Evaluating colonies of honey bees for resistance to *Varroa jacobsoni*

John R Harbo

USDA/ARS
Honey-Bee Breeding, Genetics and Physiology Research Laboratory
1157 Ben Hur Road
Baton Rouge LA 70820

ABSTRACT

The purpose of this work is to present a procedure for evaluating the growth of a population of mites (*Varroa jacobsoni* Oudemans) within a colony of honey bees (*Apis mellifera* L). The evaluation uses the equation $P_1 (rs)^n = P_2$. Where P_1 and P_2 are the initial and final mite populations, r is the rate of mite reproduction in brood cells, s is the survival of mites outside the brood cells, and n is the number of reproductive cycles of the mite. The equation describes the growth of the mite population in a colony of honey bees during a field test that can range from 9 - 16 weeks in duration. Only adult female are counted in a mite population, and all measurements are colony averages. By comparing the rates of growth of the mite populations in each colony (P_2/P_1), one can determine which colonies are more resistant to mites. The values of r, s and n can then explain how the mite population went from P_1 to P_2 in terms of measurable reproductive factors. If three stocks of bees each affect a different reproductive factor (r, s, or n), then these three selections, each with resistance to varroa mites, may be combinable into a single, more resistant stock. The numerical analysis describes which part of the mite's reproductive cycle is being affected by the bee. Therefore, it can guide the process of selection and can provide a way to measure progress during selective breeding.

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KEY WORDS

Insecta, *Apis mellifera*, mites, *Varroa jacobsoni*, population, selective breeding

INTRODUCTION

This work presents a method for selecting honey bees (*Apis mellifera* L.) for resistance to varroa mites (*Varroa jacobsoni* Oudemans). The procedure measures the growth of a population of mites within a colony of honey bees and describes how each part of the mite's life cycle contributed to that growth (Fig. 1).

A field test is the core of the evaluation. It requires 9 - 10 weeks and measures growth of mite populations in a group of honey bee colonies. For each colony in a test, the evaluation establishes the initial and final mite populations (P_1 and P_2). Population growth is then explained in more detail. For each colony, I explain how a mite population went from P_1 to P_2 in terms of the three general factors that can affect the growth of a mite population. These factors (described in Fig 1.) are (1) the rate of mite reproduction in the brood cell (r), (2) the probability (ranging from 0 - 1) that an adult female mite will survive to enter another cell (s) and (3) the number of days needed to complete one reproductive cycle of the mite (t), which is converted to n, the number of reproductive cycles during the test period. All three factors are colony averages. The measurements that are needed to calculate factors 1 - 3 (hereafter referred to as r, s, and t) are somewhat destructive of the mite population, so they are made at the end of the field test.

With this type of evaluation, resistance in bees is related to the rate of growth of a population of mites This assumes that variation in the growth of a mite population is caused by honey bees. Any resistance in honey bees that is not simply a function of the mite population (such as resistance to any microorganisms transmitted by mites) would not be detected with this method.

The equation is $P_1 (rs)^n = P_2$. Population growth of mites is $(rs)^n$, where n is the number of reproductive cycles (the duration of the field test divided by t) and rs (r times s) is the average population change per reproductive cycle. Therefore, multiplying $(rs)^n$ times the initial population (P_1) equals the final mite population (P_2). If any four of the five values are known, the remaining one can be calculated. I have developed techniques for measuring P_1, P_2, r, and n.

The result is a simplified model of mite reproduction that will describe mite reproduction within each colony of bees. It is not a complete model of the mite population as presented by Camazine (1986) and Fries *et al.* (1994) because it is limited to a brief and uniform period of reproduction for mites and bees. My model counts only the adult, female mites and uses only group averages to evaluate mite populations within a colony of bees. Although the model ignores mite migration, the design of the field test does not. The field test is designed to minimize the effects of migration by starting test colonies with equal mite populations and by keeping these colonies in an apiary by themselves, away from highly infested colonies. The design also equalizes the effect of genetic variation in mites by starting colonies with a uniform mixture of both mites and bees.

The intent of this paper is to assist those who are working to select bees for resistance to varroa mites. Data from field tests are presented as examples of how

Table 1

Field test of 24 colonies for 76 days (12 April - 27 June). All colonies started with 87 mites, 370 g of bees and no brood. The duration of the reproductive cycle of the mite was not measured, so each colony was assigned a reproductive cycle with a duration of 20 days.

