Honey Bees (Hymenoptera: Apidae) in the United States That Express Resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae)

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ABSTRACT The purposes of this study were to select honey bees, *Apis mellifera* L., for resistance to varroa mites, *Varroa jacobsoni* Oudemans, and to find a probable cause for this resistance. As a genetic source, we assembled 8 colonies that we thought had potential for resistance to varroa. Queens and drones were propagated from this group to produce 43 instrumentally inseminated queens, each queen mated to only 1 drone. Colonies from 27 of these queens were tested in Louisiana and 16 were tested in Michigan. Each colony in the Louisiana test began with 986 ± 13 g (mean ± SD) of bees and ≈290 mites; Michigan colonies began with 3,212 ± 171 bees and ≈51 mites. The populations of mites and bees were measured 10 wk later. Three of the 43 colonies had fewer mites at the end of the test than at the beginning. During the experiment, we evaluated each colony for grooming behavior, hygienic behavior, the duration of the postcapping period, and the frequency of nonreproducing mites in brood cells. Of these 4 characteristics, only nonreproduction of mites was highly related to a change in the mite population. The duration of the postcapping period was marginally related, and the other 2 characteristics were apparently unrelated to the growth of the mite population. This study showed that resistance to varroa mites is present in the honey bee population in the United States, nonreproduction of mites was highly correlated with the growth of a mite population, and nonreproduction of mites may be a valuable characteristic for selecting bees for resistance to varroa mites.

KEY WORDS *Apis mellifera, Varroa jacobsoni,* breeding, selection

Varroa jacobsoni Oudemans is an external parasite of the eastern hive bee, *Apis cerana* F., that recently (≈1950) transferred to the honey bee, *Apis mellifera* L. *A. cerana* has a natural resistance to the mite, thus varroa mites do not develop high populations in those colonies. However, mite populations can increase 100 times per year in a colony of *A. mellifera* (Fries et al. 1990) and infested colonies are usually dead in 2 yr. Beekeepers in the United States currently rely on a chemical (fluvalinate) to control varroa mites, and feral colonies (those not protected by a beekeeper) have disappeared in many parts of the United States.

Our overall objective was to develop bees that reduce or do not allow an increase in a mite population during periods when mites have the opportunity to reproduce (when brood is present in the hive). Natural resistance to varroa mites has been discovered in some populations of *A. mellifera* in Uruguay, Tunisia, Brazil, New Guinea, and Argentina (Ruttner et al. 1984, Ritter 1990, Anderson 1994, Eguaras et al. 1995). In every case, including this study, resistance in bees appears to be associated with the failure of female mites to produce progeny after they enter a brood cell (nonreproduction of mites).

Our study differed from previous work in 2 ways. First, we produced and tested populations of bees and mites in a controlled setting, whereas other workers (referenced above) evaluated natural populations that had survived varroa infestation without chemical treatment. Our field test was completed in 70 d. Second, we instrumentally inseminated each of our test queens with semen from only 1 drone. In nature, queens mate with ≈10 drones. Therefore, a natural colony has much more genetic variability than the colonies that we produced. We reasoned that multiple mating and the resulting genetic variability could mask resistant characteristics at the colony level. Also, when propagating selected stocks, the daughter queens produced from a queen inseminated with a single drone will be highly related (0.75) to the worker bees that had been tested.

Materials and Methods

Our study was an analysis of the growth of mite populations in 27 colonies in Louisiana and 16 col-

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onies in Michigan. As a genetic source, we assembled 8 colonies that we thought had potential for resistance to varroa. They were subjectively chosen for grooming behavior, hygienic behavior, a short postcapping period, or for maintaining low mite populations. Queens and drones were propagated randomly from this group to produce 43 instrumentally inseminated queens. Each queen was mated to only 1 drone. Experiments were conducted in Baton Rouge, LA, and in East Lansing, MI, but all queens were produced in Baton Rouge.

The Louisiana Test. Test colonies were established on 12 May by subdividing a large mixture of mite-infested bees into 27 populations (Harbo 1986). Each population consisted of 986 ± 13 g (mean ± SD) of bees. This uniformity was achieved by producing 2 cages of bees for each of the 27 colonies. About 500 g of bees was scooped from bees in the large cage into the first 27 cages. These were set aside and another group of 27 cages was filled with bees from the same source (each cage 14 by 22 by 16 cm). The cages had been preweighed, and the weight of bees was recorded for each cage. Cages in the 1st group were each paired with a cage in the 2nd group to achieve maximum uniformity among the 27 pairs.

