Recapping and mite removal behaviour in Cuba: home to the world's largest population of Varroa-resistant European honeybees

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Article

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Abstract

The *Varroa destructor* ectoparasitic mite spread globally and in conjunction with Deformed Wing Virus killed millions of honeybees (*Apis mellifera*) colonies. Forcing Northern hemisphere beekeepers into using miticides to avoid mass colony losses. However, in many Southern hemisphere countries widespread treatment did not occur since miticides where prohibitively expensive or a centralised choice was made not to treat, both allowing natural selection to act. This Varroa caused initial high losses before mite-resistant appeared in the honeybee populations. Initially, mite-resistance was only associated with African and Africanised honeybees. Although recently, several isolated mite-resistant European honeybee populations have appeared. Here we studied the mite-resistance in Cuba and found high rates (77%) of recapping of infested worker cells, high removal of mites (80%) and corresponding low mite fertility (*r*=.77). These are all traits found in all naturally evolved Varroa-resistant populations. We can confirm Cuba has the world's largest European mite-resistant population with 220,000 colonies that have been treatment-free for over two decades. Cuban honeybees are also highly productive, 40-70 kg of honey produced annually, and are mild mannered. Cuba has many queen rearing stations and is excellently positioned to export Varroa resistant European queens throughout the region and beyond.

Introduction

During the past 70 years the Varroa mite (*Varroa destructor)*, in association with Deformed Wing Virus (DWV) that it transmits, has spread world-wide, killing millions of *Apis mellifera* colonies, particularly in the Northern Hemisphere. This resulted in the almost universal uptake of miticides in the Northern Hemisphere to control Varroa populations. However, in some Southern Hemisphere countries the beekeepers either could not afford to treat or their central beekeeping organisation decided not to treat when Varroa arrived but allowed natural selection to run its course. Initially, these countries suffered high losses, but these losses declined after several years as honeybees adapted to the mite and became Varroa-resistant. On a much smaller scale a growing number of UK, European and USA beekeepers are taking a similar approach of ceasing treatment or collecting resistant feral colonies. A recent study[1] found that all well-established resistant populations, despite being widely dispersed across several continents, all share the same key traits which all arise from the ability of the workers in resistant colonies to detect mite-infested cells using chemical signals produced by the mite offspring[2]. This leads to increased rates of recapping and removal of infested cells; this reduces the mites' ability to reproduce and promotes increased mite infertility. Subsequently, there is a long-term decrease of mite populations and viral loads whilst there is an increase in colony survival rates[1]. The role of elevated recapping rates, consistently found in self-selected honeybee populations [3,4], is currently the best 'proxy' for a resistant population.

The largest Caribbean Island is Cuba, being 1250 km long, covering 109,884 km^2 and currently contains over 220,000 managed colonies. In the 1950's Cuban colonies were estimated at between 100,000-150,000 when Eva Crane visited Cuba in 1957[5]. In 1968 managed colonies were censed and this revealed

151,000 hives. During the 1970's and 1980's beekeeping contained to grow with State support and investments to 208,000 colonies in 1985. After this numbers fluctuated and fell to a minimum of 126,000 colonies in 2003 due to the Varroa and an economic crisis. Since then, colony numbers have steady increased reaching 221,000 colonies in 2021. All the managed colonies are currently kept by 1,900 government registered beekeepers that have always selected for productivity, hygienic behaviour and calmness, under the Centro de Investigaciones Apícolas (CIAPI) Bees Selection Program. As a result, Cuban bees are highly productive: annually averaging 45–70 Kg of honey per colony according to honey production records held by CIAPI, 80% hygienic behaviour based on removal of dead brood. In addition, a large unmanaged feral honeybee population exists due to expansive regions of flowers and the Cuban Royal palm (*Roystonea regia*) forests that cover around 25% of Cuba.

Honeybees (*A. m. mellifera*) was first introduced into Cuba from the USA (Florida) in 1768, followed by later by *A. m. ligustica*, *A. m. caucasica* and *A. m. carnica*[6]. Despite the presence of Africanized honeybees in some but not all surrounding Caribbean Islands[7], a honeybee import ban for the last 60 years has allowed this large European population to thrive in Cuba. Studies using allozyme markers[8, 9] confirmed that the Cuban honeybee population was still European, which was confirmed by finding that most mitochondrial haplotypes belong to European lineages (eg M, and C)[10]. Furthermore, this microsatellite data found Cuba has a homogeneous population of honeybees across the country, confirming the isolated nature of the population.

