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Evaluating Mating Control of Honey Bee Queens in an Africanize Area of Guatemala

Richard L. Hellmich II
United States Department of Agriculture, richard.hellmich@ars.usda.gov

Jorge Ibarra
MOSCAMED

Manuel Mejia
United States Department of Agriculture

Thomas E. Rinderer
United States Department of Agriculture

Nicholas A. Gutierrez
APHIS

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Evaluating Mating Control of Honey Bee Queens in an Africanized Area of Guatemala

by RICHARD L. HELLMICH II[1,5], JORGE IBARRA [3], MANUEL MEJIA [2], THOMAS E. RINDERER [2] and NICHOLAS A. GUTIERREZ [4]

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SUMMARY

Tests using mark-recapture of cordovan drones show that beekeepers in southwest Guatemala can control at least 70% of the matings of their queens by using drone flooding techniques. When European-derived stocks are used, such control should be sufficient to avoid most of the problems associated with Africanized honey bees. We assume that the beekeepers will monitor their colonies closely and requeen when necessary with marked queens derived from selected or imported breeding stock. With these practices beekeepers in Guatemala should be able to maintain bees more desirable than the highly Africanized bees they had previously.

Additionally, this study demonstrates the effectiveness of the beekeeper cooperative. The cooperative has facilitated beekeeper education and has resulted in increased genetic control, both of which are necessary for controlling Africanization. Beekeepers throughout Latin America should consider using similar cooperatives to reduce problems associated with Africanized honey bees.

INTRODUCTION

A queen breeding project was started in Southwest Guatemala in 1990 to help local beekeepers overcome problems caused by the Africanized honey bee (AHB). Africanized bees arrived in Guatemala in 1985 and rapidly spread through most of the county (DIGESEPE 1985). Problems similar to those in Venezuela (Hellmich & Rinderer 1991) and other Africanized countries (Spivak 1991) developed as these bees became established. By 1990 Guatemalan beekeepers had no reliable sources of European queens, and imported queens were considered by most beekeepers to be too expensive to purchase. Thus, this project was started in response to a need for quality queens. This was a cooperative effort funded by AID and APHIS and conducted by ARS and MOSCAMED scientists. The primary objective of the project was to determine if it was economically possible to produce quality naturally-mated queens in an area with AHB.

This project was inspired by a study conducted in Texas with two commercial queen producers (Hellmich & Waller 1990). The Texas study suggested that many U.S. queen producers could control 90% or more of their queens' matings through simple drone saturation procedures. These results were encouraging for U.S. queen producers who were looking for ways to avoid problems with Africanized honey bees. But the question still remains whether or not high levels of mating control are possible in Africanized areas. The Guatemalan project focused on answering this question while helping beekeepers with modest resources mitigate difficulties following Africanization.

Most U.S. queen producers have access to hundreds of colonies that can be used for producing drones. Few of the Guatemalan beekeepers, however, have more than 25 colonies. We believed that individually they had little chance of producing quality queens through drone saturation. We predicted that by working together, however, they could influence the drone population in a designated zone, and thus produce quality queens. Consequently, the second objective of the study was to determine if beekeeper cooperatives can be effective in reducing AHB problems.

In this study mating control was measured in two areas. The first was at a cooperative control zone which had 39 apiaries with 1178 colonies; the second was on a farm which had 10 apiaries and 313 colonies.

MATERIALS AND METHODS

Measuring mating control:

The effective population of drones within range of queen mating yards was estimated with a measure called Drone Equivalents (DE) (for more details see Hellmich & Waller 1990 or Hellmich 1991). The method resembles mark-and-recapture procedures commonly used by biologists. To measure DE, a single-gene recessive trait for light-brown cuticle called cordovan functions as the marker. Cordovan queens are mated from an apiary that has a known number of cordovan drones. "Recapture" occurs when a queen mates with a cordovan drone; such mating is detected by evaluating the worker progeny of the queen. Cordovan workers result from cordovan queens mating with cordovan drones; darker wild-type workers result from cordovan queens mating with wild-type drones. Cordovan test queens usually produce both cordovan and wild-type drones because most of them mate with several drones. The DE is calculated from:

$$\mathrm{DE} = \frac{\%\ \text{wild-type progeny}}{\%\ \text{cordovan progeny}} * \begin{array}{c}\#\ \text{cordovan drones}\\ \text{near mating apiary}\end{array}$$

