; Otten, 1990); abiotic/climatic conditions such as ambient temperature, humidity and altitude (De Jong *et al.*, 1984; Kraus & Velthuis, 1997); the number of bees and mites in the colony (Kovac & Crailsheim, 1988); the presence of micro-organisms or viruses associated with the mites and bees (Bailey *et al.*, 1981; Ball, 1985; Allen *et al.*, 1986; Strick & Madel, 1986; Koch & Ritter, 1991); the availability of resources such as nectar and pollen to the bee colony (DeJong *et al.*, 1984).

The physical and physiological effects of *V.jacobsoni* on individual bees have been extensively investigated and, as expected, the mites have been shown to cause considerable pathological effects (DeJong *et al.*, 1982b). Infested bees are also said to be 'restless' or 'irritated' because of the mites

which may lead to an increased respiration/metabolic rate (Grobov, 1977; Shabanov *et al*, 1978; Byzova *et al*, 1982; Boecking, 1992). Most of the studies on the effect of varroa on the metabolic reserves of bees have concentrated on only one or two factors in isolation and differences in methodology, strains of bee, climate *etc.* makes comparing them difficult. The purpose of this study was therefore to investigate the changes caused by varroa on the physical and biochemical aspects of the host. The factors given consideration were weight of emerging bees, the relationship between morphological damage and parasitosis, the effect of parasitism on host soluble protein and lipid titres, and the effect of parasitism on host carbohydrate titres. By examining all the above factors simultaneously on a large sample of emerging bees collected at the same time of year from the same geographic area, the results would give a reliable indication of the effect of varroa on summer colonies at any one time. This is the first time that the effects of varroa on honeybee protein titres (other than haemolymph proteins), carbohydrate titres and lipid titres have been investigated.

MATERIALS & METHODS

All the bees came from 10 infested hives sited at two apiaries separated by 25 km in North Devon, collected during one week in August 1996. Bees were collected at the point of emergence directly from their

capped cells. Only those bees which had started to pierce their cell cappings were used. Bees in a more advanced state of emergence were not used because some or all the infesting mites could have escaped. All the bees and their vacated cells (including the innermost surface of the cappings) were searched for mites. The parasitosis of a bee was defined as the number of adult female mites and deutonymphs^{*1} present at its emergence. Male varroa and protonymphs were not included in this study because it is difficult to locate them without spending a disproportionate amount of time. This would have involved losing many other emerging bees and female mites. Furthermore, because of their small size, it is unlikely that protonymphs and males make a great impact on the host. Bees were randomly allocated to one of the biochemical assays on the basis of their parasitosis and not on the basis of their colony origins. This ensured that no bias would occur should any inter-colony differences have existed.

Emergence weights and water content of parasitised and non-parasitised worker bees

All bees were weighed using a CahnTM 500 microbalance to the nearest 5µg, then frozen at -22°C overnight. They were then freeze dried (Edwards Super Modulyo freeze dryer) for 3 days to a constant weight and

¹ Words marked with an asterix are defined in the glossary

re-weighed to determine their total water content. Their appendages were then removed and the head, thorax and abdomen separated and weighed individually. The body compartments were assayed for either soluble protein, total carbohydrate (anthrone-positive material) or total lipid as described below. All three compartments of an individual bee were assayed for the same biochemical component, i.e. either protein, carbohydrate or lipid.

Determination of protein titres

The heads, thoraces and abdomens were individually ground-glass homogenised in 1ml distilled water and then centrifuged at 1500g for 5 minutes (MSE MicroCentaur™ Centrifuge). Haemolymph was obtained prior to freezing the bees by making an abdominal inter-segmental incision and drawing known volumes of haemolymph (1-5µl) into capillary tubes. Each haemolymph sample was then mixed with 0.1ml distilled water and centrifuged at 1500g for 5 minutes (MSE MicroCentaur™ Centrifuge). Aliquots of supernatant (head 20µl, thorax 10µl, abdomen 10µl, haemolymph 20µl) were assayed for soluble protein by the dye-binding method of Bradford (1976). A 1:4 (vol:vol) dilution of BioRad Dye Reagent (Bio Rad Labs. GmbH) was used with bovine serum albumin (Sigma[Fraction V, 98%]) as standard (Anon, 1979). After 5 mins the

sample absorbances were recorded at 595nm using a Jenway 6100 spectrophotometer against a reagent blank.

Determination of carbohydrate titres

Carbohydrate content was determined using the anthrone reagent method (Gunn & Gatehouse, 1987). The anthrone reagent (BDH, 99%) was prepared as follows: 140mg of anthrone was added to 1g of thiourea and 100ml of 66% (v/v) sulphuric acid. The solution was heated to 85°C and then cooled and stored in the dark.

The heads thoraces and abdomens were individually ground-glass homogenised in 1ml of 5% (v/v) trichloroacetic acid and then centrifuged at 1500g for 5 minutes (MSE MicroCentaur™ Centrifuge). Aliquots of supernatant (head 0.1ml, thorax 0.05ml, abdomen 0.05ml) were added to 2ml of anthrone reagent. The sample was then heated to 90°C for 20 minutes after which the absorbance was recorded at 620nm against a reagent blank using a Jenway 6100 spectrophotometer. Dilutions of a stock of 1mg/ml of glucose (AnalaR D(+)-Glucose) were used as standards.

Determination of lipid titres

The total lipid concentration in individual bees was determined using a slight modification of Van Handels' (1985) vanillin-phosphoric acid reagent

method. The heads, thoraces and abdomens were individually crushed in 2ml of (2:1) chloroform:methanol solution, and sonicated for 15 seconds at an amplitude of 30 μ m using an MSE sonicator.. Each sample was then centrifuged (MSE MicroCentaur™ Centrifuge) at 1500g for 5 minutes. 200 μ l of supernatant was taken from each sample and transferred to a clean tube from which the solvent was evaporated at 37°C in a heating block. 42 μ l of 95% (wt./vol.) sulphuric acid was then added to each sample and the tubes were then heated at 90°C for 10 minutes. Afterwards, 1ml of vanillin-phosphoric acid reagent was added (AnalaR-Vanillin, 99%) and the sample was left to stand for 15 minutes at room temperature. The absorbance was then read using a Jenway 6100 spectrophotometer at an optical density of 525nm against a reagent blank. The lipid content per bee portion could then be read directly from a calibration curve obtained using serial dilutions of 1mg/ml trioleine (SIGMA-Trioleine {C18:1,[CIS]-9}, 99%) in 2:1 chloroform methanol as a standard.

Statistical analysis

The significance of the difference between the mean values of data sets with unequal variance (as determined by the F-test [Fowler & Cohen, 1997]) was calculated using Welch's approximate t-test (Sokal & Rohlf, 1995). Regression analysis of parasitosis and other variables was carried out

using a type I model (least squares regression), whereas regression analysis of log transformed data on bee emergence weights and proportional weight tegmata was carried out using a type II model (reduced major axis regression [R.Brown pers.comms]). ANCOVA (analysis of covariance) was used to determine the significance of heterogeneity between the mean weights at emergence of deformed and non-deformed emerging bees around their regression slopes (where the covariate was the intensity of mite infestation (Sokal & Rohlf [1995]). Homogeneity of regression slopes was determined according to Fowler & Cohen (1997).

RESULTS

Emergence weights and water content of parasitised and non-parasitised worker bees

The mean(\pm se) emergence weight of non-parasitised bees (116.37 ± 0.61 mg [$n = 165$]) was significantly more than that of parasitised (min=1 mite, max=11 mites) bees (107.27 ± 0.57 mg, $n=377$) (Welch $t'_{164, 376} = 10.91 > t'_{.05} = 1.96$). Using regression analysis it was demonstrated that there was a significant negative relationship between the wet weight of workers ($n=542$) at emergence and their degree of parasitosis ($F_{1, 540} = 258.44$, $p < 0.001$) (Figure 4.1). It was calculated from the regression

equation that for every female mite (mature adult or deutonymph), the weight at emergence of the developing worker bee would decrease by ~3%.

The mean(\pm se) body water content of non-parasitised emerging worker bees was 103.28 ± 1.00 mg ($n=41$), which was significantly more than the mean water content of parasitised emerging workers (91.41 ± 0.73 mg) ($n=173$) (Welch $t'_{s, 40, 172} = 9.58 > t'_{.05} = 2.48$). The total water content of emerging worker bees was negatively associated with increasing parasitosis ($F_{1, 212} = 146.70$, $p < 0.001$), and it was estimated from the regression equation that a parasitised worker bee would lose ~3% (3 mg) of its total body water content for every parasitising mite (Figure 4.2).

Table 4.2 shows the mean wet emergence weights (mg) of worker bees in relation to increasing parasitosis by *V.jacobsoni* and the results of a GT2 (unplanned means comparison) test, carried out to determine which pairs of means were significantly different. The table shows that the mean emergence weights of non-parasitised bees were significantly greater than those of bees emerging parasitised by 2 or more mites. The table similarly shows that bees parasitised with 1 mite had significantly higher mean weights than bees which emerged parasitised by 2 or more mites.

Emerging bees could be classified according to whether or not they had morphological deformities. Of the 542 bees examined, 8.5% ($n=46$) exhibited morphological damage, which included crumpled wings and small bodies.

Three of the unparasitised bees were deformed. The emergence weights of both deformed and non-deformed (Figure 4.4) bees were negatively correlated with parasitosis (deformed bees, $F_{1,46}=27.45$, $p<0.001$; non-deformed bees, $F_{1,494}=162.4$, $p<0.001$). Although no significant difference was detectable between the slopes of the regression lines ($t_{538}=0.05$, $p>0.05$; Fowler & Cohen [1990]) of these two classes of bees, the intercept of the slope was lower for damaged bees (103.34mg) than for non-damaged bees (116.12mg). It was also found that the percentage bees which emerged damaged was positively correlated ($r_{12}=0.96$, $p<0.01$) with the intensity of infestation (Table 3).

ANCOVA demonstrated that bees which emerged deformed were significantly smaller (adj. mean=100.02mg, $n=46$) than their non-deformed sisters (adj. mean=110.97mg, $n=496$) with the same intensity of infestation, ($F_{1,539}=61.41$, $p<0.001$).

The mean(\pm se) dry weight (mg) of non-parasitised bees (19.88 ± 0.28 mg) ($n=37$), was significantly more than that of parasitised bees (17.54 ± 0.14 mg) ($n=143$) ($Z_{37,143}=7.39$, $p<0.01$). A negative relationship existed between dry emergence weights of workers and their degree of infestation ($F_{1,178}=37.05$, $p<0.001$) (Figure 4.3a). This trend was present in all three body compartments of the bee, with the heads, thoraces, and abdomens all showing highly significant weight losses (dry weight) with

increasing parasitosis (head, $F_{1, 178} = 11.87$, $p < 0.001$; thorax $F_{1, 178} = 29.69$, $p < 0.001$; abdomen $F_{1, 178} = 16.32$, $p < 0.001$) (Figure 4.3a).

The proportion of dry weight in the heads (mg head / g dry body) of bees decreased significantly in association with increased dry emergence weight (Figure 4.3b). The same trend was apparent in the thoraces of bees, except for bees exhibiting deformities at emergence, where the proportional thorax weights were independent of emergence weight (Figure 4.3c). In contrast, the proportional weights of the abdomens of non-parasitised bees and deformed emerging bees increased with increasing dry emergence weight, whereas the proportional weights of the abdomens of non-deformed parasitised bees was independent of emergence weight (Figure 4.3d).

Protein titres of emerging worker bees.

The mean(\pm se) concentration of soluble protein in non-parasitised bees was 57.44 ± 1.1 mg/g dry body weight ($n=18$), or about 1.03mg / bee (= 6% of dry weight). This was lower than other published values (3.3-14.6mg/bee [Kunert & Crailsheim, 1988]), but this could have been caused by the different method used.

The mean concentration of protein in the heads of non-parasitised bees was 151.73 ± 5.5 mg/g dry head ($n=18$). This was significantly more than the mean concentration of protein in the heads of parasitised bees (1-11

mites), which was 138.62 ± 2.44 mg/g dry head ($n=62$) ($Z_{61, 17} = 2.165$, $p < 0.05$). Moreover, a negative relationship was found between increasing parasitosis and the concentration of protein in the heads ($F_{1, 78} = 7.16$, $p < 0.05$) (Figure 4.5).

The mean concentration of protein in the thoraces of non-parasitised bees was 135.35 ± 2.21 mg/g dry thorax ($n=18$). This was significantly more than the mean concentration of protein in the thoraces of parasitised bees (1-11 mites), which was 128.5 ± 1.87 mg/g dry thorax ($n=62$) (Welch $t'_{61, 17} = 2.36 > t'_{.05} = 2.06$). No significant relationship was found between increasing parasitosis and the concentration of soluble protein in the thoraces (mg protein thorax/g dry thorax weight: $F_{1, 78} = 0.97$, $p > 0.05$) (Figure 4.6).

The mean(\pm se) concentration of protein in the abdomens of non-parasitised bees was 141.99 ± 3.21 mg/g dry abdomen ($n=18$). This was significantly more than the mean concentration of protein in the abdomens of parasitised bees (1-11 mites), which was 118.39 ± 3.32 mg/g dry abdomen ($n=62$) (Welch $t'_{61, 17} = 5.109 > t'_{.05} = 2.04$). As with the heads, a negative relationship was found to exist between the concentration of soluble protein in the abdomen (mg protein abdomen/g dry abdomen weight) and increasing intensity of infestation ($F_{1, 78} = 32.88$, $p < 0.001$) (Figure 4.7).

The mean(\pm se) concentration of protein in the haemolymph of non-parasitised bees was $11.48 \pm 1.35 \mu\text{g}/\mu\text{l}$ haem ($n=18$). However, in emerging bees, parasitised by 1-3 mites, the protein concentration was $6.2 \pm 0.42 \mu\text{g}/\mu\text{l}$ haem ($n=41$), and in emerging bees parasitised by 4-10 mites, the protein concentration was $4.88 \pm 0.9 \mu\text{g}/\mu\text{l}$ haem ($n=20$). These values were very similar to those of Schatton-Gadelmayer and Engels (1988), who also used the same protein assay, but were lower than those reported by Weinberg & Madel (1985), who employed the Lowry method to determine protein concentration. A highly significant negative relationship existed between parasitosis and protein levels in the extracted haemolymph of emerging bees ($F_{1,75} = 26.42$, $p < 0.001$). It was estimated from the regression line that a parasitosis of 1 female mite, reduced the haemolymph protein titre of the honey bee by about 10% (Figure 4.8)

Carbohydrate titres of emerging worker bees

The mean(\pm se) concentration of carbohydrate in non-parasitised bees was $72.61 \pm 6.55 \text{ mg/g}$ dry body ($n=19$), or about 1.3mg/bee (= 7.2% of dry weight), and the mean(\pm se) concentration of carbohydrate in parasitised bees (min. 1 mite, max. 11 mites) was $68.66 \pm 4.86 \text{ mg/g}$ dry body ($n=52$), which was not significantly different ($Z_{18,51} = 0.75$, $p > 0.05$). No relationship existed between increasing intensity of infestation and the concentration of

carbohydrate in the whole body ($F_{1,70} = 3.487$, $p > 0.05$), the head ($F_{1,70} = 0.12$, $p > 0.05$) (Figure 4.9) or the thorax ($F_{1,70} = 0.24$, $p > 0.05$) (Figure 4.10) of newly emerged bees.

A significant negative relationship existed between the concentration of carbohydrate in the abdomen (mg/g dry abd) and parasitosis ($F_{1,70} = 5.56$, $p < 0.01$) (Figure 4.11) but the mean concentration in the abdomens of non-parasitised bees (136.47 ± 12.4 mg/g dry abd) ($n=19$) was not significantly different from that of their parasitised sisters ($n=52$) which was $111.86 \pm$ mg/g dry abdomen ($Z_{18,51} = 1.77$, $p > 0.05$). From the regression equation, it was calculated that for every female mite (adult or deutonymph) parasitising a developing worker bee, there was about a 7.5% decrease in the total carbohydrate in the abdomen

Lipid titres of emerging worker bees

The mean(\pm se) concentration of lipid in non-parasitised emerging worker bees was 56.4 ± 1.9 mg/g dry wt ($n=10$), or 1.02mg/bee, which represented about 6% of the mass of the bee. In parasitised bees (1-4 mites) the mean(\pm se) concentration of lipid was 55.4 ± 1.57 mg/g dry wt ($n=53$). This figure equates well with other published data (1.03-1.7mg/bee [Kunert & Crailsheim, 1988]). No significant relationships existed between the concentration of lipid in the head (mg/g dry head), thorax (mg/g dry thorax)

or, abdomen (mg/g dry abdomen) with increasing parasitosis (head, $F_{1,56} = 1.24$, $p > 0.05$ [Figure 4.12]; thorax, $F_{1,56} = 0.05$, $p > 0.05$ [Figure 4.13]; abdomen, $F_{1,56} = 1.5$, $p > 0.05$ [Figure 4.14]). The concentration of lipid in deformed bees was, except in the thorax of one bee, lower, than the mean concentration of lipid in the tegmata of other non-deformed bees (Figures 4.12, 4.13 & 4.14).

DISCUSSION

Metamorphosis is an energetically highly demanding process since, at this time, the whole of the adult anatomy has to be constructed. This construction relies entirely on the reserves acquired during the larval stage. Any reduction in metabolic reserves during metamorphosis is irreversible (Hepburn *et al.*, 1979). There are two reasons for this. Firstly, pupae are physically isolated from the imagos which could furnish them with food and water (in the case of honey bees, the pupae are isolated from the rest of the hive by being enclosed within sealed cells which remain closed until their metamorphosis is complete). Secondly, honey bee pupae do not have functioning through-guts. During pupation the midgut is isolated from the mouth and anus by occlusion of the intestine both at the junction between the foregut and midgut and at the junction between the midgut and hindgut (Dade, 1962). Consequently, any loss of metabolites, due to mite feeding

activity on the developing pupae is irreversible and clearly could have severe repercussions on the normal development of the host. Indeed, my investigations have shown that varroa infestation results in significant changes in both the morphology and biochemical composition of emerging worker bees.

The emergence weights of bees was found to decrease linearly in association with increasing mite levels (Figure 4.1), which confirms the findings of many other studies (Table 4.1). This trend was evident, not only in the wet weights at emergence, but also in the dry weights of bees, suggesting that the mites are not selectively removing either water or haemolymph metabolites alone.

Although increasing parasite numbers are associated with lower emergence weights of bees, increasing parasite numbers are also associated with an increasing proportion of dry mass in the heads and thoraces (Figures 4.3b & 4.3c). In contrast, the proportional weights of the abdomens of non-deformed parasitised bees showed no change with increasing emergence weight and the weights of non-parasitised and parasitised-deformed bees showed an increase (Figure 4.3d).

These trends may be tentatively explained in relation to the organs located within the different body regions. The heads and thoraces of

emerging worker bees may contain organs which are incapable of functioning effectively if they fall below a certain mass. For example, the brain of the bee may only function fully if it consists of a minimum number of neurones, or perhaps wing muscles will only generate lift if they have a minimum number of muscle-fibres etc. Consequently, as the head and thorax compartments became smaller, the bees maintain some organs at a constant mass. Thus, the proportional mass of each body compartment increases. Therefore, the proportional weights of honeybee heads and thoraces are dependent not on parasite levels *per se*, but on the overall body weight.

It is hypothesised that the abdomens of worker bees contain organs, which, at least at the time of emergence, are not required to be of a certain size to function effectively. As a consequence, the proportion of metabolites invested in the development of the abdominal compartments in developing bees, is dependent on their availability (after the needs of the heads and thoraces have been met), and not on a minimum operative mass of organs within the abdomen. This would explain why non-parasitised bees have proportionally more mass in their abdomens as they become larger, whereas parasitised bees, (whose levels of metabolites are greatly reduced due to mite feeding behaviour), are not able to invest the same amount of metabolites in their abdomens and thus no increase is found.

Surprisingly, deformed parasitised bees were found to have proportionately more mass in their abdomens than non-parasitised bees (Figure 4.3d). This apparent paradox may be explained if it is accepted that bee deformity is frequently, if not exclusively, caused by microorganisms, particularly viruses (chapter 6). If a virus was present in the tissues of a developing bee, it could be interfering with their normal development and therefore distribution of reserves. Thus reserves which would normally be directed to the head and thorax tissues would remain within the abdomen.

