Preferential distribution of the parasitic mite, *Varroa jacobsoni* Oud. on overwintering honeybee (*Apis mellifera* L.) 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. 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 increasing numbers of bees carried 2 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 introduction in 1992 (Mobus & deBruyn, 1993). The mites spend much of their time in the brood cells, where they reproduce (De Jong, Morse & Eickwort, 1982). 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, Eguaras & Hernández (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, Koeniger & Fuchs (1986) concluded that the mites preferred the dorsal aspect while Delfinado-Baker, Rath & Boecking (1992) found that they had a strong preference for the lateral intertergites (3rd and 4th). De Jong *et al.* (1982) 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 an ongoing project on the effect of *V. jacobsoni* on overwintering bees, we have studied the distribution of the mites on clustered *Apis mellifera* – 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.* 1982). It is becoming apparent that this is linked to pathogens (viruses and bacteria etc.) which are transmitted by Varroa (Bailey & Ball, 1991; Glinski & Jarosz, 1992). 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

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Table 1. Prevalence and distribution of *Varroa jacobsoni* within a colony of *Apis mellifera* during early winter (October–December 1995) and late winter (January and February 1996)

(Number of mites observed in parentheses.)

Percentage	October–December	January and February
Uninfested bees	87·1 (1603)	63·2 (1023)
Infested bees	12·9 (237)	36·8 (595)
Parasitized bees with 1 mite	97·5 (231)	73 (434)
Parasitized bees with 2 mites	2·5 (6)	24 (143)
Parasitized bees with 3 mites	0	2·7 (16)
Parasitized 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)

(Corkins & Gilbert, 1932), they have longer lifespans (Fukuda & Sekiguchi, 1966), they have raised brain cell counts (Rockstein, 1950), their hypopharyngeal glands are hypertrophied (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 lifespan (Shabanov *et al*. 1978) and a rise in the amount of guanine in their malpighian tubules (DeJong *et al*. 1982, citing Ionescu-Varo & Suciu, 1978). We also investigated 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 AND METHODS

Distribution of mites on the bees

Between October 1995 and February 1996, groups of 50–100 bees were collected using a pooter from the top bars of a naturally infested overwintering colony of *A. mellifera mellifera* × *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, 0·01 and 0·1 μl of geraniol (Sigma, UK 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 min, 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

Pattern of infestation

During the period October–December there was usually only a single mite per infested bee but between January and February many more bees were found to carry more than 1 mite. Increases were also recorded in the numbers of mobile mites, the percentage of infested bees and the mite : bee ratio (Table 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.

Distribution of the mites on the bees

Analysis of the pooled data for all observed mites, indicated that they showed a highly significant preference for the abdomen over the rest of the body (χ^2_4, $P < 0·0005$), 77·7 % being found there (Fig. 1). On the abdomen, mites had a highly significant preference for the space between the 3rd and 4th true abdominal tergites (Fig. 2) (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). Also, mites had

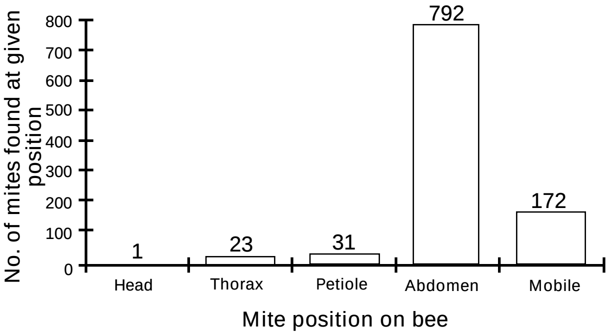

Fig. 1. Number of *Varroa jacobsoni* ($n = 1019$) found at each location on the body of overwintering *Apis mellifera* ($n = 3458$). Pooled data gathered between October 1995 and February 1996.

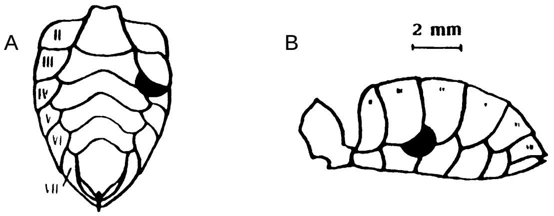

Fig. 2. Abdomen of *Apis mellifera* (A) ventral aspect, (B) left lateral aspect; showing the preferred attachment site of *Varroa jacobsoni* on the overwintering host between the left 3rd+4th tergites. (Re-drawn from Snodgrass, 1956.)