On 12 May, the 2 cages of bees, a caged queen, and 5 combs were placed into a standard Langstroth hive that could contain 10 frames. The worker bees were immediately released from their cages, but the hives remained closed for at least 24 h (with screen over the entrances). Entrances were opened at night. Queens were released on 16 May. Colonies were given only combs with worker-sized cells (a total of 10 frames) for brood and honey production.

Each of the 27 colonies began the test with ≈289 varroa mites. This number was determined from 4 samples of bees that had been collected from the large cage as it was being subdivided. Known weights of bees (≈150 g) were collected at the beginning, middle, and end of the bee distribution. Mites were separated from bees by heat (40°C for 2 d) followed by ether, and then by an alcohol wash to be certain that all mites had been counted. The mite populations in the 4 samples were 26, 27, 29, and 35 mites per 100 g of bees.

Mite populations and bee populations in each colony were measured again at the end of the experiment on 18 and 25 July (63 and 70 d after queen release). Two dates were chosen because all measurements could not be collected on all 27 colonies at 1 time. On the night before the evaluation, the colonies were screened so that all adult bees were inside. The next morning each colony was weighed with and without bees to establish the weight of the adult bees in each colony. To remove adult bees from their hive, bees were shaken and brushed from the frames into an empty hive body that was placed at the colony location. The hive equipment was then reassembled and weighed without bees. After a sample of adult bees (≈150 g) was taken from the cluster of bees in the combless box, the bees were reunited with their hive and the process was repeated with the next colony. The number of mites in the 150-g bee samples was determined as before and used to calculate the number of mites living on the adult bees in each colony. To measure the number of adult female mites in brood cells, we first calculated the number of cells in each colony that could contain mites. These included all cells of capped brood plus those that would be capped within the next 12 h. The area of capped brood was measured with a wire grid (each square of the grid was 2.5 cm on a side). The number of squares was multiplied by 23.6 to calculate the number of cells of capped brood and then by 1.04 to include the number of cells within 12 h of capping. By counting the number of foundress females per sample of 200 capped cells (50 cells were counted on each side of 2 frames), we estimated the number of adult female mites in the brood cells of each colony. The total mite population for each colony was the sum of mites on bees and in brood.

During the experiment, we evaluated each colony for the duration of the postcapping period, the frequency of nonreproducing mites in brood cells, hygienic behavior, and grooming behavior. By correlating these measurements with the final mite populations, it was possible to determine which, if any, of these characteristics were associated with changes in the mite population.

Duration of Capped Period. Queens were released in the late afternoon so that the initial capping of the cells could be witnessed 7.5–8 d later during daylight hours. The number of capped cells was recorded in each colony beginning in the early morning ≈7.5 d after queen release. A time was established for the 1st capped cell in each colony by examining colonies at 4-h intervals throughout the day. Eleven days later, the colonies were examined for newly emerged worker bees, beginning in the early morning and continuing to the following morning as needed. Because these teneral bees (with softer bodies, matted hair, and slower movement than older bees) were the 1st bees to emerge as adults in these colonies, they were distinctive for the 1st hours after emergence. As with counting capped cells, we counted newly emerged adults and established the time when the 1st bee emerged in each colony. When cells had been capped or adults had emerged before the 1st colony examination in the morning (colonies were not checked at night), a rate of 8 cells per hour was used to establish the times of 1st cell capping or 1st emergence of adult bees in a colony.

This technique probably underestimates the mean capped period for the worker bees in the colony. The standard deviation was ±5 h for the capped period of worker bees from queens inseminated with semen from a single drone (Harbo 1992). Because ≈8 cells were capped per hour during the 1st hours of egg laying, there is a high

probability that the capped period of 1 of the 8 cells would be at least 1 SD below the mean, thus causing the estimate of the capped period to be ≈5 h less than the mean.