In a commercial setting the DE determination does not distinguish between feral and managed wild-type drones. This means that a queen producer cannot evaluate mating control because the feral baseline is unknown. An estimate of the feral drone population in terms of Drone Equivalents (DE_{feral}) would solve this problem. Thus two types of DE determinations are necessary:

1) DE_{total} - conducted in mating apiaries within mating range

[1] Department of Zoology & Genetics, Iowa State University, Ames, Iowa 50011

[2] USDA-ARS Honey Bee Breeding, Genetics & Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, Louisiana 70828

[3] MOSCAMED, Oficina 602, 6 Calle 1-36, Zona 10, Guatemala C.A.

[4] APHIS, Oficina 404, 6 Calle 1-36, Zona 10, Guatemala C.A.

[5] To whom correspondence should be addressed. Research conducted while working for USDA-ARS.

of both managed and feral drones.

2) DE_{feral} - conducted in remote apiaries within mating range of only feral drones except for the test cordovan drones. Remote apiaries are located 8-10 km from managed colonies in areas that have terrain and flora similar to those near the normal mating apiary. Only mating colonies with cordovan virgin queens and drone source colonies that produce only cordovan drones are present in a remote apiary.

The DE_{feral} establishes a baseline of feral drone influence. A measure of the influence of managed drones is estimated by removing the influence of feral drones. In terms of DE this is calculated from:

$$DE_{managed} = DE_{total} - DE_{feral}$$

The mating control percentage then is calculated from:

$$\% \text{ control} = DE_{managed} / DE_{total} * 100$$

Experimental Design:

These experiments were conducted from November 1990 through March 1991 in a predominately Africanized area near the southwest coast of Guatemala adjacent to the Mexican border in the Department of San Marcos (Fig. 1). This area is a very humid, warm, subtropical forest (Holdridge classification in

Figure 1. The study sites Catarina, San Antonio and Mundo Nuevo were located in southwest Guatemala near the border with Mexico.

García 1987) in the foothills southwest of a chain of volcanos that crosses the country from west to east. Rubber (Hevea brasilienisis) and coffee (Coffea arabica) are principal crops grown in the region.

The queen production program with the San Antonio beekeeper cooperative (COOPRAM R.L.) was conducted near the village of San Antonio Las Flores (Fig 1). In addition to the mating apiary, 34 cooperative members had 1178 colonies which were located in 39 apiaries (Figure 2). The second experimental site was located near Malacatan on the Mundo Nuevo farm (Figure 1). Two beekeepers managed 10 apiaries with 313 colonies on this farm. One apiary was used for mating queens (Figure 3).

The remote apiary was located near Catarina (Figure 1) which was 8 km from the nearest managed colony. Terrain and flora in this area were similar to those in San Antonio and Mundo Nuevo. Only cordovan drones and queens were present in this apiary. Drone brood and wild-type drones were eliminated from the mating colonies.

All the colonies used in this experiment were either from the apiaries of the San Antonio cooperative, Mundo Nuevo, or MOSCAMED. Local beekeepers propagated test queens, established temporary apiaries, and provided experimental mating and drone source colonies.

Cordovan virgin queens were produced from cordovan breeder queens. (Breeder queens had been instrumentally inseminated with semen from cordovan drones.) Four lines of unrelated breeders were selected to reduce inbreeding problems. Separate lines were used to produce test queens and queens for the drone source colonies.

Drone source queens were mated at the San Antonio and Mundo Nuevo mating apiaries approximately four months prior to the mating experiment. Although these cordovan queens mated randomly, presumably with wild-type drones, they still produced only cordovan drones due to the drone's haploid nature. Progeny samples (100 workers) were taken from 10 of these queens. Only wild-type workers were found which suggests that no cordovan drones were in the area prior to the experiment.