The weight at emergence of honeybees is important, as it influences their ability to contribute to the normal functioning of the colony. Park (1925) found that the minimum flying weight of Italian bees (*A.mellifera ligustica*) was about 82mg (normal range=81-151mg [Winston, 1987]). Bees which were lighter than this were unable to fly, presumably because they had underlying morphological and/or biochemical deficiencies. Kerr & Hebling (1964) reported that the age at which a bee began new activities within the nest was linked to their weight. 'Heavy' honeybees, like bumble bees, began new activities at an earlier age than smaller lighter bees i.e. the heavier bees changed to an ontogenetically more advanced type of worker earlier than the smaller lighter bees. They also reported that heavier bees (95.8mg) had heavier brains (2.3mg) than lighter bees (69.8mg and 1.8mg

brains), although they did not take into consideration that proportionally there was little distinction, with heavy and light bees having brains which were 2.4% and 2.6% of their body weights respectively. These figures lend support to the hypothesis that brain volume is maintained irrespective of the size of the bee. Brain morphology, particularly that of the mushroom bodies in the protocerebrum is believed to be associated with learning and 'higher-order sensory integration' in insects (Erber *et al.*, 1987). Withers *et al.* (1993) found that the, so called, 'age-dependent' behaviour of bees was linked with changes in the mushroom bodies of the brain, which in foragers, were found to have a neuropil volume 14.8% larger than that of 1 day old bees. However, the overall volume of the mushroom bodies did not differ between foragers and nurse bees because the Kenyon cells were 29.3% smaller in foragers than in 1 day old bees. Moreover, these differences were linked with foraging experience and not age *per se*. This was clearly demonstrated in an experiment in which a cohort of same-age bees were isolated from older workers so that some of the young bees were forced to become 'precocious foragers' as young as 4 days old (in contrast to the normal 21-24 day age of foragers). These 'precocious foragers' had mushroom bodies indistinguishable in size from normal aged workers. Therefore the weight of a bee at emergence does not influence the size of the brain, neither does the age of the bee.

In contrast to Kerr & Hebling (1964), who found that heavy bees (>111.2mg) forage earlier than lighter bees (<92.2mg), Schneider & Drescher (1987) reported that parasitised bees (mean emergence weight = 89-103mg) began foraging earlier than their non-parasitised sisters (mean emergence weight = 114mg) even though they were smaller and lighter in overall body weight. This suggests that body weight alone does not determine when flight activity begins, unless brain size is independent of body size as proposed.

Because the emergence weight of bees is known to be associated with subsequent behaviour, other physiological parameters which may be responsible for activating the behavioural changes must also be influenced together with weight. How could this be so? If a bee is underweight at emergence, it may be assumed that it is physiologically altered (e.g. abnormal titres of enzyme(s), metabolic reserves, water *etc.*), which in turn could influence the rate of development (*i.e.* anatomical changes associated with age polyethism [e.g the increase in size of the neuropil volume and a decrease in the size of Kenyon cells as a bee starts to forage]). The rate of development could increase or decrease, depending on the nature of the regulatory mechanisms. A reduction in key enzymes/enzyme

regulators/hormones could decrease the normal rate of development, or alternatively, their absence could actually allow an otherwise limited physiological process to proceed, or proceed at a faster rate. Thus varroa parasitosis could cause undeveloped bees to fly earlier than would otherwise be expected by their size and age.

Age-associated behavioural changes (age or temporal polyethism) in worker bees are accompanied by internal morphological changes such as the development of wax glands and the regression of head glands (Winston, 1987). Glandular development would make demands on the metabolic reserves of the bees which, especially in bees with a paucity of reserves due to varroa parasitosis, may exceed their availability. Thus the precocious flying attempts observed in parasitised bees combined with their depleted metabolic reserves, may help to explain the increased incidence of bee drifting in parasitised apiaries which is thought to be due to a reduced ability to navigate (Ruano *et al.*, 1991). By flying earlier in their lives than is normal for workers, infested bees could be taking to the wing before the brain / orientation organs, had fully developed. This in turn, could have a serious negative effect on the bee population which would rapidly dwindle as bees became lost and separated from their colony. Interestingly, Schneider & Drescher (1988) reported that only 20% of non-parasitised bees got lost

during their first flight, whereas, 36% of parasitised bees got lost. Not surprisingly, a rapid dwindling of bee numbers, which cannot be accounted for by deaths within the hive or its immediate vicinity, is associated with varroa infestation before colony collapse (S. Martin pers comms; pers. obs.). Additionally, by increasing the rate at which inter-colony drifting occurs, the mites would be improving their chances of infesting other bee colonies.

A factor which was not considered during this study, nor to my knowledge has ever been considered is the interrelation between cell size, emergence weight and parasitism by varroa. It has been shown by Kulzhinshaya (1955) that the size and weight of a bee at emergence can be correlated with the size of the cell in which it developed. Large cells are linked with the development of larger bees. Old comb, tends to have smaller cells than new comb because of the successive build up of exuviae, being at their smallest after 20-30 generations use. Consequently old cells yield smaller imagos, on average, than new comb (Nowakowski, 1969). Furthermore, worker bees that develop within drone cells (which are larger, both in diameter, depth and therefore volume) tend to be larger than the workers that emerge at the same time from worker cells (Tuenin, 1927; Nogueira & Gonçalves, 1982). One possible reason is that the mislocated worker larvae receive ~8 times more food in drone cells than they would

normally receive in worker cells (Taber & Poole, 1973). Therefore, the combined effects of comb cell size and varroa parasitosis on the emergence weights of bees should be investigated to determine whether the presence of both 'treatments' have an effect. It would also be interesting to know whether the mortality of varroa was higher in smaller cells than in larger cells, as the mites behaviour in the cell influences their survival and the survival of the progeny (Donzé & Guerin, 1994). This would be a worthwhile investigation because evidence exists which shows varroa prefer to parasitise large cells (Abdellatif, 1965). Whether or not this may be linked to their propensity to parasitise drones in preference to workers, or as a new adaptation for life on *A. mellifera* is undetermined. If small cell size, when linked to parasitism, was found to cause an extra weight loss in developing brood, and/or be linked with mite reproductive success, it would then be important to determine whether it would be viable (in view of cost) to advise beekeepers to use new comb where possible if hives are infested with varroa, so that the bees would not be additionally compromised by small cells.

These investigations have shown that there was a 3% decrease in the water contents of an emerging parasitised bees, for every female mite (deutonymph or adult female) present during its development. This represents about 3% of the the bees total water volume. Water loss would

occur as a consequence of the mites feeding on the haemolymph of the host. Haemolymph depletion (which was discussed above), according to both Daly *et al.* (1988) and Koch & Ritter (1991), will result in the hydrostatic pressure of the haemocoel being lowered. Therefore, successful wing flexion by the imago may be compromised leading to wing deformities- these are frequently encountered in varroa infested bees. In this study, and other published work (Table 4.1), the number of bees exhibiting morphological damage can be correlated with an increase in parasitosis. However, a high percentage of deformed bees only became apparent with a parasitosis of four or more mites (Table 4.1). This may be an artefact of smaller data sets at higher levels of infestation as opposed to a true trend.

This study demonstrates for the first time that mite infestation, causes a reduction in the protein titres of emerging bees, other than those of the haemolymph alone. The greatest loss of proteins occurred from the abdomen, followed by the head. A reduction in haemolymph proteins was also found, in agreement with that of the other studies (Table 4.2). It was found that a varroa infestation of 1-3 mites caused a 46% reduction in haemolymph protein titres, and an infestation of 4-10 mites resulted in a 57% reduction in haemolymph protein titres. A varroa infestation of 1-6 mites has previously been shown to cause a 30-50% reduction in the total haemolymph

proteins of emerging worker bees (Gliński & Jarosz, 1984; Weinberg & Madel, 1985; Schatton-Gadelmayer & Engels, 1988). Similarly, a depletion of drone haemolymph proteins has also been reported to be associated with varroa infestations, but the extent of the depletion is reportedly lower, at about 12%, and was curiously found to be independent of the number of parasitising mites (Weinberg & Madel, 1985). A possible explanation for this is that the level of protein in the drone haemolymph is maintained at the expense of the spermatocytes, which are also developing during the pupal stage (Weinberg & Madel, 1985). Alternatively, proteins may be diverted from the fat bodies of the drones.

Schatton-Gadelmayer & Engels (1988) reported that the 74kda fraction of bee haemolymph was significantly depleted in bees infested with three or more mites. The 74kda fraction corresponds with the arylphorin fraction of bee blood (Ryan *et al.*, 1984), which are storage proteins synthesised during the larval stage, and from which imaginal tissue is synthesised during metamorphosis (Telfer *et al.*, 1983). Depletion of this fraction would no doubt have severe consequences on the normal metamorphosis of parasitised bees. Schatton-Gadelmayer & Engels (1988) and Gliński & Jarosz (1988a + b) both reported that *V.jacobsoni* infestation

not only caused a reduction in the total protein levels of bees but, more specifically, in the lysozyme fraction. They postulated that the reduction in lysozyme resulted from the introduction of exogenous materials into the haemolymph (possibly proteases) which would normally result in the bee mounting an immune response which would increase the lysozyme titres (see chapter 6). However, in varroa infested bees, the lysozyme levels are reduced to almost zero (Gliński & Jarosz, 1988a + b). Consequently, varroa infestation reduces the efficacy of the bees cell-free immunity although it is unclear at present how this occurs.

This investigation showed no significant loss of protein from the thoraces of worker bees, even with parasite loads of up to 11 mites. Haydak (1935), reported no significant difference in the nitrogen content of the head or thorax compartments of emerging bees which had been reared on pure carbohydrate diets (no protein) as compared with bees reared on normal diets (inclusive of pollen-derived protein). However, in the protein-deprived group, the nitrogen contents of their abdomens was significantly reduced. Consequently, he proposed that the abdomen contains an organ/organs which acted as a reserve store for protein (probably the fat body) which could be mobilised when the protein levels of the bee were low. This could

account for an apparent lack of effect of varroa on the thoraces of bees whereas the abdomen showed a highly significant loss of protein.

At emergence, parasitised bees are often observed with morphological damage (see Figure 1.10), which according to Kolev & Shabanov (1989) may be associated with protein depletion. Protein depletion alone, however, is an unlikely explanation for the deformities, because even highly parasitised bees (which would certainly have depleted protein levels) frequently emerge without deformity, although their abdomens are smaller.

A similar observation was made by Haydak (1935), who reported that an inadequate protein supply to developing bees resulted in hypotrophied abdomens, but no other deformities were reported. Not only are the abdomens smaller as a result of varroa infestation, but other organs such as the hypopharyngeal glands (Schneider & Drescher, 1988), the seminal vesicles and mucus glands of drones (Drescher & Schneider, 1988) are also smaller. Abdominal hypotrophy can be explained on the basis of the hypothesis that proteins are diverted from the abdomen to the heads and thoraces. Thus deformities only become apparent when viruses are present, which result in an abnormal protein distribution due to their competing for resources.

My study is the first demonstration that varroa infestation causes a reduction in the carbohydrate titres of emerging worker bees, the abdomen being the compartment from which the majority of the carbohydrate was depleted. Two important functions of carbohydrates in insects are to act as metabolic reserves and as cell-surface markers. In insects, and in particular in honey bees, carbohydrates are found in the haemolymph at high concentrations (Chippendale, 1978), so provided bees have access to honey, carbohydrate losses due to parasitism are unlikely to be limiting to adult bees. However, in pupae (which do not have access to food of any kind other than that stored from the larval stage) any loss of carbohydrate could have a significant impact. A 50% reduction in the number of spermatozoa produced by drones parasitised by three or more mites has been recorded (Schneider & Drescher, 1987). In addition to which, the sperm do not mature normally and thus may not be capable of fertilising female gametes (DelCacho *et al.*, 1996). The incomplete development of sperm was attributed to a lack of surface glycoproteins, which are important in cell-cell recognition and sperm-egg fusion (DelCacho *et al.*, 1996).

A review of the literature (Table 4.1) shows a very large range in the percentages of emerging bees which exhibit morphological damage. For example, DeJong *et al.* (1982b) reported 6% of all emerging bees were

damaged, whereas Marcangeli *et al.* (1992b) reported a 29% damage rate. Damage is associated not only with highly-parasitised bees, but also with bees which have low parasitoses (1-3) or in some cases no parasites at all! During this investigation an emerging bee with 10 mites (adult females and offspring) was observed with no obvious morphological damage, although it had a reduced head protein titre and water contents. Daly *et al.* (1988) reported a similarly non-deformed bee with a parasitosis of 8 mites. This suggests that secondary infections may also have a role in the causation of morphological damage, and that deformity is not simply due to removal of metabolites.

During this study it was found that, not only did bees decrease in weight with increasing parasitosis, and that with increasing parasitosis, an increasing percentage of bees emerged deformed, but also that the mean weight of damaged bees was 10% less than their non-damaged sisters at all levels of parasitosis (Figure 4.4). This pattern was also reported by Getchev (1994), again suggesting that damaged bees differ in some crucial way from non-damaged bees, resulting in their pathology being more severe.

During this study, no evidence was found to show that varroa infestation causes a reduction in the lipid concentration of emerging honey bee workers. This finding concurs with that of Brouwers (1982), but differs

from that of Drescher & Schneider (1988) who reported a 25% decrease in the diameter of fat body cells of emerging bees parasitised by three or more mites. The latter study, however, measured changes to trophocyte size and not lipid concentration. The apparent lack of effect of varroa infestation on the lipid levels of bees is probably due to the effect of metamorphosis on the final lipid reserves of the emerging bees. Czoppelt & Rembold (1970) and Hepburn *et al.* (1979) both found that the lipid levels of honey bees decreased during the course of metamorphosis by 20-30%. This is to be expected as lipids are the principal substrates oxidized during pupation. Consequently, the lipid levels of bees at emergence are very low, even in non-parasitised bees. Therefore, although the mites may have caused a reduction in lipid levels (selective uptake of haemolymph devoid of lipid molecules is untenable), this would be masked by the normal paucity of lipids normally present in honey bees after metamorphosis. Perhaps, the decrease in diameter of trophocytes reported by Drescher & Schneider (1988) correlates with Haydaks (1957) description of the normal condition of 1 day old bee fat bodies as being 'poor'. Alternatively, the vanillin-phosphate assay may not be sufficiently accurate at these low levels to detect a difference in the concentration, although this is unlikely.

An interesting observation was made however that deformed bees appeared to have lower concentrations of lipid in their tegmata on average

than their non-deformed sister bees. The lower lipid levels may go some way to explain why deformed bees always emerge lighter than their non-deformed sisters. Additionally, deformity is associated with some other physiological changes in the host not directly due to varroa infestation alone.

The underdevelopment and metabolic deficiencies prevalent in emerging varroa infested bees may, to some extent, be reversible. Kovac & Crailsheim (1988), citing the related findings of Kunert & Crailsheim (1988), who investigated weight increases in newly-emerged bees (but not in relation to varroa) suggested that parasitised bees 'under optimal hive conditions' might also be able to compensate for the weight and protein loss caused by varroa during development. It is difficult to know how a parasitised colony could ever be said to have 'optimal conditions', but perhaps at an early stage of infestation some semblance of 'normal' pollen levels / temperature *etc.* would prevail. This hypothesis receives some support from unrelated work carried out by Haydak (1937), who reported that worker bees which emerged after being raised on pure carbohydrate diets were lighter than normally fed workers (pollen included in their diet). However, when bees were supplied with protein after emergence, they rapidly increased in weight and their nitrogen contents rose to normal levels. Thereafter, the young bees commenced normal brood rearing activities and the brood they tended

developed normally. This suggests that even though protein levels may be lower than normal (due either to the effect of poor nutrition on larvae or due to protein depletion by varroa) some restitution can take place if the newly emerged bee had access to proteins at emergence.

A study by Schneider & Drescher (1987), however, did not find any restitution in the weights of parasitised bees over a period of seven days after their emergence. Moreover, the study by Kunert & Crailsheim (1988) cited by Kovac & Crailsheim (1988), was not related to varroa parasitosis and no attempt has been made to further investigate whether any weight recovery occurs naturally after the parasite has dismounted voluntarily from the host. Also, the normal development/weight gained by individual organs will be more important in enabling a bee to carry out its colony-tasks rather than overall weight gain. Consequently, even though weight restitution may occur, it may not do so in such a way as to alter the course of the pathology. Moreover, weight gain may occur more as a result of re-hydration after emergence than as a result of a true gain in metabolites.

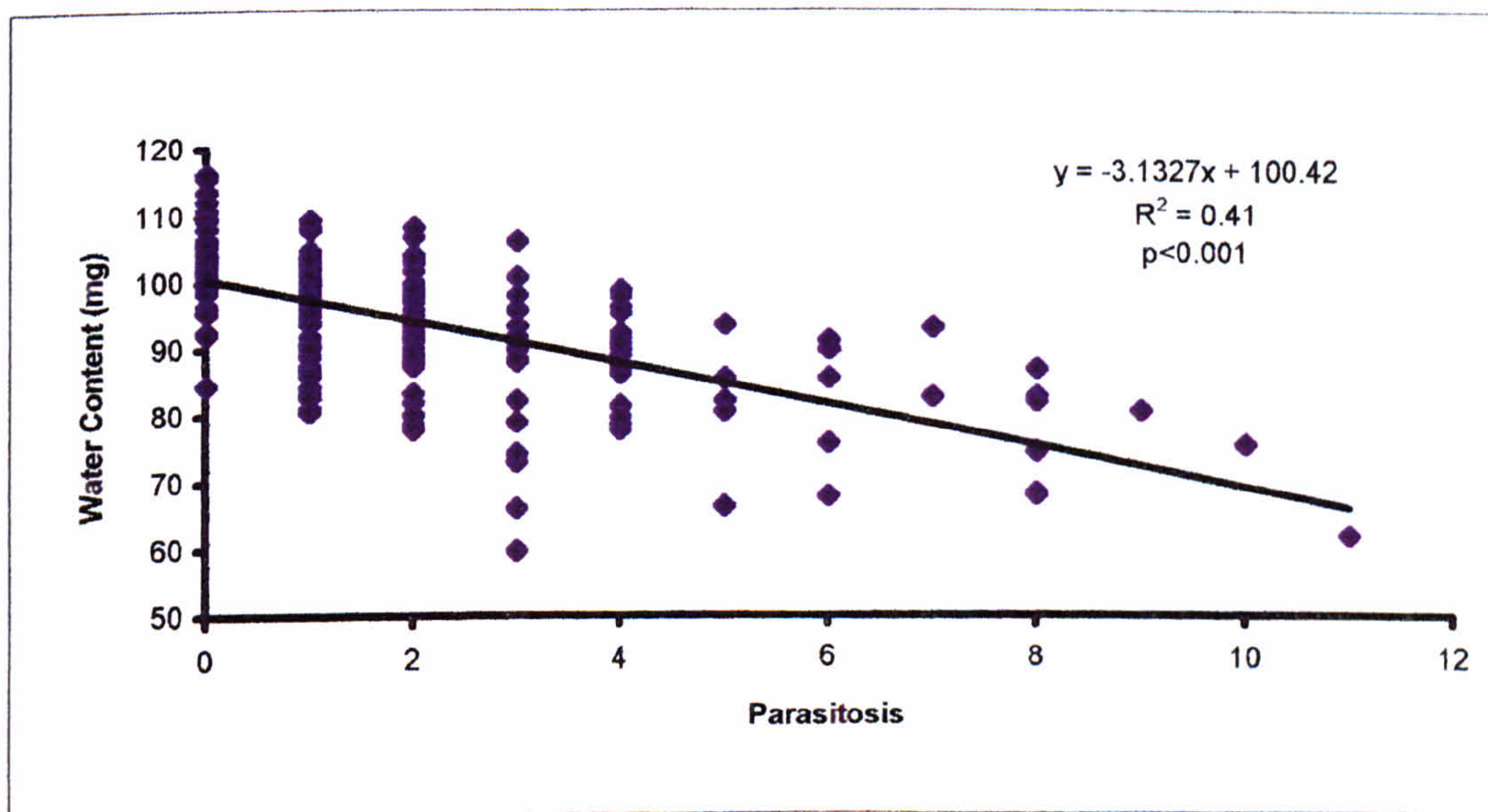
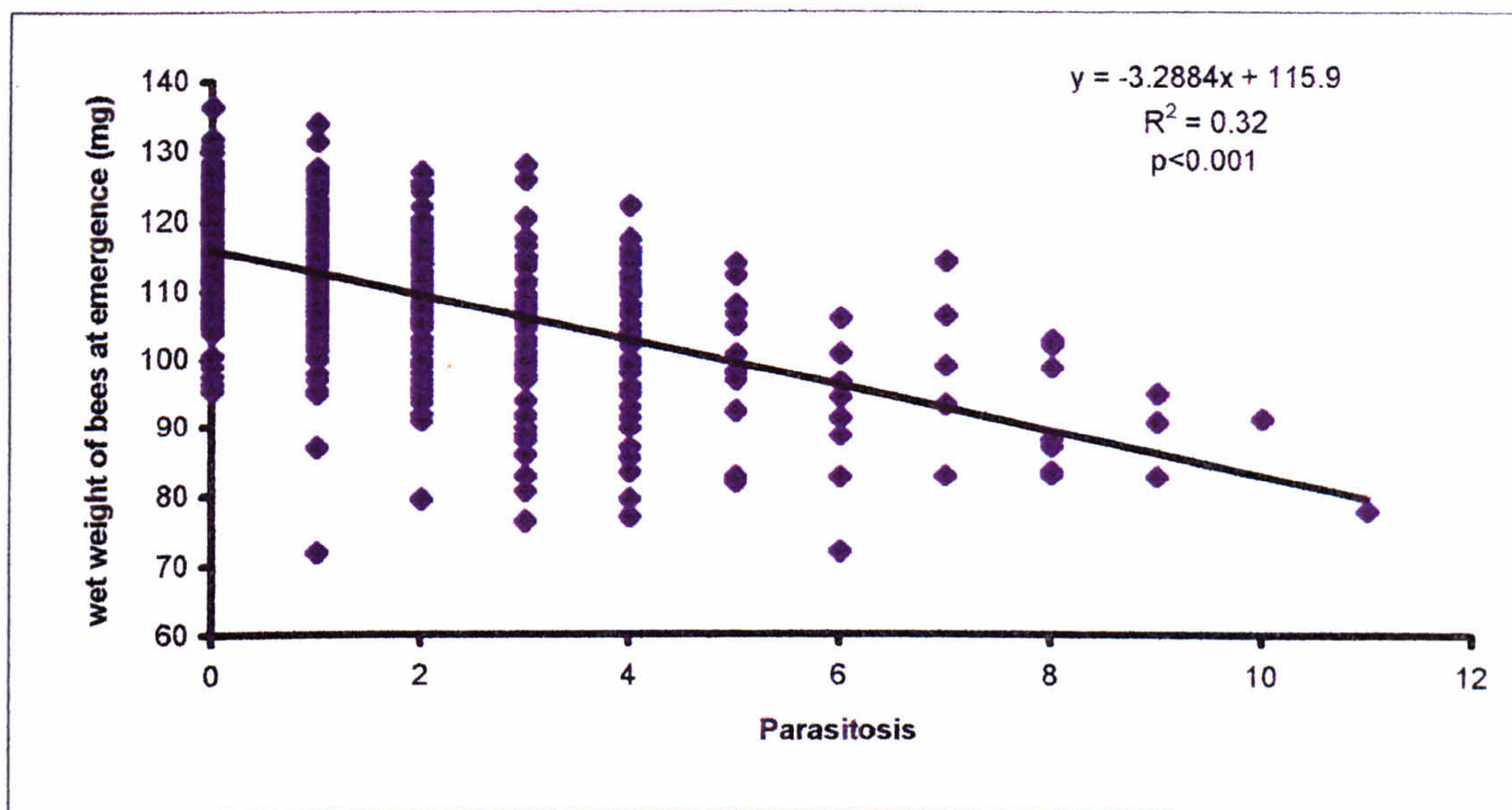
This investigation has clearly demonstrated that varroa infestation results in a significant reduction in the level of water and metabolites in developing bees. The depletion of metabolic reserves principally occurs in the abdomen and is correlated with the degree of infestation, as is an increasing

frequency of appearance of morphological deformity. Nevertheless, my investigation has also shown that a depletion in metabolic reserves alone is not sufficient to explain the appearance of deformity, and consequently it is proposed that other factors must be present, such as the introduction of exogenous material (e.g. saliva together with its constituents), or pathogens (e.g. microbes or viruses) may also have a significant role. This hypothesis is further explored in chapters five and six.