	Initial mite Pop. (P_1)	Pop. Increase per repr. cycle (rs)	Duration (in days) of repr. cycle (t)[1]	Final mite pop. (P_2)
Group mean	87	1.67	20	508 ± 207[2]
Best colony[3]	87	1.32	20	224
Worst colony	87	2.08	20	1042

[1]With a reproductive cycle of 20 days, there were 3.4 reproductive cycles for mites in this experiment (when starting with no brood, the first reproductive cycle was assigned to be 27 days).
[2] Mean ± standard deviation.
[3] In this and in other tables, best and worst colonies in the experiment refers to overall growth of the mite population, column 4 divided by column 1 (P_2 / P_1). They may not be best or worst in columns 2 and 3.

I conducted evaluations in Louisiana. The field evaluations validate the procedure and give an indication of the results that can be expected. An evaluation system is useful not only in selecting stock, but it can also provide a measurement of progress and can serve to maintain the characteristics that have been selected.

GENERAL PROCEDURE FOR FIELD EVALUATION

Establishing test colonies

Each field test began with about 20 mated queens whose progeny were to be evaluated for resistance to varroa mites. About 25 kg of bees were collected from miscellaneous colonies, stored for about 24 hours in a large cage and then subdivided into cages as package bees (Harbo 1986). A caged queen, four broodless combs, and caged bees (ca. 1 kg) were placed into each of about 20 hives with entrances screened. Entrances of the hives were opened after sundown on the day after installing the bees. Queens were released about 1 day later. Additional combs were added as needed.

Mite populations were measured in the initial populations of bees by measuring the number of mites per gram of bees in four samples from the large cage. The number of mites were counted in each of the samples which consisted of ca. 150 g of bees. There are a number of methods for counting mites on adult bees (De Jong *et al.* 1982). However, I prefer a method that does not kill the bees (Harbo unpublished). This method involved putting caged bees into a 40° incubator (50 ± 10% RH) and feeding the bees water and candy made of powdered sugar and honey (2 : 1). Mites dropped from the caged bees within 48 hours. To verify that all mites had been removed from the bees during the 48 hours at 40°, ca. 50 grams of bees were collected from about half of the cages. These bees were checked for mites with an ether roll and alcohol wash. No mites were found.

Evaluating colonies

Mite populations were measured again at the end of the test. The weight of the bees in each colony was measured by weighing the hive with and without bees. The number of mites on adult bees was measured by collecting a known weight of bees (ca. 150 g) from each colony and measuring the mite populations with the heat treatment described above. The number of mites on all adult bees was then calculated from the number found in the sample.

Unlike the initial mite population which was entirely on adult bees, the final mite populations are also in brood cells. Therefore, these populations were estimated by measuring mites per cell of brood and the number of brood cells per colony. As with all mite counts in these evaluations, only adult female mites were counted (in this case only foundress females in the cells). This was done by uncapping 200 cells (50 cells on each side of two brood combs) and counting the number of foundress females. As long as bee cells were examined two or more days before adult bees emerged, foundress females were easily discernible from their daughters.

By measuring the area of capped brood with a wire grid, I calculated the number of capped brood cells in each colony. Because mites enter worker cells 0 - 24 h before capping (Fuchs & Muller 1988, Infantidis 1988), the brood cells that are infested by mites include not only the capped brood but also the cells that will be capped within the next 24 hours. With mites entering cells 0 - 24 hours prior to capping, 12 hours is a reasonable average time. Therefore, brood that would be capped within the next 12 hours (about 4% of the capped brood if brood is being produced at a uniform rate) was added to the number of capped cells to arrive at the number of cells that can contain mites. The number of adult mites/200 cells (described above) was then used to calculate total mite population in brood.

Table 2

Field test of 22 colonies for 65 days (26 June - 31 Aug.). All colonies started with 140 mites, 1 kg of bees, and no brood. The duration of the reproductive cycle was estimated for the mites in each colony in this experiment.

	Initial mite pop. (P_1)	Pop. incr. per repr. cycle (rs)	Duration (in days) of repr. cycle (t)	Final mite pop (P_2)
Group mean	140	2.12	23.1	1008
Best colony	140	1.64	—[1]	325
Worst colony	140	2.56	20.6	1947

[1] Duration of the reproductive cycle for this colony was difficult to measure because the colony contained very little capped brood on 31 August (summer dearth in Louisiana). The number of reproductive cycles for the mites in this colony was estimated at 1.7.

Other considerations for field tests

(1) Colonies were not highly infested with mites at the beginning of the evaluation. (2) There were no highly infested colonies nearby. (3) There was no drone comb in the colonies. I tried to eliminate all drone brood. However, removing drone cells after the mites had entered could kill mites and thus lower the estimate of population growth, so a few drone cells were tolerated in most experiments. (4) Colonies remained reasonably small (beginning with 0.5 - 1 kg and ending with less than 2.5 kg of bees). (5) All colonies were in a single hive body containing no more than 10 combs (each 20 x 43 cm). This made it easier to weigh colonies with and without bees at the end of the test and thus measure the final bee population. Lastly, combs of honey were replaced with empty combs if the brood area became restricted.