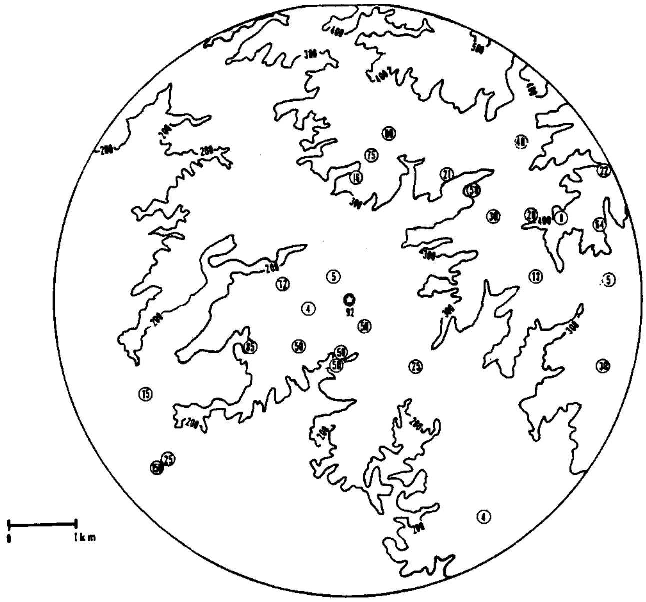

Figure 2. Contour map of the area surrounding the San Antonio mating apiary (star). Number of full sized colonies at each apiary within a 4.5 km radius is indicated in the circles.

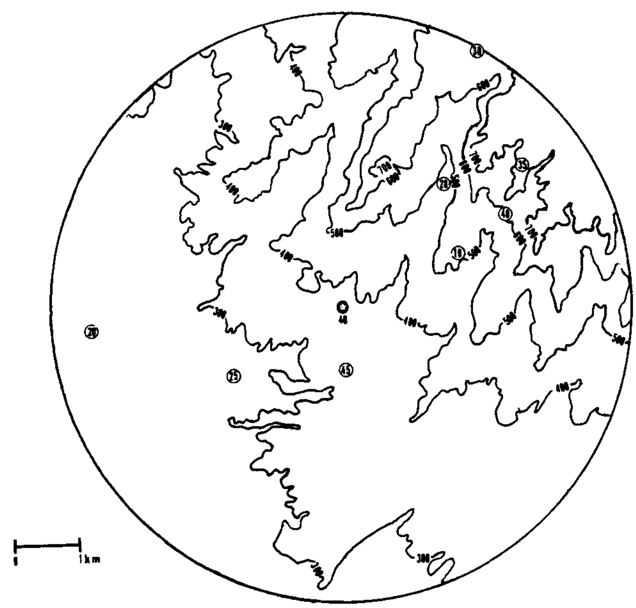

Figure 3. Contour map of the area surrounding the Mundo Nuevo mating apiary (star). Number of full sized colonies at each apiary within a 4.5 km radius is indicated in the circles.

The number of cordovan drones present in each of the mating apiaries was estimated three times at approximately six-week intervals. Each time, a random sample of colonies was chosen for inspection. Drones were counted during a complete colony inspection which occurred prior to drone flight.

Mature queen cells that contained cordovan virgins about to emerge were introduced into 20-30 mating colonies at each apiary. These virgins sampled the drone population when they mated with the cordovan and wild-type drones in the area. Progeny from these queens were collected by placing combs with emerging brood into nylon mesh bags within the hives. Cordovan and wild-type bees were counted among samples of approximately 200 workers.

