Project Report 1994

TITLE:	**Africanized Honey Bee Research Project 94-G8**
PRINCIPAL INVESTIGATOR:	**Robert E. Page, Jr.**
COLLABORATORS:	**Robbin Thorp, Kim Fondrk, Greg Hunt**
ADDRESS:	**Department of Entomology University of California Davis, CA 95616**
PHONE:	**(916) 752-5455**

Objectives

Objective 1: develop methods to maintain and produce commercial honey bee stocks that are free from the influence of Africanization

Objective 2: develop programs that will allow selective breeding and stock improvement of resident honey bee populations following Africanization

Objective 3: determine the efficacy of and develop methods for improving the genetic composition of feral honey bee populations following Africanization

Objective 4: develop improved methods for analyzing mitochondrial and nuclear DNA in order to determine the range and degree of Africanization throughout California

Objective 5: develop better breeding techniques including instrumental insemination

Objective 6: develop new apicultural practices for commercial beekeeping

Where are the Bees?

In late October, the first Africanized honey bees were detected in California. These bees were from an established colony located near Blythe. Africanized bees were expected in California in early Spring, but their spread has apparently slowed down. Why? There are at least three hypotheses:

1. **The bees have reached their natural, climatic range**. It is expected that eventually these tropically-adapted Africanized bees will reach a climate for which they are not suited. This has apparently happened in Argentina where their southern expansion halted just south of Buenos Aires. This hypothesis is unlikely to explain the slow spread of Africanized bees into California, however, because their spread throughout the deserts of northern Mexico and south-eastern Arizona has been very rapid. The deserts themselves do not seem to impede their expansion.

2. **The bees have interbred with Europeans and the Africanized traits have become diluted.** This is not a likely explanation because the first Africanized colony identified in California was "highly" Africanized, based on current identification procedures. In addition, it had African-type mitochondria, demonstrating that it is part of a continual maternal lineage of feral colonies stretching back to its original importation into southern Brazil in 1956. There is little evidence for a genetic "dilution" of the Africanized bees by feral or commercial European colonies.

3. **The feral, Africanize population is being reduced by the parasitic mite, *Varroa jacobsoni*.** This is the most likely explanation. We studied the spread of this recently-introduced parasitic mite by examining samples of worker honey bees taken from 208 feral colonies in 1990. These colonies were distributed throughout California. We resampled 124 of the nest sites for Varroa in 1993 and 1994. There were no Varroa mites detected in any of the samples from 1990, suggesting that the feral population was not severely infested at that time. However, by 1993, 75% of all of the sampled nest sites located in the Sacramento Valley were empty, and all of the occupied nest sites with surviving colonies had severe infestations. We found a similar result when we sampled feral colonies from southern California, near Riverside. From these and other data, we estimated that in areas of California with intensive use of honey bees for pollination, the feral population had been reduced by Varroa to about 13% of its original size.

Africanized bees show some resistance to Varroa, relative to European bees. In our studies conducted in Mexico, we have found that brood and adults from European colonies are about twice as likely to get parasitized by Varroa than brood and adults from Africanized colonies. However, Africanized bees are not immune to Varroa. Beekeepers in the state of Vera Cruz, Mexico are suffering severe Varroa damage to their Africanized commercial colonies. It seems likely that Africanized feral colonies are also suffering. If this is true, then we can hope that the feral population of Africanized bees in California ultimately will be reduced due to Varroa parasitism and cause fewer problems for commercial beekeeping. But, only time will tell.

What has been the impact of Varroa on commercial beekeeping? We studied the rates of infestation of commercial hives and the rate of Varroa population growth throughout a year. Our results were staggering. In temperate climates, where similar studies have been conducted, Varroa populations grow in colonies about 10 fold per year. In the Central Valley of California, Varroa populations grow about 286 fold per year, or about 30 times faster than in more temperate climates. We also found that during two times of the year, May-June and October-November, colonies are being infested by large numbers of adult mites. These episodes of infestation correspond to periods of the year when honey bee colonies are very active in robbing each other. This means that beekeepers must treat their hives **at least** twice each year with the miticide, fluvalinate. These two treatments increase their operating costs by at least $10.00 per hive, per year.

Pollen Hoarding Selection

We are continuing our selection program for increased pollen stores and pollen collecting. After 5 generations of selection, our high strain colonies stored more than 6 time as much pollen as colonies from our low strains (Fig. 1). We are now in our 7th generation.

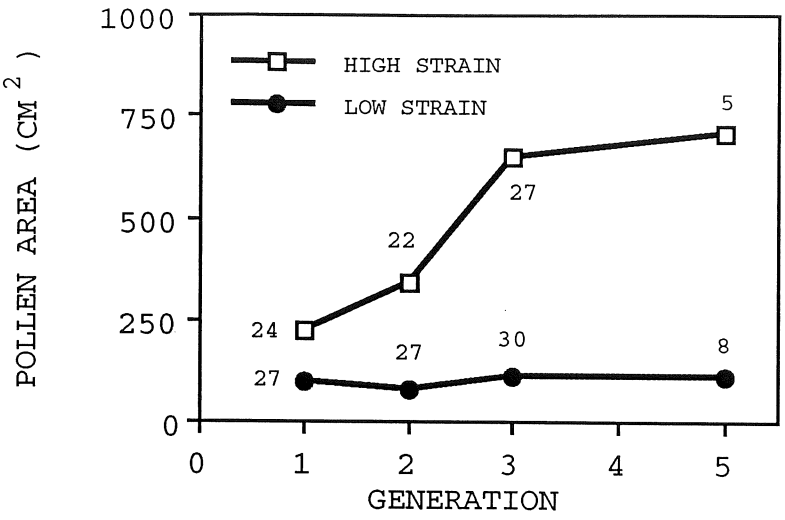

Figure 1. Results of five generations of two-way selection for high and low pollen hoarding strains of honey bees. Open boxes represent average areas of stored pollen for colonies of high strain bees; closed circles, averages of colonies of the low strain. Strain averages differed significantly in all generations. The number of colonies evaluated for each strain, each generation is shown by each symbol. Generation 4 results are not shown because colonies were tested under different conditions and the results are not equivalent. Evaluations were made by directly measuring the amount of pollen stored in each comb within the nest.