P.T.O

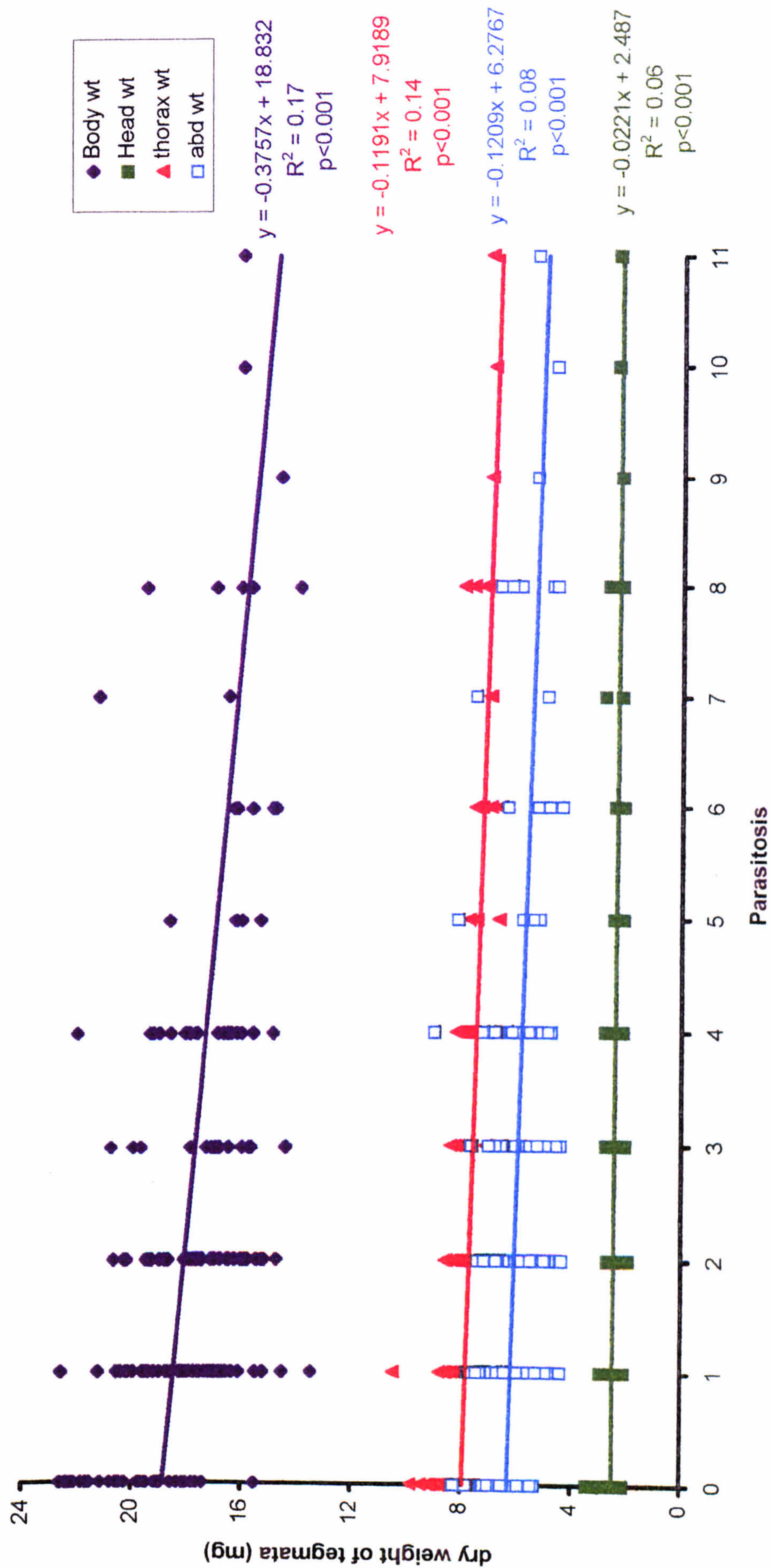
Figure 4.1 The relationship between increasing numbers of *Varroa jacobsoni* (1-11 mites) infesting developing worker honeybees (*Apis mellifera*) and their weight (mg) at emergence.

Figure 4.2 The relationship between increasing numbers of *Varroa jacobsoni* (1-11 mites) infesting developing worker honeybees (*Apis mellifera*) and their water content (mg) at emergence.



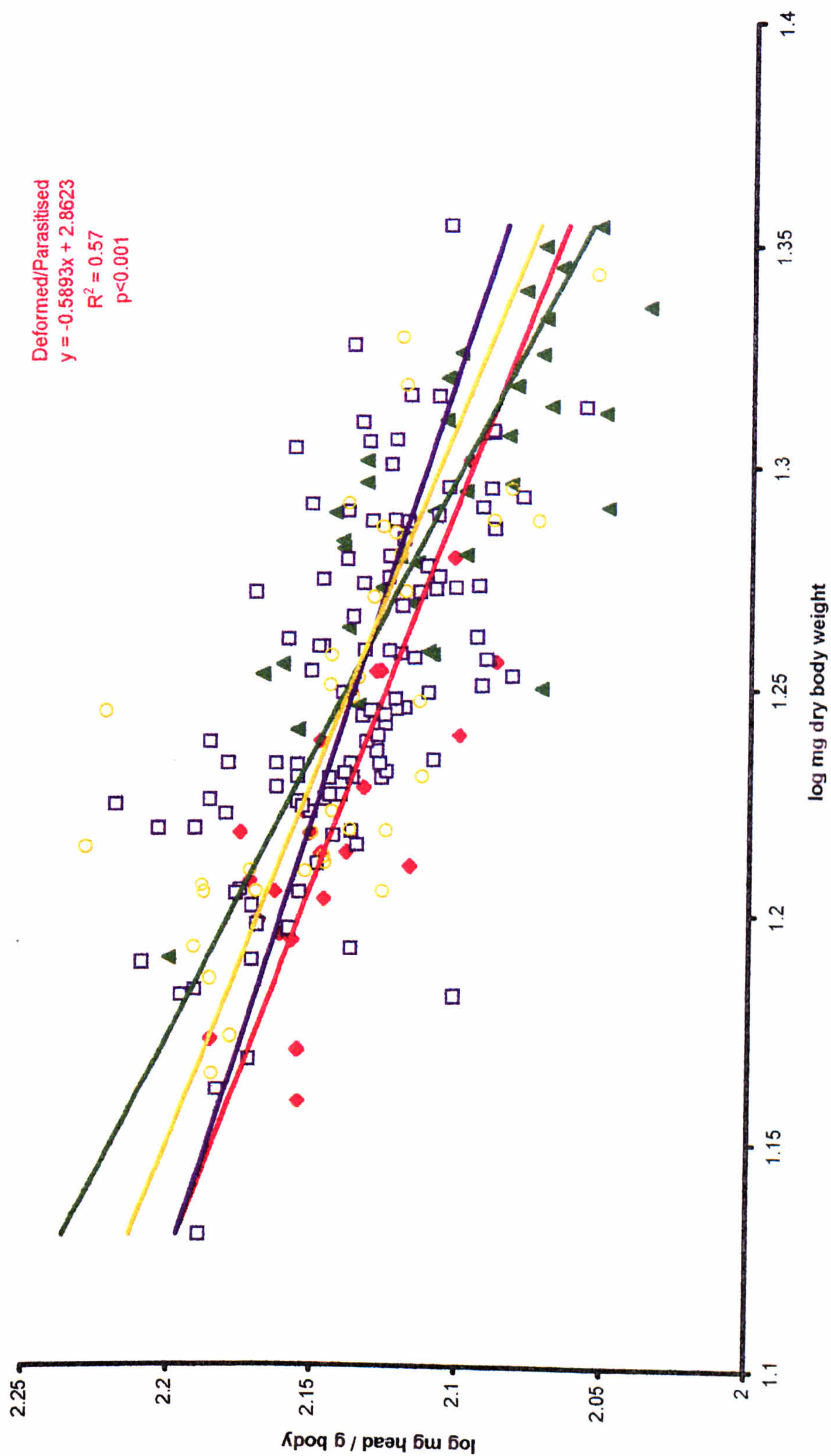
P.T.O

Figure 4.3a The relationship between increasing numbers of *Varroa jacobsoni* (1-11 mites) infesting developing worker honeybees (*Apis mellifera*), and the dry weight (mg) of their heads, thoraces and abdomens at emergence.



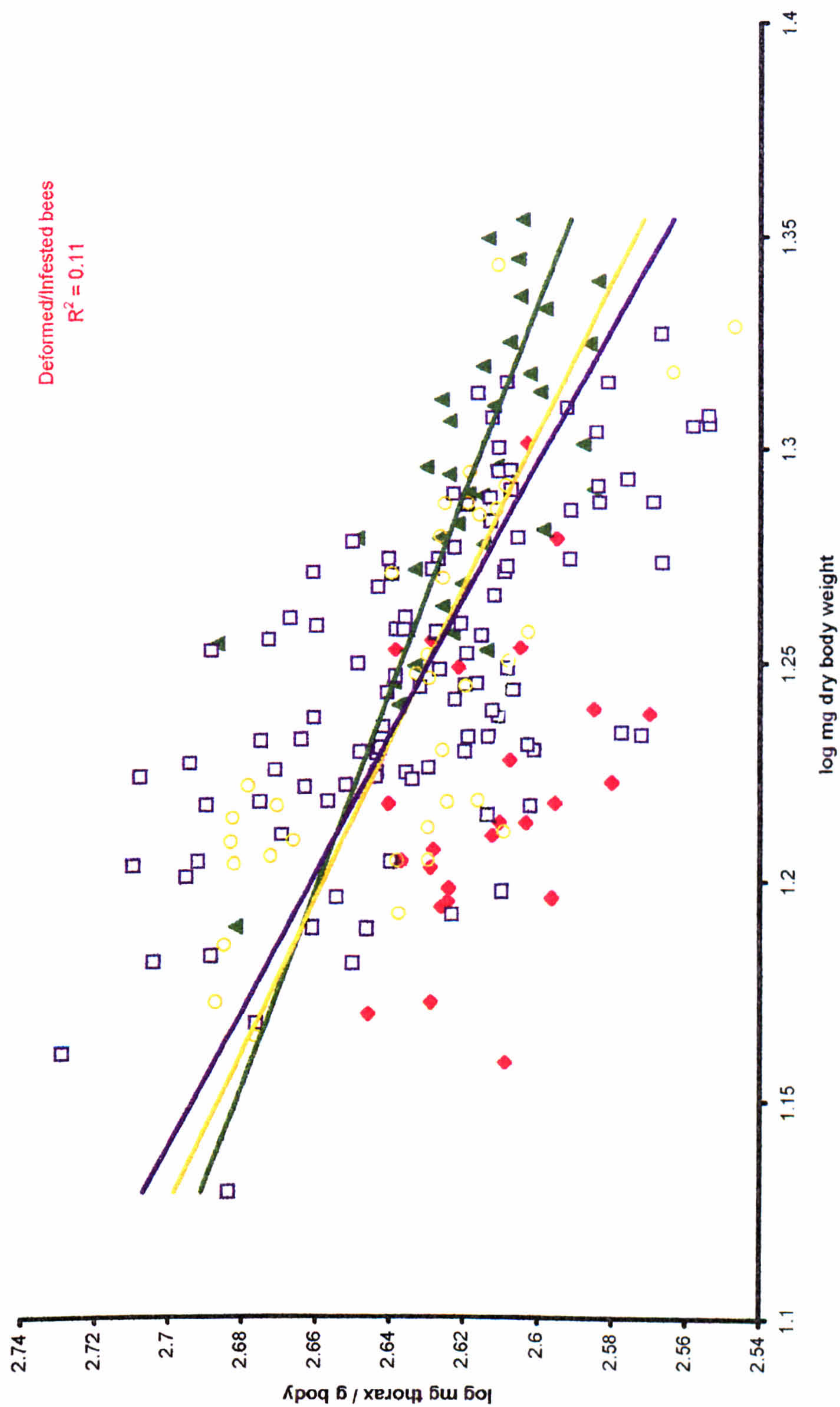
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Figure 4.3b The relationship between the dry weight of emerging worker honeybees (*Apis mellifera*) and the proportional dry weight of their heads (log mg head / g dry body weight).



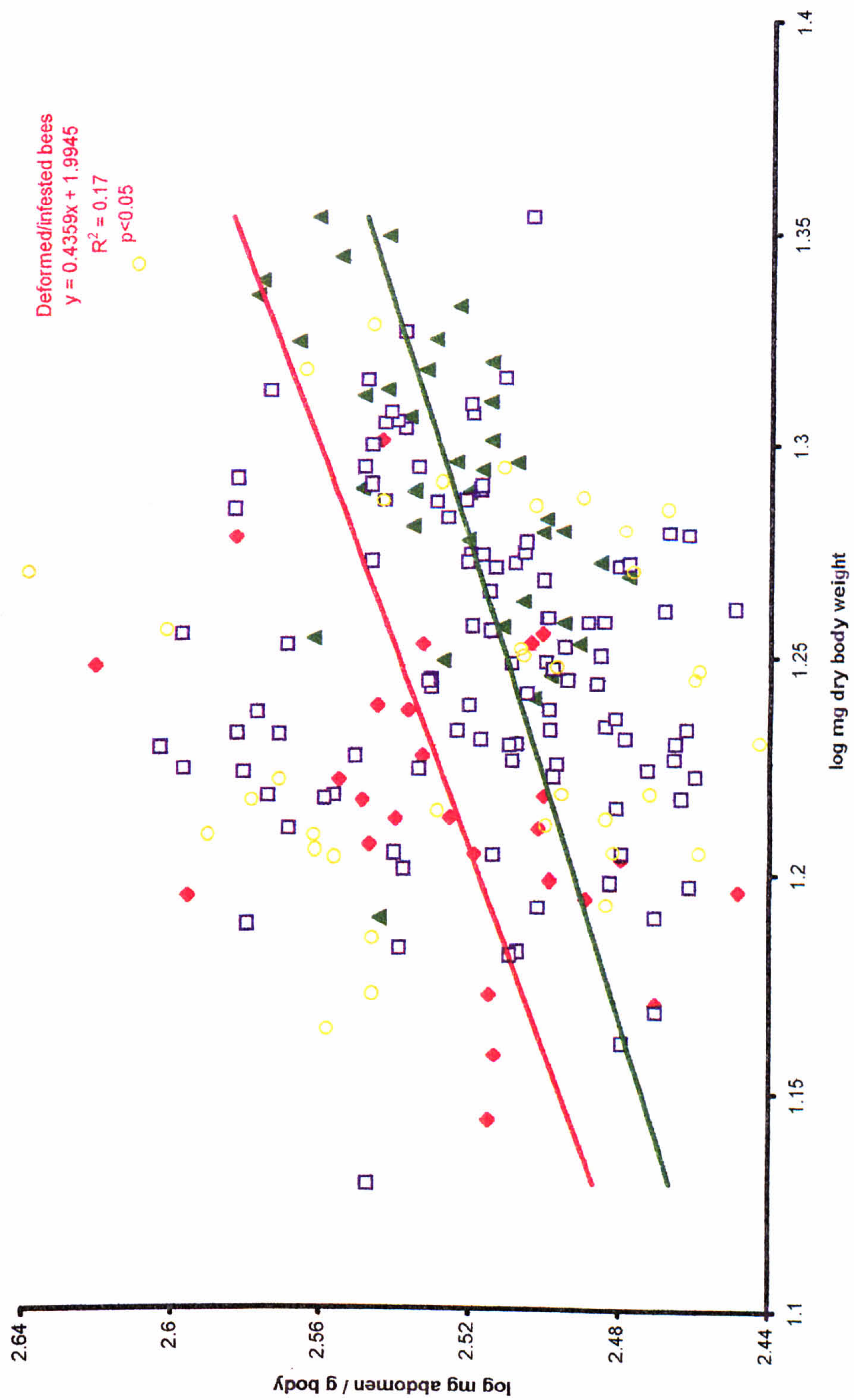
P.T.O

Figure 4.3c The relationship between the dry weight of emerging worker honeybees (*Apis mellifera*) and the proportional dry weight of their thoraces (log mg thorax / g dry body weight).



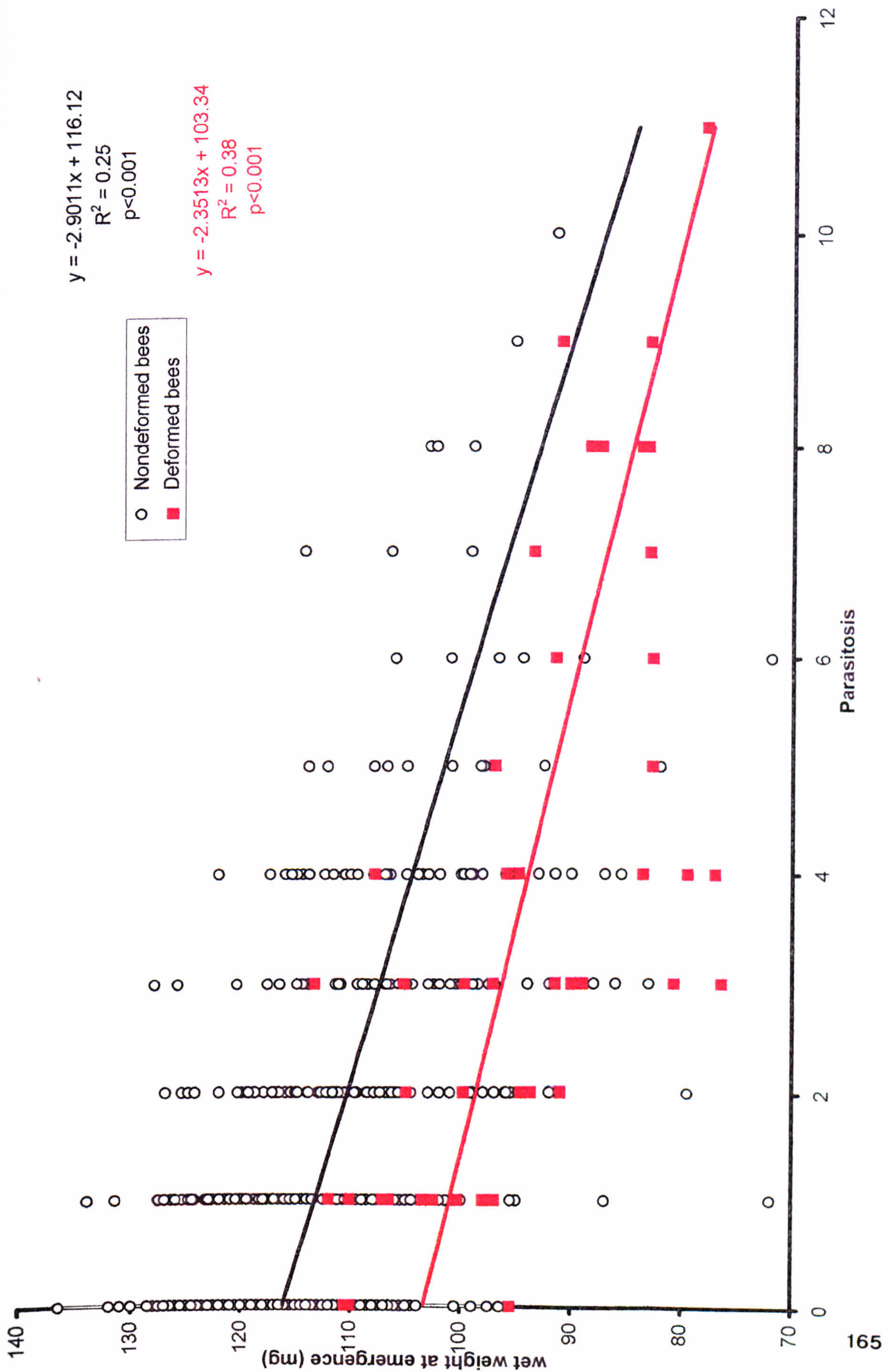
P.T.O

Figure 4.3d The relationship between the dry weight of emerging worker honeybees (*Apis mellifera*) and the proportional dry weight of their abdomens (log mg abdomen / g dry body weight).