RESULTS

Feral Baseline Determination:

The average percentage of cordovan progeny produced by the cordovan test queens at the remote apiary Catarina is presented in Table 1. The 4.5% value for the first mating, for example, suggests that queens mated with feral (wild-type) and cordovan drones in about a 21:1 ratio. There were approximately 600 cordovan drones present in the apiary when these queens mated (Table 1). Thus the influence of feral drones on the matings, DE $_{feral}$, was equivalent to approximately 12,700 cordovan drones (21.2 x 600). DE $_{feral}$ determinations from both matings suggest that the average influence feral drones had on the matings at this remote apiary was 9,600 DE ± 4,400 ($\bar{x}$ ± standard deviation; Table 1). We are 95% confident that feral drone influence in this part of Guatamala during February and March fell between 3,500 and 15,700 DE.

Evaluating Mating Apiaries:

The mating apiary at the San Antonio Cooperative had a DE $_{total}$ value of 39,600 ± 12,100 which puts mating control at about 76% when DE $_{feral}$ = 9,600 (95% confidence interval 69 to 80%). The mating apiary at Mundo Nuevo had a DE $_{total}$ value of 31,000 ± 12,400 (Table 1). The mating control estimate for this apiary when DE $_{feral}$=9,600 is approximately 69% (95% confidence interval 56 to 76%).

Mating control changes with the feral population of drones as depicted in Figure 4. This graph shows these changes for the DE $_{total}$ means representing Mundo Nuevo and San Antonio mating apiaries.

DISCUSSION

This study represents the first time mating control of honey bee queens has been measured in an Africanized area, and the results

Table 1

Location, approx. date queens mated	#Cord. test queens	%Cord. Workers	Wild-type/Cord.	Estimated # Cord. Drones in (n) col.	Drone Equivalents (DE)	$\bar{X}$ ± SD
Catarina					**DE $_{feral}$**	
27 FEB	5	4.5	21.2	600 (5)	12,700	
13 MAR	6	8.5	10.8	600 (5)	6,500	9,600 ± 4,400
San Antonio					**DE $_{total}$**	
2 NOV	19	12.4	8.1	4,800 (42)	38,900	
8 NOV	3	15.3	6.5	4,800 (42)	31,200	
28 DEC	6	14.7	6.8	5,000 (42)	34,000	
17 JAN	7	22.1	4.5	5,000 (42)	22,500	
24 JAN	4	11.8	8.5	5,000 (42)	42,500	
25 FEB	7	11.4	8.8	5,500 (42)	48,400	
11 MAR	4	9.3	10.8	5,500 (42)	59,400	39,600 ± 12,100
Mundo Nuevo					**DE $_{total}$**	
19 NOV	8	12.4	8.1	5,100 (42)	41,300	
9 DEC	6	16.9	5.9	5,100 (42)	30,100	
20 DEC	11	14.4	6.9	5,100 (42)	35,200	
17 JAN	10	21.5	4.7	5,300 (42)	24,900	
21 JAN	11	22.9	4.4	5,300 (42)	23,300	
24 JAN	10	10.6	9.4	5,300 (42)	49,800	
25 FEB	4	43.5	2.3	5,300 (42)	12,200	31,000 ±12,400

Table 1. Mean percentage of cordovan workers counted from approximately 200 workers from each of the (n) queens that were mated at various dates in remote apiary in Catarina and established apiaries in Mundo Nuevo and San Antonio. These percentages were used to derive total ratios of wild-type progeny to cordovan progeny. Drone Equivalent (DE) values for the three locations DE $_{feral}$ at Catarina *, DE $_{total}$ at San Antonio and Mundo Nuevo) were calculated by DE = (ratio of wild-type progeny / cordovan progeny) X (estimated # of cordovan drones). Mean DE (± standard deviation) were calculated for the three locations.

***Ratio at Catarina was adjusted (1 was subtracted) to account for cordovan drones that were used in the experiment. This adjustment was not made for San Antonio and Mundo Nuevo because their cordovan drones were considered to be part of the managed drone population.**

are encouraging. Beekeepers were able to influence 70% or more of their queens' matings by pooling colony resources in designated zones. When this project started nearly all the beekeepers in Southwest Guatemala had Africanized colonies that were considered unacceptable. Now there is optimism that quality queens can be produced with drone saturation procedures. Frequent requeening of colonies with quality queens is considered by many scientists and beekeepers to be an essential first step toward reducing problems with AHB.