In previous reports, we demonstrated that high strain colonies have significantly more pollen foraging activity than low strain and commercial colonies. Now, we are locating genes on honey bee chromosomes that are responsible for the observed differences. So far, we have found two major genes that collectively are responsible for 59% of the total observed variance between our high and low strains. Our study used only 38 colonies and, as a consequence, our markers are not close enough to the genes to be effective in marker-assisted selection. However, this summer we repeated the study using 159 colonies. We are now constructing a new genetic map and are hopeful that we will find markers that are very close to the these two major genes.

Prospects for Honey Bee Certification

Currently, the only official method of identification and certification of Africanized honey bees is USDA-ID morphometrics. This method measures 21 different body parts of 10 workers from a colony and subjects these measurement data to a computerized statistical analysis. The analysis then classifies the colony from which the workers were sampled as either European or Africanized. We tested the efficacy of this system for detecting colonies of varying degrees of Africanization ranging from highly Africanized to pure European. We found that the USDA-ID morphometrics were only able to detect highly Africanized colonies, hybrid colonies were classified as European. We performed defensive behavior tests on these same colonies and found that hybrid colonies were extremely defensive, like the highly Afrricanized bees. We conclude from these studies certification of commercial honey bee colonies as free from Africanization will not be feasible. Extremely defensive hybrid colonies will be classified European and will not be subject to any restrictions. Colonies should not be evaluated on the basis of their pedigrees. What matters is behavior, a trait that beekeepers should be alert to and ready to requeen objectionable colonies.

ECONOMIC ENTOMOLOGY
Monday Nov 07 08:32 AM
Allen Press • AViiON System
GALLEY 53
ecen 88 109 Mp 53
File # 09tx

NOV 0 9 1994

I have checked this proof.
I have marked all changes or
corrections I wish to be made.
Signed:
Date:

Colony Performance of Selected Honey Bee (Hymenoptera: Apidae) Strains Used for Alfalfa Pollination

DAVID M. GORDON,[1] JOHN F. BARTHELL,[1] ROBERT E. PAGE, JR.,[1, 2] M. KIM FONDRK,[1] AND ROBBIN W. THORP[1]

J. Econ. Entomol. 88(0): 000–000 (1995)

ABSTRACT High and low pollen-hoarding strains of honey bees were selected based on quantity of pollen stored in combs. Performance of strains in commercial alfalfa seed pollination is reported. After three generations of selection, colonies with instrumentally inseminated high-strain queens stored significantly more pollen (2.4-fold) than low-strain colonies. Similarly, colonies from naturally mated, outcrossed high-strain queens stored more pollen (2.4-fold) than outcrossed low-strain colonies. Selection did not change preferences for sources of pollen. After four generations of selection, colonies with naturally mated high-strain queens outcrossed with commercial drones stored significantly more pollen (1.4-fold) than commercial colonies. Rates of queen acceptance (54 and 61%) and overwintering survival (61%) in commercially managed colonies were surprisingly low, indicating 37% queen survival during 10-mo period. Overwintered outcrossed high-strain colonies were more populous than commercial colonies at the beginning of almond bloom.

KEY WORDS *Apis mellifera*, selection, pollen-hoarding

HONEY BEES ARE important pollinators of crops. In California, ≈47 crops, worth $1.8 billion annually, are pollinated by honey bees (Page 1992). Approximately half of California beekeepers depend more on the income they receive from pollination services than from the income they receive from honey production (Gordon et al. 1986). Two California crops that require large-scale use of honey bees for pollination are almonds and alfalfa. High colony densities must be used for alfalfa pollination because honey bees avoid triggering the flower mechanism that effects pollination (Vansell & Todd 1946, Reinhardt 1952, Bohart 1957, McGregor 1976) and often visit other species blooming in the vicinity of the target crop (Stanger & Thorp 1976).

Previous studies have demonstrated that pollen collecting and hoarding are traits that can be selected in honey bees (Nye & Mackenson 1965, 1968, 1970; Mackenson & Nye 1966, 1969; Hellmich et al. 1985). In 1990, R.E.P. & M.K.F. (unpublished data) initiated selection for pollen-hoarding behavior using California commercial bees (see also *Material and Methods*). Their program was based on the methods of Hellmich et al. (1985) and was designed to increase the pollination activities of commercial colonies in California. Two-way selection for quantities of stored pollen rapidly resulted in the production of high and low pollen-hoarding strains that differed significantly in areas of stored pollen after a single generation of selection. By the third generation of selection, the high-strain colonies stored an average 6-fold more pollen than low-strain colonies ($P < 0.001$; Mann–Whitney U test). High-strain colonies also had 37% more pollen foragers ($P < 0.01$; Mann–Whitney U test), however, high- and low-strain colonies had equal total numbers of foragers. When generation 3 workers of the high and low strains were reared together in the same colonies, the high-strain workers were 87% more likely to forage for pollen than were workers from the low strain ($P < 0.0001$; G-test for heterogeneity; from R.E.P., K. D. Waddington & M.K.F., unpublished data).

