Heritability in Honey Bees (Hymenoptera: Apidae) of Characteristics Associated with Resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae)

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ABSTRACT This study uses sibling analysis to measure the heritability in honey bees, *Apis mellifera* L., of characteristics that have been associated with resistance to the mite, *Varroa jacobsoni* Oudemans. Twenty-eight uniform colonies of bees were established on 13 May in Baton Rouge, LA, each with 1 kg of mite-infested bees and a queen. The 28 unrelated queens in these colonies were divided into 7 groups of 4 based on the insemination of 4 queens with the same mixture of semen from 1 of 7 sire colonies. After worker progeny from these queens had replaced the initial bee populations, a colony was related as a full sister to the other 3 colonies in its sire group and unrelated to the other 24 colonies. Heritability (h^2) was 1.24 for proportion of mites in brood, 0.65 for hygienic behavior, 0.89 for the duration of the capped period, 0.46 for suppression of mite reproduction, and 0.00 for physical damage to mites (measured by the presence of physically broken or dented mites on the bottom board). These results suggest that it should be possible to enhance the expression of 4 of these 5 characteristics with selective breeding of bees, thus reinforcing confidence in our ability to breed honey bees for resistance to *V. jacobsoni*.

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

Varroa jacobsoni OUDEMANS is an external parasite of *Apis cerana* F. and the honey bee, *Apis mellifera* L. These mites feed on the hemolymph of immature and adult bees.

Varroa jacobsoni has a reproductive cycle of ≈19 d. Just before a brood cell is capped and as the bee larva in the cell approaches maturity, a female mite enters the cell to reproduce. This reproductive opportunity lasts for ≈12 d while the host bee in the cell progresses through its late larval and pupal stages. All male mites and immature female mites die when the host bee removes the cell capping and exits the cell as an adult. Only adult female mites survive outside the cell, and they spend ≈7 d on adult bees before repeating the reproductive cycle and entering another brood cell.

Varroa jacobsoni usually destroys a bee colony within 2 yr unless they are controlled by a beekeeper. The most widely used method of controlling *V. jacobsoni* is to place plastic strips impregnated with the pesticide fluvalinate inside the bee colony. However, mites recently have become resistant to fluvalinate (Lodesani et al. 1995), so there is a demand for bees that are resistant to mites.

The purpose of this study is to measure the heritability in bees of characteristics that are related to the growth of mite populations. Selective breeding for mite resistance then can proceed with the characteristics that are heritable.

Heritability (h^2) has been measured on many characteristics of bees. Heritability is the proportion of the observed variance (among a group of bee colonies in this case) for which differences in heredity are responsible (Lush 1945). If a characteristic has an h^2 close to 1, then the characteristic can be changed rapidly with selective breeding. If $h^2 = 0$, selective breeding will fail. As a general rule, selective breeding can proceed if h^2 is >0.25. Examples of heritability measurements on honey bees are as follows: (1) duration of capped period = 0.8 (Moritz 1985) and 0. 61 (Harbo 1992), (2) pupal weight = 0.65 (Milne and Friars 1984), and (3) longevity of caged bees = 0.32 (Rinderer et al. 1983) and 0.20 (Milne 1985).

We used sibling analysis (Collins 1986) to determine the heritability of 8 measurements that had been associated with the growth of mite populations. Two of these measurements are characteristics of honey bees (hygienic behavior and duration of capped period). Three describe the behavior or physiology of mites (proportion of mites in brood cells, mite reproduction, and physical damage on the body of the mite), and 3 are direct measures of mite populations (change in total mite population, mites per 100 cells of capped brood, and mites per 1,000 bees). We measured heritability in honey bees, not heritability in mites. Therefore, even a measurement of mite physiology, such as egg production of mites, is examined as to whether it is affected by a heritable component of the honey bee.