P.T.O

Figure 4.4 The relationship between increasing numbers of *Varroa jacobsoni* (1-11 mites) infesting developing worker honeybees (*Apis mellifera*) and the weight (mg) at emergence of deformed and non-deformed bees.

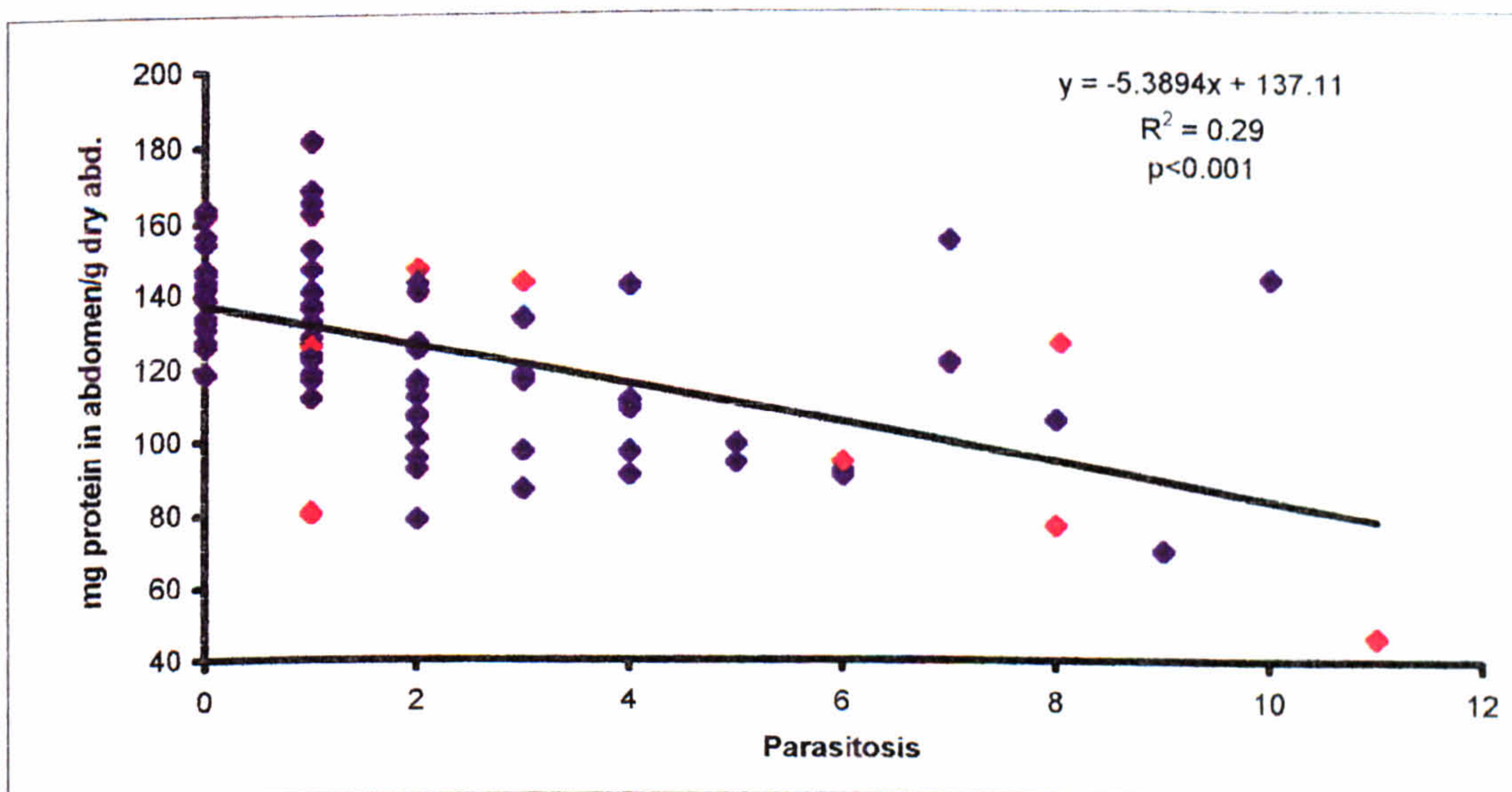
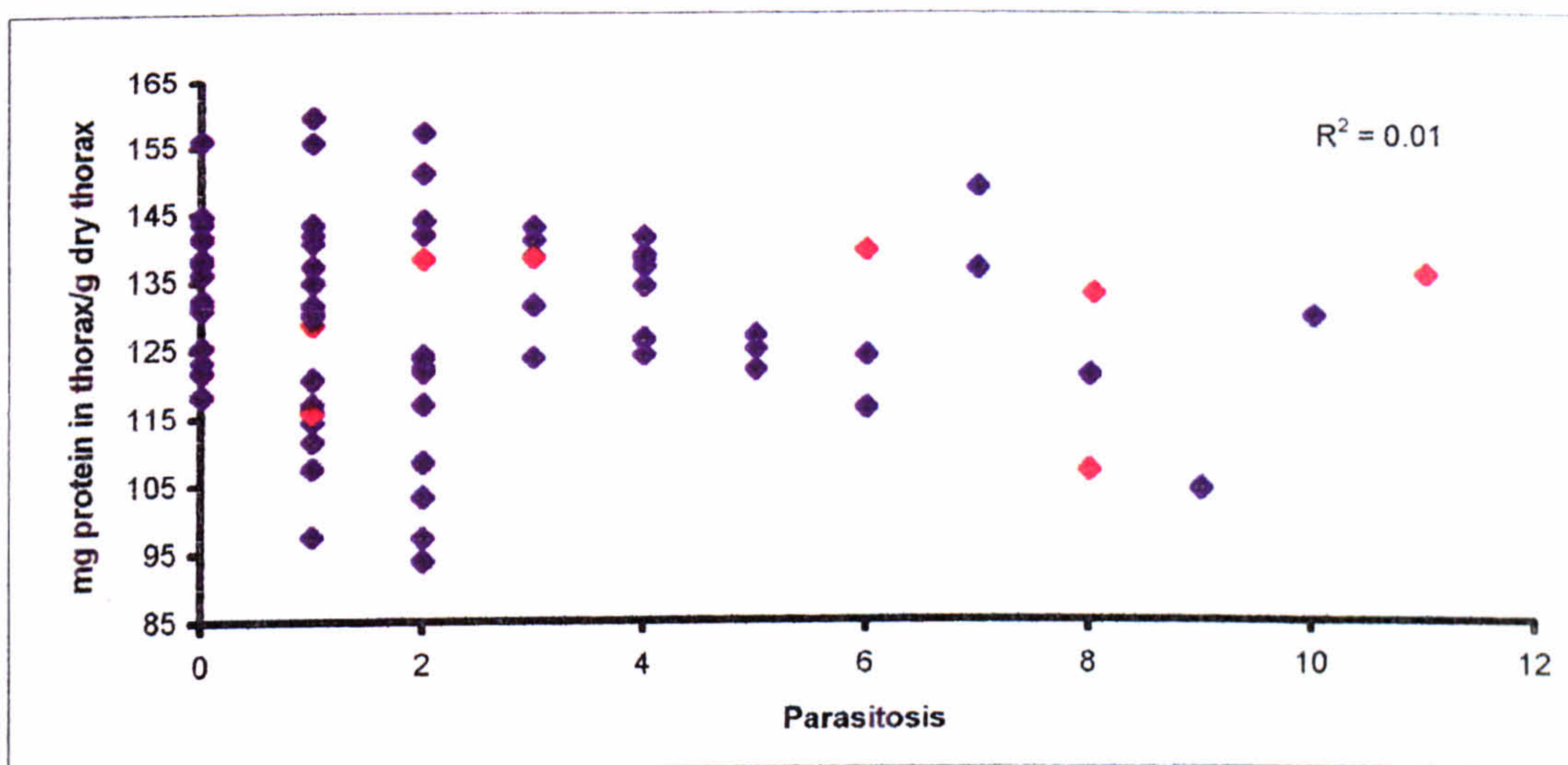
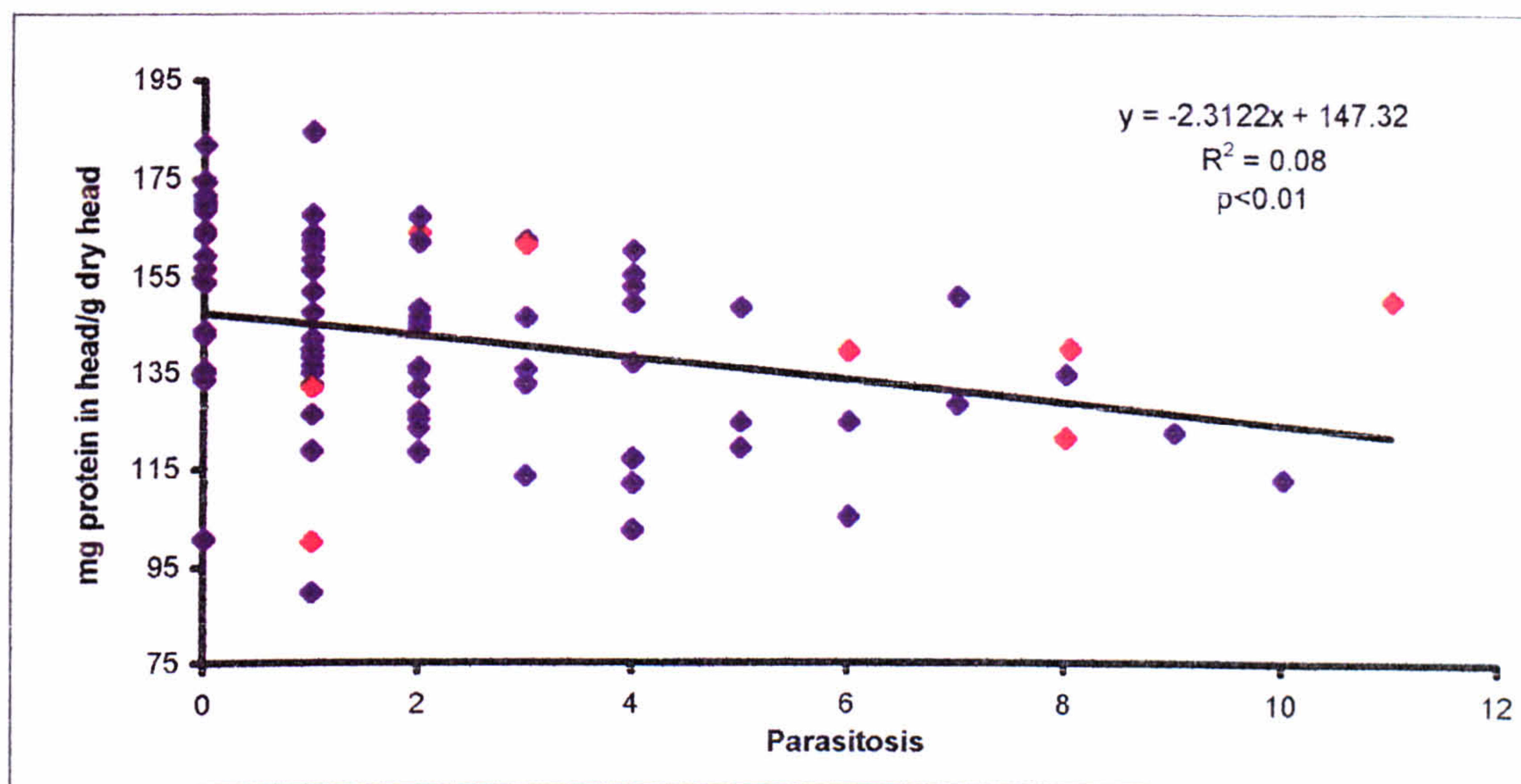


P.T.O

Figure 4.5 The relationship between increasing numbers of *Varroa jacobsoni* (1-11 mites) infesting developing worker honeybees (*Apis mellifera*) and the mass of soluble protein in their heads (mg protein in head / g dry head). Bees which were deformed at emergence = red, those non-deformed = blue.

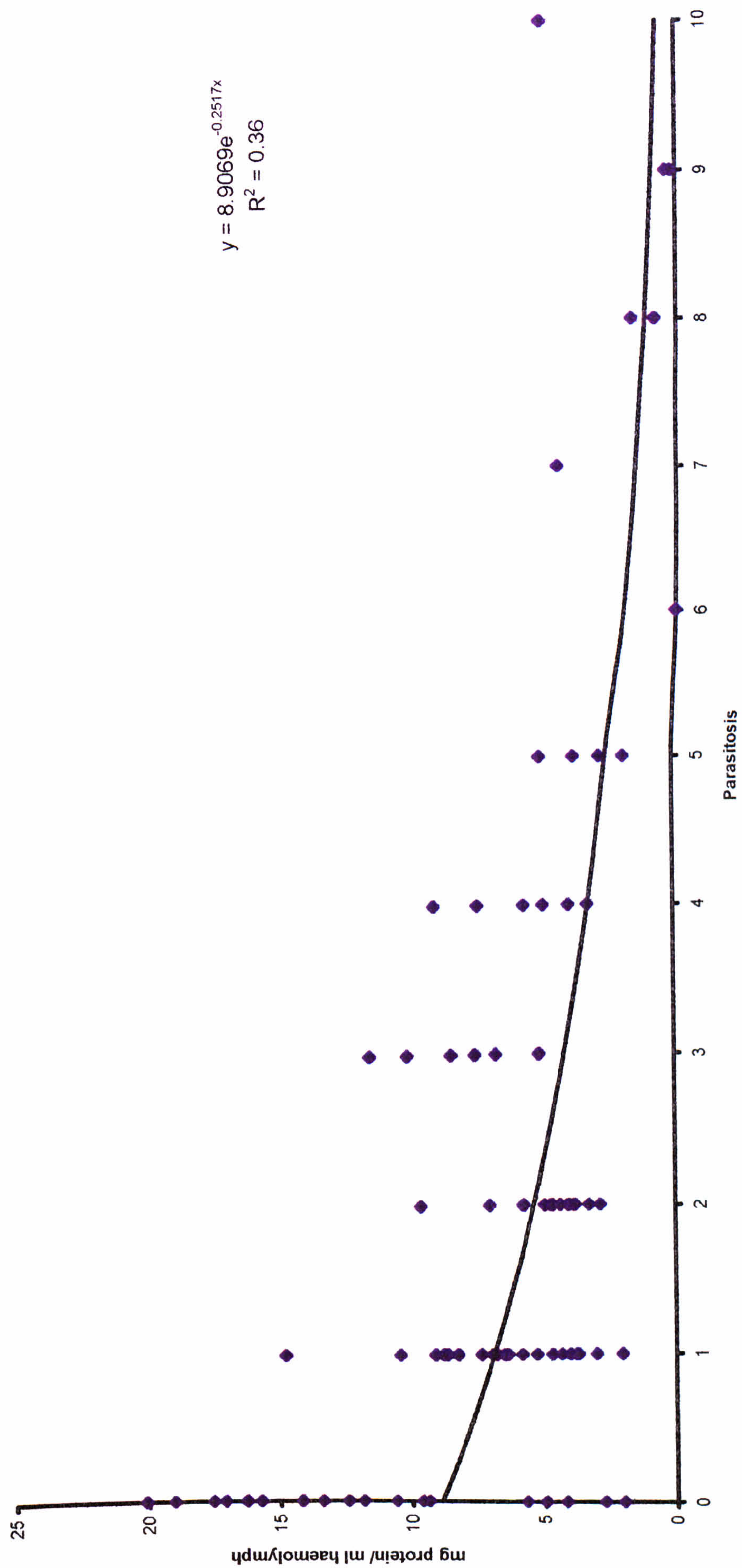
Figure 4.6 Scattergram of the mass of soluble protein in the thoraces (mg protein in thorax / g dry thorax) of *Apis mellifera* parasitised with 1-11 *Varroa jacobsoni*. Bees which were deformed at emergence = red, those non-deformed = blue.

Figure 4.7 The relationship between increasing numbers of *Varroa jacobsoni* (1-11 mites) infesting developing worker honeybees (*Apis mellifera*) and the mass of soluble protein in their abdomens (mg protein in abdomen / g dry abdomen). Bees which were deformed at emergence = red, those non-deformed = blue.



P.T.O

Figure 4.8 The relationship between increasing numbers of *Varroa jacobsoni* (1-10 mites) infesting developing worker honeybees (*Apis mellifera*) and the mass of soluble protein in their haemolymph (mg protein in haemolymph / ml haemolymph).



P.T.O

Figure 4.9 Scattergram of the mass of carbohydrate in the heads (mg carbohydrate in head / g dry head) of *Apis mellifera* parasitised with 1-8 *Varroa jacobsoni*. Bees which were deformed at emergence = red, those non-deformed = blue.

Figure 4.10 Scattergram of the mass of carbohydrate in the thoraces (mg carbohydrate in thorax / g dry thorax) of *Apis mellifera* parasitised with 1-8 *Varroa jacobsoni*. Bees which were deformed at emergence = red, those non-deformed = blue.