The selection studies reported by R.E.P. & K.D.E. (unpublished data) were conducted in almond orchards during peak bloom periods (February–March) and in the University of California Davis Arboretum, during the summer. Here we present evaluations of these same stocks used for commercial alfalfa pollination. This study was also designed to involve the commercial queen production and pollination industries in the use of a selected strain of honey bees in anticipation of the need to control the genetic lineage of commercial honey bee stocks after the imminent arrival of Africanized honey bees in California.

Materials and Methods

Source of Bees. Two-way, colony-level selection for the amount of pollen stored in wax combs

[1] Department of Entomology, University of California, Davis, CA 95616.
[2] To whom reprint requests should be addressed.

0022-0493/95/000–000$02.00/0 © 1995 Entomological Society of America

ECONOMIC ENTOMOLOGY
Monday Nov 07 08:32 AM
Allen Press • AViiON System
GALLEY 54
ecen 88 109 Mp 54
File # 09tx

was performed for five generations using bees derived from commercial colonies in California. Selection procedures and the mating system employed were the same as those reported by Hellmich et al. (1985). In 1990, 127 commercial honey bee colonies were evaluated. Colonies were arbitrarily selected from among several apiaries of several beekeepers and broadly represented the genetic variability present in California commercial populations. Evaluations were made of the area of comb within the nest that contained stored pollen. Ten colonies, each containing the highest and lowest stores, respectively, were selected as founding parents of the high and low strains. Queens of five high- and five low-strain colonies were designated queen mothers, constituting five sublines within each selected strain. Sublines were designated A–E and Q–U for the low and high strains, respectively. The other five colonies of each selected group provided drones for instrumental insemination. Each drone source colony was paired with a queen mother. Virgin queens were raised from each subline and at least 10 were instrumentally inseminated with semen from single drones from the designated drone source. Workers within colonies derived from these queens constituted generation 1.

For subsequent generations, all surviving colonies were evaluated for stored pollen, the single superior performing colony within each subline was identified, and virgin queens and drones were raised from the queens of these colonies. Matings were made between sublines within pollen strains: for example, virgin queens of subline A were mated to drones of subline B, B to C, and so on. Each generation, the matings changed; for example, matings in generation 2 were A to C, B to D, and so on. Colonies used for selection were maintained and evaluated in single-story Langstroth hives. The first two generations of selected strains were not tested in alfalfa, and the results of those studies will be reported separately (R.E.P. & M.K.F., unpublished data, see introduction).

Third-Generation Studies. *Selection Procedure.* Third-generation workers originated from second-generation queens that were reared from one first-generation high-line queen and one first-generation low-line queen and mated to drones in two ways: virgin queens emerged in cages and each was instrumentally inseminated with semen from four drones derived from unrelated queens within the respective (high or low) sublines; and virgin high and low queens were emerged in nuclei and allowed to mate with drones of unselected northern California commercial stock. Sixty-one open-mated (outcrossed) queens were produced, marked with enamel paint, and given to a beekeeper. He then introduced the queens (using his standard introduction methods) into his commercial colonies that were located in citrus orchards on 4–5 May 1991. The beekeeper managed all colonies identically and was blind to the sources of the queens. Marked, instrumentally inseminated queens were introduced into colonies maintained at the University of California Davis Bee Biology Facility on 15 May.

Performance in Alfalfa. Twenty-four instrumentally inseminated and 61 outcrossed colonies were placed on a commercial alfalfa seed production field near Mendota, Fresno County, on 1 June and were evaluated 45 d later on 16–17 July 1991. Outcrossed colonies were placed in three apiaries ≈200–500 m apart in a single 65-ha field. The standard practice of beekeepers is to pack many colonies side-by-side on cotton trailers. Outcrossed colonies were placed on the ground ≈10 m from the cotton trailers in all three apiaries, with each colony separated by ≈1 m. The instrumentally inseminated colonies were placed near one of the outcrossed apiaries. To reduce mixing of forager populations (Jay 1966), colonies were arranged in pairs ≈1 m apart, with entrances within pairs facing opposite directions. High- and low-strain colonies were distributed randomly within each set of experimental colonies.

Only queenright colonies were used for statistical analyses. Colonies from high and low pollen strains that did not contain appropriately marked queens were not analyzed. All evaluations were performed blindly with respect to the origins of the colonies. The area of adult bees, brood, and honey on each frame was estimated to the nearest 0.1 frame by visual inspection (McGregor & Rowe 1979). Pollen was estimated with the aid of a 6.45-cm^2 wire sampling grid (Nolan 1925). Although reported as areas of adult bees, these data may be converted to estimates of total numbers of workers (Burgett & Burikam 1985). Outcrossed colonies were evaluated in the field on 16 July. The instrumentally inseminated colonies were transported to the University of California Davis the night of the 16 July and evaluated the following morning.