Nonreproduction in Brood Cells. We examined purple-eyed bee pupae because at this stage of host development, it is too late for mites to begin producing progeny, and progeny in the cells are too young to be mistaken for a foundress female. We counted at least 20 infested cells of worker brood from each colony. Dead mites or those with no offspring were counted as non-reproducing. Some cells may have had immature mites, none of which would have matured. However, these cells were counted as reproducing to eliminate any arbitrary decisions.

Hygienic Behavior. Hygienic behavior is sometimes called nest cleaning behavior and because it is a task of adult bees, it is important to wait until progeny from the test queen have replaced the original population of bees. This requires ≈40 d. The test was made by cutting a square of sealed brood (≈60 cells) from each colony. The squares were frozen for 24 h, returned to the colony, and evaluated 24 h later for removal of the dead brood (Newton et al. 1975).

Grooming Behavior. Physical damage found on mites that fall to the bottom of the colony is referred to as "grooming behavior." As with hygienic behavior, grooming behavior could not be tested until the adult bee population consisted of progeny from the test queen. Oiled freezer paper was placed on the bottom of each colony for 3 d. The paper served as a trap because live mites that fall onto the paper will die quickly from contact with the oil. The oiled paper was placed on a hardboard (35 by 45 cm) and covered with an 8-mesh hardware cloth. The hardware cloth was raised above the paper to keep the bees from touching the paper or the trapped mites. The mites were examined under a microscope for physical damage, usually missing legs or dents in the idiosoma.

The Michigan Test. The Michigan portion of the experiment used the same basic design as the Louisiana test. The following describes only those aspects that differed from the Louisiana test.

The initial population of bees was taken from commercial package bees as well as from local colonies (to ensure mite infestation) and put into a large cage. About 450 g of bees was put into preweighed cages, the type used for commercial packages. Three subsamples of bees were taken to establish the level of mite infestation and the weight of individual bees within the uniform population.

Colonies were established on 19 June by placing the caged bees within an empty hive body that was temporarily placed above a hive containing 9 combs (each 13 by 42 cm) and a caged queen. As in the Louisiana test, caged bees were released immediately before placing the lid on the newly established colony. Screened entrances for the hives were opened at night (20 June) and queens were released 22 June. The established populations of bees were determined by photographing each side of each comb near the beginning (day 7) and at the end (day 69) of the experiment. Bees were counted from transparencies that were projected onto a screen.

Table 1. Experimental data from population tests in Michigan (MI) and Louisiana (LA), 1995

	Location	Range	Mean ± SD
Final mite population[a]	LA	238–2,170	873 ± 421
	MI	24–528	220 ± 162
Final bee population	LA	1,182–2,780[b]	1,982 ± 342
	MI	3,316–9,391[c]	6,115 ± 1,917
P_2/P_1 (final/initial mite populations)	LA	0.82–7.51	3.02 ± 1.46
	MI	0.47–10.35	4.31 ± 3.17
Mite increase per reproductive cycle[d]	LA	0.95–2.23	1.36 ± 0.25
	MI	0.60–2.31	1.59 ± 0.50
Capped period, h	LA	267–286	275 ± 5
	MI	270–286	276 ± 5
Nonreproducing mites, %	LA	0–100	27 ± 27
	MI	0–100	22 ± 33
Hygienic behavior (% removal in 24 h)	LA	6–100	61 ± 30
	MI	0–100	33 ± 35
Grooming behavior (% damaged mites)	LA	0–35	15 ± 8
	MI	0–50	26 ± 16

Colonies in Michigan began with 3,200 bees and 51 mites; colonies in Louisiana began with 986 g of bees and 289 mites. Experimental periods ranged from 63 to 70 d.

[a] Mite population counts consisted of adult females only (mites on adult bees and foundress females in brood cells when there was brood present).

[b] Values in grams.

[c] Values in number of bees.

[d] A reproductive cycle of the mite (as described by Harbo 1996) is the time spent in a brood cell (≈12 d) plus the time spent on adult bees (≈6 d, but highly variable) until a mite reenters a cell. Increase in the population of mites per reproductive cycle is the number of mites at the end of a reproductive cycle divided by the number at the beginning.