CALCULATING REPRODUCTION

The most basic calculation is the overall growth of the mite population in each colony. This is simply the final mite population divided by the initial mite population (P_2/P_1). Based on the equation $(P_1 (rs)^n = P_2)$, the overall growth of the mite population (P_2/P_1) equals $(rs)^n$. The next step is to solve for rs by calculating n.

Measuring n

Before one calculates the number of reproductive cycles (n), one must calculate the duration of the reproductive cycle of the mites in each colony. The duration of a reproductive cycle is the average time from when a mite enters a cell until it enters another cell (time spent in the cell plus time spent on adult bees). Not all mites will have the same time for their reproductive cycle and the length may change with the age of the mite. However, only the group average is important. The time spent in a worker cell is quite uniformly 12.5 ± 0.5 days (0.5 day in uncapped brood plus about 12 days in capped brood). The ± 0.5 days is based on 2 standard deviations about the mean for the capped period of bees in Louisiana (Harbo 1992). The duration of capped brood of European bees in Germany and Louisiana averaged 285 hours (Büchler and Drescher 1990, Harbo 1992). However, the average time spent between emerging from one cell and entering another may be quite variable. Fries *et al.* (1994) estimate this time to be about 6 days during periods when brood is available.

I estimated the length of the reproductive cycle of mites (t) for each colony by comparing the number of mites on adult bees with the number of mites in brood cells (both were measured at the end of the experiments). For example, if 60% of the mites were in cells, I calculated the average generation time of the mites in that colony as 12.5 (the time mites spend in cells)/0.60 or 20.8 days (Otten 1991).

The number of reproductive cycles in the test was then calculated from the estimate of the length of the reproductive cycle for mites in a colony (divide the duration of the experiment by the length of the reproductive cycle). However, most of these tests were started in bee colonies with no brood. Since mites cannot begin their reproductive cycle until bees have been producing brood for about 7 days, the mites in a colony that begins with no brood were assigned 27 days for their first reproductive cycle.

The worst colony in Table 2 is used as an example of calculating n. The test has run for 65 days and the colony has a reproductive cycle (t) equal to 20.6 days. Therefore, $n = 1 + (65 - 27)/20.6 = 2.8$ reproductive cycles

Calculating rs

As described in Fig. 1, rs is the population growth of mites per reproductive cycle. It is a very useful number. An rs of 1 equals no change in the mite population; numbers >1 indicate population increase and <1 indicate population decline.

Table 3

Table 3. Field test of 15 colonies for 62 days (3 Jan. - 6 Mar.). This experiment began with established colonies rather than with package bees. Therefore, populations of mites were estimated for each colony at the beginning of the experimental period.

	Initial mite pop. (P_1)	Pop. incr. per repr. cycle (rs)	Duration (in days) of repr. cycle (t)	Final mite pop. (P_2)
Group mean	134	1.56	19.3	620
Best colony	134	1.22	15.9	298
Worst colony	137	1.93	16.7	1580