Despite the 60-year honeybee import ban, in 1996 Varroa was first detected in Matanzas province in Western Cuba and further investigations found the mite in and around La Habana city. The mite was predicted to have entered Cuba a couple of years earlier[11], potentially via shipping or illegal. queen imports. In 1997 around 8000 colonies died, all infested with Varroa, and by 1998 the mite had spread to the seven western provinces. Movement of bees between major regions was prohibited, some mite control via drone-brood trapping was used but more losses were suffered before Varroa resistant bees appeared, several years after the mite invaded Cuba. Thereafter, no mite treatments have been administered for over 20 years[12] making the Cuban population the largest Varroa-resistant European honeybee population in the world.

Previous research [13] found DWV is in 100% of apiaries and only the Korean haplotype of Varroa was found in Cuba [12]. Therefore, the situation in Cuba, with respect to the bees, mites, and virus, is similar to that found across the Northern hemisphere. Therefore, the aim of this study was to investigate if the traits found in other resistant populations from other countries i.e., increased recapping and mite removal along with reduced ability of Varroa to reproduce, were present in the worker brood of the Cuban honeybee population. In addition, recapping and mite reproduction was measured in drone brood.

Results

Honeybee data:

In December 2021 a total of 6923 worker and 1906 drone brood cells were investigated from the 32 and 16 colonies respectively that were managed in six locations across western Cuba (see methods Fig. 5). The average Varroa-infestation rate of worker (13% SD ± 10%) and drone (40% ± SD 18%) sealed brood was significantly different (d.f.= $_{[15, 31]}$ t = 6.8909 p = .00001), as expected.

Recapping data:

To provide baseline data, 3090 worker brood cells from five Varroa-naïve colonies from Kauai, Hawaii had a mean recapping rate of 3.6% (± 4.2 SD) and median of 1.3% due a single outlier colony in which the recapping rate was 10.7%. In comparison, the average recapping rate in Cuba for infested worker brood was 72% (± 21SD), while non-infested cells were recapped 33% (± 33SD), which again are significantly different (df $_{30,24}$ t = -6.9304, p = .00001). Whereas, in the 1906 drone brood the recapping levels of 52% (infested) and 34% (non-infested) were not significantly different (df $_{10,10}$ t = -1.7208, p = .101) due to the high level of inter-colony variably (Fig. 1).

The weighted average recapping rate of infested worker brood for all previous studies is 55% in mite resistant colonies[1]. Whereas the weighted average for all the Cuban colonies was 63%. Hence, each of the six locations in Cuba can be classed as highly resistant since all six locations were above the average and median of all previous studies (Fig. 2).

The diameter of the recapped hole is significantly smaller in the baseline Varroa-naïve Kauai colonies than Cuban worker cells non-infested colonies. Whereas recapped sizes in infested cells were significantly larger than non-infested worker brood, although there was no significant difference in recapping size between infested and non-infested drone brood (Fig. 3)

The average colony recapped size in of Varroa-naïve worker cells, Kauai were significantly smaller than non-infested Cuban worker sealed brood (d.f [4, 30] t = 2.981, p = .0054), whereas there was a significant larger recapped area in infested than non-infested cells ($df_{24, 29}$ t = 5.53239, p = .00001) in Cuban worker brood. However, in drone sealed brood, no significant difference in recapped size ($df_{10,10}$ t = -0.8728, p = .196) was found between non-infested and infested cells.

Mite removal:

In March 2022 a total of 200 mites and 200 control sham openings were performed on worker brood from ten colonies in the CIAPI apiary. There were significantly more artificially mite infested cells removed than sham control openings (df $_{9,9}$ t= -3.5135, p = .00248). In fact, over 35% more (Fig. 4.), since 81% of the mite-infested cells were removed while 45% of the controls were removed. Of the 38 mite-infested not removal 36 (95%) were recapped, while of the 111 control cells not removed 80 (72%) were recapped. The removal rates of the mites were consistent (except one outlier) across the ten colonies, the removal of control cells was highly variable (Fig. 4). In 14 (7%) cells the mite was missing although since they all had been recapped, they probably escaped during the period the cell was open. A total of 26 mites (23%) were found in the remaining 111 control cells at the end of the experiment.