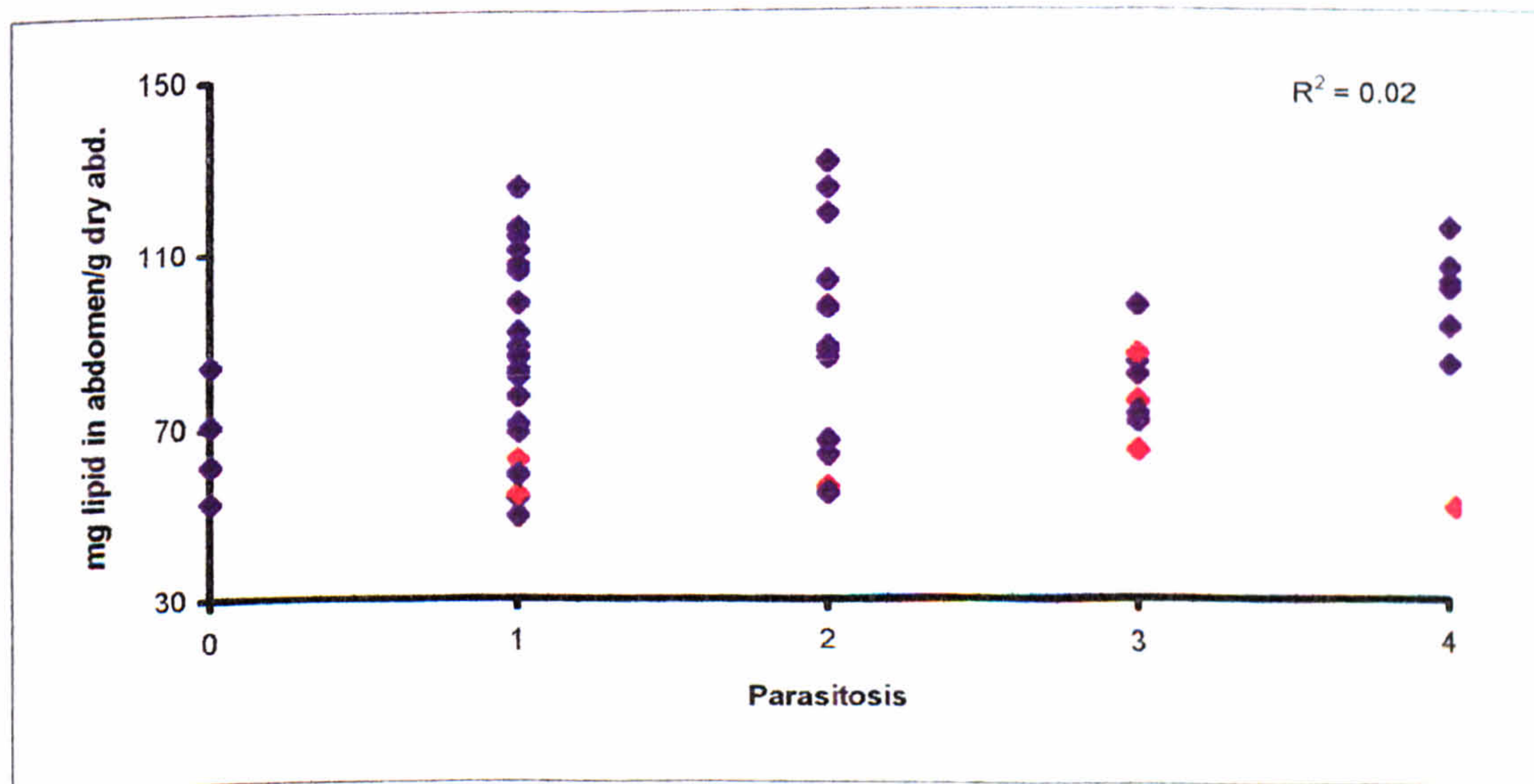
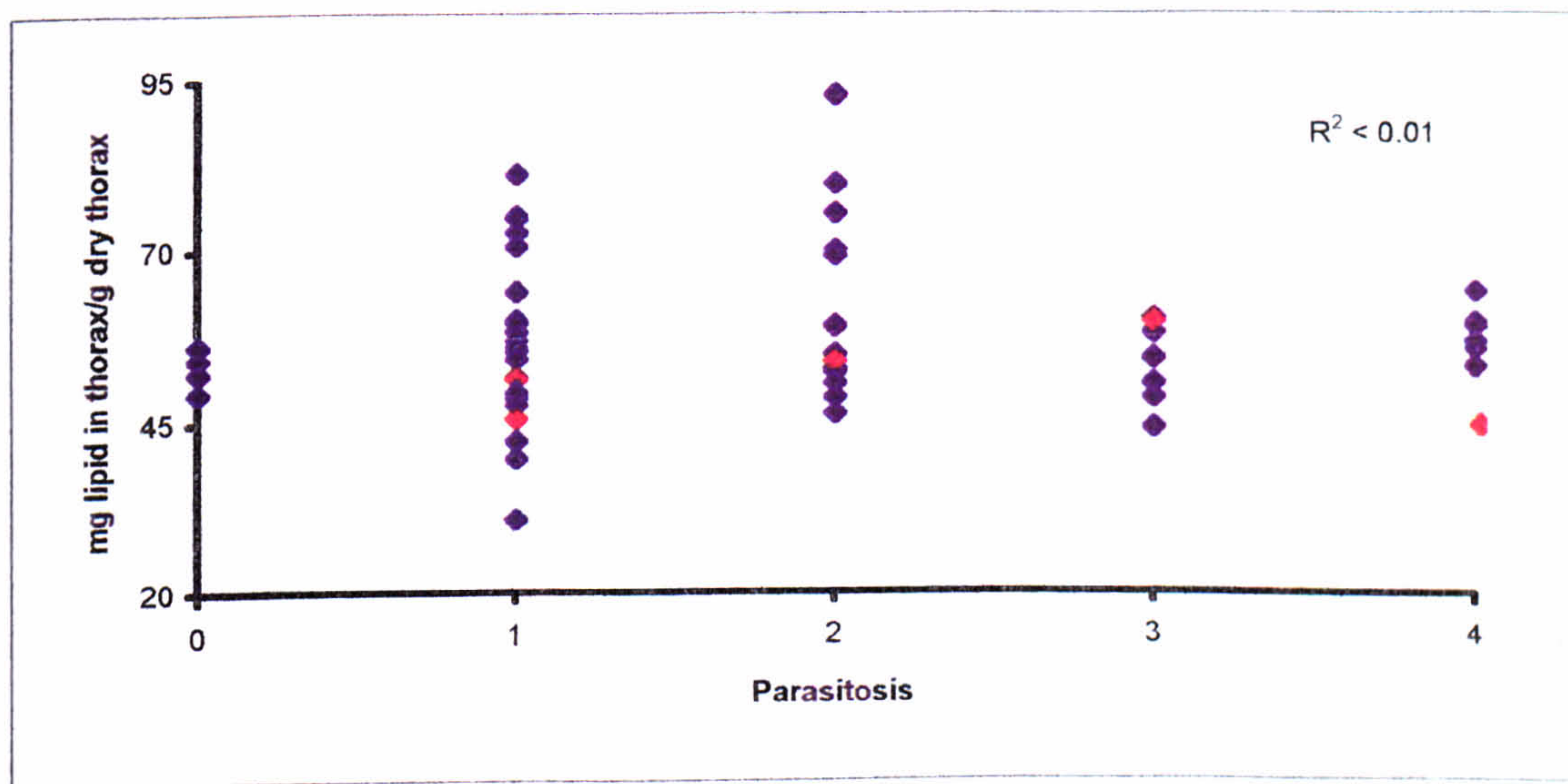
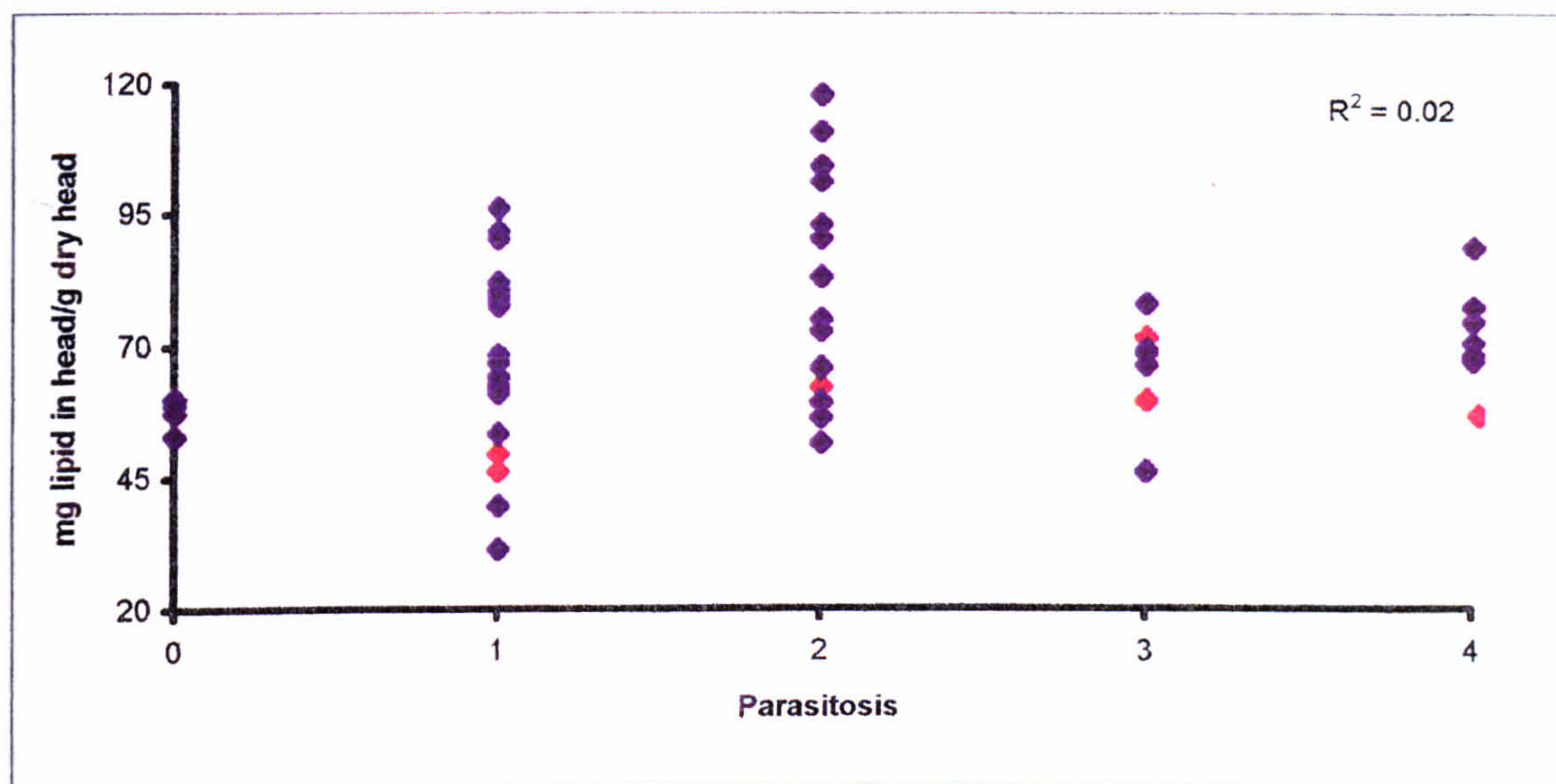
Figure 4.11 The relationship between increasing numbers of *Varroa jacobsoni* (1-8 mites) infesting developing worker honeybees (*Apis mellifera*) and the mass of carbohydrate in their abdomens (mg carbohydrate in abdomen / g dry abdomen). Bees which were deformed at emergence = red, those non-deformed = blue.

P.T.O

Figure 4.12 Scattergram of the mass of lipid in the heads (mg lipid in head / g dry head) of *Apis mellifera* parasitised with 1-4 *Varroa jacobsoni*. Bees which were deformed at emergence = red, those non-deformed = blue.

Figure 4.13 Scattergram of the mass of lipid in the thoraces (mg lipid in thorax / g dry thorax) of *Apis mellifera* parasitised with 1-4 *Varroa jacobsoni*. Bees which were deformed at emergence = red, those non-deformed = blue.

Figure 4.14 Scattergram of the mass of lipid in the abdomens (mg lipid in abdomen / g dry abdomen) of *Apis mellifera* parasitised with 1-4 *Varroa jacobsoni*. Bees which were deformed at emergence = red, those non-deformed = blue.



P.T.O

Table 4.1 Literature review concerning the effect of *Varroa jacobsoni* infestation on *Apis mellifera*. Data is given on weight loss, percentage bees emerging with morphological damage, percentage haemolymph lost and percentage haemolymph proteins lost by the host during their development. nr = not recorded.

Authority	Parasitosis	% weight lost	% bees damaged	% haemolymph lost	% haemolymph protein lost
Choi & Woo, 1974	6	Pupae = 10%	nr	nr	nr
Sadov, 1976	nr	nr	nr	nr	15-20%
Grobov, 1977	2 or 3	15-20%	nr	nr	nr
Anshakova <i>et al.</i> , 1978	nr	20%	Workers = 3 Drones = 40	nr nr	nr nr
Schneider, 1978	1 > 3	9.6%	nr	nr	nr
	3 <	22%	nr	nr	nr
Beetsma <i>et al.</i> , 1980	nr	5.2%	nr	nr	nr
Domazkaja, 1980	4	nr	nr	nr	39.2-51.5%
DeJong <i>et al.</i> , 1982b	0	0	0	nr	nr
	1	6.3-6.5%	0	nr	nr
	2	10.6-10.9%	0	nr	nr
	3	11-14.5%	2.6	nr	nr
	4	16.4-18.4%	5.1	nr	nr
	5	16.2-19.2%	7.7	nr	nr
	6	21.3-21.9%	40	nr	nr
	7	23.9%	60	nr	nr
	8	25.3-25.4%	50	nr	nr
	9	24.3%	100	nr	nr
Glinski & Jarosz, 1984	larvae 1-3	nr	nr	nr	34.4%
	larvae 1-3	nr	nr	nr	55.6%
Weinberg & Madel, 1985	Workers 1>3	nr	nr	pupae 23.7%	27%
	Workers 4>6	nr	nr	pupae 40%	50%
	Drones 1>3	nr	nr	pupae 18.9%	11.3%
	Drones 4>6	nr	nr	pupae 21.9%	12.3%
	Drones 7>	nr	nr	pupae 31%	12.1%
Schneider & Drescher, 1987	Workers 1>3	9.6%	nr	nr	nr
	Workers 3<	21.6%	nr	nr	nr
	Drones 1	3.1%	nr	nr	nr
	Drones 3<	14.1%	nr	nr	nr
Schatton-Gademayer & Engels, 1988	1	6.6%	0	nr	nr
	2	12.7%	0	nr	nr
	3	14.6%	0	nr	nr
	4	17.6%	0	nr	nr
	5	21.6%	20%	nr	nr
	6	24.1%	30%	nr	60%
	7>10	28.7%	89%	nr	76%
Kolev & Shabanov, 1989	Drones slight	nr	nr	nr	60%+
	Drones severe	nr	nr	nr	83-97%
Romaniuk & Wawrzyniak, 1991	nr	0.18	nr	nr	nr
Marcangeli <i>et al.</i> , 1992	1>7	nr	29%	nr	nr
Getchev, 1994	nr	7.6-9.5	68%	nr	nr
	nr	18.2-23%		nr	nr
Sadov, 1976	2>3	Pupae 3.6%	nr	nr	nr
Romaniuk <i>et al.</i> 1988, cited by Getchev, 1994	Queens 1-2	0.5-2.3%	nr	nr	nr
Koch & Ritter, 1991	0	nr	6%	nr	nr
	1	nr	15%	nr	nr
	2	nr	26%	nr	nr
	3	nr	38%	nr	nr
	4	nr	33%	nr	nr
	5	nr	50%	nr	nr
	6	nr	41%	nr	nr
	7	nr	61%	nr	nr

Parasitosis	n	Mean Weight (mg)	% weight loss	
0	165	116.38		a
1	133	113.05	2.9	a
2	96	108.84	6.5	b
3	57	103.21	11.3	c
4	50	102.84	11.6	c d
5	14	100.61	13.6	b c d e
6	10	94.06	19.2	c d e f
7	5	99.32	14.7	b c d e f g
8	7	92.44	20.6	c d e f g
9	3	89.73	22.9	not determined
10	1	91.5	21.4	not determined
11	1	78	32.9	not determined

Table 4.2 Relationship between increasing *Varroa jacobsoni* (1-11) parasitosis and the mean emergence weight of worker honeybees (*Apis mellifera*) and the percentage weight lost by parasitised bees. Means emergence weights followed by the same letter are not significantly different, as determined by a GT2 test.

Parasitosis	Number of bees at emergence		% bees damaged
	not deformed	deformed	
0	162	3	1.8
1	124	9	6.8
2	91	5	5.2
3	49	9	15.5
4	43	7	14
5	12	2	14.3
6	8	2	20
7	3	2	40
8	3	4	57.1
9	1	2	66.7
10	1	0	0
11	0	1	100

Table 4.3 Relationship between increasing *Varroa jacobsoni* (1-11 mites) parasitosis and the number of worker honeybees (*Apis mellifera*) which emerged ($n=543$) exhibiting morphological deformities ($n=43$).

VARROA JACOBSONI AS A TRANSMITTER
OF EXOGENOUS SUBSTANCES BETWEEN
HONEYBEES



SUMMARY

Varroa jacobsoni which were permitted to feed on live [U¹⁴C]-labelled bees acquired the label within 24 hours. Assuming from the morphology of the mouthparts that varroa, like many other gamasids, consumes liquid-food, then it can be calculated that they ingest 0.67µl of haemolymph over 24 hours. Mites were also found to feed on dead bees. This may have some bearing on enhancing the ability of mites to survive if their host bee dies.

It was also demonstrated that ¹⁴C was transmitted to a previously nonradioactively-labelled bee when a mite which had fed on a previously radiolabelled bee changed hosts. This would provide a direct mechanism whereby varroa can transmit exogenous material (saliva or regurgitated gut contents) between bees. This finding lends strong support to the suggestion that varroa is a vector of pathogenic micro-organisms and/or viruses.

Key Words: *Apis mellifera*, *Varroa jacobsoni*, honeybees, feeding, disease transmission, mite survival, radioactive glucose, mite saliva.

INTRODUCTION

When *Varroa jacobsoni* Oud. was first described by Oudemans in 1904 (Oudemans, 1904) on *Apis cerana*, he suggested that it might be a parasite. Some 60 years later, Saltchenko (1966) stated that it is definitely a haematophagous parasite of bees and later still, Akwatanakul & Burgett (1975) warned that it was a prospective pest of honeybees of world-wide significance. Today, varroa infestation is associated with the death of individual bees (Anshakova *et al.*, 1978) and ultimately, with the decline of honeybee colonies (Grobov, 1977; Shabanov, *et al.*, 1978). Many factors are involved in causing the mite-associated pathology but their relative importance is uncertain. For example, varroa can be present in colonies for many years with no apparent ill effect (S.Martin pers.comms.), but the mites can cause serious physical and physiological damage to individual bees (Ritter, 1986; Daly, *et al.*, 1988; Needham, 1988; Strick & Madel, 1988b; Koch & Ritter, 1991; Marcangeli, *et al.*, 1992b; chapter 4). Additionally, varroa has been shown to act as a vector of some viral and microbial diseases (Bailey, 1965; Allen *et al.*, 1986; Ball, 1989; Koch & Ritter, 1989; Bruce *et al.*, 1991a; Gliński & Jarosz, 1992; Faucon *et al.*, 1992; Liu, 1996; chapter 6).

Although it has been known for many years that varroa are haematophagous, it has remained uncertain whether they introduce exogenous material (e.g. saliva) into their host while feeding (Ball, 1985; Gelbe & Madel, 1988). If saliva or regurgitated gut contents are introduced into a host whilst feeding then the potential for disease transmission will be enormously increased. Effective contaminative transmission of bacteria or viruses depends on how stable the pathogen is outside the host. Bee viruses are unlike the majority of known insect-viruses as they are not embedded in crystalline matrices of protein (forming polyhedra) (Bailey & Ball, 1991), consequently, "they do not survive long outside the living tissue in which they multiply" (Bailey & Ball, 1991). So, if varroa only act as 'contaminative transmitters', the bee viruses may not survive long enough on the mouthparts between feeding episodes and therefore will not retain their infectivity. On the other hand, if it can be shown that material is introduced into the host then, the viruses would not be exposed to the external environment and therefore may retain infectivity between mite feeding episodes.

Adult female *V. jacobsoni* move between hosts during both the summer (Kuenen & Calderone, 1997) and winter months (chapter 3). The frequency of their inter-host transfer (probably once a week in winter; and every 10-30 days in summer, between each reproductive cycle) and the time elapsing

between each feeding episode (no more than 24hrs in winter and several times a day in summer [Donzé & Guerin, 1994]) would suggest they are a reliable and effective means of spreading viruses through a bee colony and between bee stages (pupae and adults).

The following experiments were undertaken to determine the effectiveness of varroa as a vector of foreign material between overwintering adult honeybees. The aims were, first, to quantify the amount of haemolymph varroa removes from live and dead bees, and second to establish whether varroa transmits exogenous material between hosts.

These experiments were carried out using [^{14}C]-Glucose. Glucose levels are generally low in the haemolymph of insects (Wigglesworth, 1974). When glucose is injected into the haemolymph, it is rapidly taken up by the fat body cells and synthesised into trehalose, which diffuses out of the fat cells and into the haemolymph (Rees, 1977). When in the haemolymph, trehalose is moved to the tissue where it is to be utilised and moves into the cells. Once in the cells it is hydrolysed into two molecules of glucose by trehalase (Rees, 1977). This is not the case in honeybees where glucose levels are unusually high (ca. 180mM) and trehalose levels are comparatively low (ca. 30mM) (Chippendale, 1978). In honeybees the principal carbohydrates in the haemolymph are fructose and glucose, with glycogen

being the main storage carbohydrate. Glycogen is found in the fat body cells and in muscle cells (Neukirch, 1982). Due to the abundance of glucose in bee haemolymph and its centrality in many biochemical pathways, e.g. glycogenesis, glycolysis, respiration (TCA cycle), pentose phosphate pathway, lipogenesis, fatty acid/glycerol formation *etc.* (Rees, 1977; Chippendale, 1978), it was selected as the carrier molecule for the [^{14}C]-label. By entering a number of biochemical pathways the [^{14}C] would rapidly be incorporated into a wide range of haemolymph constituents without the concern of it being selectively partitioned to one region and effectively lost from the haemolymph.

All experiments were carried out on overwintering bees collected from a single hive between October and March 1997.