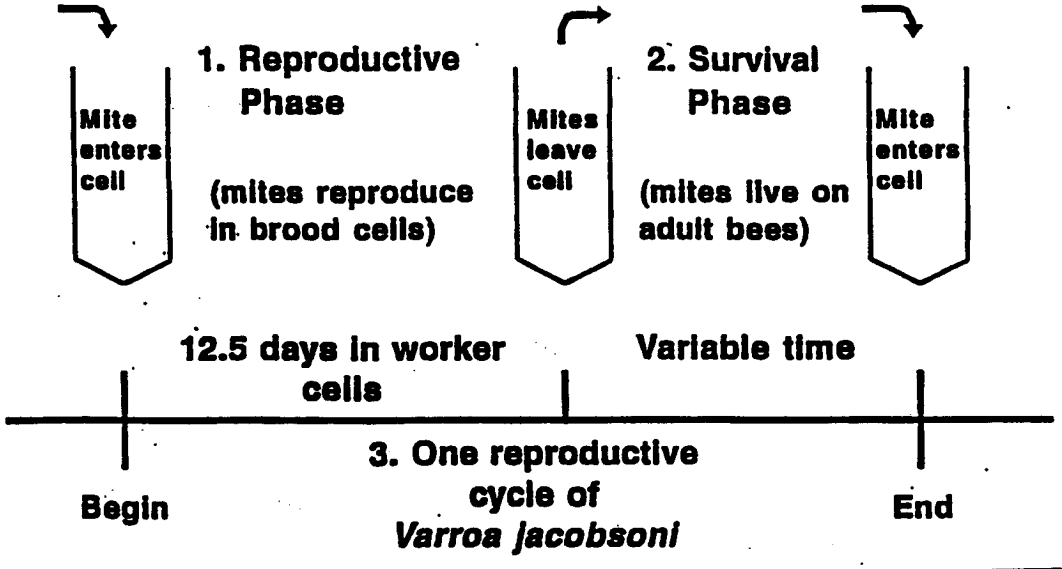

Figure 1. Three distinct functions within a reproductive cycle of *Varroa jacobsoni* that affect the growth of a mite population and can thus be used to evaluate mite resistance in a colony of bees. All measurements are group averages for adult female mites in a colony. Number 1, reproduction (r), is the number of adult female mites that leave the cell per mite (always an adult female) that enters. Number 2, survival (s), is the probability (ranging from 0 - 1) that a female mite will survive to enter another cell. Number 3 is the duration of the reproductive cycle (t). In short, r and s are two independent phases of reproduction that affect the mite population and t is the time frame in which they occur. Since the time spent in the cell is fixed to the development time of the bee, the time spent outside the cell is the main cause of variation for the duration of the reproductive cycle. Population growth per reproductive cycle equals rs (r x s). Population growth for any period of time is $(rs)^n$ where t is used to compute the number of reproductive cycles (n) during any time period wherein the mites have continuous opportunity for reproduction.

Now that n is known, the basic equation can be rearranged for calculating rs. The equation becomes: $rs = \sqrt[n]{(P_2/P_1)}$. Again using the worst colony in Table 2 as the example where n has been calculated to be 2.8: $rs = \sqrt[2.8]{13.9} = 2.56$. Note that all these data and the calculations can be handled on nearly any spreadsheet program. However, when using a computer it is simpler to write $\sqrt[2.8]{(P_2/P_1)}$ as $(P_2/P_1)^{(1/2.8)}$.

Calculating r *and* s

When rs is already estimated, only one of the factors needs to be measured and the other can be calculated. I suggest measuring r, reproduction in the cell.

I estimated r by counting the number of foundress females per 200 cells in the early pupal or prepupal stage, caging the queen in the colony, and then (8 - 10 days later) allowing about 500 bees to emerge from that comb in an incubator. The queen was caged so that no

Table 4

Field test of 12 colonies (3 Jan. - 16 Feb.). This experiment began with caged bees (derived from a single source) that were established 18 Nov. but queens were kept caged until 3 Jan. Populations of mites and bees were estimated for each colony at the beginning and end of the 44-day experimental period. The duration of the reproductive cycle of mites was estimated for each colony.

	Initial mite pop. (P_1)	Pop. incr. per. repr. cycle (rs)	Duration (in days) of repr. cycle (t)	Final mite pop. (P_2)
Group mean	140	1.22	27.9	204
Best colony	150	0.93	30.2	134
Worst colony	40	1.95	16.9	153

uncapped brood existed on the comb when it was put into the incubator (otherwise mites in uncapped cells could leave the cell if the unfed larva crawls out or dies). A 3 - frame hive body holding one or two brood combs was placed in an incubator. The hive body was screened on the bottom and set on a proportionally sized bottom board that was painted white on the top surface. Any mites that fell from the newly emerged bees would be collected on an oiled paper below the screen and were added to the subsequent mite count. All the bees that emerged in the incubator were counted and placed into a cage. Using the 40° C heat technique described earlier, I measured the number of adult mites on these bees. Mites per bee in the final test divided by foundress females per cell in the initial test equals r.

I am searching for a better way to estimate r because the method above is tedious and inaccurate. Sampling error is the problem. For example, if 21 foundress female mites were counted from a sample of 300 cells (7%), the 95% confidence interval for the true mean would range from 4.4 to 10.5% (Steele and Torrie (1980). Moreover, this error occurs twice (when counting mites in brood cells and again when counting mites from adult bees) because there are two separate samples. Two other methods eliminated this sampling error by using a single sample of bees and mites. One method examined mites from newly emerged bees and attempted to discern the young, adult females from the foundress females. I used body color to separate young from old mites, and I was not satisfied with the result. The third method allowed about 400 adult bees to emerge in an incubator. I measured the number of mites on those adult bees and then estimated the number of foundress female mites based on evidence (fecal accumulations of the mites) that remained in the cells from which the bees had emerged. These cells can be identified by the presence of a bee pupal skin at the base of the cell. It was important to remove the newly-emerged adult bees before they become nest cleaners and thus before they could destroy the evidence in the cells.