Varroa mite data:

Data on mite-reproduction was collected from 688 worker and 350 drone single infested sealed brood cells that aged from white-eye (85 hours post-capping) to the resting stage. Of these, 195 workers and 57 drones were grey pad (240 hours post-capping) or older. In addition, 31 worker and 46 drones infested by two or more mites and were grey pad (300 hours post-capping in drones) to resting stage were used of reproductive success calculations. Overall an average of 0.77 new viable (mated) offspring were produced in worker cells and 1.6 in worker cells (Table 1).

Table 1
Various Varroa reproductive classifications in Cuban worker and drone sealed brood cells. The minimum pupal age category is also given. Pale eyes (po) ≈ 100 hrs in workers and 120 hrs in drone. Grey pads (gp) ≈ 240 hrs in workers and 300 hrs in drones.

Classification	worker	drones
Viable mothers > po	72%	71%
Non-repo mothers > po	8%	11%
Viable offspring > gp live male + S	51%	65%
Viable offspring > gp in multple-infested cells	32%	49%
Total fertility in all cells > gp	47%	53%
Reproductive rate > gp single infested cells	0.87	1.96
Reproductive rate > gp multiple infested cells	0.49	1.42
Total Reproductive rate in all cells > gp	0.77	1.6

The number of viable offspring produced in singly infested worker cells aged 240 hours post-capping or older in recapped (mean = .88) or non-recapped (mean = .85) cells were not significantly different ($t_{133, 60}$ =0.18276, p = 0.855). The result is the same when all infested worker cells (single and multiple) are considered ($t_{157, 67}$ =0.71157, p = 0.477), indicating recapping *per se* has not direct effect on mite mortality.

Discussion

We confirmed at Cuba is home to the world's largest European honeybee population that has become Varroa-resistant, with an estimated 220,000 colonies maintained without treatment for over two decades [12], despite the presence of the K-haplotype of the mite[13] and the widespread occurrence of DWV [12]. Hence, the Cuban honeybee population is the first major case of Varroa-resistant European bees occupying an entire country of a large size (109,884 km^2). Although, an increasing number of varroa-resistant European honeybee populations are occurring throughout the Northern hemisphere[15] they still consist of small, isolated populations within a country. For example, the second largest known area of European Varroa-resistant honeybees is in North Wales, UK where 104 beekeepers manage around 500 honeybees over an area of 2500 km^2 without treatment for over a decade[16].

It has long been established those sub-Sharan African and Africanised honeybees are Varroa-resistant and both populations cover much larger areas than Cuba, but these honeybee races are not capable of thriving in temperate regions or are rejected by beekeepers in Northern hemispheres. However, both African/Africanised and European honeybees all appear to have evolved the same resistance mechanism[1] and Cuban honeybees follow this pattern showing high recapping behaviour and mite removal behaviour (Figs. 1, 4).

The strongest evidence that increased recapping behaviour is a direct response to the presence of Varroa is the very low recapping rates in Varroa-naïve colonies. This is evidenced by the recapping baseline data that has now been collected from four different Varroa-naïve (Varroa free) honeybee populations (Australia, UK [2 populations] and Hawaii [this study]) all producing similar results (Fig. 1). Across the four populations, a total of 9542 worker cells from 15 colonies have been studied with an average recapping rate of 2.0% (+ SD 3.2). Interestingly, only two of the colonies had atypical recapping rates of 8.5% and 10.7% from Australia and Kauai respectively. This may suggest increased sensitivity in these colonies as no obvious causes e.g., wax moth or dead pupa were detected in either colony. The data summary in Fig. 1 indicates that even in Varroa-treated populations the workers are still able to detect mite infested cells, but the average consistently falls significantly below that found in resistant populations. That is, in non-infested worker cells recapping rates are significantly higher in resistant populations in comparison to susceptible populations (Fig. 1) $t_{4, 5}$ =-4.185, p = .0023 as well as for infested cells $t_{4, 5}$ =-6.905, p = .00007.

The ability of Cuban honeybees to detect infested cells causes high recapping levels but also high removal rates of artificially mite-infested cells. A mean removal rate of 81% is among one of the highest recorded in *Apis mellifera* [1]. The average control rate of 45% is driven by three colonies that all removed more than 75% of the controls, while the average of the remaining seven colonies was 28%. During the mite-removal studies in March 2022 natural Varroa infestation were 23% above the 13% recorded in the previous December 2021. This is due to decreasing worker brood rearing caused by a shortage of nectar during the annual dry season. During this time an increase in hygienic behaviour in the colonies[17], which could help explain the higher-than-expected removal of control cells.

