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ATTRACTION OF VARROA JACOBSONI, PARASITE OF APIS MELLIFERA BY ELECTRICAL CHARGES

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Abstract—Precise measurement of the electrical charges carried by honey bee workers allows one to investigate the role of this abiotic factor in bee contamination by the ectoparasitic mite *Varroa jacobsoni*. A metallic cylinder charged with four different intensities (chosen in the range measured on living bees) of either positive or negative sign was used as a lure. The mite's movements in the vicinity of the cylinder was videotaped and subsequently digitized. Spatial and temporal dimensions of the paths were computed by a specially designed analysis programme. The frequency and nature of the contacts with the lure were also noted.

A two-way ANOVA indicated no significant differences in the characteristics of the paths between charges of different intensities. However, the charge sign was found to influence the following characteristics: immobility, velocity, turning angle standard deviation and sinuosity. In addition, the frequency with which the mite contacted and climbed on the cylinder was higher in the case of negative charge. We suggest that the mites are not merely passively attracted towards the lure by the action of electrical forces. Rather, the detection of charges triggers a change in the movements of the animal which increases the probability to contact its host.

Key Word Index: Electrical charges; host finding; trajectometry; Varroa jacobsoni; Apis mellifera

INTRODUCTION

The genus Varroa and the species Varroa jacobsoni (Mesostigmata: Acari), identified by Oudemans (1904), have been classified as a "parasite of Apis indica F. Java." and included in the family of Laelaptinge and thereafter in a newly created family of Varroidae (Delfinado and Baker, 1974). In the 1960s, this ectoparasite began to contaminate the European honey bee, Apis mellifera L., and it is today considered as one of the major pests of the honey bee causing serious damages to the colonies of this species (Poliakov et al., 1975; Cavalloro, 1989). The gravity of this epizootic disease is due to the parasitism of both adult bees and brood. Adult mated female mites settle on workers or drones, suck the haemolymph and contribute to the transmission of bee pathogens. The reproduction of V. jacobsoni is in close synchronization with the life cycle of the honey bee (Haenel and Koeniger, 1986; Colin, 1989). Adult females of V. jacobsoni leave the adult bee and enter the brood cell 24 h before sealing. Egg-laying of the mite begins with the bee metamorphosis. Hatching, nymphal stages and mating of young adults of *V. jacobsoni* take place before emergence of the bee imago. Transfer of bee pathogens also occurs during mite reproduction and frequently leads to the death of the bee pupa.

One of the main tasks when studying the biology of a parasite is to identify the mechanisms responsible for the detection of its host. In most parasites, visual, vibratory, thermal and chemical stimuli have been shown to be involved in host detection (see Alberti and Crooker, 1985; Altner and Loftus, 1985; Sabelis and Dicke, 1985; Sonenshine et al., 1986). However, electrical charges have so far never been considered as a possible way of mediating host finding by a parasite.

One can hypothesize that a parasite's movements may be altered by an electrical field generated by its host in two different ways. First, a parasite and its host could carry charges of opposite signs. According

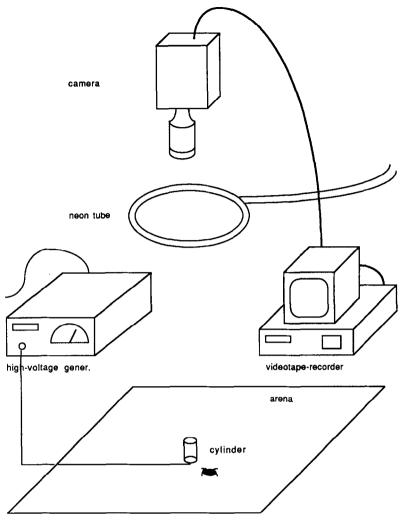


Fig. 1. Experimental setup.