An example of measuring r and then separating r and s from rs is taken from a colony in the group described in Table 3 where rs had been calculated as 1.38. There were 10 adult mites per 200 cells of brood, and I counted 42 adult mites from 502 bees that emerged in an incubator. Thus, $r = 1.67$. Since $s = rs/r = 1.38/1.68$, $s = 0.83$.

EXAMPLES OF FIELD EVALUATIONS

Tables 1 - 4 report results from four field evaluations conducted in Baton Rouge Louisiana. Since r and s were not calculated for all colonies, these data were not included in the tables.

Population growth of mites per reproductive cycle (rs) was highly variable among colonies of bees. The average for the 73 colonies was 1.66, but the test colonies were not randomly chosen so this value does not necessarily represent the honey bee population in Louisiana. However, these data confirm the contention that mite populations can increase 100 fold within one year (Fries *et. al.* 1990). For example, an $rs = 2$ (which is not uncommon) and a generation time of 19 days (typical) would increase a mite population by a factor of 128 in 7 reproductive cycles (about 133 days). Moreover, this is in worker brood only.

RECOMMENDATIONS

Duration of field test

The duration of the evaluation period should be at least 60 days and probably no longer than 110 days. Advantages of a short period of evaluation are (1) there is less time for beekeeping variables (queen supersedure, drone production, robbing of weak colonies, swarming, etc.) to enter the experiment, (2) colonies remain small and manageable, and (3) one can avoid seasons when brood production and thus mite reproduction is vari-

able.

A period of 44 days (test in Table 4) is too short. For in a colony that begins with no brood, the first complete reproductive cycle of mites involves brood of the test queen, but not her adult progeny. Since it takes 20 days for progeny of the test queen to begin emerging as adult workers and 2-3 weeks more before these bees constitute a majority of the worker population, about 40 days must pass before one can fully evaluate resistance in the progeny of a test queen.

In Louisiana, a 70-day field test could be done anytime between 1 Feb. and 1 August and possibly between 20 Aug. and 31 Oct. In short, avoid periods of broodlessness or greatly diminished brood production.

Biological evaluation

Certain biological evaluations (such as those reviewed by Büchler 1994) can and should be included in the context of a field test. In this way, specific characteristics of the bees (such as hygienic behavior, grooming behavior or duration of capped period) can be correlated with one of the factors in the field test. Many of these tests involve the physiology or behavior of worker bees, and in those evaluations, the tests should be done after the worker population has changed to consist of progeny of the test queen (at least 40 days after the start of the experiment). If a cause and effect relationship can be established between a biological characteristic and a change in the mite population, selective breeding can be done by measuring only the specific biological characteristic.

Modifications

The evaluation technique can be modified. Some of the procedures may be too cumbersome for one who may simply want to screen queens for possible resistance to varroa mites. To simplify the field evaluation, one can cage each queen about 20 days before the end of the test. By doing this, there is no brood to evaluate at the end of the experiment, so the final mite population is entirely on the adult bees. Of course, by not measuring mites in brood, one cannot estimate the length of the mite's reproductive cycle, but as long as there is sufficient time, the queens could be released and the mite activity can be given a more complete analysis.

Selection

This paper focuses on an evaluation system that enables one to evaluate a colony before mites can destroy it. Therefore, there is no need to allow colonies to die in the name of selecting for mite resistance.

Queens for test colonies can be each instrumentally inseminated with a single drone, inseminated with two or more drones, or allowed to mate naturally. The latter will probably result in slower progress, but Hydak & Tanquary (1940) demonstrated that selection can be effective when queens are allowed to mate naturally.

One possible strategy in selection is to isolate three characteristics of bees that cause resistance at each of the three areas (reproduction, survival, and the length of the reproductive cycle) and then combine these characteristics into a single stock.

Objectives

My immediate objective is to develop stocks of honey bees that limit mites to an *rs* value equal to or less than 1 (zero population growth for mites) when reproducing in worker brood. I think that this can be done. Moreover, I think that many people have described such colonies. In some cases, perhaps mites reproducing in drone brood eventually overwhelmed these colonies or queen supersedure changed their genetic composition and caused them to lose their high level of resistance.

My ultimate goal is for bee breeders to establish selection procedures wherein mite resistance can be selected and maintained at many locations. I suspect that resistance to varroa mites is widespread and that resistant stock can be assembled from bees in numerous places throughout the world. If many people develop stocks with mite resistance, we can retain much of the genetic variability in our honey bee population as we gradually reduce our dependence on chemicals to control varroa.

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