The reproductive ability of Varroa to produce viable i.e., mated, female offspring (r) in infested worker cells in resistant colonies in South Africa [4] (r = 0.9), Brazil [4] (r = 0.8), Mexico [18] (r = 0.73), Europe[3] (r = 0.84) are all similar to the 0.87 found in Cuba (this study). In Cuba 'r' reduces to 0.77 when both single and multiple infested cells are considered. This reduction in mite reproduction, relative to susceptible colonies that have values of r greater than one, it directly linked to the increased ability of resistant workers to both detect and remove, by cannibalisation, the infested pupa. Hence, this ensures the invading mite fails to reproduce [1] or reducing mite fertility but to the recapping process [3]. Although, in this study no significant difference was found in the reproduction of Varroa in recapped or non-recapped cells supporting the findings of two previous studies [4, 14]. Therefore, recapping may be playing a minor role in resistance. However, recapping remains the best indicator or 'proxy' of resistance within a honeybee population since it's easier, quicker, and it requires less skill to measure recapping rates than mite removal rates.

Despite the current focus on what is happening in worker cells, studies focusing on the role of recapping in drone brood are still in their infancy with and currently, data is only available from South Africa [4] (Fig. 1) and now Cuba (this study). Interestingly, both studies indicated no significant difference in recapping rates between infested and non-infested brood. This is caused by some colonies performing no recapping of drone brood, while some colonies do recap cells but in a non-targeted manner. Whereas there is a significant increase in the size of the recapped area between infested (3.1mm) and non-infested (2.3 mm) in worker cells (Fig. 3). This does not occur in drone brood, as it appears the holes are entirely exploratory. However, the lack of removal of infested drone brood may be playing an important role in mite-resistance (see below).

The mite infestation of worker cells is currently various between 23 – 13% in Cuba (this study), roughly 25 years after it was first detected (1996). Whereas, in Mexico and Brazil infestation rates of worker brood have fallen from around 20% in 1996/1999 down to 4% in 2018/19. Although, Varroa was first detected in Brazil much earlier, in 1972 [19] and the Africanised honeybees adapted to the mite and spread northward replacing the susceptible European colonies. Therefore, we predict that the worker infestation rate in Cuba will continue to fall to reach over the next 20 years, especially if high mite-removal rates persist. Correspondingly, we would expect to see the infestation rates of the drone brood currently at 40% to remain high as mites potentially avoid reproduction in worker cells. This potentially is a key but currently overlooked part of the resistance mechanism. Since an empirical model [18] indicated that negative mite population growth occurs in (resistant) Africanised honeybee colonies only when the initial drone cells are present. This is thought to arise because mites also show a tenfold preference to reproduce in drone cells (which comprises only 1–5% of all the honeybee brood) and they soon become overcrowded as the mite population increases. This leads to inter-mite competition for the limited food and space, causing an increase in mite mortality [20], resulting in negative reproductive success for mites entering these overcrowded drone cells. Thus, mite population growth in drone brood cells is limited by a density-dependent mechanism. In Cuba it has been observed that strong colonies typically with drone brood do not weaken during the drought season, whereas colonies without drone brood and weak often die during the drought (APP personal comm).

Although Cuban beekeepers have been aware of their mite-resistant honeybees for decades, Cuba's situation has only recently come to light [12,10]. The main reason for Varroa-resistance in Cuba is due to the centralised decision to allow natural resistance to evolve, as also was done successfully in South Africa[21], rather than becoming locked into using miticides as has happened throughout the Northern hemisphere. The CIAPI and Veterinarian Services central decision to 'not treat' was greatly assisted by all

Cuban beekeepers being professional, reregistered and embedded within a strong locally based beekeeping community where colony movement and exchange of queens is within each province.

There is also a large feral population and due to Cuban's sub-tropical climate, queens are replaced annually in managed colonies because of almost continuous egg-laying, similar to honeybees in Hawaii. This rapid queen turnover speeds up natural selection relative to honeybee populations in more temperate climates. Finally, Cuba's 60-year ban on honeybee importation has helped isolate the country from been invaded by Africanized bees which has occurred in many nearby regions (eg. Mexico, Southern USA, Puerto Rico, neighbouring Dominican Republic[7] and Haiti (D. Macdonald, Apiary Inspector, Min. of Agi BC, Canada, pers. Comm.). Cuba has many managed European colonies coupled with many queen rearing stations. These colonies are productive and mild mannered. Thus, Cuba would be in an excellent position to become a vital export site for Varroa resistant European queens throughout the region and beyond if the external political pressures on the island ease.