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Of special interest was the suppression of mite reproduction. This experiment evaluated both the immediate brood effects of this characteristic reported by Camazine (1986) and a delayed expression of this characteristic. In the delayed expression, suppression of mite reproduction was not evident when mites went through their 1st reproductive cycle in colonies that would ultimately suppress mite reproduction ≈2 mo later. Fuchs (1994) attributed this delayed effect to attributes that the mite attained before it entered a brood cell.

Materials and Methods

General Design. We produced a group of 28 colonies to estimate heritability with sibling analysis. The colony was the experimental unit in the analyses. The 28 colonies consisted of 7 groups of 4 colonies each. Each colony was related as a full sister to the other 3 colonies in its group (average relatedness of 0.5 among the worker populations) (Moritz et al. 1987) and was unrelated to the other 24 colonies.

The experiment was designed so that growth of the bee and mite populations were independent. Mites needed (and had) a constant supply of bee brood so that their reproductive cycles were not interrupted. Mites apparently did not alter the growth of bee populations during the 10-wk test period because there was no relationship between the growth of bee and mite populations ($R^2 = 0.002$; $F = 0.05$; df = 1, 26; $P = 0.83$). At the end of the test, the colonies had 19,300 ± 2,750 (mean ± SD) adult worker bees and 1,649 ± 618 adult female mites.

Source of Queens and Matings. Before the test, 28 mated queens were chosen as queen sources and 7 were chosen as drone sources (sire colonies). All 35 were unrelated and were chosen from colonies in the Baton Rouge area. Two of the sire colonies were known to suppress mite reproduction. One queen was produced from each of the 28 queen sources, and semen was collected from at least 40 drones from each sire colony. Semen from each sire colony was diluted 1:1 with saline, mixed (Harbo 1990), and used to inseminate 4 of the queens. Thus, we produced 7 groups of 4 mated queens. Worker populations produced by the progeny of these queens would be related as full sisters to the other 3 colonies in its sire group (Moritz et al. 1987) and unrelated to colonies in other sire groups.

Source of Worker Bees and Mites. We established uniform populations of bees and mites for the colonies by collecting a large population (>30 kg) of mite-infested bees into a cage with a volume of ≈340 liters (Harbo 1986, Harbo and Hoopingarner 1997). The bees were collected from miscellaneous colonies in the Baton Rouge area. Bees and mites in the large cage were subdivided into 28 populations, each consisting of 1,002 ± 8 g of bees and ≈784 *V. jacobsoni* in smaller cages that would be placed inside the hive when the test colonies are established. The number of mites was determined from 4 samples of bees that were collected from the cage as it was being subdivided. We poured the samples of bees (≈330 ml each) directly into wide-mouthed, 0.95-liter (1 qt) canning jars that had been preweighed. Bees died a few minutes after capping the jar. Each jar was reweighed to establish the weight of the bees. Ethanol (70%) was added to each jar, bees were shaken in the alcohol, and mites were separated from bees with a basket and a tray (Harbo and Zuhlke 1988). We rewashed the samples with alcohol until no mites were found in the tray (usually 4 washings). The samples contained 77.5 ± 6.8 (±SD) mites per 100 g of bees.

Establishing Colonies. We started the 28 test colonies on 13 May with caged populations of bees and mites (described above), a caged queen, and 5 combs (each 20 by 43 cm) in a standard hive that could hold 10 combs. Colonies were placed in an apiary that contained no other bees. We immediately opened the cages to allow free movement of the worker bees within the hives, but the hives remained closed until the following night. Hives were opened after dark on 14 May, and queens were released on 15 May. We added combs as needed for the duration of the test but provided only combs with worker-sized cells to minimize drone production and thus eliminate the complication of mites preferring and having a higher rate of reproduction in drone cells.

Measurements During the Test. We evaluated each colony for the duration of the capped period, hygienic behavior, physical damage to mites, and the frequency of nonreproducing mites in brood cells.