Statistical Analyses. Data were evaluated with analysis of variance (ANOVA) using a general linear model procedure and SAS Institute (1979) software. Analyses consisted of linear regressions with location (Michigan or Louisiana) as a block effect because there was no significant interaction between location and the characteristic being tested. The same trend was found at both locations. Development time, hygienic behavior, grooming behavior, and nonreproduction of mites were each analyzed with P_2/P_1 (final/initial mite population) as the dependent variable. Because the experimental periods were not equal in all colonies in Louisiana (some were evaluated for 63 d and others for 70 d, as explained above), the colonies with different test periods were evaluated as separate experiments and treated as location blocks in the analyses. The R^2 procedure (SAS Institute 1979) was used to compare single and multiple combinations of the independent variables with the dependent variable.

Table 2. Linear regression analyses between each of 4 characteristics of bees (nonreproduction of mites in cells, duration of capped period, hygienic behavior, and grooming behavior) and the population change of mites (P_2/P_1 as described in Table 1)

Source	df	Mean square	F	P	R^2
Location	2	0.02	0.07	0.93	—
Nonreproduction[a]	1	9.5	36.3	0.0001	0.53
Error	35	0.26	—	—	—
Location	2	19.1	2.0	0.15	—
Development time	1	15.7	3.3	0.07	0.18
Error	37	4.7	—	—	—
Location	2	7.3	1.4	0.25	—
Hygienic behavior	1	2.6	0.5	0.48	0.10
Error	39	5.1	—	—	—
Location	2	11.6	2.3	0.12	—
Grooming behavior	1	3.0	0.6	0.45	0.10
Error	39	5.1	—	—	—

Interactions between the characteristic and test location (Louisiana or Michigan) were not significant, so location was used as a block effect in the analyses. Because half of the colonies in the Louisiana test were terminated after 63 d and the rest after 70 d, the time periods were treated as 2 separate locations in these analyses.

[a] Although highly significant as a linear relationship (P = 0.0009), a logarithmic curve (natural log of Y) best described the relationship between nonreproduction and the growth of the mite population (see Fig. 1). The other 3 analyses showed no logarithmic trends and are presented as linear analyses.

Results and Discussion

Three of the 43 colonies (1 in Louisiana and 2 in Michigan) had fewer mites at the end of the test than at the beginning. The mite populations in these colonies declined during a time when there was a doubling of the bee populations (Table 1). Clearly, these 3 colonies expressed resistance to varroa mites.

Of the 4 characteristics tested, only nonreproduction of mites was highly correlated with changes in the mite population (F = 36.3; df = 2, 35; P = 0.0001; R^2 = 0.53 [Table 2]). The equation for the logarithmic regression is $Y = 4.5*0.928^x$ (Fig. 1). Multiple regression of all 4 variables added only 0.04 to the R^2 of nonreproduction alone. Therefore, the other 3 variables contributed very little to describing changes in the mite populations that had not already been described by nonreproduction.

Although not supported by results of this study, grooming behavior, hygienic behavior, and development time may yet be important. Development time (Table 2) with an R^2 of 0.18 was similar to the R^2 of 0.23 reported by Büchler and Drescher (1990) and was marginally significant. Although hygienic behavior was unrelated to changes in mite populations (Table 2), Spivak (1996) showed that hygienic behavior is effective in removing varroa mites. Our bees were never selected for their ability to detect and remove mites in cells, and bees that detect and remove frozen pupae do not always have the ability to detect and remove mite-infested pupae (Spivak 1996).

Perhaps these mechanisms of resistance are effective only when a colony possesses these traits at a very high level: postcapping periods that are similar to that of African bees (Moritz 1985), grooming behavior in excess of the maximum of 50% that we recorded, and perhaps a high level of the specialized hygienic behavior for detecting and removing mites from cells. Nonreproduction had little effect when it occurred at levels <30% (Fig. 1).

This study suggests that nonreproduction may be an important mechanism whereby *A. mellifera* halts or limits reproduction of varroa mites. The exact cause of nonreproduction is unknown, but early events in the capped brood are known to be essential for the reproduction of mites. A female mite needs contact with a late 5th-instar bee if she is to initiate oocyte development (Milani and Chiesa 1990). Vitellogenesis in the mite begins ≈10–15 h after the cell is capped and continues for ≈10 h (Steiner et al. 1994). Vitellogenesis continues for subsequent eggs, but a mite does not initiate oocyte development if she is placed into a brood cell 24 h after the cell is capped (Steiner et al. 1994).

