

Scientific note

A scientific note of an application of isotope ratio mass spectrometry to feeding by the mite, *Varroa jacobsoni* Oudemans, on the honeybee, *Apis mellifera* L.

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The parasitic mite, *Varroa jacobsoni* Oudemans, feeds on hemolymph and on developing pupae and adult honey bees, *Apis mellifera* L., causing loss of weight in workers [6], shortening the life span of foragers [5] and reducing blood protein levels [9]. Though mites have been shown to take up honey bee proteins [8] and various techniques for rearing mites in vitro on artificial diets have been attempted [3, 4, 7], it is difficult to assess feeding frequency because *V. jacobsoni* does not exhibit physogastry after feeding. We therefore developed a novel approach, using ¹³C labeling.

A total of 450 *A. mellifera* honey bee workers were removed from colonies maintained at the Honey Bee Laboratory at Oregon State University. Approximately 112 workers were kept in each of four small cages without food for a period of 6 h. Female mites removed from drone brood combs of infested colonies were inoculated at a rate of one mite/adult worker bee. Bees in three of the four cages were then allowed to feed on a glucose solution (1:1 by volume) labeled with a stable isotope (D-glucose-1-¹³C, 99 atom % enrichment) for a period of approximately 24 h.

A control cage was similarly manipulated but fed unlabeled sugar.

Every 6 h, samples of bee hemolymph (ca. 10 µL) and of adult female mites (six to eight per sample) were taken for ¹³C analysis. Hemolymph and mite samples were placed in 6 × 4 mm tin capsules, combusted to CO₂, and analyzed for ¹³C abundance using standard methods [1, 2]. Stable isotope analysis was performed with an isotope ratio mass spectrometer (Europa Scientific, Crewe, UK) operated at the Stable Isotope Research Unit (Department of Crop and Soil Science, Oregon State University). All data are expressed in delta (δ) notation:

$$\delta^{13}\text{C} (\text{‰}) = 1000 \cdot (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}$$

where δ¹³C is the difference in the ¹³C/¹²C ratio of the sample (R_{sample}) relative to the ¹³C/¹²C ratio of the PDB calcium carbonate standard (R_{standard}) expressed as parts per thousand. Positive δ¹³C values mean that the sample is enriched in ¹³C relative to the PDB standard; negative δ¹³C values mean that the sample is depleted in ¹³C relative to the PDB standard.

Mean values of hemolymph of bees feeding on ¹³C-glucose ranged from 8.1 ± 3.3 to

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Table I. $\delta^{13}\text{C}$ detected in *V. jacobsoni*.

Time	Cage 1	Cage 2	Cage 3	Control
6 h	-17.77*	-27.71	-30.92	-31.38
12 h	-28.05	-32.79	-29.14	-31.32
18 h	-26.89	-26.94	-27.88	-37.58
24 h	-24.59*	-24.18*	-35.93	-29.35

Units are in parts per thousand.

* Samples that fell outside 95 % confidence intervals (-26.72 and -38.09).

$29.9 \pm 12.7\text{‰}$ compared with $-20.2 \pm 1.8\text{‰}$ for controls. Mites were labeled less highly with lower uniformity than bee hemolymph (table I). Ten of 12 mite samples were more enriched with ^{13}C than the control mites, three significantly so. The statistical significance of these results would have been enhanced by using uniformly (every carbon) labeled ^{13}C -glucose, which would have amplified the signal six-fold.

Our preliminary results suggest that frequency of feeding, which is the inverse of the rate of feeding (here three positive cages over 12 per 24 h), occurs 1/4 per day or once every 4 days per cage of six to eight mites. This result is consistent with a report of *V. jacobsoni* ingesting bee macromolecules between 12 and 48 h [8]. Our study is the first to introduce and demonstrate ^{13}C labeling in arthropods. The technique may also be of great value in mark-recapture experiments, such as ^{14}C radio-labeling, fluorescent dyes, rubidium labeling, etc. Most notably, ^{13}C labeling is non-toxic and non-radioactive. The next step in this investigation would be to repeat the study longitudinally, assess rates of feeding of individual mites, and then document the actual rate of transfer of material from host to parasite, thus estimating parasite load.

Note scientifique sur l'application de la spectrométrie de masse du rapport isotopique à l'alimentation de l'acarien *Varroa jacobsoni* Oudemans sur l'abeille *Apis mellifera* L.

Eine wissenschaftliche Notiz zur Anwendung von Isotopenverhältnis-Massenspektroskopie auf die Nahrungsaufnahme der Milbe *Varroa jacobsoni* Oudemans an der Honigbiene *Apis mellifera* L.

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