Methods & Materials

Recapping Study

A total of 37 colonies were sampled in December 2021 from six locations spread across 250 km of Western Cuba (Fig. 5). From each colony a patch of sealed worker or drone brood, if present, containing approximately 300 cells was cut out of the frame. In the laboratory using binocular microscopes and ring lights we investigated if each cell had been recapped, by carefully inverting the cell cap and estimating the size of the recapping on a scale from 1–5, which in worker brood equates roughly to a 1–5 mm scale. The age of the pupae was recorded using changes in eye or body colour [22] and if infested each of the mite stages were recorded[22]. Mite exuviate indicated the presence of an adult male or female and was important in determining multiple invaded cells and the number of mated female offspring. No baseline data can be collected in Cuba since no Varroa-free regions exist. Therefore, baseline recapping rates were collected from the Varroa-free colonies from the Island of Kauai (Hawaii) since it lies at a similar latitude to Cuba, has a similar sub-tropical climate and is home to European honeybees. From each of five Kauai colonies around 600 worker brood cells per colony were studied for recapping rate and size of recapped area estimated on the 1–5 scale.

Mite Removal Study

The removal rates of ten colonies from CIAPI (Fig. 5, location 3) were studied during March 2022. For each colony 20 mites collected from sealed drone brood within 2–3 days of capping and the mother mites individually placed used a fine paint brush into 20 worker sealed brood cells that were within 1–2 days of been sealed over. In addition, 20 control sham openings were also conducted. The positions of all the manipulated cells were recorded on an acetate sheet. The logic behind using mites from young drone brood is there will have already received the stimulus to start egg laying [23] since the mite-offspring produce the compounds detected by the worker honeybees [2]. Eight days after the mites were inserted the ten frames were removed and the number of infested and control cells removed were recorded, along with the recapping levels of unremoved cells.

Data Analysis

For each colony we measured the recapping rate and hole size of infested and non-infested cells in all cells older than 85 hours post-capping (white eye stage), the brood infestation rate using all pupae. These values were then standardized by calculating percentages. However, if fewer than 5 mite-infested cells were present in a colony then the data on infested recapped cells and size of recapped were excluded, to avoid the effects of a small sample size. All data was then averaged across all colonies and values compared to previous studies. As the resulting recapping and mite-removal data were all normally distributed (Kolmogorov-Smirnov Test of Normality), parametric statistics are used throughout.

To ensure a sufficient sample size we pooled the mite data from all the colonies knowing that all Cuban honeybees are considered genetically similar [10]. Both single and multiple infested worker or drone cells reproductive values were calculated separately to allow direct comparisons with previous studies. The mite development Figs. 2[4, 25] were used to check if the development timings of Varroa found in Cuba are similar or not to that found previously.

Declarations

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Author contributions (names must be given as initials)

SJM and APP devised the study, all authors collected samples, IG, GW and SJM conducted the recapping study, ARL conducted the mite removal experiment, SJM and ARL analysed the data and drafted the manuscript with input from APP, all authors were involved in comments and improving the manuscript.

Data availability statement

All raw data will be available on request from the corresponding author.

Disclosure statement

The author declare that they have no potential conflict of interest in relation to the study in this paper.