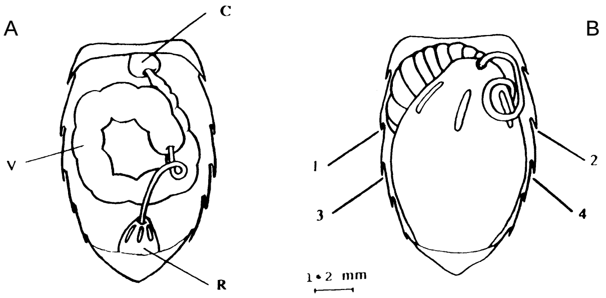

Fig. 3. Dorsal aspect of dissected *Apis mellifera* gaster showing the positions of the internal organs, (A) in summer, (B) in winter. C = crop, V = ventriculus, R = rectum. 1, 2, 3 and 4 = Positions occupied by *Varroa jacobsoni* (in decreasing order of preference, respectively) on adult overwintering *A. mellifera*. (Re-drawn from Dade, 1962.)

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, $P < 0{\cdot}0005$).

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).

When 2 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}, $P < 0{\cdot}005$). When 3 mites were recorded on a bee, the combinations of positions occupied varied greatly (Table 2).

When bees were dissected it became obvious that the mites were preferentially locating themselves at a site very close to the central ventriculus (Fig. 3). The

Table 2. Pooled data (October 1995–February 1996) on the distribution (as %) of *Varroa jacobsoni* on *Apis mellifera* when 1, 2 and 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 found.)

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 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.)

Volume of geraniol (μl)	Number of mites 'attracted' by chemical	Number of mites 'repelled' by chemical	Significance
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	$\chi^2_1 = 9·48$, $P < 0·005$

midgut is naturally coiled to the left (personal observations) but in winter bees it is further displaced sideways and upwards by the grossly swollen rectum (Snodgrass, 1956 and personal observations). 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 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). Furthermore, the mites must have been transferring from dead/dying bees before these were separated from the cluster. These results contrast with those of Ritter (1988) who stated 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 (Bowen-Walker & Gunn, manuscript in preparation). However, we found that a dead bee placed in the centre of the cluster could take up to 48 h 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 10 km 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 by Fernández *et al.* (1993) on *A. mellifera* in Argentina, so our 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), we found relatively few mites on the thorax of bees. They suggested that the mites preferred the thorax during cold weather because this would be the warmest region. This seems an unlikely explanation because Esch (1960) has demonstrated that there is no difference in thoracic and abdominal temperatures of overwintering bees.

Hoppe & Ritter (1988) stated that mites are most commonly found between the thorax and abdomen of older bees. They suggested that this was because the mites were avoiding geraniol released from the Nasanov gland which they said had a strong repellent effect. By contrast, we found mites were attracted towards low levels of geraniol although, in agreement 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, our 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, Koeniger & Fuchs, 1986). 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. We are 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). 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 (Bowen-Walker & Gunn, manuscript in preparation) – 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 the majority of the 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 the summer 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 (1991) citing King, 1933). Wax is produced by the wax mirrors, of which there are 4 pairs that lie at the anterior of sternites 4–7. The presence of active wax mirrors would increase the thickness of the layer of tissue through which the mites access 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 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 midgut probably contains higher concentrations of many nutrients 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. 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) and must therefore rely to a large extent on absorbing amino acids present in the bees' haemolymph. However, Hymenoptera are also well known for the exceptionally high amino acid concentrations in their haemolymph (Mullins, 1985) so unless there are certain ones which are essential to the mite but low within the general circulation this is also unlikely. Possibly there are certain vitamins and/or cofactors which are absorbed in this region then rapidly bound to other molecules/metabolized 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 2 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. This may reflect a relict behaviour from the time the mites parasitized *A. cerana* when, as mentioned above, any mite which exposed itself would be extremely vulnerable to removal by grooming (Fries *et al*. 1996).

Our observations, assuming they are typical, might explain why hives which are infested but otherwise apparently healthy in autumn often suffer severe losses over winter (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, there was an increasing prevalence of infestation and an increase in the proportion of bees carrying 2 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 with which *V. jacobsoni* has been implicated. 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 emphasize 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, as no honey will be collected for many months after acaricide application, the risk of residues is reduced considerably.

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