Drone saturation that produces high levels of mating control, however, is ineffective if drone colonies are not headed by quality queens. Accordingly, beekeepers from the San Antonio cooperative began requeening colonies with European queens from their mating apiary shortly after the project began. (Breeder queens were imported from the U.S.) They first requeened their most defensive colonies, especially those within two km of the mating apiary. Although these queens mated freely, they produced only European drones. Consequently, quality drones were produced within a couple months after the colonies were requeened, and mating control approached the 75% level predicted from this study.

We told the beekeepers that 75% mating control should not be considered an upper limit. It can be improved either by increasing managed drones or by decreasing feral drones. With this in mind the beekeepers have started to give queens derived from European stock to local beekeepers who were not in the cooperative. They also have switched from killing drones in their colonies to encouraging drone production. Many have put the equivalent of one or two drone combs in each requeened colony. At the time of the study drone production was low and very variable. Colony surveys indicated that there were approximately 123 $\pm$ 187 (n=20) drones per colony at San Antonio and 120 $\pm$ 105 (n=25) colonies per colony at Mundo Nuevo. Several of these colonies had no drones. The beekeepers should be able to increase these numbers appreciably with proper colony management. Such efforts would be rewarded. Just a doubling of drone production, for example, theoretically would increase mating control from 76% to 86% at San Antonio and from 69% to 82% at Mundo Nuevo.

The feral population of bees also is influenced by activities of the beekeepers. In this part of Guatemala collecting and selling a swarm is worth approximately ten days labor. The beekeepers from the San Antonio cooperative, for instance, captured several swarms and hived a number of feral colonies during the project. In each case the bees were requeened with queens from the cooperative apiary. Because of a demand for feral bees, we expect that the DE_{feral} in the San Antonio and Mundo Nuevo areas may be lower than the 9,600 value that was found near the remote location, Catarina. (There were no beekeepers near Catarina.) If this is true then the actual mating control would be higher than indicated. For example, if villagers and beekeepers locate, hive and requeen half of the feral colonies near San Antonio, mating control would increase to approximately 88%.

These considerations are optimistic for mating control. We believe that a combination of increasing managed drones and decreasing feral drones in these areas could result in mating control greater than 90%. On the other hand, there probably are areas in Latin America where feral populations of AHB are substantially higher than those that were found in this study. Acceptable levels of mating control might not be possible in such areas.

There are two major challenges to attaining and maintaining high levels of mating control.

The first challenge is to requeen frequently. Requeening in an Africanized area is a task that never ends. Colonies must be requeened regularly to reduce introgression of undesirable genes. Presently, the goal of the cooperative is to requeen colonies every other year. If biannual requeening is insufficient, then cooperative members hope to increase queen production so that colonies can be requeened annually.

The second challenge is to maintain acceptable stock for breeding. We have recommended that they try two strategies;

1) select breeders from existing stock;
2) continue to import breeders.

We have taught the technicians methods to evaluate colonies for selection purposes. They use a patch-dragging test over the brood nest to assay for stinging behavior. (Many variations of this test are possible. We dragged a leather patch over the brood nest for 30 seconds, then later counted the number of stings.) The technicians also select colonies based on honey production. Once a candidate colony is located, it is moved to an evaluation apiary near the mating apiary for further tests before it is considered for breeding. We have recommended that they continue to develop these methods to produce stock suited for their needs and to reduce the need for imported stock. We believe that selection, which could include some genetic materials from Africanized bees, will allow the cooperative to continue to produce quality queens. If necessary these stocks could be augmented occasionally with imported stock.

Success of the queen breeding program at San Antonio perhapcan be measured best by the members of the cooperative. Once these beekeepers were hesitant to requeen colonies, now they are eager to replace their Africanized stock. Every member is pleased with the quality of the queens produced at the cooperative apiary.