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Figures

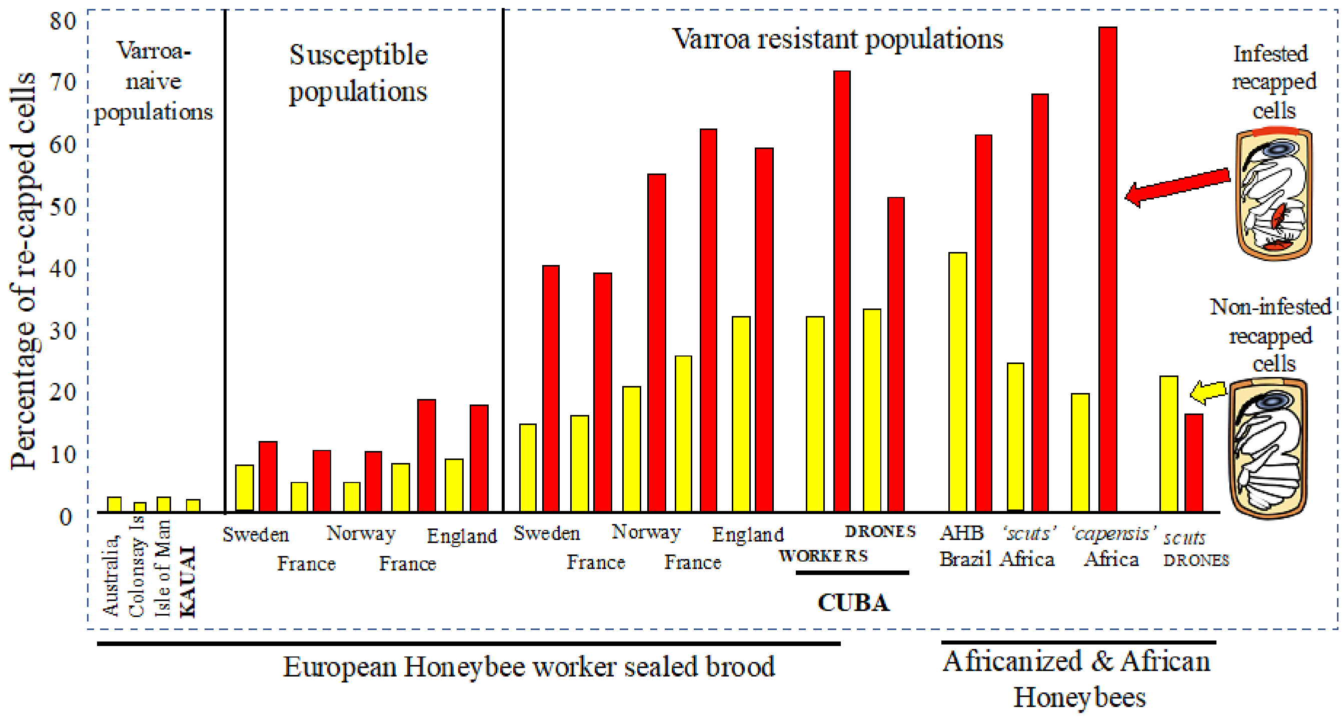

Figure 1

Recapping rates from both worker and drone showing levels in non-infested (yellow) or infested (red) cells from this (in bold) and previous studies[3,4,14].

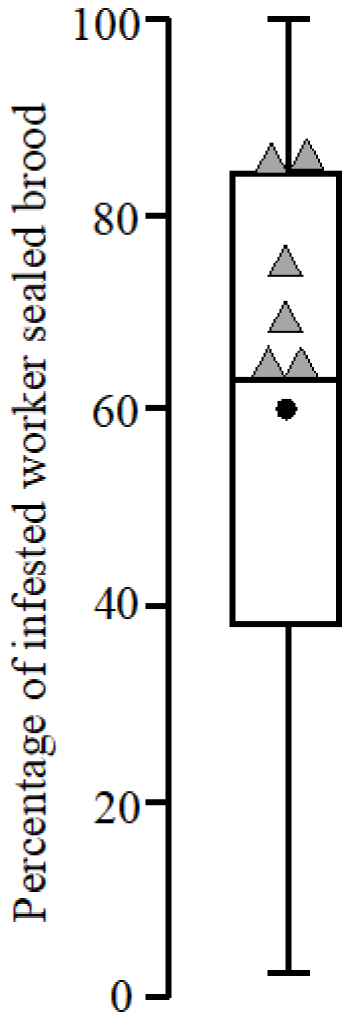

Figure 2

A comparison of the percentage of recapping of infested cells from the six Cuban locations (triangles) in comparison with the 101 data points from all other resistant *A. mellifera* colonies studied around the world according to[1] shown as a box plot.