to the Coulomb's law, the parasite would then be passively attracted by its host by the mere effect of electrostatic forces, in the same way as a pollen grain is electrostatically attracted by a foraging bee (Corbet et al., 1982). If this hypothesis is valid, the electrical charges carried by forager bees could explain the mechanism responsible for the sometimes observed sudden "jumps" of V. jacobsoni from an inert support to their host bees. In our previous experiments, we found that bee net charges range from -300 to +500 pC and that the minimal electrical charge that a mite should carry to be attracted over a distance of 3 mm, would be at least ±8 pC (Colin et al., 1991). Second, the parasite may be sensitive to electrical charges and modifies its behaviour after detection of the electrical field generated by its host (Colin and Richard, 1990). The separation of the mite from the bee is a common occurrence at the emergence of the bee imago or on the decline of the colony. Such a sensitivity to

electrical field has been shown to occur in some arthropods. For example, Edwards (1960) and Watson (1984) have observed a reduction of the movements of adult *Drosophila* placed in electrical fields and a local preference of *Aedes aegypti* larvae or *Musca domestica* for the anode or cathode has been noted by Riordan (1971) and Perumpral et al. (1978).

Since the complete elimination of mites is impossible in an apiary even by chemicals, a biological control based on the disturbance of the host-parasite relationships might reduce the level of the mite population without injury to the bee colony. The aim of the experiment presented here is first to analyse the effects of electrical charges, variable in sign and in intensity, on the characteristics of the mite path, second to compare the mite path before and after walking on a charged cylinder (Edwards, 1962) and third to state the conditions of attraction of the mite to a charged lure.

Table 1. Mean values and standard deviations of the parameters in the control group and in the groups in the presence of positive and negative charges

Sign (param.)	Positive		Control		Negative		
	Mean	SD	Mean	SD	Mean	SD	Unit
Immobility	26	19	25	17	16	11	%
m d2p	0.62	0.24	0.61	0.22	0.76	0.21	mm
max d2p	1.67		1.65		1.95		mm
Velocity	2.06	0.38	1.97	0.32	2.24	0.36	mm/s
m dsp	8.8	6.2	6.4	4.3	9.7	6.0	mm
max dsp	18		14		20		mm
Turning angle	312	50	312	56	296	57	deg
Segment heading	253	66	225	68	246	71	deg
Azimuth SD	40		40		41		deg
Straightness	1.07	0.62	0.75	0.43	1.10	0.62	mm E. 1
Sinuosity	35.3	5.0	39.6	4.2	38.5	6.1	$\operatorname{deg} \operatorname{mm} E_{\cdot}^{2} - \frac{1}{2}$
Laterality	-0.013	0.214	-0.121	0.158	-0.019	0.184	5

m, mean; max, maximum; SD, standard deviation; d2p, distance between two successive points; dsp, distance of each point to the starting point.

MATERIALS AND METHODS

In order to study the sole effects of any electrical field generated by the bees, experimentally we chose a metallic lure providing a radial electric field. To compare movement alterations in electrically opposite conditions precisely, we resorted to the trajectometry technique and mite paths were measured according to a number of criteria and indexes proposed by various authors for the spatial or spatio-temporal analysis of trajectories (Kitching, 1971; Batschelet, 1981; Bovet and Benhamou, 1988).

Experimental setup (Fig. 1)

To simulate the charges carried by the bees, we used a 1 cm high and 0.7 cm wide steel cylinder, fixed in the centre of a small arena and connected to a high voltage generator providing a steady electrical charge to the cylinder. To avoid the discharge of the mite, the arena floor was a flat smooth insulated surface. A 35 cm dia circular neon lamp lighted the arena uniformly. A video CCD camera equipped with a macro lens was connected to a videotape recorder and used to film the movement of the mite on the arena floor at a frequency of 25 pictures per second. The camera field was 140 mm dia and corresponded to 256 pixels, which gave a resolution of 0.55 mm/pixel. A white paper disc was glued onto the cylinder top to obtain a uniform background.