MATERIALS & METHODS

a) Validating the effectiveness of the injection methodology

In a preliminary investigation, worker bees ($n=126$) were removed at random from the top-bars of a hive, anaesthetised by chilling at -22°C for ~2 minutes and then restrained on a cork platform using entomological pins. Thereafter they were injected with UV-sterilized insect Ringer's solution to assess the effect of the technique on mortality rates. 58 bees were injected with $8\text{ }\mu\text{l}$ ($\pm 1\%$ of syringe volume) insect Ringer's solution (composition per

litre = NaCl, 8.1g; KCl, 0.74g; CaCl₂, 0.22g; MgCl₂, 0.2g; NaHCO₃, 0.33g; NaH₂PO₄, 0.78g) using a Hamilton™ syringe [10µl -700 series] with a sharpened needle [inner diameter =114µm; outer diameter =470µm] fitted with a Hamilton™ Chaney adapter. All bees were injected between the 3rd and 4th dorsal abdominal tergites. The remaining 68 bees were not injected. Thereafter the bees were transferred to an Apidea™, which was maintained at 33°C (~ central cluster temperature, LeConte & Arnold, 1980; Winston, 1987) and 55-80% relative humidity (R.H.) in a Sony Gallenkamp™ incubator and supplied with sucrose solution (1:2 wt:vol) and water. After 5 days the mortality was noted.

b) The effect of 0.65mmol glucose on honeybee mortality

48 bees were used, 27 bees were injected with 8µl of 0.65mmol D-glucose solution as described previously and the remaining bees ($n=21$) were not injected. The bees were placed in an Apidea™ for 5 days as described above and their mortality was noted.

c) Labelling *A. mellifera* with U¹⁴C-glucose.

Bees were radioactively labelled as follows: bees were anaesthetised as above and then injected with 8µl of 0.65mmol D-[U¹⁴C]-Glucose (Amersham Life Sciences, Specific Activity 10.8GBq/mmol). As the bees

were recovering after the injection, a drop of sucrose solution (1:2 wt./vol.) was placed onto their out-stretched mouthparts. This improved recovery and reduced mortality rates. The bees were then transferred to an Apidea™ which was maintained as described previously. At intervals of 0, 2, 24 and 48 hrs post-injection, 20 bees in groups of 5 ($n=30$) were removed from the Apidea™ and their radioactivity levels determined as described below (whole bee preparation), to determine the turnover of the ^{14}C . In addition, 1 μl of haemolymph was extracted from 10 of the bees 24hrs post-injection, and the radioactivity levels were determined. Each 1 μl sample of haemolymph was analysed separately to determine how the haemolymph radioactivity levels changed over time.

d) Determination of the levels of [U^{14}C]-glucose in whole bees, bee haemolymph and in mites

Mites and bees were crushed individually in 1ml and 2ml of Ecoscint™ respectively using Eppendorff™ pestles. 1ml Ecoscint™ was added to each 1 μl haemolymph sample and vortex mixed using a Gallenkamp spinmix. The assay containing the crushed mites, bees or haemolymph were read for 5 minutes per sample in a Liquid Lumex scintillation counter against both blank Ecoscint™ controls and non-radioactively labelled mites, bees or an equivalent volume of haemolymph in Ecoscint™. The scintillation counter

was set to read β -radiation which is the type of radiation emitted during the decay of ^{14}C -sources (Isaacs *et al.*, 1991).

e) Labelling *V. jacobsoni* with U^{14}C -glucose

Individual, naturally-parasitised bees carrying 1 or 2 mites were radioactively labelled as described previously. After 24 hours the bees were removed from the ApideaTM and their mites ($n=219$) collected. The mites were then used as described below.

f) Feeding of *V. jacobsoni* on dead bees

In chapter 3, strong circumstantial evidence was presented which suggested that adult mites continue to feed on their hosts after these had died. The purpose of this experiment was to confirm that feeding on dead hosts occurs and to quantify the associated feeding activity. Parasitised adult overwintering bees ($n=34$) were killed by crushing the head and thorax and were then left for 24hrs at 33°C and 55-80% R.H. On the following day, the bees onto which mites remained attached ($n=24$; some mites had moved between bees during this time) were radioactively labelled as described previously. Two bees (of $n=24$) and their mites ($n=30$) were then placed in sealed cylindrical plastic containers (diameter= 35mm, height =28mm) with 25 ventilation holes made using pins and kept at 33°C and 55-80% R.H. in a

Sony Gallenkamp™ incubator for 24 hours. The bees and mites were subsequently analysed separately for radioactivity as described previously.

g) The transmission of $U^{14}C$ glucose between adult bees by *V.jacobsoni*.

Mites labelled with $U^{14}C$ glucose ($n=219$) were used to artificially infest non-radioactively labelled adult overwintering worker honeybees (2-7 per bee; $n=44$). Two artificially infested bees at a time were confined together for 24 hours in a cylindrical plastic container (dimensions as described previously) lined with absorbent paper (to take-up faecal material) and supplied with sugar solution (1:2 wt.:vol). The bees were maintained at 33°C and 55-80% R.H. in a Sony Gallenkamp™ incubator. Bees ($n=14$) were kept in pairs because preliminary experiments indicated that bees died rapidly if kept singly. After 24 hours, the bees and mites were separated. The bees were killed by chilling at -22°C for 10 minutes and then washed in 1ml of 1% v/v Decon 90™ solution for 1 minute before being dissected into head, thorax, abdomen and gut. Decon 90™, a non-ionic detergent, was used to remove contaminating materials from the cuticle of the bees (physical cleaning could have resulted in damage to membranes and the release haemolymph material). This removed any mite faeces which may have contained the radioactive label from the surface of the bee. The liquid in

which the bee was washed and the compartments of the dissected bee were analysed separately for their radioactivity-levels.

A further 30 bees were treated as above but their intestines were not dissected out and were analysed *in situ* together with the abdomen.

RESULTS

a) Validating the effectiveness of the injection methodology

24% of the bees injected with Ringer's solution and 21% of the non-injected bees died during the 5 days after commencement of the experiment. The difference in mortality was not significant ($\chi^2_1=0.26$, $p>0.05$), indicating that the injection technique and volume of fluid injected were not overly disruptive.

b) The effect of 0.65mmol glucose on honeybee mortality

No significant difference in mortality was noted over 5 days between the glucose injected (26%) and control (29%) group. This indicated that the concentration of glucose (0.65mmol) used to radioactively label bees would not be deleterious ($\chi^2_1=0.14$, $p>0.05$).

c) Labelling *A. mellifera* with $U^{14}C$ -glucose.

The detectability of the radioactive label once injected into the bees rapidly decreased over 24 hours. This was probably due to metabolic processes which either removed the ^{14}C from the bees body, for example as expired CO_2 , or converted it into compounds which became incorporated into the host tissue resulting in its ultimate removal from easy detectability e.g. by incorporation into muscle. The loss followed a curvilinear pattern, with the greatest loss occurring in the first few hours, gradually becoming smaller over 48 hours (Figure 5.1).

1 μ l of honeybee haemolymph had a β -level of 44678 ± 3472 CPM 30 minutes after injection with 8 μ l of D- $[U^{14}C]$ -glucose ($n=5$). This has fallen to 2220.26 ± 556.5 CPM after 24hrs post injection ($n=5$).

e) Labelling *V. jacobsoni* with $U^{14}C$ -glucose

A total of 219 *V. jacobsoni* were successfully radiolabelled and were subsequently transferred onto a non-labelled second host. Of these mites, 158 (72%) survived for 24 hours on the new host. The remaining 61 mites (28%) died during their association with the new host (the mites may or may not have fed on the second host).

Non-labelled control mites taken from the same colony had a mean(\pm se) background radioactivity β -level of 23.2 ± 0.35 CPM

(min=17.4CPM; max=29.8CPM; $n=62$). Of the mites which survived on their new hosts, 15 (9%) had a β -level at or below maximum background level. This suggests that they may not have fed or had fed very little on their first host after it was radiolabelled. The mean (\pm se) β -level in the remaining mites ($n=143$) was 110 ± 5.9 CPM. A Mann-Whitney U-test confirmed that the mites which had fed on the radiolabelled bees had a median β -level (CPM) significantly higher than that of the median background β -level ($U_{62,158} = 169.5$, $p < 0.0001$).

When all mites which had been radiolabelled in this way (both in practice infection-runs and in this experiment) were pooled ($n=305$), a mean(\pm se) β -level of 137.28 ± 9.69 CPM was obtained (trimmed mean [where the upper and lower 5% of the data is removed] = 110.06 CPM). This data had a large spread about the mean ($sd = 169.17$). Moreover, the data was strongly skewed to the right ($g_1=4.45$, $p < 0.001$), and a kurtosis test confirmed that the data was significantly leptokurtic in its distribution (where a curve has more items near the centre and at the tails, with fewer items in the shoulders relative to a normal distribution [Sokal & Rohlf, 1995]) ($g_2=28.74$, $p < 0.001$) (Figure 5.2). This strongly suggests that although the mites feed at a fairly constant rate (at least once every 24 hrs to ingest the radioactive label) over time, some of the highly radiolabelled mites had very

high β -levels ($>500\text{CPM}$, $n=10$) and may have been contaminated. Mites could have become contaminated by radioactive material on the surface of the host. This source of contaminating material could arise if the injection wound did not occlude fully, allowing some of the radioactive label to leak onto the surface of the host. Alternatively, these bees may have been feeding at or soon after the $[\text{U}^{14}\text{C}]$ -glucose was injected into the haemolymph, and therefore picked up a high radioactive dose.

f) Feeding of *V. jacobsoni* on dead bees.

30 mites were used in this experiment. 6 died within 24 hrs exposure to dead bees (Group A), 5 died 24-48 hrs exposure to dead bees (Group B), 14 live mites were removed for scintillation counting after 24hrs exposure to dead bees (Group C), and the remaining 5 live mites were removed for scintillation counting after 48 hrs exposure to dead bees (Group D).

Group-A mites had a mean radioactivity level of 593.63CPM ; group-B mites had a mean radioactivity level of 1066.48CPM ; group-C mites had a mean radioactivity level of 1473.97CPM , and group-D mites had a mean radioactivity level of 1548.08CPM .

The median radioactivity levels (CPM) of mites which had fed on dead bees for 24hrs (1473.97CPM) was significantly higher ($\times 10$) than the

median radioactivity levels (CPM) of mites which had been feeding on live bees for the same length of time (84.4CPM) ($U_{305, 14} = 39630$, $p < 0.001$).

To confirm this observation, and to try and explain it; dead ($n=10$) and live ($n=7$) bees were injected with $8\mu\text{l}$ of ^{14}C -glucose (as described above) and left for 24 hrs. Thereafter they were divided into their respective body compartments (head, thorax and abdomen). Each compartment was scintillation counted separately. It was found that the median β -counts (CPM) of the heads and thoraces of dead and live bees were not significantly different (Head, $U_{10, 7} = 75$, $p > 0.05$; Thorax, $U_{10, 7} = 107$, $p > 0.05$). However, the median β -count (CPM) of the abdomens were significantly different ($U_{10, 7} = 120$, $p < 0.005$). Thus, dead bees retained significantly more radiolabel in their abdomens than did live bees (Table 5.1).

If it is accepted that the mean β -level of mites after feeding on radiolabelled bees is a good approximation of the amount of ^{14}C imbibed over 24 hours, it is possible, albeit crudely, to calculate the mean amount of haemolymph that mites consume ($1\mu\text{l}$ of bee haemolymph [after 24 hrs] had a β -level of $2220.26 \pm 556.5\text{CPM}$). For mites to obtain a β -level of 1277.96CPM then they would have to consume about $0.67\mu\text{l}$ of bee haemolymph over 24 hrs (or 165% of the mite's body weight over 24hrs).

There are two factors, however, which introduce error into this calculation. First, the mean β -levels in the bees fell significantly during the 24 hours post-injection (Figure 5.1). Second, mites would not retain all the ^{14}C they acquired, some would be egested along with other faecal material and some would be lost as CO_2 . Thus it is likely that the figure of $0.67\mu\text{l} / 24\text{hrs}$ is an underestimate of the volume of haemolymph consumed.

g) The introduction of foreign material into adult bees by *V.jacobsoni* during its normal feeding activity.

A significant difference ($U_{48,44} = 3033$, $p < 0.0001$) was found between the median background β -levels of unlabelled bees 22.9CPM ($n=48$) and bees which had been fed upon by radioactively labelled mites 46.6 CPM ($n=44$). The radioactively labelled bees had a median β -level of 46.6 CPM ($Q1=32.35$, $Q3=59.35$), which was x2 greater than that of the background.

DISCUSSION

Sadov (1976, cited by Smirnov, 1978) injected varroa parasitised bees (life-cycle stage of bees not stated) with radioactive ^{90}Sr which was subsequently found within the female mites, indicating that they had fed on the bees. In a later study, Gelbe & Madel (1988), using ^3H -valine injected into honey bee pupae, found that varroa acquired the tracer during their feeding activities. Gelbe & Madel (1988) also found that the ^3H -tracer became incorporated into the fat bodies, haemolymph and “cephalic” glands of the bee pupae if the mites were moved onto unlabelled pupae, suggesting that the mites introduced exogenous material into the pupae.

A number of more recent studies have shown that the amount of haemolymph imbibed by the mites is fairly consistent, irrespective of the time of year. Romaniuk & Wawrzniak (1991) state that “one female of *V.jacobsoni* daily takes twice as much haemolymph than it weighs itself”. As a female mite weighs about 0.345mg, the haemolymph removed would correspond to approximately 0.7 μl per 24 hrs (approximated from the fact that 1 μl of pure water at 4°C and 760mmHg has a weight of 1mg). The estimated volume of 0.7 μl / 24hrs, however, would be slightly greater, as haemolymph is denser than water. Thus a smaller volume would be needed to attain the same mass. Schatton-Gademayer & Engels (1988, citing Tewarson, 1983) state that “during the act of sucking, adult female mites

take up about 0.1 µl of host blood", but do not mention how long an "act of sucking" lasts nor how frequently they occur. Assuming an 'act of sucking' is equivalent to a 'feeding episode' of Donzé & Guerin (1994), one of which they report occurs approximately every 3 hours and lasts about 3 minutes, then the mites would consume 0.8 µl haemolymph / 24 hrs. Tewarson (1983) found that adult female varroa ingested 0.75 µl of host haemolymph / 24 hrs. In contrast, Moritz (1980, cited by Tewarson, 1983) having fed bees with syrup containing ^{32}P , calculated that the mites consumed 0.25 µl in 24 hrs. Smirnov (1978, citing Sadv, 1976) stated that "the amount of haemolymph consumed in two hours in spring and summer by the starved females was 0.08-0.14 mg" (i.e. 0.08-0.14 µl / 2hrs). Unfortunately, once again no indication of the frequency of mite feeding was given. Therefore it is difficult to compare Sadv's work directly with that of the other studies. However, Sadv's (1976) data would be comparable to the other studies if the mites continued to imbibe similar volumes of haemolymph to those recorded but in accordance with the feeding frequency observed by Donzé & Guerin (1994). That is, if the mites imbibe 0.08-0.14 µl haemolymph following regular breaks which last for 3 hours, then in 24 hrs Sadv's mites would consume 0.64-1.12 µl. Thus, despite the use of very different techniques and different bees at different stages of development, estimates of haemolymph consumption are remarkably consistent. This may result from the rigidity of

the idiosomal shield which makes it difficult for the mite to take in large blood meals. The only distension possible is by flexion of the ventral sclerites.

The amount of radioactivity taken up by the mites as determined in this study (0.67 μ l / 24hrs) is far in excess of that which would be indicative of simple mouthpart contamination. Moreover, the SEM micrographs of the mouthparts of varroa (see introduction) did not display large quantities of debris which would be necessary to account for this level of radioactivity in the hosts. Therefore, this study suggests that varroa must be introducing material into the host, such as saliva or regurgitated gut contents, to account for the elevated β -levels.

These experiments not only confirm that varroa introduces exogenous material into their hosts, but that such transmission occurs between live adult bees, and also between dead bees and subsequent live hosts. Although the nature of the material introduced into the bee tissue has not been precisely determined, Gelbe & Madel (1988) suggested that it may have been saliva. This hypothesis was proposed on the basis that droplets of saliva could be observed on the mouthparts of the mites, although no

information was given how they would have discounted the liquid being regurgitated gut contents.

The introduction of exogenous material into hosts by haematophagous ectoparasites is a well-known phenomenon, but what is less well established is the role of saliva in haematophagy (Ribeiro, 1987) and how it is linked to the morbidity and mortality of the host. The possibility of material being inoculated into honeybees by varroa during feeding was first proposed by Ball (1985), but whether the salivarial route is a viable route of disease transmission has never previously been satisfactorily answered (chapter 6).

Saliva in varroa is produced by the paired antero-dorsal salivary glands (Gelbe & Madel, 1988), which are present in all female varroa stages and are composed of two histochemically distinct sections which produce secretions of differing composition. Whole salivary gland preparations have been found to contain water, glycogen, mucopolysaccharides, neutral fats, phospholipids, fatty acids, saturated glycerides, unsaturated lipids, basic proteins, tyrosine, tryptophan and histone (Gelbe & Madel, 1988). It is peculiar that glycogen and neutral fats are present in the saliva of mites as their loss would represent a waste of resources. Similarly, in a study of the salivary gland secretions of the tick *Amblyomma americanum*, Sauer *et al.*

(1993) (citing Shipley *et al.*, 1993) identified phospholipids, diacylglycerols, sterols, free fatty acids and triacylglycerols, which would also appear wasteful. However, they suggested that the composition was consistent with the highly membranous structure of salivary glands, which would result in complex metabolites being lost together with membrane fragments. Moreover, because the methodology employed by Gelbe & Madel (1988) involved using whole gland preparations, many of the components reported may have been intra-cellular in origin, and would not normally contribute to the composition of the secretions. In addition to the substances listed by Gelbe & Madel (1988) as contributing to saliva composition, it has also been suggested that other, “undetermined substances or proteases that initiate specific alterations in blood protein spectra of bees”, are also present in varroa salivary secretions (Gliński & Jarosz, 1988a).

The role of saliva in varroa remains conjectural. In most haematophagous arthropods, at least those of vertebrates, the saliva contains factors that help locate blood vessels and prevent coagulation (Titus & Ribeiro, 1990). This role for varroa saliva is highly unlikely for two reasons. Firstly, bees have poorly coagulating blood (Grégoire, 1955; Gilliam & Shimanuki, 1970). Secondly, the haemolymph of honeybees is not enclosed in discrete blood vessels, but instead is distributed throughout large haemocoel sinuses

(Snodgrass, 1956). So to obtain a blood-meal, varroa only needs to pierce the intersegmental membranes, no effort is required in locating blood vessels first.

A possible reason for saliva production is for the purposes of osmoregulation. Both ticks (Edney, 1977) and the mite *Haematogamasus ambulans* (Young, 1959) produce copious amounts of saliva when blood-feeding. The purpose of the saliva is to eliminate excess water (and inorganic salts, such as Na⁺ etc) which is returned back into the host (Evans, 1992). By not using the malphigian tubules to excrete the excess water, the rectum will not fill-up rapidly, thereby requiring frequent defecation, which would result in contamination of the area which the mite preferentially occupy on adult bees (chapter 2). If mites were to deposit faeces at their attachment site, especially if it was very liquid like that of aphid excreta, this could compromise their ability to remain attached to the host. Moreover, if faeces was to accumulate about the mites, this may draw attention to their presence from grooming bees, resulting in their displacement or even death. Thus the production of relatively dry guanine pellets, as opposed to liquid faeces (which would normally be expected from a hamatophage), may be an adaptation for life on hygienic honeybees.

Weinberg & Madel (1988) reported that drones infested by varroa had elevated haemolymph protein levels. This contrasted with the depletion in haemolymph proteins in worker bees which they and many others (Table 4.1) have reported. A possible explanation for this could be linked to the difference in response by drones and workers to substances introduced by the mites when feeding. Gliński & Jarosz (1984) found that in drones parasitised by varroa, the level of haemolymph oenocytes was 12 times higher than normal. However, in parasitised worker bees, the oenocyte level only reached at most half the level of drones, being x4-6 times higher than normal. Oenocytes are believed to be involved in the secretion of immunologically-active substances (Gliński & Jarosz, 1984). Consequently, if varroa introduce substances into the haemolymph of bees during feeding, these may stimulate the immune system of bees to mount a defence. The oenocytes activity may account for the rise in protein levels of drones parasitised by varroa.

The abdomens of dead bees retained more radioactive material than did the abdomens of live bees. There are two possible reasons for this. Firstly, the haemolymph of insects is located in large open sinuses and is circulated anteriorly, by the dorsal heart and aorta, towards the head. Once in the head, haemolymph returns posteriorly via the thorax. As the haemolymph moves posteriorly, glucose is absorbed by the tissue and is subsequently

metabolised and some is lost via the tracheal system as CO₂. Consequently, mites feeding on live bees would have access to less radioactively labelled haemolymph than those feeding on dead bees as the circulatory system would be disrupted in dead bees. More importantly, live bees would readily utilise glucose, unlike dead bees during metabolic processes, which in fact would probably occur at an increased rate in response to being handled and disturbed (Figure 5.1).