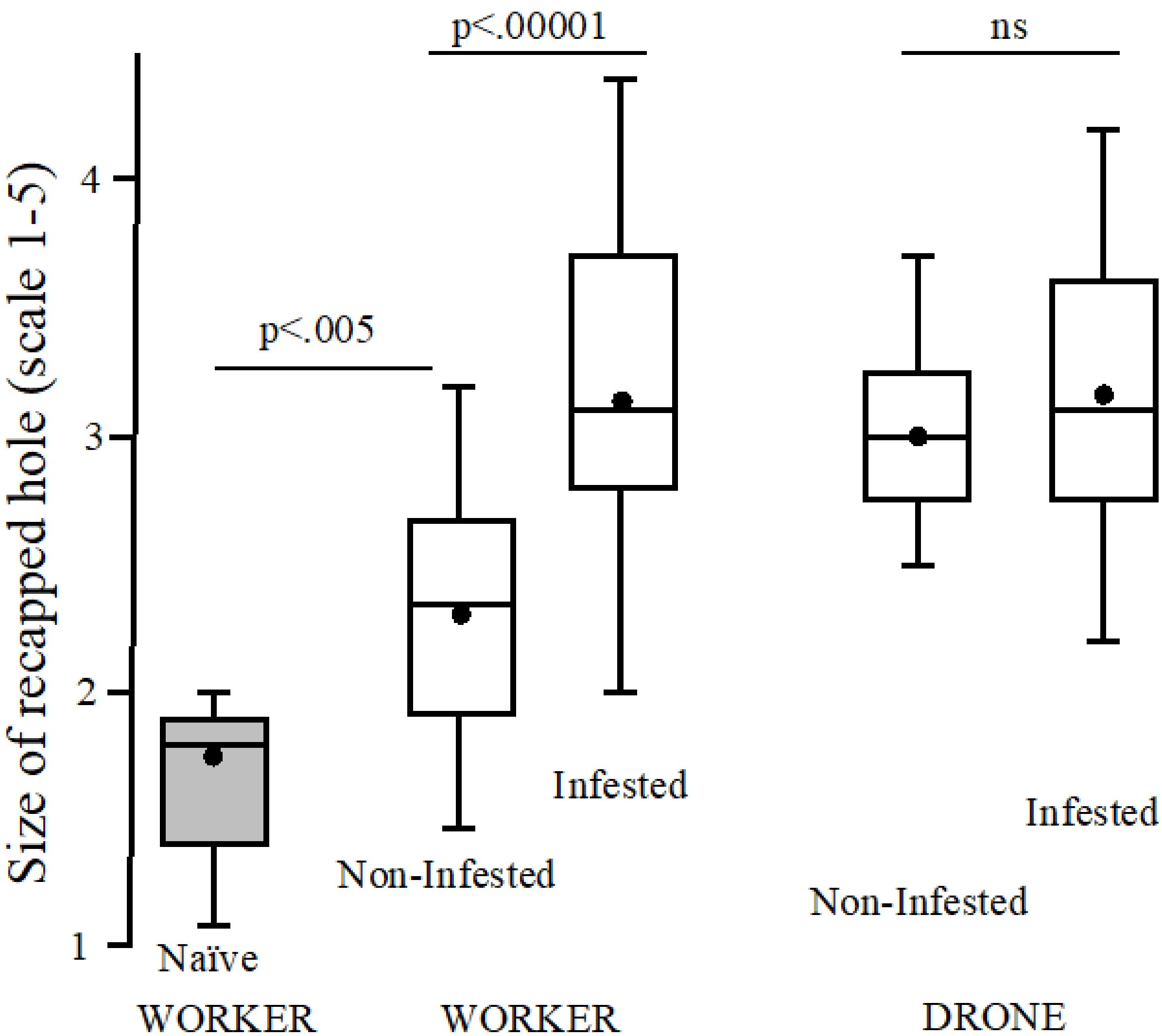

Figure 3

Size of recapping holes in Varroa-naïve worker cells (Kauai) compared with Cuban workers and drones sealed brood that were infested or not.