Image analysis

Video recordings were analysed in real time by a video digitizer (MID[®] DGI 1) installed on a microcomputer (Bull[®] Micral 30), which calculated mite coordinates frame by frame (i.e. 25 pairs of coordinates per second). The mite only covered a few pixels on the screen. Because of its slow locomotory rate and the low spatial resolution of the image analysis, the changes of direction between successive steps were

limited to only a few directions. To avoid this bias, the paths were thus smoothed by taking the centre of gravity of 10 successive points. Although this method sometimes produces functionally unrealistic large changes of direction for a mite (Tourtellot *et al.*, 1991), it is still valid as long as one only uses it to compare the paths of the same species in different stimulus conditions.

The reduced coordinate files were then processed by a specially designed path analysis programme computing various path characteristics related to the spatial and temporal dimensions of the trajectories (Fourcassié et al., 1985).

Characteristics of the paths

The main parameters describing each path were:

- (i) number of points;
- (ii) percentage of immobility (imm);
- (iii) distance between two successive points (d2p) in mm (maximum, mean, standard deviation);

Table 2. F-values and associated probabilities given by a two-way ANOVA on sign and intensity

	Sign		Int	ensity	Sign*Intensity		
	F	P	F		F	P	
imm	12.1	0.003	2.5	0.063	2.6	0.114	
vel	6.2	0.015	1.6	0.205	1.2	0.305	
t.a. SD	22.3	0.0001	1.4	0.247	2.5	0.061	
m dsp	0.7	0.414	2.5	0.064	0.5	0.684	
stght	0.2	0.641	2.2	0.093	1.0	0.380	
sinu	6.6	0.012	2.8	0.046	1.2	0.329	
df		1		3		3	

imm, percentage of immobility; vel, velocity; t.a. SD, turning angles standard deviation; m dsp, mean distance of each point to the starting point; stght, straightness; sinu, sinuosity; df, degrees of freedom.

Bold characters show significant difference for which P < 0.02.

- (iv) velocity (vel) in mm/s: distance between two successive points during mobile phases only;
- (v) distance of each point to the starting-point (dsp) of the path in mm (maximum, mean, standard deviation);
- (vi) turning angle (t.a.) between two successive path segments in degrees (angle ranging between 0 and 180°, turn to the left; angle ranging between 180 and 360°, turn to the right) mean, standard deviation;
- (vii) segment heading in the coordinate system, in degrees; mean, standard deviation;
- (viii) azimuth of each point in relation to a coordinate system fixed on the starting point in degrees (mean, standard deviation).

In addition, some of these measures were associated to compute various indexes.

A straightness index (stght) was defined as the ratio of the straight distance between the first and last point of the path to the square root of the total distance covered by the animal (Bovet, unpublished). To minimize the importance of the last point of the path, we took the mean dsp in place of the straight distance between the first and last point. This index can thus be superior to 1.

A sinuosity index (sinu) was computed based to the definition given by Bovet and Benhamou (1988): ratio of the standard deviation of the turning angles to the square root of the mean step length.

A laterality index was calculated as the ratio of the difference between the number of clockwise turning angles and the number of counter-clockwise turning angles to the total number of turning angles (null angles not included).

Attraction to the lure

The attraction to the lure was also measured by the frequency and the nature of the physical contacts with the cylinder (touching or climbing).

Measurement of the charge of the mite

The mite charge was estimated by using a special sensor, conceived for the measurement of the charges carried by bees and connected to an electrometer (Colin et al., 1991). The measured mites were not included in the behavioural analysis, because they were almost completely discharged after walking through the sensor.

Experimental procedure

Ninety-six adult females of V. jacobsoni, collected on adult worker honey bees were tested in the presence of electrical charges ranging from -500 to +500 pC induced respectively by -1000 to +1000 V

voltages. Mites were divided into eight groups of equal size (n = 12) and each group was submitted to one charge in value or in sign: ± 125 , ± 250 , ± 375 , ± 500 pC. For the control group the cylinder was connected to the mass. The control group of 10 mites was filmed before the generator was switched on.