Varroa enter worker cells approximately one day prior to their being capped over, thereafter, the mites feed on the pupae and reproduce. The worker pupal stage lasts 8-9 days, and as this study has demonstrated, the mites consume 0.67µl / 24 hrs, so it can be calculated that one female mite will consume about 5-6µl of haemolymph during the time the bee is developing. This rate of depletion of haemolymph represents approximately a 4-5% loss in weight of honeybees parasitised by one female mite, which corresponds quite well with the 3% decrease in weight actually observed (Table 5.2). Similarly, this rate of haemolymph depletion, would result in a parasitosis of 11 mites causing a predicted 50-57% decrease in weight of emerging bees. The predicted weight loss is however far in excess of the 33% weight loss actually recorded. An explanation for this would be inter-mite competition at the feeding site (Donzé *et al* [1996], citing Donzé,

1995). As the number of mites parasitising a single bee increases, the competition between mites to access the feeding site will also increase. This may result in each mite either having less time to feed or its feeding being disrupted. Consequently mites would only be feeding sub-optimally in multiple-infested cells, giving rise to a smaller weight loss in the host than predicted. If mites consumed 5-6 μ l haemolymph per day, this would cause a loss of 57-69 μ g of haemolymph protein during development as a result of each mite. It is unfortunately not possible to compare the last figure with those obtained for protein depletion of the haemolymph in chapter 4 because the total volume of haemolymph was not determined.

During this study, it was shown that not only do varroa feed on live bees, but that they also feed on dead bees. The latter observation support the hypothesis proposed in chapter 3, namely, that to extend their survival time, mites remain with dead hosts, and feed on them, until another live bee comes close enough and onto which they can then transfer. This takes advantage of the cleaning behaviour of honeybees.

This study has also shown that *V.jacobsoni* not only feed on the haemolymph of bees, but, as is the case with many other haematophagous

invertebrates, also introduces material acquired from previous hosts when feeding. This is an important finding and provides a firm mechanism whereby pathogens can be vectored between bees by varroa. This is further investigated in chapter 6.

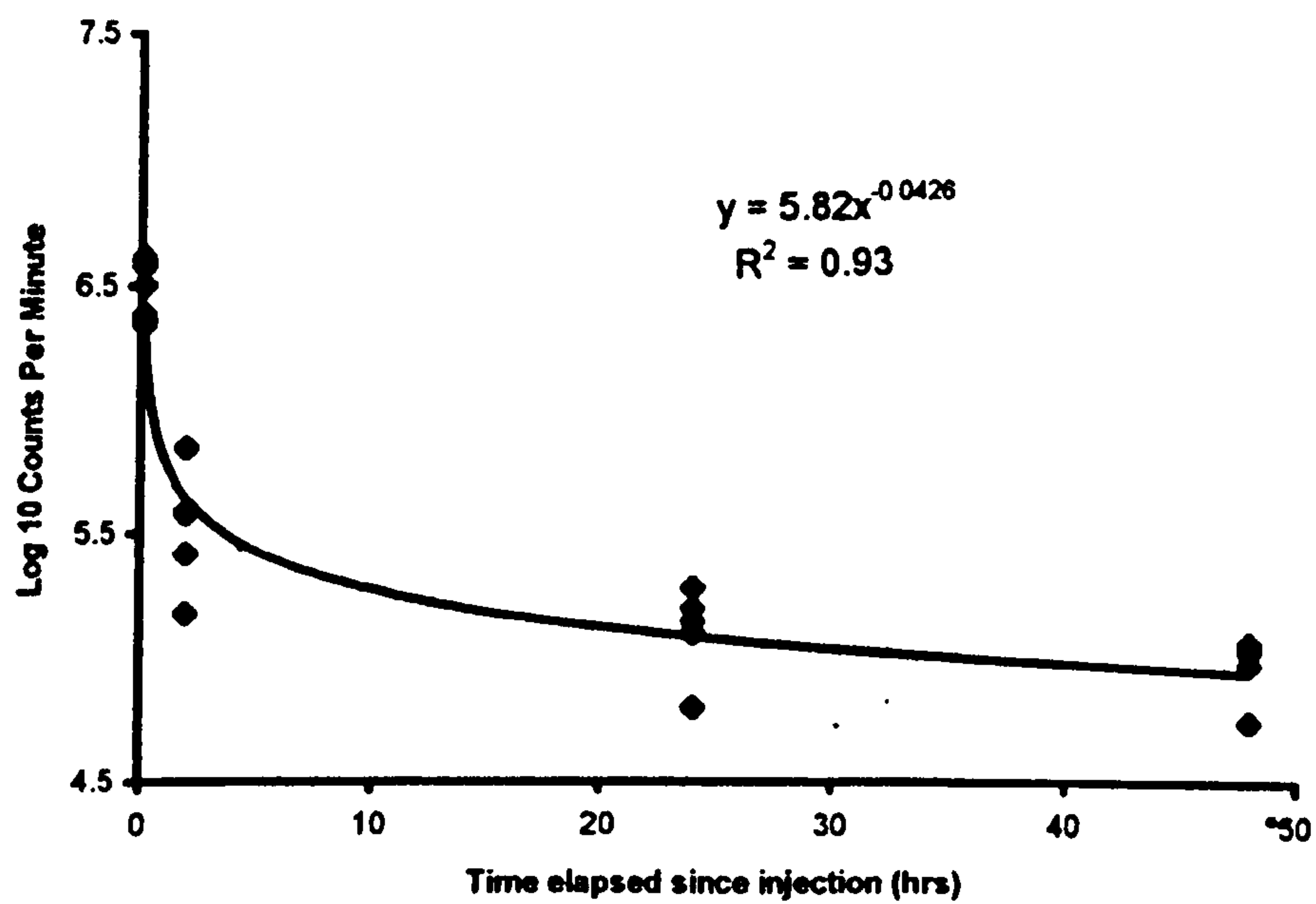


Figure 5.1 Changes in the radioactivity levels (expressed as counts per minute) of *Apis mellifera* (n=20) over time (48hrs), following their injection with 8 μ l of D-[U¹⁴C]-glucose.

Region of bee	Median Beta Levels of Dead bees (CPM)	Median Beta levels of Live Bees (CPM)	Mann-Whitney test for significance
Head	10897 (<i>n</i> =10)	40885 (<i>n</i> =7)	<i>p</i> >0.05
Thorax	159778 (<i>n</i> =10)	134782 (<i>n</i> =7)	<i>p</i> >0.05
Abdomen	663073 (<i>n</i> =10)	225502 (<i>n</i> =7)	<i>p</i> <0.005

Table 5.1 Mean β -levels in the heads, thoraces and abdomens of live honeybees (*n* = 7) and dead honeybees (*n* = 10) 24hrs after being injected with 8 μ l of D-[U¹⁴C]-glucose. The significance was determined by means of a Mann-Whitney U-test.

THE TRANSMISSION OF DEFORMED WING
VIRUS BETWEEN HONEYBEES BY *VARROA*
JACOBSONI



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SUMMARY

Under field conditions, *Varroa jacobsoni* were shown to be highly effective vectors of deformed wing virus (DWV) between bees. Adult female mites obtained from honeybee pupae naturally infected with DWV contained virus titres many times in excess of those found in their hosts and, beyond that, which might be expected from a concentration effect. It is therefore possible that DWV may be capable of replicating within the mite.

Bees which tested positive for DWV exhibited characteristic morphological deformity and / or they died during pupation. Asymptomatic bees had much lower virus titres than those which were deformed or had died during pupation. It is therefore suggested that for DWV to cause pathology it must be present in pupae above a certain concentration. The amount of DWV vectored by *V.jacobsoni* will depend on the level of mites' infection, which will in turn depend on whether they had fed previously on dead or deformed bees and also on the rate of replication of the virus within the mites. Consequently, developing bees infested with a large number of mites could suffer a high incidence of deformity if the mites are heavily infected or harbour an especially virulent strain of virus.

A positive relationship was found between increasing numbers of mites on individual bees and the incidence of morphological deformity and death. This probably reflected the large number of viral particles transmitted

by the mites, which resulted in many multiply-infested bees dying before emergence.

These results demonstrate the importance of the role of viruses when considering the pathology of *V. jacobsoni* and that much of the pathology previously associated with the effects of mite feeding could be attributed directly to secondary pathogens vectored by *V. jacobsoni* (chapter 5).

Key words: *Varroa jacobsoni*, honey bees, *Apis mellifera*, deformed wing virus, vector, bee damage, bee death, disease transmission.

INTRODUCTION

In 1979, Batuev reported that laboratory experiments had demonstrated for the first time that *Varroa jacobsoni* acted as a vector of Acute Paralysis Virus (APV) between adult honey bees. However, in 1985, Ball proposed that in the case of APV, mite transmission could not entirely account for the differential rates of infection seen between the different bee stages, although the mites were suggested to be activating the viruses which would enhance their being spread by the feeding activities of bees. Later in 1988, Ball & Allen obtained evidence which suggested that varroa may be acting as a vector of APV between adult bees and brood.

In 1989 evidence was obtained (Ball, 1989) to support the idea that varroa was a vector of other viruses. Amongst the viruses under investigation was the Japanese strain of Egypt Bee Virus, now called Deformed Wing Virus (DWV). DWV (serologically related to Egypt Bee Virus) was known to result in deformity, reduction in emergence size and mortality of infected honey bees (Ball, 1989; Bailey & Ball, 1991), symptoms previously associated with mite feeding. Due to the methodology of the investigation (artificially infecting mites, by feeding them on virus inoculated pupae), and due to their small sample sizes, doubts remain about the validity of the findings to the situation in the field and the extent to which vectoring

occurred, although the ability of the mites to vector viruses could not be easily disputed any longer.

V. jacobsoni is associated with the appearance of deformed bees in colonies of *A. mellifera* and colony death normally occurs within 3-5 years of initial infestation (Korpela *et al.*, 1992). Bee deformity includes malformed appendages (crumpled/vestigial wings), shortened abdomens, reduced weight at emergence (DeJong *et al.*, 1982b; Schatton-Gadelmayer and Engels, 1988), possibly a reduction in life-span (Kovac and Crailsheim, 1988) and eventually colony effects such as patchy/irregular brood and dwindling bee numbers (Shimanuki *et al.*, 1994; DeJong, 1988).

Several theories have been proposed to explain these effects. Varroa is known to feed on the haemolymph of developing larvae and has been shown to deplete total haemolymph volume and haemolymph protein titres (Gliński and Jarosz, 1984; Weinberg & Madel, 1985; chapter 4). Visible bee deformity could therefore be attributed to both reduced hydrostatic pressure during development and to a reduction in protein availability during development (Daly *et al.*, 1988). Consequently with increased parasitoses there would be a greater probability of emerging bees exhibiting deformity (DeJong *et al.*, 1982b). Unfortunately, bees with very few or no mites can be found exhibiting the same deformities as highly parasitised bees, and highly parasitised bees exhibiting no deformities are also regularly recorded (Hara

et al., 1986; Marcangeli *et al.*, 1992b; Bowen-Walker, pers. obs.). This casts serious doubt on the ability of mite numbers alone to explain bee deformity and colony collapse.

Another theory is that bee deformity is related to microbial septicaemia, caused by micro-organisms transmitted by varroa (Shabanov, 1984; Strick & Madel, 1988a; Gliński and Jarosz, 1992; Liu, 1996). Again, other evidence has not been supportive (Koch and Ritter, 1989; Koch and Ritter, 1991) or is in direct opposition (Alippi *et al.*, 1995) to the hypothesis.

Under experimental conditions, varroa transmits certain viruses (e.g. Acute Paralysis Virus and Cloudy Wing Virus) (Batuev, 1979; Ball & Allen, 1988) raising the possibility these might be involved in causing bee deformity. DWV causes deformity, reduction in emergence size and mortality of infected honey bees, symptoms commonly associated with mite feeding behaviour. Furthermore, because female mites feed regularly and move between both bee brood and adult bees (chapter 3) they have the potential to act as vectors of pathogenic organisms.

The purpose of this investigation was to provide evidence that under natural field conditions, mites

- 1) acquire and carry viruses
- 2) act as vectors of these viruses

3) that the pathology associated with varroa is linked to the viruses they carry and not necessarily their feeding activities.

MATERIALS AND METHODS

All experiments were conducted in May and June 1997 in Devon, (U.K). Six untreated *Apis mellifera* colonies were used: three source colonies and three target colonies. Source colonies were severely infested with varroa (daily mite drop, 30+mites) and many bees (both emerging and older) exhibited noticeable morphological deformity, such as crumpled/vestigial wings, and some bees appeared to be small. The target colonies had undetectable varroa populations (no mite drop over five days) and the bees exhibited no morphological deformities. All colonies were within the same apiary. The target colonies being recent introductions to the apiary, accounting for their low mite populations.

Transmission of Deformed Wing Virus by *V. jacobsoni*

Collection of mites from source colony

Frames of emerging brood were removed from the source colonies and female mites were collected from both deformed and non-deformed emerging worker bees. The mites were kept overnight on damp tissue at

33°C in a Gallenkamp™ incubator before introduction into the target bee cells the next day.

Introduction of mites into the target colony brood

The queens of the target colonies were marked, caged onto drawn worker comb, and released following oviposition. Brood development was monitored daily. On the day of capping between one and six mites from the source colonies were introduced into the recently sealed cells. Inoculation was accomplished by making a small incision in the capping using a pin, inserting a mite which had mounted a seeker (flamed immediately before picking up each mite) into the incision, and then melting a small piece of beeswax from the same frame over the incision. Control cells were prepared in the same way but no mites were introduced into the cells. Each cell was identified by being marked on an overlying sheet of acetate pinned to the frame.

The frame was then inserted into a Gallenkamp™ incubator maintained at 33°C and 55-60% relative humidity (monitored by two hair hygrometers) until the bees were due to emerge. Frames were not returned to the hives, owing to the possibility of the bees rejecting the manipulated cells (S. Martin, personal observation). Developing bees into whose cells the

mites were introduced were termed 'target hosts'; the original emerging bees from which the mites were collected were termed 'source hosts'.

Emerging target bees, classified as being deformed or non-deformed, were collected at emergence together with their infesting mites and frozen (-20°C) for later viral analysis. This procedure was repeated three times, the mites being drawn from the same three source colonies, but each frame of target brood came from a different hive.

Identification of Deformed Wing Virus in *V.jacobsoni* and target hosts

Due to the nature of the morphological deformity (deformed wings, reduction in overall size and mortality of brood [Ball, 1989]) mites were tested for DWV, as other known viruses in the U.K (e.g. Slow Paralysis Virus and Cloudy Wing Virus) do not show similar symptoms (Bailey & Ball, 1991). The mites and target bees were analysed by the indirect ELISA technique of Allen *et al.* (1986) as modified by B.Ball (pers.comm.) for the identification of DWV. All analyses were conducted at IARC Rothamstead, Harpenden.

Whole bees and whole mites were homogenised in 1ml and 0.5ml respectively, of extraction buffer, pH 7.4 (per 100ml: 2g polyvinyl pyrrolidone [44000 Av Mol Wt], 500µl 10% Tween 20, 0.2g ovalbumen, 10ml stock phosphate-buffered saline{stock-PBS}[1.4M NaCl, 0.01M

KH_2PO_4 , 0.08M $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.02M KCl]) using a Pro 2000 electric homogeniser. Carbon tetrachloride (100 μl) was added to each homogenate to remove fatty material and the samples were centrifuged at 8000rpm for 10 minutes in a 5417C Eppendorff™ centrifuge. Aliquots of the supernatants were then diluted with extraction buffer (1:40 for bees, 1:20 for mites) and assayed for the presence of DWV as detailed below.

Cliniplate polystyrene microtitre plates (Labsystems) were coated with 200 μl of DWV-specific F(ab')₂ fragments (1:4000) in 0.0125M sodium carbonate buffer (pH 9.6), and incubated at 30°C for 4 hours. The plates were rinsed with 10% stock-PBS containing 0.05% Tween 20 {Tween-PBS} in a Skatron A/S Microwash III. Diluted bee or mite supernatant (200 μl) was then added to each well and the microtitre plates were incubated at 4°C overnight. The plates were then rinsed with Tween-PBS after which 200 μl of DWV-specific IgG in extraction buffer (4 $\mu\text{g ml}^{-1}$) was added to each well. The plates were then incubated at 30°C for 3 hours, after which they were rinsed with Tween-PBS and 200 μl of protein A-peroxidase conjugate in extraction buffer (25 $\mu\text{g ml}^{-1}$) was added to each well. The plates were incubated at 30°C for 3 hours after which they were rinsed with Tween-PBS and 200 μl of a peroxidase substrate (per 20ml: 2ml sodium acetate-citric acid buffer, 200 μl 3,3',5,5'-tetramethyl benzidine in dimethylsulfoxide, 20 μl hydrogen peroxide) was added to each well. The plates were incubated in the

dark at 20°C for 20 minutes, after which the reaction was stopped by the addition of 50µl of 3M H₂SO₄. Finally, the optical density of each well at 450nm and 690nm was recorded against a reagent blank using a Titertek Multiskan MCC/340 MKII microtitre plate reader equipped with Titresoft II software.

The antiserum used is not known to cross-react with other bee viruses (B.Ball pers.comm.). Bees were analysed for DWV individually, but all the mites within the same cell were pooled for analysis. DWV research is presently in its infancy and no standard curve for absorbency against virus particle concentration exists (B.Ball pers.comms)

RESULTS

Bee deformity in source and target colonies

462 mites in 3 trials were introduced into a total of 179 recently capped worker cells. Of these, 97 (54%) survived to emergence. By contrast all the bees in randomly opened, non-experimental cells on the frames ($n=674$) survived. All of the control bees emerged ($n=30$) and were non-deformed, even though one bee had a natural (non-experimental) parasitosis of 4 mites. With an increasing experimental parasitosis, fewer nondeformed bees emerged, more bees died, and more bees emerged with morphological deformities (Figure 6.1). Source host deformity was associated with target

host deformity; thus the target host was more likely to be deformed if the source host was deformed and less likely to be deformed if the source host was not deformed ($\chi^2_1=4.42$, $p<0.05$) (Table 6.1).

Identification of Deformed Wing Virus in *V. jacobsoni* and target hosts

High DWV-ELISA optical densities were recorded in all deformed and dead bees and in all the mites that were infesting dead and deformed bees (Figure 6.2). Low or undetectable DWV levels were recorded in uninfested and infested but non-deformed bees (Figure 6. 2). Interestingly, those mites recovered from non-deformed bees exhibited a wide range of optical densities, which were essentially platykurtic (Sokal & Rohlf, 1995) in their distribution ($g_{2n=17} = -2.05$). There were highly significant associations (i) between a bee being infected with DWV and emerging deformed ($\chi^2_1=38.2$, $p<0.001$) (Table 2), (ii) between bee deformity and the presence of DWV in their parasitising mites ($\chi^2_1=22.6$, $p<0.001$) (Table 6.3), and (iii) between a bee being infected with DWV and its parasitising mites being infected with DWV ($\chi^2_1=13.45$, $p<0.001$).

The mean weight of adult female *V. jacobsoni* is $0.34\pm0.01\text{mg}$ ($n=5$), while that of an emerging worker honeybee is $116.4\pm0.61\text{mg}$ ($n=165$) (Bowen-Walker, unpublished). Using these data, and the mean DWV-ELISA optical densities (measured in optical density units) for 5 singly infested bees

(2.623 ± 0.221 OD units) and their associated mites (2.891 ± 0.115 OD units), it was possible to compare, albeit crudely, the relative amounts of virus present (estimated as optical density units/mg live weight) in the mites and in the bees. As an *a priori* our calculation assumed that the standard curve for the DWV-ELISA would be of the typical sigmoid-shape (Allen *et al.*, 1986; Clark & Barbara, 1987), and that the measurements fell on the linear portion of the curve. The results suggested that the mites contained virus particles that led to an average absorbency of 85.03 OD units/mg live weight and that the bees contained virus particles that lead to an absorbency of 0.904 OD units/mg live weight.

DISCUSSION

There are two principal mechanisms whereby *V.jacobsoni* could spread viruses within and between bee colonies. First, the mites feeding activities could potentiate inapparent virus infections already present in the bees, i.e., until activated by the mites the viruses do not cause noticeable pathology and their levels are low or undetectable. Second, the mites could act as vectors of viruses by directly transmitting them between bees, for example, in their saliva or in regurgitated gut contents.

These results prove that *V.jacobsoni* can act as a highly effective vector of DWV. The evidence for this is, first, that the mites are capable of

acquiring DWV from infected bees - all the mites feeding on deformed bees themselves tested positive for the virus. Second, it was found that a bee was more likely to die or emerge deformed if the mite feeding on it had previously fed on a deformed bee. This association would be baseless if the mites were not capable of transmitting DWV between hosts. If *V.jacobsoni* acted solely as a potentiator of DWV then the introduction of mites into the target bee cells would have resulted in little more than 13% deformity among the emerging bees. That is, the level of deformity would be similar to the natural incidence of virus among the bees in the target colony (13%).

The existence of a low frequency of DWV within the colony indicates that its transmission can also be independent of *V. jacobsoni*. This would explain the occasional outbreaks of DWV in the UK before the arrival of *V. jacobsoni* in 1992 (B.Ball pers. comm.). The absence of bee deformity among naturally infected bees could result from either the virus normally being inhibited by the bee immune system or because, for some reason, the virus might only be replicating slowly.