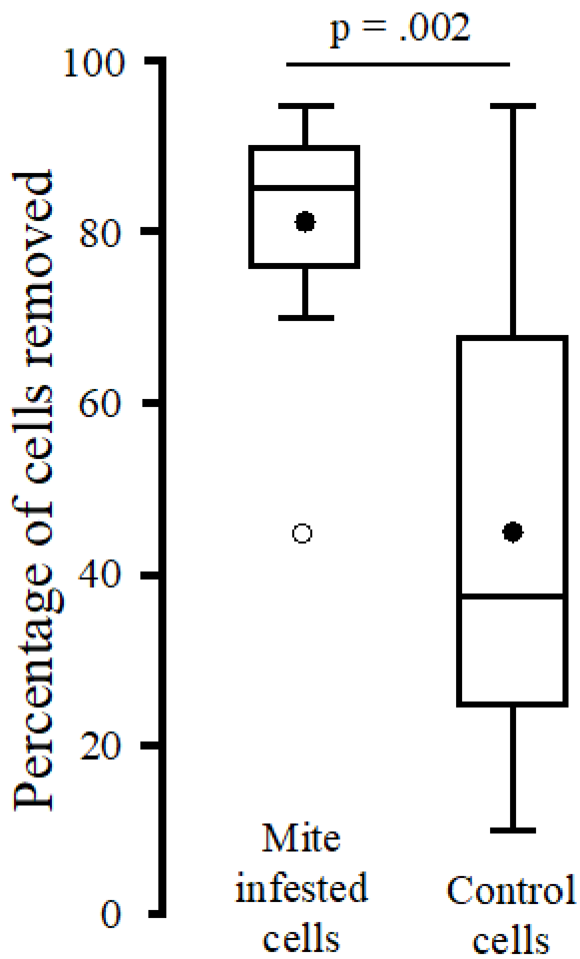

Figure 4

Percentage of removal in mite infested and control cells indicating the difference between the two groups and shows a large variation among the ten control colonies, relative to the mite infested cells.

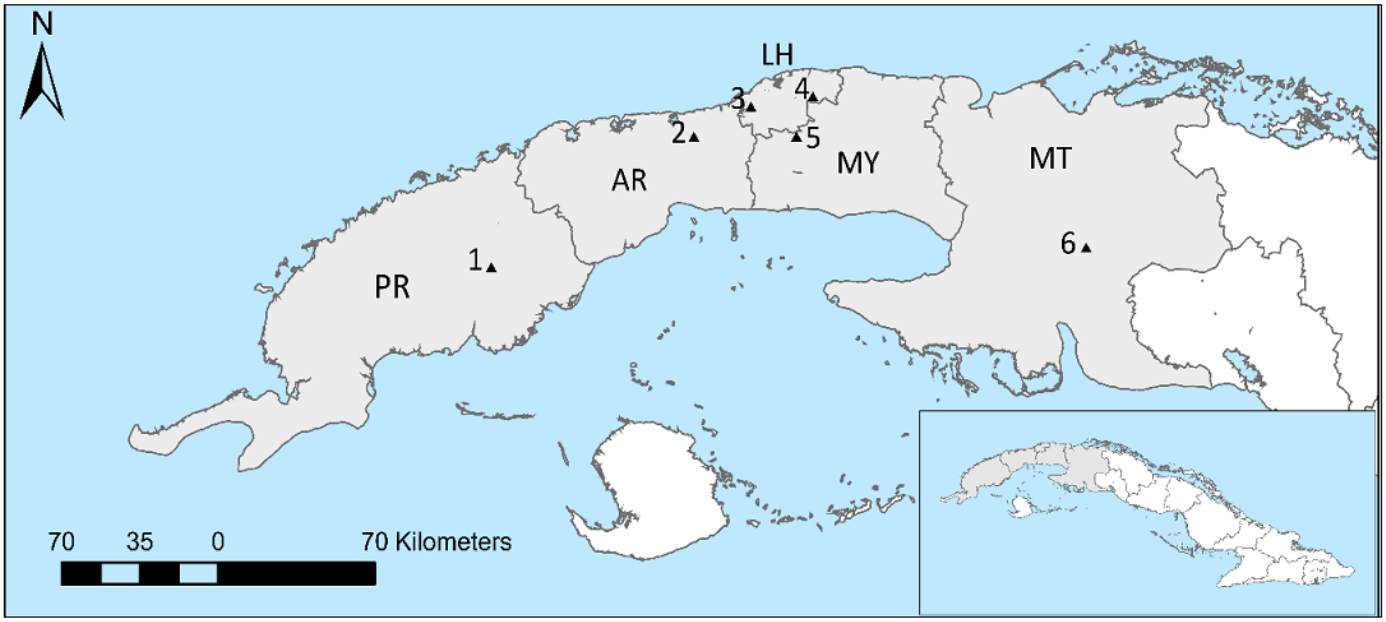

Figure 5

Black triangles represent the approximate locations of the six sampling apiaries in Western Cuba. Letters indicate the name of the provinces: Pinar del Rio (PR), Artemisa (AR), La Habana (LH), Mayabeque (MY), Matanzas (MT)