Each individual was carefully dropped at about 1.5 cm from the cylinder centre and filmed during an average 50 s. This corresponded to the maximal size allowed for the coordinates files. When counting the touching or climbing events, the duration was increased by 40 s in the presence of charged cylinder. Video recording was interrupted when the animal left the camera field or when it climbed on the cylinder, and was resumed when it came back on the insulated arena floor.

For technical reasons, pathways with less than 30 distinct points, were excluded from statistical analysis, except for the comparison of the percentage of immobility and for the evaluation of attraction towards the lure.

RESULTS

The characteristics of the paths in the absence and in the presence of a positive and negative charge are presented in Table 1.

Influence of the intensity and the sign of the charge (Table 2)

A two-way (sign and intensity) analysis of variance indicated no differences at the significance level 0.02 for the "intensity" factor, but significant differences were observed for charge of opposite sign in immobility, velocity, turning angle standard deviation and sinuosity values (see Scherrer, 1984).

The groups corresponding to variable intensity were pooled in two groups (negative and positive sign) and compared with the first control group of 10 mites by a Wilcoxon-Mann-Whitney test at the significance level 0.05 (Table 3). In the presence of negative charges, the mites moved faster (2.23 vs 1.99 mm/s in the control-group) and seemed to stay

Table 3. Comparison of the groups of negative and positive signs to the control group

		Negative Control	Positive Control
imm	<i>Z</i> =	1.63*	-0.08
vel	Z =	-1.86	-0.50
t.a. SD	Z =	-0.12	-2.32
sinu	Z =	-0.26	-2.46
numb.		45/10	46/10

Mann-Whitney Z 0.05 = -1.645.

*Z = -1.63, P = 0.051.

Bold characters show significant difference for which P < 0.05.

Table 4. Attraction of the mites to the lure

Sign (param.)	Positive			Negative			Control		
a	27			40				10	
b	23				47			0	
c			1		1.5			_	
e.c. (param.)	125	250	375	500	125	250	375	500	
a	8	33	33	33	45	42	50	25	
b	0	25	25	25	40	80	33	33	
С	_	1	1	1	2	1.75	1.5	i	

a, % of individuals touching the lure within 90 s of observation; b,
% of "a" individuals climbing on the lure; c, mean number of climbing events per "b" individual; e.c., electric charge in pC;
Control group within 50 s of observation.

motionless a shorter time (16 vs 25% of immobility in the control-group at P=0.051). In the presence of positive charges, the turning angle standard deviation was 50° (control-group: 56°) and the sinuosity decreased to 35.4 (control-group: 39.6). Within a period of $90 \, \text{s}$, a larger number of mites reached the negatively charged lure ($40 \, \text{vs} \, 27\%$ in the case of a positive charge and 10% in the control-group filmed during $50 \, \text{s}$ per mite). Touching was followed by climbing on the metallic cylinder twice more frequently, when it carried negative charges as when it carried positive charge. Moreover, about 50% of the mites climbed twice on the lure (Table 4).

Success in lure finding was also independent of the intensity of the charge; the highest frequency (50%) of successful mites was found for $-375 \,\mathrm{pC}$.

Influence of the charge of the mite

In several cases, filming was resumed after the mites climbed on the cylinder and after a while, climbed down on the arena surface. In this condition, the charge of *V. jacobsoni* thus always became of the cylinder sign. The characteristics of six pathways were compared before and after climbing on the negatively charged cylinder (Table 5). The mean distance to the starting point and the straightness were significantly increased after walking on the cylinder.

Table 5. Average values of the paths characteristics before and after walking on the negative charge

	Before	After	z
imm (%)	11	8	-1.48
vel (mm/s)	2.40	2.45	-0.31
t.a. SD (deg)	57	50	-1.63
dsp m (mm)	8	21	-1.99
stght	0.84	2.38	-1.99
sinu	37.4	32.6	-1.15

Wilcoxon matched pairs Z0.05 = 1.645. Bold characters show significant difference for which P < 0.05. The charge on the mite

The average charge of the mite was 1.3 ± 4.8 pC. A proportion of 47% had a charge ranging between -1 and +1 pC. The positively charged mites were more numerous (41%) than the negatively charged ones (12%) (Fig. 2).