Both the genotype of the brood (Camazine 1986) and the conditions that affect the female mite before she enters a brood cell (Beetsma and Zonneveld 1992, Fuchs 1994) have significant effects on nonreproduction. We exchanged combs of eggs and young larvae between bee colonies with reproductive and nonreproductive mites. In the colonies with a high level of nonreproduction, mites that entered the brood from colonies with highly reproductive mites had 64% nonreproduction; mites that entered brood from their own colony had 83% nonreproduction. Conversely, in colonies with highly reproductive mites, mites entering the brood from colonies with nonreproductive mites had 18% nonreproduction; mites entering brood from their own colony had 8% nonreproduction. These observations are inadequate to stand alone but support conclusions by Fuchs (1994), who reported that the conditions to which an adult female mite are exposed before or during reproduction (a colony effect) more strongly determine non-reproduction than the genotype of the brood. However, our observations also are consistent with data by Camazine (1986), so there may be a weaker but possibly an important effect caused by the genotype of the larva.

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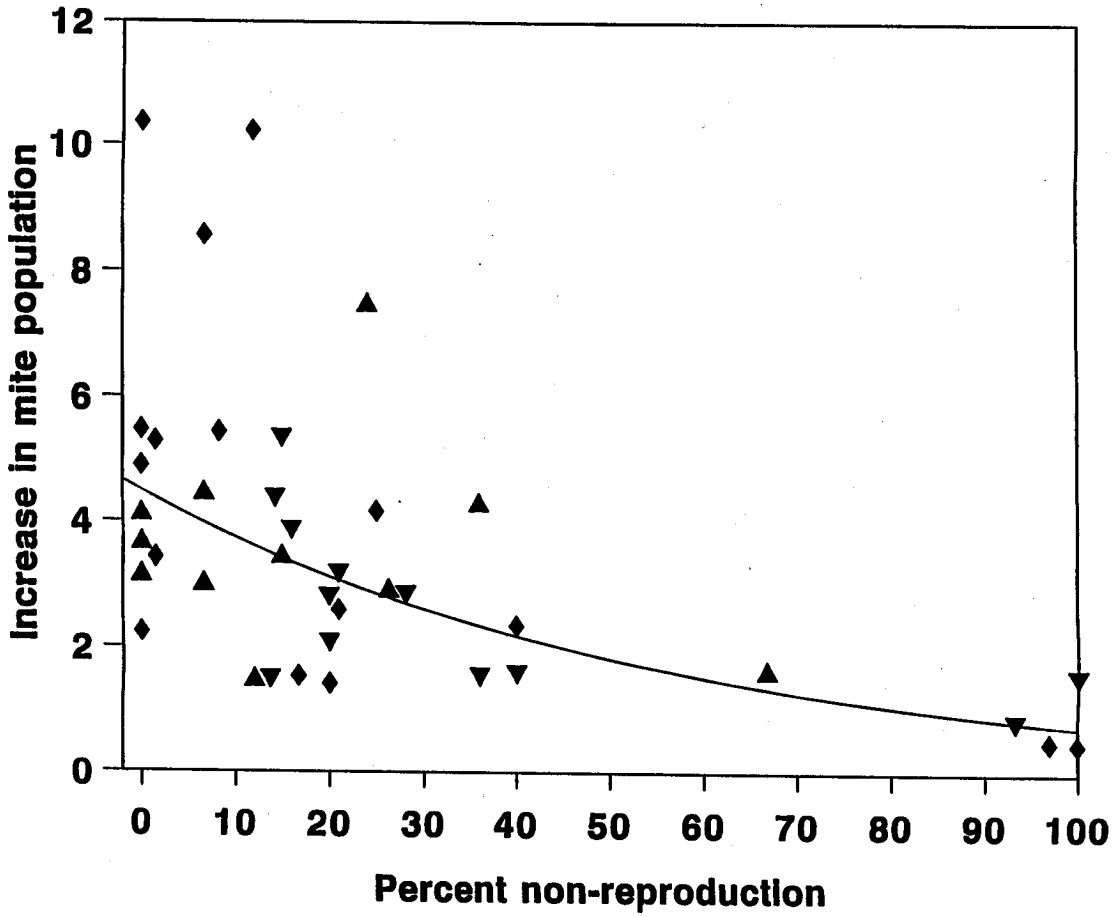

Fig. 1. Relationship between population growth of mites (final mite population/original mite population) and percentage of mites that failed to reproduce in brood cells of worker bees. The regression equation is log Y = 1.5 − 0.018x or Y = 4.5 * 0.982^x, R^2 = 0.53. A ▲ or ▼, colony from Louisiana test (63- or 70-d test periods, respectively); ♦, colony from Michigan test (69-d test period).