Pollen Preference. The types of pollen collected by colonies were compared by sampling foragers returning to 12-high and 12 low-strain instrumentally inseminated colonies on 16 July. Returning foragers were sampled between 0800 and 1000 hours to avoid collecting bees engaged in orientation flights. Colony entrances were blocked with screen for 1 min and all foragers returning to the hive were vacuumed off the entrances for 3 min. Bees were vacuumed into wire cages and quickly killed by placing the cages into dry ice. Dead bees were transferred to petri dishes and kept frozen until they were examined in the laboratory. Pollen loads were categorized by color and then removed. Subsamples of pollen from each category were examined microscopically and compared with a reference collection to confirm the pollen source.

Queen Acceptance. Marked queens of the outcrossed high and low strains were supplied to the beekeeper who introduced them on 4–5 May. The rate of acceptance of those queens by colonies was determined by examining 46 colonies of both high

and low strains for the presence of marked queens 72 d later, on 16 July.

Fourth-Generation Studies. *Selection Procedure.* Colonies with fourth-generation workers were produced from high-strain third-generation queens that were provided to five commercial queen producers. The queens were raised according to the standard commercial practices of each queen breeder and outcrossed through natural mating with drones from their commercial stocks. Marked queens were pooled and mixed before distribution to four beekeepers so each beekeeper received equivalent numbers from each queen producer. Beekeepers introduced queens into colonies located in citrus groves using their standard methods. All colonies within these apiaries, including those with high-strain queens, were managed identically before and after queen introduction. Colonies were transported into alfalfa fields after sufficient time for queen establishment.

Performance in Alfalfa. In total, 896 outcrossed high-strain fourth-generation colonies and 890 commercial colonies were placed in two 130-ha alfalfa fields, ≈8 km apart, near Corcoran, Kings County, on 19 May 1992. Colonies were distributed among 12 apiaries per field. Pairs of pallets containing four colonies each were placed side-by-side within apiaries, which contained from 40 to 104 colonies. Treatments were segregated into the eastern and western halves of each field. One field contained only high-pollen bees in the eastern half, and only commercial bees in the western half. The other field had the opposite configuration. Colonies were not distributed randomly throughout apiaries or fields, because the amount of drifting of foragers into other colonies that occurs under commercial pollination conditions in alfalfa would most likely have obscured differences between treatments. Drifting occurs because there are few distinct landmarks for orientation and colonies are concentrated in apiaries at high densities.

Queen Acceptance and Survival. In total, 1,040 outcrossed queens, color-coded by queen producer, were introduced into colonies from 10 to 14 April 1992 for the alfalfa study described above. The rate of queen acceptance in 56 colonies of the outcrossed high strain was assessed on 19 May. After the end of the alfalfa pollination season, the beekeeper with the highest queen introduction success was chosen for a colony reevaluation the following spring. Fifty-one high-pollen strain colonies with the same management history were examined in an almond orchard near Dunnigan (Yolo County) on 13 February 1993. Because all 51 colonies had contained marked queens when evaluated during the alfalfa study, this provided an estimate of overwintering survival of those accepted queens. Queen survival from the date of original introduction in April 1992 until February 1993 (10 mo) was determined by multiplying the rate of queen acceptance by the rate of overwintering survival of those accepted queens.

Colony Strength at Winter's End. We expected that high pollen colonies would be more populous at the end of winter. As a preliminary assessment of the potential differences in early-season colony strength that may accompany selection for high pollen hoarding, 40 outcrossed high pollen and 64 commercial colonies with the same management history were evaluated on 5 March 1993. All of these colonies belonged to the beekeeper described in the preceding paragraph, and had been moved into two almond orchards ≈10 km apart just before evaluation. On 2 March, 114 commercial colonies with unknown (but presumably similar) management histories were evaluated in three almond orchards located near the orchard with the high pollen strain. Adult bee populations were evaluated by counting the number of frames covered by workers (cluster count; Nasr et al. 1990). The bee populations were converted to square centimeters so the results could be compared with previous studies in alfalfa. All evaluations were made while the clusters were still intact, before the onset of daily foraging (≈0900 hours).

Statistical Tests. Stored pollen data for both generations was $\log_e$ transformed to meet assumptions of normality (based on Bartlett's test, Sokal & Rohlf 1981). Statistical tests are reported for transformed data, but means are reported in square centimeters. Third-generation colony performance was tested with one-tailed *t*-tests. Product–moment correlations were calculated among the colony variables. Differences among types of pollen collected by colonies was assessed with a replicated test for goodness-of-fit (*G* statistic; Sokal & Rolf 1981). For the fourth-generation colony performance, the two evaluations were analyzed separately by two-way analysis of variance (field and strain). A repeated measures procedure was not used because it was not possible to evaluate the same colonies in both evaluations. Sample sizes were increased for the second evaluation, and some colonies used in the first evaluation had to be eliminated, because they did not meet our evaluation criteria (queenright). Colony strength at winter's end was tested with one-tailed *t*-tests, because we had predicted that the high pollen strain would have larger bee populations.

Results

Third-Generation Performance in Alfalfa. Areas of adult bees, brood, and honey did not differ significantly between either instrumentally inseminated or outcrossed high- and low-strain colonies (Table 1). However, in instrumentally inseminated colonies, the high strain stored 2.4-fold more pollen than the low strain ($t = 2.52$, df = 22, $P < 0.02$). For outcrossed colonies, the high strain also had 2.4-fold more stored pollen than the low strain ($t = 2.95$, df = 23, $P < 0.01$).