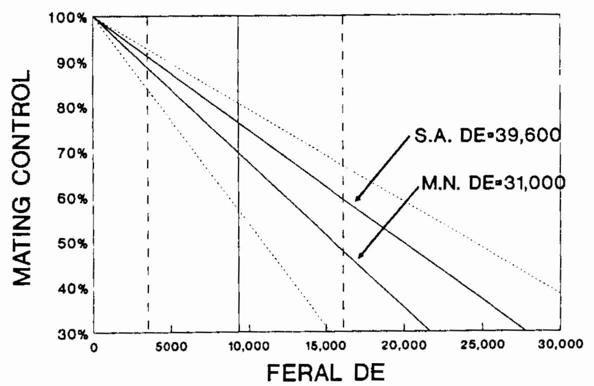

Figure 4. Estimated percentage of mating control for San Antonio (S.A.; average DE_{total} = 39,600 and Mundo Nuevo (M.N.; average DE_{total} = 31,000) apiaries when the feral population of drones (FERAL DE) ranges from 0 to 30,000. The sloping dashed lines represent 95% confidence limits for the two apiaries. The lower 95% confidence limit of San Antonio coincidently is equal to the Mundo Nuevo mean, and the upper 95% confidence limit of Mundo Nuevo is equal to the San Antonio mean. The vertical dashed lines represent the 9,600 average and the 95% confidence limits (3,500 and 15,700 represented by DE feral) which were estimated at the remote apiary. For example, estimated control at the San Antonio apiary when DE_{feral} = 9,600 is approximately 76% and when DE_{feral} = 5,000 is approximately 90%.

We believe that this quality will improve as a higher percentage of cooperative colonies are requeened. Cooperative members take pride in the fact that the gentleness of their queens is known throughout the southwest of Guatemala. Presently there is a higher demand for their queens than they are capable of supplying.

Clearly these results are good news for U.S. beekeepers. The previous study in Texas (Hellmich & Waller 1990) suggested that U.S. queen producers could attain 90-95% mating control without substantially modifying existing practices. These queen producers in Texas achieved these results with European bees through rigorous requeening and drone saturation procedures. This study suggests that similar levels of mating control will be possible in the U.S. when Africanized bees become established in queen production areas. Of course U.S. beekeepers, like the Guatemalan beekeepers, will face challenges to requeen frequently and to maintain breeding stock.

An important lesson that cooperative members taught us is that beekeepers achieve much more by working together than they do by working alone. Besides improving mating control these beekeepers also benefitted by using cooperative resources to educate its members. Because of the success of this cooperative, it is being used as a model for other beekeeper cooperatives in Guatemala that are being started by MOSCAMED, DIGESEPE, and the United Nations. We encourage other beekeepers in Africanized areas to follow their example.

Perhaps U.S. beekeepers also could benefit by working together to maintain quality bees when Africanized honey bees become established.

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REFERENCES

DIGESEPE 1985. Bee Agriculture Section.

García L.E. 1987 (Coordinator). Republica de Guatemala: Perfil Ambiental. Tomo II. Contrato Universidad Rafael Landívar/AID-Guatemala/ROCAP. No. 596-0000-C-00-3060-00. Guatemala City. pp. 46-47

Hellmich, R.L. & T.E. Rinderer. 1991. Beekeeping in Venezuela. In *The "African" honey bee*. Eds. M. Spivak, D.J.C. Fletcher and M. D. Breed. Westview Press, Inc. Boulder, Colorado. pp. 399-411.

Hellmich, R.L. & G.D. Waller 1990. Preparing for Africanized honey bees: Evaluating control in mating apiaries. Am. Bee J. 130:537-542.

Hellmich, R.L. 1991. Continuing commercial production after the arrival of Africanized honey bees. In *The "African" honey bee*. Eds. M. Spivak, D.J.C. Fletcher and M.D. Breed. Westview Press, Inc. Boulder, Colorado. pp. 187-197.

Spivak, M. 1991. The Africanization process in Costa Rica. In *The "African" honey bee*. Eds. M. Spivak, D.J.C. Fletcher and M.D. Breed. Westview Press, Inc. Boulder, Colorado. pp. 137-155.