Duration of Capped Period. A brood cell is normally capped ≈8 d after a fertilized egg is laid, and it remains capped for ≈12 d until the worker bee emerges as an adult. However, there is significant variation among a group of colonies in the average duration of the capped period (Harbo 1992). We made this measurement during the 1st wk of the test when the colonies contained only worker bees from the original population. Worker bees were not daughters of the queen or sisters to the larvae they were feeding and capping.

Queens were released in the late afternoon so that the initial capping of the cells could be detected 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. The colonies were examined 11 d later (beginning 18.5 d after queen release and in some colonies as late as the following morning) until newly emerged worker bees were detected (described by Harbo and Hoopingarner 1997). This technique probably underestimates the mean capped period for worker bees by ≈5 h (Harbo and Hoopingarner 1997).

Mite Reproduction. This is an estimate of the proportion of mites that fail to produce viable progeny after they enter a cell to reproduce. The estimate for each colony was based on whether or not mites produced viable progeny in 30 mite-infested cells. This was repeated at 3 different time periods (at 3 wk, when all colonies contained the original, uniform population of bees and mites; at 6 wk, when the colonies were in

transition; and at 10 wk, when the genotype of each colony had stabilized). We determined whether or not a mite was producing viable progeny by examining bee pupae that were purple-eyed and older, because at this stage of host development (8–11 d after the mite has entered the brood cell) the remaining capped period is too short for a mite egg (purple-eyed stage) or a protonymph (when examined at the tan body stage of the bee pupa) to mature and thus survive (Ifantidis 1983, Delfinado-Baker 1984). Only cells that had been invaded by 1 female mite were evaluated for mite reproduction. Nonreproducing mites consisted of (1) a dead adult mite with no offspring, (2) a live adult mite with no offspring, or (3) a mite that had no female progeny old enough to mature before the host bee became an adult.

Hygienic Behavior. Hygienic behavior was the rate at which adult bees removed freeze-killed brood from sealed cells. We evaluated this characteristic after the 6th wk of the experiment to ensure that we evaluated adult progeny of the test queens. We tested hygienic behavior by cutting a square of capped brood (≈60 cells) from each colony, freezing the squares for 24 h to kill the brood, returning the squares to their respective colonies, and then counting the number of freeze-killed bee pupae that were uncapped and removed by the adult bees within the next 24 h (Newton et al. 1975).

Physical Damage to Mites. Physical damage to mites may be caused by the activities of adult bees. Therefore, this test was conducted after the 6th week of the test when the adult populations consisted of progeny of the test queen. Plastic-coated freezer paper was covered with oil (canola oil brushed onto the side of the paper that was not plastic-coated) and placed on the bottom of each colony for 2 d. The oiled paper served as a trap because live mites that fall onto the paper will die from contact with the oil. The oiled paper was placed on a masonite board (35 by 45 cm) and covered with a 3-mm mesh screen which was spaced ≈5 mm above the paper. The screen allowed the mites to fall through but kept the bees from touching the paper or the trapped mites. The mites were examined under a microscope for fractured bodies, broken or missing legs, and dents in the idiosoma (oval, dorsal plate that covers the entire mite).

Measurements at the End of the Test. We ended the test on 24 July, 70 d after queen release. At that time we weighed the bees in each colony and estimated the number of mites on adult bees and the number of mites in brood cells. With these data we calculated the final mite population because mites are either on the adult bees or in the brood. Our estimates of mite populations included only adult, female mites (Harbo 1996).

The colonies were screened on the night before the measurement so that all adult bees were inside. Each colony was weighed the next morning with and without bees to establish the weight of the adult bees. We sampled the adult bees (in a jar as described earlier) so that we could count the number of mites per sample weight of adult bees.