In the outcrossed high strain, there were significant correlations between bees:brood and bees:

ECONOMIC ENTOMOLOGY
Monday Nov 07 08:32 AM
Allen Press • AViiON System
GALLEY 56
ecen 88 109 Mp 56
File # 09tx

Table 1. Comparisons of colony performance between high and low pollen hoarding honey bee strains after three generations of selection

	Adult bees	Brood	Pollen	Honey	*n*
		Instrumentally inseminated (evaluated 17 July)			
High	5,883 ± 588.3	2,787 ± 187.2	494 ± 86.1	6,610 ± 451.6	13
Low	5,940 ± 435.1	2,730 ± 191.7	208 ± 24.6	6,320 ± 733.7	11
P	0.941	0.836	0.020**	0.731	—
t	−0.075	0.21	2.52	0.35	—
df	22	22	22	22	—
		Outcrossed (evaluated 16 July)			
High	5,981 ± 400.6	3,680 ± 276.7	720 ± 119.9	4,831 ± 268.8	15
Low	5,605 ± 445.7	2,957 ± 196.5	296 ± 64.4	5,047 ± 454.8	10
P	0.544	0.067	0.007**	0.665	—
t	0.62	1.92	2.95	−0.44	—
df	23	23	23	23	—

Colonies with instrumentally inseminated and naturally mated, outcrossed queens were evaluated after 45 d of commercial alfalfa pollination. Mean areas ± SEM (cm^2). Unpaired one-tailed *t*-test. **, Significant difference.

pollen (Table 2). There were no significant correlations between any variables in the outcrossed low strain. Pooling the outcrossed high and low strains resulted in significant correlations between bees: brood, bees:pollen, and brood:pollen.

In the instrumentally inseminated high strain, there was a significant correlation between bees: honey, whereas the low strain had significant correlations between bees:honey and bees:pollen. Pooling the instrumentally inseminated high and low strains resulted in a significant correlation only between bees:honey. No variable pair in either strain exceeded a correlation of 0.79, and only 7 of the 30 total pairs exceeded 0.50 (Table 2).

Pollen Preference. The proportion of pollen foragers returning with alfalfa pollen was not significantly different between the instrumentally inseminated high- and low-strain colonies ($G = 2.12$, df = 1, $P > 0.05$). However, both strains collected slightly more nonalfalfa pollen. Out of 120 low-strain foragers, 41 carried alfalfa pollen (34%), whereas 79 carried other pollen (66%). Out of 240 high-strain foragers, 101 carried alfalfa pollen (42%), whereas 139 carried other pollen (58%). Overall, out of 360 high- and low-strain foragers, 142 (39%) carried alfalfa pollen. In addition to alfalfa, pollen loads contained pomegranate, *Punica granatum* L., and a mixture of asteraceous types. Pomegranate trees lined some nearby roads, and fields of safflower, Asteraceae, were blooming in the vicinity.

Fourth-Generation Performance in Alfalfa. There were no significant interactions in either evaluation for any of the variables. High-strain colonies (H) stored more pollen than commercial colonies (C) during both evaluations, but significantly more only for evaluation II. Means for strains by fields are provided in Table 3. For evaluation I, the high pollen strain ($n = 21$) had 1.3-fold more pollen than the commercial colonies ($n = 20$; $F = 1.80$; df = 1, 37; $P = 0.188$; 985 ± 134 H versus 764 ± 109 C; 1.2-fold more in the north field, and 1.4-fold-more in the south field). In evaluation I, there were no significant differences between strains for brood ($F = 1.34$; df = 1, 37; $P = 0.254$; 5,943 ± 317 H versus 6,457 ± 292 C), or bees ($F = 3.01$; df = 1, 37; $P = 0.091$; 13,191 ± 894 H versus 11,203 ± 645 C).

For evaluation II, the high strain ($n = 32$) had significantly more pollen (1.4-fold) than commercial colonies ($n = 31$; $F = 5.24$; df = 1, 59; $P = 0.026$; 1,116 ± 107 H versus 817 ± 76 C; 1.6-fold more in the north, and 1.2-fold more in the south field). The commercial strain had significantly more brood (1.68-fold) than the high strain ($F = 7.25$; df = 1, 59; $P = 0.009$; 5,826 ± 211 H versus 6,803 ± 294 C; 1.2-fold more than the high in the north, and 1.4-fold more in the south field). There

Ali edit OK?? -Ed.

Table 2. Correlations among bees, brood (B), honey (H), and pollen (P) within two strains of honey bees after three generations of selection for high and low pollen hoarding

	High and Low			High			Low		
	B	H	P	B	H	P	B	H	P
				Instrumentally Inseminated					
Bees	0.20	0.65*	0.34	0.37	0.74*	0.38	−0.11	0.67*	0.79*
Brood	—	0.22	0.32	—	0.29	0.45	—	0.17	0.09
				Outcrossed					
Bees	0.55*	0.07	0.50*	0.65*	0.13	0.69*	0.27	0.04	−0.11
Brood	—	−0.10	0.50*	—	0.14	0.47	—	−0.49	−0.04

Colonies were evaluated after 45 d of commercial alfalfa pollination. *, $P < .05$.