The spread of *V. jacobsoni* into a new area is often associated with an increased prevalence of viral diseases, including DWV, which were previously not reported or rare (Ball, 1993). However, there is usually a lag period of about a year or more between the arrival of *V. jacobsoni* and

reports of colony damage (Martin, 1997b). This indicates that *V.jacobsoni* infestation does not immediately or inevitably result in honeybee pathology.

The outcome of infestation will depend on numerous factors such as

- (i) the number of mites in the colony,
- (ii) the presence and level of natural viral infection among the bees,
- (iii) the prevalence of virus in the mite population,
- (iv) the pathogenicity of the strain of virus,
- (v) the susceptibility of the bees (and possibly also the mites) to the virus,
- and
- (vi) the amount/caste of brood present in the colony, which in turn will influence the mite population dynamics.

In these experiments uninfested non-deformed bees and infested non-deformed bees had similar DWV-ELISA optical densities. Again, this shows that *V. jacobsoni* infestation does not always result in bee deformity.

However, dead and deformed bees invariably expressed optical densities considerably in excess of those of the non-deformed bees. This suggests that it is the level of DWV present in the bees which determines whether they are deformed at emergence rather than just the presence or absence of virus.

Mites collected from dead and deformed bees exhibited similar optical densities to those of the bees they were infesting. Furthermore,

calculations indicated that weight for weight the mites contained much higher levels of virus than the bees. This could arise from two possible mechanisms. First, the virus particles may somehow be concentrated within the mites. However, this would require an exceptionally efficient and hitherto unknown process to account for such high optical densities in the mites. Alternatively, once the mites have acquired DWV from the bees the virus may be replicating within them as well. Such replication has not previously been described in other viruses transmitted by *V. jacobsoni* but considering the close affinity between the mites and the bees, which extends to the mites absorbing and utilising unmodified bee proteins (Tewarson & Engels, 1982), it would appear to be at least a plausible explanation, which warrants further investigation. The ability of viruses to replicate successfully within several unrelated hosts is well documented e.g., rabies virus (class V, Rhabdoviridae) and yellow fever virus (class IV, Flaviviridae) (Dimmock & Primrose, 1994).

Additional circumstantial evidence of DWV replication within *V. jacobsoni* is the finding of extremely high optical densities in some of the mites collected from non-deformed bees. These bees had low virus levels and it is unlikely that the mites could have succeed in obtaining their levels by concentration effects alone.

The relationship among honeybees, DWV and *V. jacobsoni* is obviously complex but it is possible to postulate a progression of events on the arrival of the mites in a colony. Having infested a colony, *V. jacobsoni* will either acquire DWV from naturally infected (but asymptomatic) bees or bring it with them from their previous colony. The viruses may then replicate within the mites and, ultimately, high levels of DWV are transmitted to the brood. This may help to explain the positive correlation found between increasing numbers of mites infesting developing bees and an increasing percentage of deformed emerging bees, which has also been reported by DeJong *et al.* (1982b) and Marcangeli *et al.* (1992b). With increasing mites on developing bees, there would be an increased probability that a developing bee would receive enough DWV from either a single mite with a high virus titre (e.g., one that had previously fed on a dead or deformed bee) or from the combined contributions of several mites with low virus titres. Where two or more mother mites enter a cell, only one needs to be carrying virus, to cross- infect the other(s) and the offspring by infecting the common food source i.e., the developing bee. Ultimately, there would be a depletion in the bee population, both adult and brood, and the colony may collapse .

These results emphasise the need for beekeepers to manage their colonies in such a way as to keep mite numbers as low as practically possible,

to prevent bee drifting and robbing (to reduce the movement of mites and their associated viruses), and to ensure that colonies enter winter in an optimal condition (e.g. with ample food stores, and well ventilated) and in a good location (e.g. sheltered).

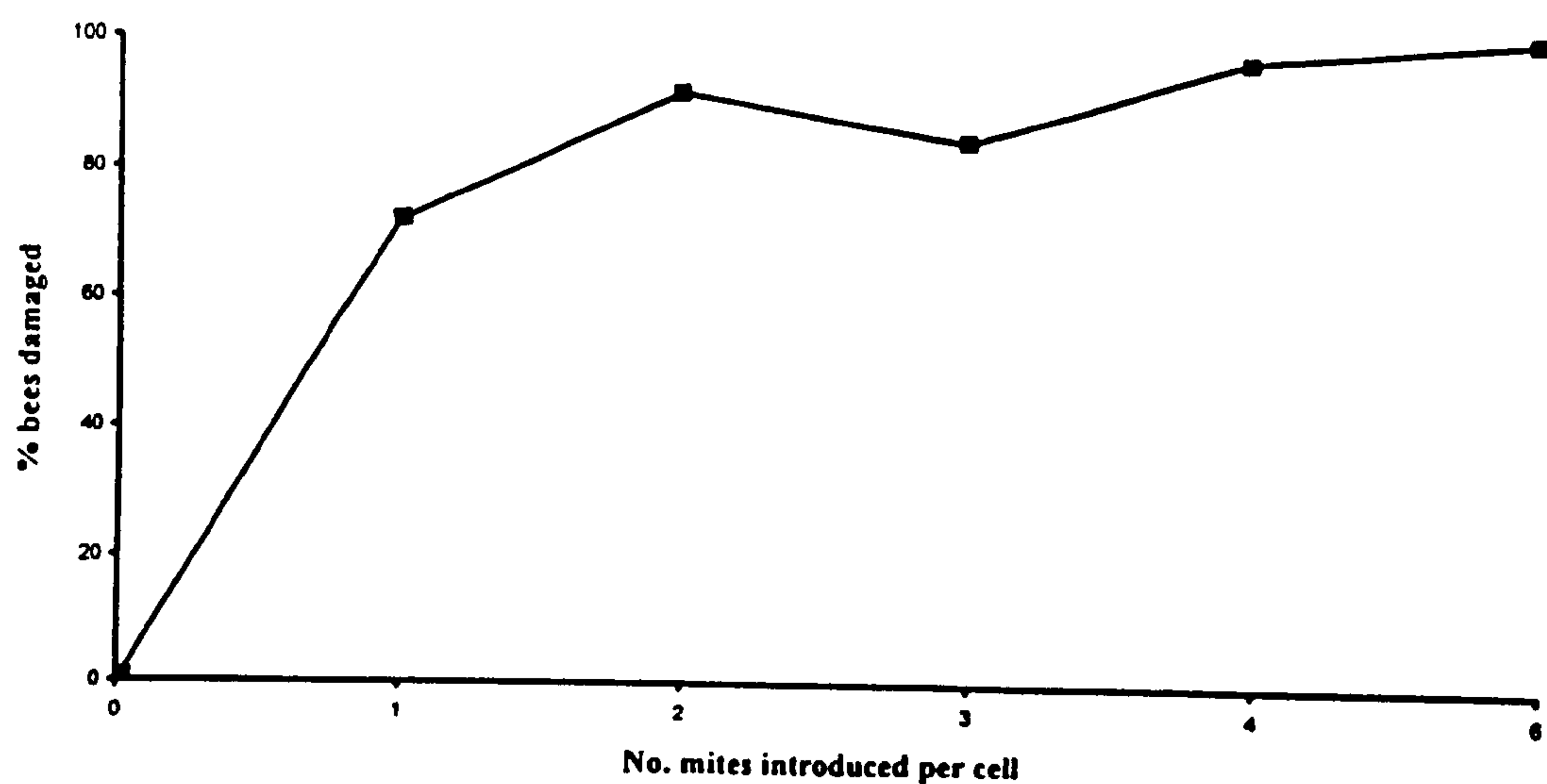


Figure 6.1 The relationship between the number of *Varroa jacobsoni* introduced into developing *Apis mellifera* cells and the percentage of bees which emerged deformed or died.

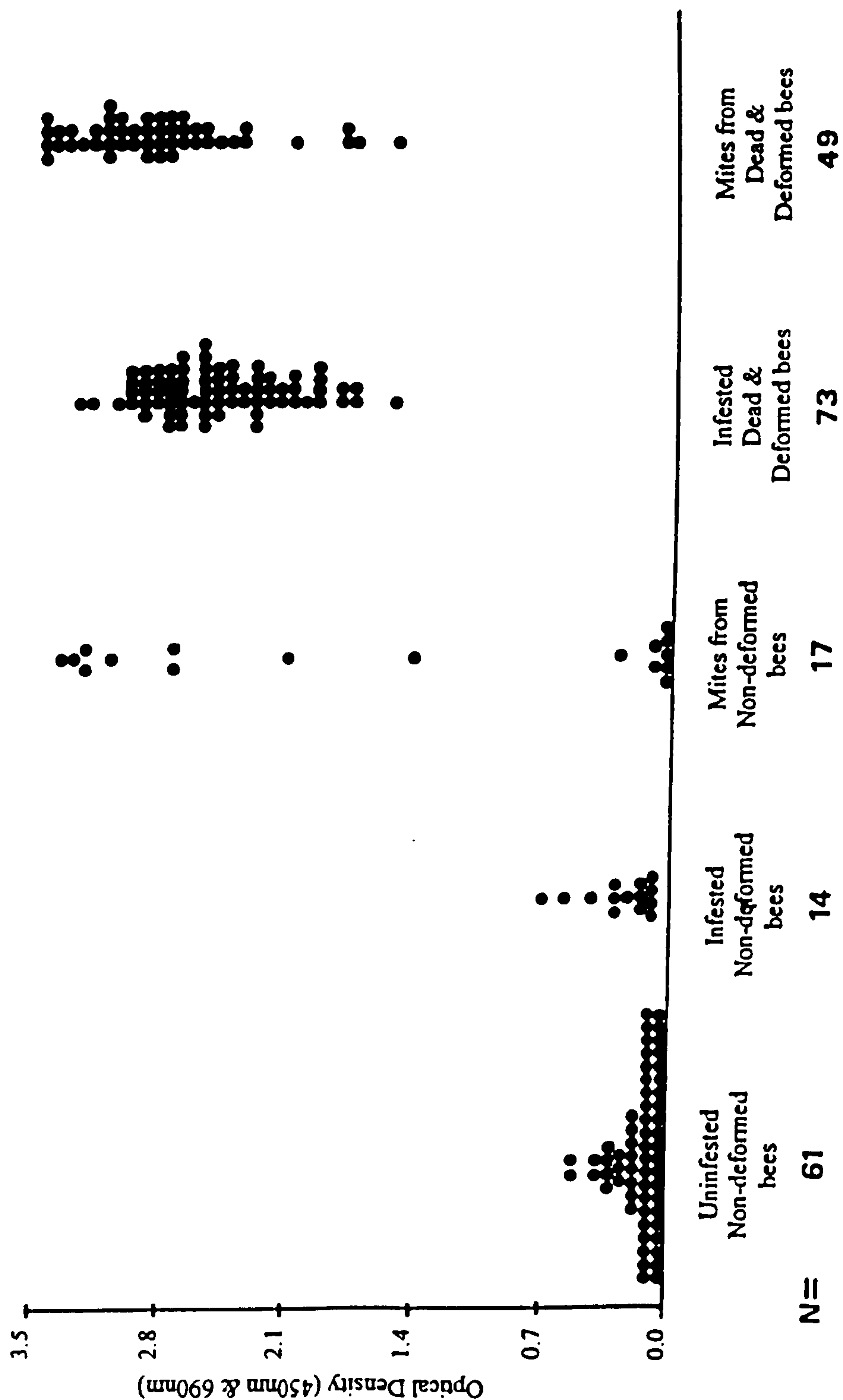


Figure 6.2 Deformed Wing Virus-ELISA optical densities measured at 450nm & 690nm of (i) Uninfested non-deformed bees, (ii) Infested deformed bees, (iii) Mites removed from deformed bees, (iv) Deformed & Dead bees and (v) Mites removed from deformed & dead bees.

		Source <i>Apis mellifera</i> deformed?	
		Yes	No
Target <i>Apis mellifera</i> deformed?	Yes	82% n=23	43% n=13
	No	18% n=5	57% n=17

Table 6.1 The percentage deformity recorded in emerging worker *Apis mellifera* parasitised by *Varroa jacobsoni* when the mites had previously infested deformed and non-deformed honey bees.

		Levels of DWV in emerging <i>Apis mellifera</i>		
		DWV Absent	DWV Likely	DWV Present
<i>Apis mellifera</i> deformed?	Yes	0% n=0	1% n=1	99% n=72
	No	0% n=0	57% n=8	43% n=6
Uninfested non-damaged bees		44% n=27	43% n=26	8% n=8

Table 6.2 The relationship between Deformed Wing Virus-ELISA optical densities measured at 450nm & 690nm in worker *Apis mellifera* and their deformity at emergence. DWV-ELISA optical densities were placed into the following groups according to Kemeny (1991), (i) DWV Absent = below 1.5 x mean plate background [mpb], (ii) DWV Likely = 1.5 x mpb to 0.2 OD units above mpb, (iii) DWV Present = higher than 0.2 OD units above mpb.

Levels of DWV in mites removed from emerging *Apis mellifera*.

		DWV Absent	DWV Likely	DWV Present
<i>Apis mellifera</i> deformed?	Yes	0% n=0	0% n=0	100% n=49
	No	35% n=6	6% n=1	59% n=10

Table 6.3 The relationship between Deformed Wing Virus-ELISA optical densities measured at 450nm & 690nm in *Varroa jacobsoni* removed from emerging *Apis mellifera* which were deformed or non-deformed. DWV-ELISA optical densities were placed into the following groups according to Kemeny (1991), (i) DWV Absent = below 1.5 x mean plate background [mpb], (ii) DWV Likely = 1.5 x mpb to 0.2 OD units above mpb, (iii) DWV Present = higher than 0.2 OD units above mpb.

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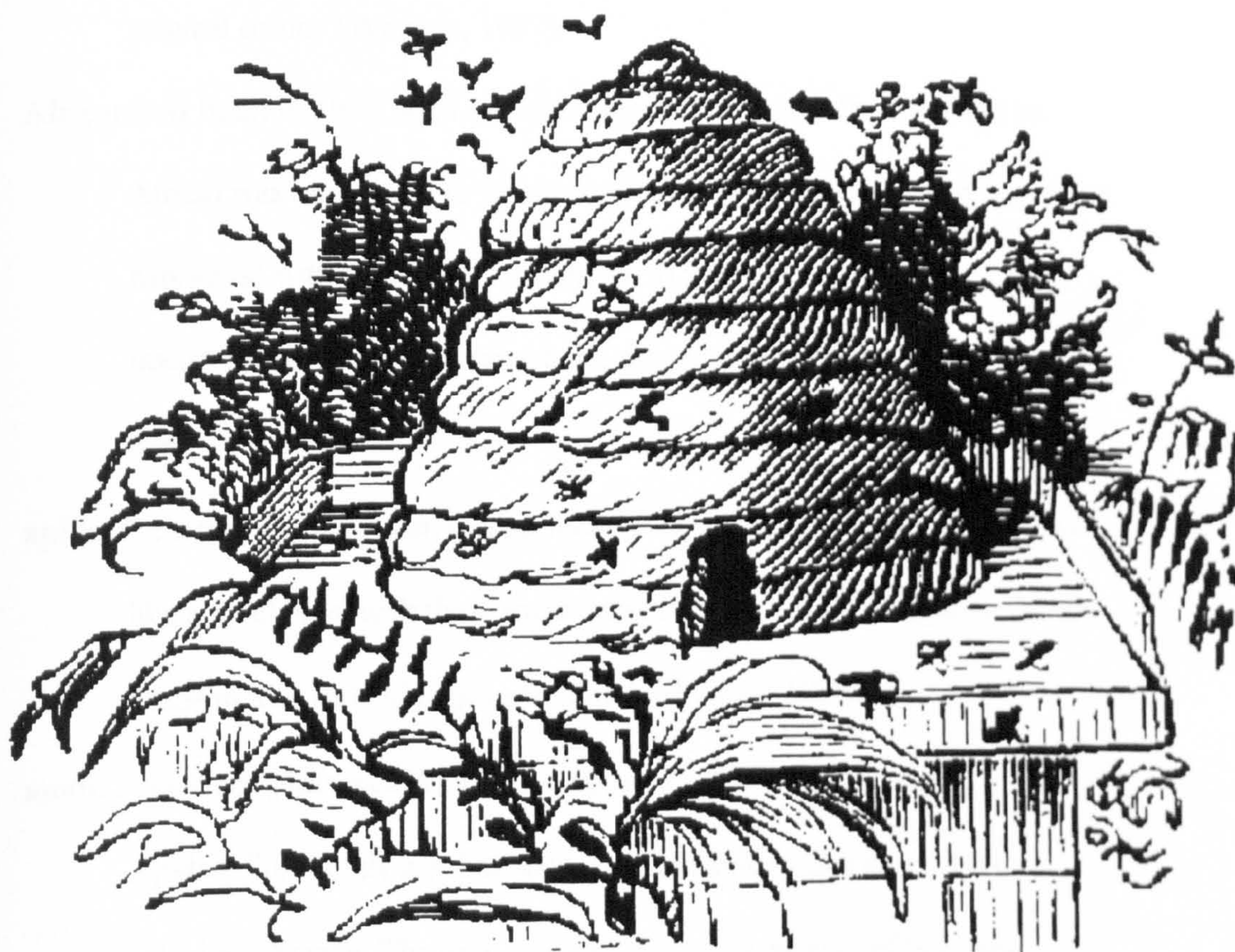
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GLOSSARY



allopatric: species or populations that occupy habitats which are geographically non-overlapping, and that do not occur together in nature (Allaby, 1992).

absconding: the abandoning of a nest by a colony which forms a swarm and presumably re-establishes itself elsewhere, following disturbance or an inadequacy of resources. Few or no workers are left behind in the original colony (Winston, 1987).

Africanized bees: in 1956, *Apis mellifera scutellata* (originally restricted to Africa) was introduced to South America. This African race of honeybee hybridised with the native *A. mellifera*, to produce uncharacteristically aggressive bees, subsequently called “Africanized bees” (Winston, 1987).

apidea™: a polystyrene queen mating nucleus-hive. Internally, the apidea™ is like a small hive, with three small frames and a space for sugar solution. Made in Switzerland Brückenstrasse 6 CH-3005 BERN.

apini: honeybees (*Apis mellifera*) are assigned to the taxonomic family-Apidae. This family is subdivided into subfamilies, one of which is called the Apinae. The two extant highly social groups of bees are assigned (melliponines and *Apis*) to the subfamily Apinae. Apini is a tribe within the subfamily Apinae, and the only living representatives are in the genus *Apis* (Culliney, 1983).

deutonymph: a stage in the life-cycle of mites proceeding the protonymph stage and preceding the adult (or tritonymph stage if present) stage.

Each stage is delineated by a molt (Snow, 1970).

epizoötic: a term applied to a disease which affects large numbers of animals (colonies in this case) over a large area at the same time and spreads with great rapidity (West, 1988).

eusocial: Applied to the condition, or to the group possessing it, in which individuals display all of the following three traits: co-operation in caring for the young; reproductive division of labour, with more or less sterile individuals working on behalf of individuals engaged in reproduction; and overlap of at least two generations of life stages capable of contributing to colony labour. (Wilson, 1979).

gnathosoma: anterior trophic-sensory structure in acari comprising of the first two segments of the prosoma^{*} (cheliceral and pedipalpal segments) (Evans, 1992).

heterochromatization: (where the chromatin becomes highly condensed and becomes transcriptionally relatively inactive [Maclean, 1989]).

idiosoma: in acari, the whole body excluding the gnathosoma^{*} (Evans, 1992).

impaternate: where the male genome makes no contribution to the overall genotype and resulting phenotype of an organism.

opisthosoma: the posterior tegmata of the Arachnida, comprised of a variable number of segments (Evans, 1992).

parasitosis: the number of individual mites infesting a bee.

plastron: a gill constructed from a layer of air trapped onto a part of the body surface by physical projections such as hair.

podospermy: where sperm cells are introduced into the coxal pores (located on coxa III) of the female by the male utilising modified chelicerae (Hänel, 1988 citing Athias-Henriot, 1969).

prosoma: : the anterior tegmata of the Arachnida, comprised of six segments.

Each segment bears an appendage as follows - I chelicerae, II pedipalps, III-VI legs (Evans, 1992).

protonymph: the stage in the life-cycle of a mite immediately preceding the deutonymph stage (Snow, 1970).

pseudo-arrhenotok(ous)y (also referred to as parahaploidy or paternal genome loss, where diploid male embryos either undergo expulsion of the paternal genome at some early stage of embryogenesis or undergo heterochromatization* of the paternal genome with subsequent retention of the male genome in somatic cells [Wrensch *et al.*, 1993]).

spermatodactyl(a): chelicerae of male acarids, modified into non-trophic tube-like structures, which facilitate sperm transfer to the female.

stenophagous: applied to organisms with a highly specialised diet (Allaby, 1992).

supercedure: the replacement of an old queen with a younger queen, without any accompanying swarming.

Sympatr(ic)y the occurrence of species or other taxa together in the same geographical area (Allaby, 1992).

telmophag(ous)y: feeding from a pool of blood following a haemorrhage (Lavoipierre, 1965).

xeric: relating to, or growing in, dry conditions (Hanks, 1985).

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