Mite Populations. Our estimate of the total number of mites on adult bees was calculated from the weight of the bees, the weight of the sample, and the number of mites in the sample. To estimate the total number of adult female mites in brood cells, we first calculated the number of cells in each colony that could contain mites. Mites could be inside a capped brood cell or in a cell that would be capped within the next 24 h (we considered 12 h before capping to be an average time of entry). 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 (the number of cells per square) to calculate the number of cells of capped brood and then by 1.04 to include the number of cells that would be capped within the next 12 h. (Because the duration of the capped period is 12 d, 1/12 of the capped cells in a colony were capped each day, and at that rate of brood production, the number of cells that would be capped in the next 1/2 d would be ≈1/24 of the total or 4%.) After establishing the number of cells in a colony, we counted the number of mites in 200 cells by opening 50 cells in a horizontal row on each side of 2 combs.

Proportion of Mites in Brood Cells. Mites in the colony are either on adult bees or in the brood cells. When a smaller proportion of the mite population is in the brood cells, then a smaller proportion of the mites in that colony are reproducing, and the growth of the mite population will decline.

Statistical Analyses. Variance components were calculated with analysis of variance (ANOVA) for completely randomized design in which the main treatment (sire) was a random variable (PROC Mixed, SAS Institute 1997). The following equation was used for each calculation of heritability:

$$\frac{2\sigma^2_{SIRE}}{\sigma^2_{SIRE} + \sigma^2_{COLONY}}$$

where σ^2_{SIRE} is the variance estimate for sire and σ^2_{COLONY} is the variance estimate for residual error (or within-sire variance). The standard error for each estimate of heritability was calculated by the method of Swiger et al. (1964).

Results and Discussion

Four characteristics associated with the growth of mite populations had heritability (h^2) of >0.25 (Table 1). These were mite reproduction, proportion of mites in brood, hygienic behavior, and the duration of the capped period. Therefore, one should be able to intensify the expression of those characteristics through selective breeding.

Suppression of Mite Reproduction. The occurrence of nonreproductive mites was a heritable characteristic at 2 different time periods (the 1st and the last), which suggests that there may be 2 separate components—an immediate effect and a delayed effect (both described in the introduction). The 1st mea-

Table 1. Heritability of colony characteristics based on sibling analysis of 28 colonies during a 70-d test period, 15 May—24 July

Characteristic	Range[a]	$h^2 \pm SE$
Suppression of mite rep.		
June 2[b]	8–52%	0.38 ± 0.58
June 23[b]	15–48%	0.06 ± 0.48
July 24[b]	9–48%	0.46 ± 0.59
Hygienic behavior	4–91%	0.65 ± 0.61
Physical damage to mites (total)	4–35%	0.00 ± 0.45
Dents in body	1–11%	0.00 ± 0.45
Broken legs or bodies	0–26%	0.17 ± 0.52
Capped period, h	268–290	0.89 ± 0.59
Proportion mites in brood	39–82%	1.24 ± 0.49
Mites per 100 cells of brood (24 July)[c]	4–30.5	0.28 ± 0.56
Final mite population[d]	534–3,389	0.17 ± 0.52
Mites per 1,000 bees (24 July)[e]	26–198	0.01 ± 0.46

[a] Range of data from the 28 colonies in the test. This experiment was not designed to produce colonies that were highly resistant to mites.

[b] Suppression of mite reproduction combines the following 3 components: (1) dead foundress mite in a cell with no progeny, (2) live foundress mite with no progeny, and (3) live foundress mite with progeny produced too late to mature.

[c] Number of adult foundress female mites, based on counts of 200 cells of capped brood per colony.

[d] Total number of adult female mites in brood cells and on adult bees. Because all colonies started with the same number of mites, this is an expression of the growth of the mite populations (final population/original population).

[e] All adult female mites in the colony (including foundress females in brood cells). Bees refer to all adult bees in a colony.

surement, 2 June, occurred when no adult bees or mites had yet been produced in the test colonies, so the larval and pupal bees (encountered by the mites after they entered the cells to reproduce) were the only portions of the colonies that contained the genetic relatedness of the experimental design. The mites and the adult bees were from the original population and were therefore uniform among all colonies. Other factors affecting mite reproduction were certainly present, but their effects should be equal or random. Because mite reproduction was heritable during this 1st observation, we concluded that larvae or pupae suppressed mite reproduction. Mite reproduction had low heritability during the transition period (early July measurement), but was again heritable at the 3rd measurement (Table 1).