ECONOMIC ENTOMOLOGY
Monday Nov 07 08:32 AM
Allen Press • AViiON System
GALLEY 57
ecen 88 109 Mp 57
File # 09tx

Table 3. Means ± SEM (cm^2) for strains by fields between naturally mated, outcrossed fourth-generation high pollen hoarding strain and commercial honey bees

	High pollen hoarding strain		Commercial bees	
	North	South	North	South
	Evaluation I (4, 9, 10 June)			
Pollen	751 ± 105.0	1,295 ± 252.3	612 ± 115.2	949 ± 186.6
Brood	5,341 ± 391.3	6,744 ± 408.1	6,010 ± 394.4	7,002 ± 381.7
Adult bees	12,980 ± 1,252.1	13,470 ± 1,330.5	10,895 ± 935.9	11,578 ± 910.1
n	12	9	11	9
	Evaluation II (17–18 June)			
Pollen	1,140 ± 170.1	1,094 ± 138.0	699 ± 126.1	943 ± 71.7
Brood	5,687 ± 331.2	5,947 ± 274.4	6,832 ± 341.5	6,771 ± 499.0
Adult bees	11,055 ± 1,027.7	10,173 ± 628.9	11,196 ± 635.4	10,941 ± 1,057.0
n	15	17	16	15

Colonies were placed in two commercial alfalfa seed fields (north and south) for pollination on 19 May 1992, and evaluated twice (I and II). Statistical differences between strains and fields are summarized in the *Results* section.

was no significant difference in adult bees between strains (F = .290; df = 1, 59; P = 0.592; 10,587 ± 582 H versus 11,073 ± 597 C).

Comparing fields, during evaluation I, the south (S) field colonies (n = 18) had significantly more pollen (1.6-fold; F = 4.65; df = 1, 37; P = 0.038; 1,122 ± 157.9 S versus 685 ± 77.3 (north field [N]) and brood (1.2-fold; F = 8.94; df = 1, 37; P = 0.005; 6,873 ± 272.9 S versus 5,661 ± 280.8 N) than the north field (n = 23). There was no significant difference in adult bee populations between fields during evaluation I (F = 0.262; df = 1, 37; P = 0.612; 12,525 ± 815 S versus 11,983 ± 805 N).

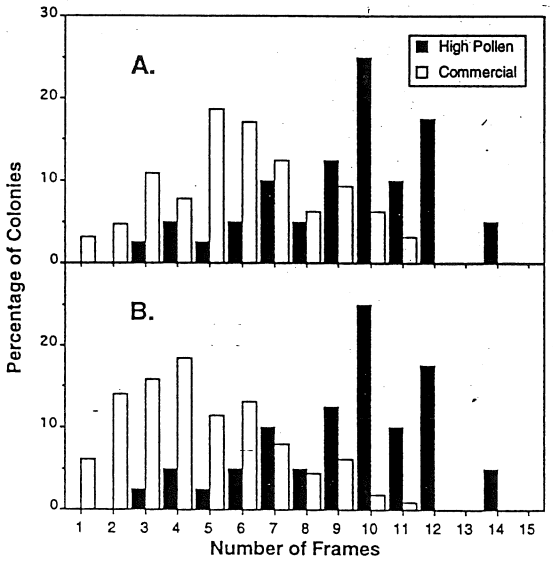

Fig. 1. Frequency distributions for the number of frames of bees contained in honey bee colonies evaluated in March 1993 in almond orchards. Colony strength of the fourth-generation high pollen hoarding strain headed by naturally-mated outcrossed queens is compared with commercial colonies with the same management history (A) and unknown management histories (B).

For evaluation II, there were no significant differences between the north (n = 31) and south (n = 32) fields for pollen (F = 2.39; df = 1, 59; P = 0.128; 1,023 ± 80.6 S versus 912 ± 110.7 cm^2 N), brood (F = 0.074; df = 1, 59; P = .786; 6,334 ± 281 S versus 6,278 ± 257 N), or bees (F = 0.453; df = 1, 59; P = 0.504; 10,533 ± 591 S versus 11,128 ± 586 N).

Queen Survival in Commercially Managed Colonies. Acceptance of outcrossed third-generation high- and low-strain queens 2 mo after they were introduced by a commercial beekeeper was 54% (25 of 46). Overall acceptance of fourth-generation outcrossed high-strain queens 5 wk after introduction by commercial beekeepers was 61% (34 of 56), although acceptance rates varied from 20 to 91% among the four beekeepers involved. Survival through the winter of accepted fourth-generation queens was 61% (31 of 51). Average survival of fourth-generation queens in commercially managed colonies over a 10-mo period (April 1992 to February 1993) was 37%.

Colony Strength After Winter. High-strain colonies had 1.6-fold more adult bees (9.3 frames or 14,435 ± 647 cm^2) than the commercial colonies (5.9 frames or 9,167 ± 478 cm^2) that had the same management history (t = 6.65, df = 102, P < .0001; Fig. 1A). High-strain colonies had 2.0-fold more adult bees than the commercial colonies (4.6 frames or 7,169 ± 341 cm^2) that had unknown management histories (t = 10.50; df = 1, 52; P < 0.0001; Fig. 1B).

Discussion

Nye & Mackenson (1965, 1968, 1970) selected a strain of honey bees that preferentially collected alfalfa pollen to improve alfalfa seed production. However, commercial use of this alfalfa specialist strain was unsuccessful and the program was abandoned (Martin 1975). Our approach has been to select for increased pollen-hoarding rather than preference for a pollen source, an approach previously used by Hellmich et al. (1985). Because

ECONOMIC ENTOMOLOGY
Monday Nov 07 08:32 AM
Allen Press • AViiON System
GALLEY 58
ecen 88 109 Mp 58
File # 09tx

honey bee colonies are typically rotated through a variety of crops, selecting a generalist pollinator strain should be more beneficial for growers and beekeepers than producing bees that specialize on single crop species.