References Cited

Anderson, D. 1994. Non-reproduction of *Varroa jacobsoni* in *Apis mellifera* colonies in Papua New Guinea and Indonesia. Apidologie 25: 412–421.

Beetsma, J., and K. Zonneveld. 1992. Observations on the initiation and stimulation of oviposition of the *Varroa* mite. Exp. Appl. Acarol. 16: 303–312.

Büchler, R., and W. Drescher. 1990. Variance and heritability of the capped developmental stage in European *Apis mellifera* L and its correlation with increased *Varroa jacobsoni* Oud. infestation. Apic. Res. 29: 172–176.

Camazine, S. 1986. Differential reproduction of the mite, *Varroa jacobsoni* (Mesostigmata: Varroidae), of Africanized and European honey bees (Hymenoptera: Apidae). Ann. Entomol. Soc. Am. 79: 801–803.

Eguaras, M., J. Marcangeli, M. Oppedisano, and N. Fernandez. 1995. Mortality and reproduction of *Varroa jacobsoni* in resistant colonies of honey bees (*Apis mellifera*) in Argentina. Bee Sci. 3: 174–178.

Fries, I., A. Aarhus, H. Hansen, and S. Korpela. 1990. Development of early infestations by the mite *Varroa jacobsoni* in honey bee *Apis mellifera* colonies in cold climates, pp. 73–74. *In* W. Ritter [ed.], Proceedings of the International Symposium on Recent Research on Bee Pathology, 5–7 September, 1990, Gent, Belgium, Janssen Farmaceutica, Belgium.

Fuchs, S. 1994. Nonreproducing *Varroa jacobsoni* Oud. in honey bee worker cells − status of mites or effect of brood cells? Exp. Appl. Acarol. 18: 309–317.

Harbo, J. R. 1986. Effect of population size on brood production, worker survival and honey gain in colonies of honeybees. J. Apic. Res. 25: 22–29.

1992. Breeding honey bees (Hymenoptera: Apidae) for more rapid development of larvae and pupae. J. Econ. Entomol. 85: 2125–2130.

1996. Evaluating colonies of honey bees for resistance to *Varroa jacobsoni.* Bee Sci. 4: 100–105.

Milani, N., and F. Chiesa. 1990. Some stimuli inducing oviposition in *Varroa jacobsoni* Oud., pp. 27–33. *In* W. Ritter [ed.], Proceedings of the International Symposium on Recent Research on Bee Pathology, 5–7 September, 1990, Gent, Belgium, Janssen Farmaceutica, Belgium.

Moritz, R.F.A. 1985. Heritability of the postcapping stage in *Apis mellifera* and its relation to varroatosis resistance. J. Hered. 76: 267–270.

Newton, D. C., G. C. Cantwell, and E. P. Bourquin. 1975. Removal of freeze-killed brood as an index of

nest cleaning behavior in honeybee colonies (*Apis mellifera* L). Am. Bee J. 115: 388, 402, 406.

Ritter, W. 1990. Development of varroa mite population in treated and untreated colonies in Tunisia. Apidologie 21: 368–370.

Ruttner, F., H. Marx, and G. Marx. 1984. Beobachtungen über eine mögliche Anpassung von *Varroa jacobsoni* an *Apis mellifera* L. in Uruguay. Apidologie 15: 43–62.

SAS Institute. 1979. SAS user's guide. SAS Institute, Cary, NC.

Spivak, M. 1996. Honey bee hygienic behavior and defense against *Varroa jacobsoni*. Apidologie 27: 245–260.

Steiner, J., F. Dittmann, P. Rosenkranz, and W. Engels. 1994. The first gonocycle of the parasitic mite (*Varroa jacobsoni*) in relation to preimaginal development of its host, the honey bee (*Apis mellifera carnica*). Invertebr. Reprod. Dev. 25: 175–183.

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