It is at this 3rd (last) time period that we (Harbo and Hoopingarner 1997, Harbo and Harris 1998) and other researchers (Fuchs 1994, Martin et al. 1997) have found the highest levels of suppressed mite reproduction. At this time, the entire colony consists of progeny of the test queen and the mites have been through ≥2 reproductive cycles. This delayed suppression of mite reproduction has gained the most attention and is certainly important. However, based on data from this study (Table 1), it also should be possible to select for nonreproducing mites during the 1st brood cycle, a time when we have not yet seen suppression of mite reproduction at a high level.

Duration of the Capped Period. We already knew that the duration of the capped period was heritable (Moritz 1985, Harbo 1992), and this characteristic has been related to mite populations (Büchler and Drescher 1990). However, we did not know that this characteristic would still be heritable (have an acceptably high h^2) using a different method of measurement, but it was (Table 1). The method of measuring a characteristic affects the value of h^2. Imprecise measurements will increase overall variance and therefore will reduce h^2, sometimes to zero. Conversely, if a characteristic is truly nonheritable, more accurate measurements cannot create an h^2 that is >0.

Hygienic Behavior. Hygienic behavior was heritable ($h^2 = 0.65$, Table 1), and Spivak (1996) found a relationship between hygienic behavior and the removal of mites from brood cells. In a field test, colonies with queens selected for hygienic behavior had fewer mites than did the control colonies (Spivak and Reuter 1998). Therefore, hygienic behavior is a good candidate for selective breeding of bees for resistance to *V. jacobsoni.*

Proportion of Mites in Brood Cells. The proportion of mites in brood cells has been related to the growth of mite populations (Danka et al. 1997, Harbo et al. 1997). It was important to learn that the proportion of mites in brood is heritable ($h^2 = 1.24$, Table 1) because this characteristic would complement any of the other 4 characteristics to enhance resistance to mites further. In contrast, the suppression of mite reproduction, hygienic behavior, and duration of the capped period all affect the same process, the success of mites in the brood cell (Harbo 1996).

Physical Damage to Mites. Physical damage to mites would complement most other mechanisms of resistance because it occurs when mites are on adult bees. Unfortunately, this characteristic has never been related to a reduction in mite populations in *A. mellifera* and was not heritable in this test. Currently it provides a poor prospect for selective breeding. We noticed that one of the components (dents on the idiosoma) may not be caused by adult bees. Some young adult female mites had dents on their idiosoma before they left a cell for the 1st time. Therefore, those dents were probably caused by leg movements of the pupal or teneral adult bee inside the cell. When physical damage to mites was limited to broken legs and bodies, heritability was marginal (Table 1)—too low to encourage selective breeding but not low enough to dismiss.

Nonspecific Characteristics. The last 3 characteristics in Table 1 provide guidance for nonspecific selection for resistance. Measuring mites per 1,000 adult bees probably would fail to yield any progress. Measuring the final mite population (the growth of mite populations) is marginal and probably would yield very slow progress. Of the 3, the best measurement was the number of mites per 100 cells.

However, we do not recommend selection for nonspecific characteristics, although these characteristics are direct measures of what we are trying to accomplish—low populations of mites. Low heritability suggests that progress would be slow when trying to enhance these general characteristics, perhaps because the growth of a mite population is affected by

many unrelated characteristics. When multiple and diverse effects are involved, selective breeding that is based only on a final result becomes inefficient because it attempts to select many diverse characteristics on the basis of the sum of their effects.

We think that the best approach to producing resistant bees is to identify the specific characteristics of resistance, enhance those that are heritable, and then combine the characteristics, when possible, into a single colony of bees. We recommend 3 specific characteristics—suppression of mite reproduction, hygienic behavior (based on this study and that of Spivak 1996), and proportion of mites in brood cells.

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