We have assumed that alfalfa pollination is improved using a high pollen strain because colonies that hoard more pollen tend to collect more pollen as well. In almonds, for example, outcrossed colonies of high-strain bees exhibited 1.6-fold more pollen foraging activity than comparable, unselected commercial colonies (R.E.P. & ~~K.M.F.~~, unpublished data). The resulting high pollen-hoarding colonies should be more effective at setting seed in these crops than commercial bees because pollen foragers are more effective pollinators than nectar foragers in both alfalfa and almond crops (Vansell & Todd 1946, Thorp 1979).

These studies assessed pollen collecting performance of colonies used for commercial alfalfa pollination. Factors that complicate field studies such as these include very high densities of colonies, pesticide applications to crops, drifting between colonies by returning foragers, and large quantities of more attractive crops blooming in the area. Despite the stressful conditions that commercial alfalfa seed production is known to place on colonies, the high pollen strain consistently stored more pollen than either the low-strain or commercial colonies in three studies during a 2-yr period. The lack of significant differences between strains in honey, brood, or adult bee populations indicated that we successfully selected for the pollen foraging trait and not other traits such as brood production or disease susceptibility.

Several problems surfaced during evaluation I in 1992 that probably contributed to the significant differences between the two fields, but not between strains. An insecticide was applied to the north field before evaluation I. The south field had been irrigated just before evaluation I, which prolonged our sampling time in that field. Irrigation is also known to decrease foraging by workers for nectar and possibly for pollen (Teuber & Thorp 1987). Despite the complications that occurred during evaluation I, the high-strain colonies stored 1.3-fold more pollen than the commercial colonies. To improve our ability to detect significant differences between strains, we increased the sample size for evaluation II. During evaluation II, large, nearby tracts of blooming safflower drew our bees away and probably caused drifting between the high-strain and commercial colonies. We were still able to detect significant differences even though drifting may have reduced the magnitude of the differences between the strains.

Evaluation of pollen loads suggested that the selection has not changed preferences for sources, only the amount of pollen that colonies collected. Although it has been shown that pollen stores are positively correlated with the quantity of adult bees and brood (McLellan 1978), correlations between the three variables were generally low and were inconsistently significant within strain types (Table 2). It is clear that the increased pollen storage in the high-strain colonies compared with the commercial colonies was not simply the result of larger brood or adult populations because they did not differ significantly between strains in either of the third-generation studies nor in evaluation I of the fourth generation. When a significant difference in brood was detected between strains during evaluation II, the commercial colonies had 1.7-fold more brood than the high strain, but 40% less stored pollen.

Although the studies were not initially designed to assess queen survival, it became evident that queen acceptance and survival was low under commercial pollinating conditions. We attribute most of this low survivorship to differing queen introduction techniques because there was more than a 4-fold difference among beekeepers for queen acceptance rates, although there was also considerable overwintering mortality. There is little information available for comparing these findings, although under carefully monitored conditions in Mexico (Guzman-Novoa & Page 1994a), queen survivorship was nearly twice the averge reported here. If this low survivorship of queens accurately reflects levels found in commercial apiaries, then beekeepers will face difficulties when Africanized bees invade California (Taylor 1988). A frequent turnover of queens in commercial colonies would increase the likelihood that Africanized drones would mate with replacement queens. Such introgression of Africanized genes would produce a rapid, conspicuous, and undesirable effect. Recent studies in central Mexico have demonstrated that defensive behavior in Africanized bees has a genetic component that dominates hybrids of the Africanized and European races (Guzman & Page 1993, 1994b). Because Africanized honey bees are not suited to commercial pollination (Loper & Danka 1991), it will be essential to maintain preferred queens in commercial colonies. This will require a cooperative effort among researchers, queen producers, beekeepers, bee brokers, and growers similar to the one employed during these studies.

Those high-strain colonies that were followed through the winter were more populous than commercial colonies at the beginning of the almond bloom (Fig. 1). It is unknown if this early-season colony strength is related to pollen gathering abilities or other factors. Because the queens in the commercial colonies were unmarked, it is not known if they were older than the high-strain queens. However, a logical consequence of producing more pollen stores would be that those colonies would be stronger going into and coming out of winter. Preliminary evidence suggests that the high pollen strain may have characteristics that are favorable for pollination.

ECONOMIC ENTOMOLOGY
Monday Nov 07 08:32 AM
Allen Press • AViiON System
GALLEY 59
ecen 88 109 Mp 59
File # 09tx

Acknowledgments

M. Rosso coordinated activities between beekeepers, growers, queen producers and researchers, and evaluated queen acceptance for the fourth-generation study. His advice and field assistance were invaluable to this project. J. Aguirre Lazarraga, C. Dullum, E. Guzmán-Novoa, M. Humphries, A. Kraus, and D. Nielsen provided field assistance. The American Salyer Ranch and Silviera Ranches provided the alfalfa fields and some logistic support to conduct the experiments. Funding was provided by contracts from the California Department of Food and Agriculture, the Alfalfa Seed Production Board of California and Salyer's Seed Company.

References Cited

Bohart, G. E. 1957. Pollination of alfalfa and red clover. Annu. Rev. Entomol. 2: 355–380.

Burgett, M. & I. Burikam. 1985. Number of adult honey bees (Hymenoptera: Apidae) occupying a comb: a standard for estimating colony populations. J. Econ. Entomol. 78: 1154–1156.