DISCUSSION

The trajectories of *V. jacobsoni* were generally characterized by a highly convoluted and meandering aspect, with no remarkable directionality (Kitching, 1971). The mites tended to move in a stop-and-go fashion with long pauses, interrupting bouts of sinuous displacements, allowing the animal to disperse only very slowly from its initial location. Since the values of the parameters used to describe the trajectories did not vary significantly within a period of 15 days, we assume that the latter characteristics are typical of *V. jacobsoni* pattern of movements in a neutral environment.

Because just after leaving the cylinder the mite and the lure carried charges of the same sign, the best example of possible action of electrical forces was given by the comparison of the mite trajectories before and after having contacted the charged cylinder. Changes were mainly observed in the parameters or indexes describing the direction of the path. On

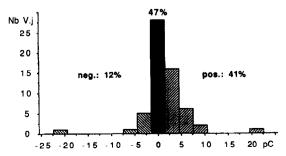


Fig. 2. Histogram of the distribution of electrical charges carried by V. jacobsoni.

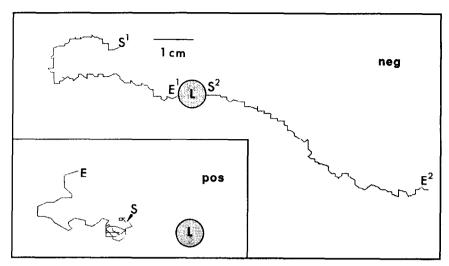


Fig. 3. Graphic representation of different mite paths, neg, in the presence of a negatively charged lure (L), S^1 , E^1 are the starting and end point before climbing on the lure. S^2 , E^2 are the starting and end point after climbing on the lure. The differences between S^1-E^1 and S^2-E^2 paths occur in the straightness and in the mean distance of each point to the starting point, pos, in the presence of a positively charged lure. The two paths are plotted on identical scales.

one hand, the increase in the straightness and in the mean distance to the onset of the path corresponds to a regular movement away from the cylinder. On the other hand, the absence of changes in the velocity, the sinuosity and the percentage of immobility seems to indicate that the repulsive electrical forces existing between the mite and the cylinder only play a minor role, if any, in the movements of the mite (Fig. 3).

A negatively charged cylinder was more attractive to the mite than a positively charged or neutral cylinder. A first explanation could be provided by the charge of the animal itself which was more frequently positive (Fig. 2). The mite would then be guided only by the low intensity electrical forces created between itself and the cylinder. A second explanation could be that the presence of a negatively charged object in the mite's surroundings would have an "arousal" effect which would increase its restlessness, resulting in shorter pauses in its movements and in a higher linear and angular velocity (see Bell, 1985; Sabelis and Dicke, 1985). Within a comparable period of time, this in turn would increase the probability for the mite to contact a negatively charged as opposed to a positively charged or neutral object. In this case, one can imagine that the contamination of the colony or the recontamination of bees would begin by the negatively charged insects. The increase in velocity and mobility we observed favours the second explanation. An electrical field would thus provide the parasite with a non-directional cue that could act in two different ways: either simply by increasing its restlessness and thus by shortening the time required to find its host, or by a process of "cross-channel

potentiation" (see Bell, 1990) i.e. a change in the threshold of one of its sensory modalities (e.g. kairomone or temperature sensitivity) that will enhance its host detection capabilities. Along the same line, Erickson (1982) has for example, provided evidences in the honey bee of an enhancement of the odour receptor function in the presence of an electrical field.

In conclusion, although no direct evidences of the precise role of electrical charges in the detection of individual bees by V. jacobsoni were provided in this paper, our results clearly show that the behaviour of the mite is altered by the presence of an electrical field in its surroundings and thus suggest that electrical charges are likely to influence the physiology of the sensory organs of invertebrates. We believe that this could give some clues as to a way of disturbing the relationship between the mite and its host. This could possibly be used in the biological control of this pest or in the improvement of the chemical therapy of the colony.

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