Gordon, D. M., S. J. Locke, M. E. Nasr, T. L. Tyler & T. C. Webster. 1986. A survey of current beekeeping practices in California: implications for Africanized honey bee control. Am. Bee J. 126: 799–803.

Guzmán-Novoa, E. & R. E. Page. 1993a. Backcrossing Africanized honey bee queens to European drones reduces colony defensive behavior. Ann. Entomol. Soc. Am. 86: 352–355.

1994a. Genetic dominance and worker interactions affect honey bee colony defense. Behav. Ecol. 5: 91–97.

1994b. The impact of Africanized bees on Mexican Beekeeping. Am. Bee J. 134: 101–106.

Hellmich, R. L., J. M. Kulincevic & W. C. Rothenbuhler. 1985. Selection for high and low pollen-hoarding honey bees. J. Hered. 76: 155–158.

Jay, S. C. 1966. Drifting of honeybees in commercial apiaries. III. Effect of apiary layout. J. Apic. Res. 5: 137–148.

Loper, G. M. & R. G. Danka. 1991. Pollination tests with Africanized honey bees in southern Mexico, 1986–88. Am. Bee J. 131: 191–193.

Mackenson, O. & W. P. Nye. 1966. Selecting and breeding honey bees for collecting alfalfa pollen. J. Apic. Res. 5: 79–86.

1969. Selective breeding of honey bees for alfalfa pollen collection: sixth generation and outcrosses. J. Apic. Res. 8: 9–12.

Martin, E. C. 1975. The use of bees for crop pollination, pp. 579–614. *In* The hive and the honey bee. Dadant, Hamilton, IL.

McGregor, S. E. 1976. Insect pollination of cultivated crop plants. U.S. Dep. Agric. Agric. Handb. 496.

McGregor, S. E. & J. B. Rowe. 1979. Honey bee colony quality for alfalfa pollination. Am. Bee J. 119: 700–703, 761–765.

McLellan, A. R. 1978. Growth and decline of honeybee colonies and inter-relationships of adult bees, brood, honey and pollen. J. Appl. Ecology 15: 155–161.

Nasr, M. E., R. W. Thorp, T. L. Tyler & D. L. Briggs. 1990. Estimating honey bee (Hymenoptera: Apidae) colony strength by a simple method: measuring cluster size. J. Econ. Entomol. 83: 748–754.

Nolan, W. J. 1925. The brood rearing cycle of the honey bee. U.S. Dep. Agric. Bull. 1348.

Nye, W. P. & O. Mackenson. 1965. Preliminary report on selection and breeding of honeybees for alfalfa pollen collection. J. Apic. Res. 9: 61–64.

1968. Selective breeding of honey bees for alfalfa pollination: fifth generation and backcross. J. Apic. Res. 7: 21–27.

1970. Selective breeding of honey bees for alfalfa pollen collection: with tests in high and low alfalfa pollen collecting regions. J. Apic. Res. 9: 61–64.

Page, R. E. 1992. How Africanized bees will affect California agriculture. Calif. Agric. 46: 18–19.

Reinhardt, J. F. 1952. Some responses of honey bees to alfalfa flowers. Am. Nat. 86: 257–275.

Sokal, R. R. & F. J. Rohlf. 1981. Biometry, 2d ed. Freeman, San Francisco.

Stanger, W. & R. W. Thorp. 1976. Honey bees in alfalfa pollination. Univ. Calif. Coop. Ext. Leafl. 2382.

Taylor, O. R. 1988. Ecology and economic impact of African and Africanized honey bees, pp. 29–41. *In* G. R. Needham, R. E. Page, M. Delfinado-Baker & C. E. Bowman [eds.], Africanized honey bees and bee mites. Wiley, New York.

Teuber, L. R. & R. W. Thorp. 1987. The relationship of alfalfa nectar production to seed yield and honey bee visitation, pp. 25–30. *In* Proceedings, Alfalfa Seed Production Symposium. USDA and Univ. Calif. Coop. Ext. Davis, CA.

Thorp, R. W. 1979. Honey bee foraging behavior in California almond orchards, pp. 385–392. *In* D. M. Caron [ed.], Proceedings, Fourth International Symposium on Pollination. Md. Agric. Exp. Stn. Spec. Misc. Publ. 1.

Vansell, G. H. & F. E. Todd. 1946. Alfalfa tripping by insects. J. Am. Soc. Agron. 38: 470–488.

Morphometric Techniques Do Not Detect Intermediate and Low Levels of Africanization in Honey Bee (Hymenoptera: Apidae) Colonies

ERNESTO GUZMÁN-NOVOA,[1] ROBERT E. PAGE, JR.,[2,3] AND M. KIM FONDRK[2]

Ann. Entomol. Soc. Amer. 87(5): 507–515 (1994)

ABSTRACT We tested three different morphometric methods used to identify Africanized honey bee (*Apis mellifera* L.) colonies and determined the correlative relationships of their associated discriminant scores and colony defensive behavior. Workers within and between experimental colonies varied in the percentage of their genotype that was of African origin. Morphometric scores of colonies were compared with two defensive behavior traits: the time it took for the first worker in a colony to respond to, and sting, a moving leather-patch target, and the total number of stings received in the target during a 60-s interval following the first sting. All identification methods correctly classified all of the colonies that were presumed to be 100% Africanized or European. However, <45% of the hybrid samples were scored as Africanized. In all cases, as the level of Africanization decreased, so did the sensitivity and accuracy of the method. Correlations between morphometric scores and defensive behavior were significant when extreme genotypes were included in the analyses, but no method correlated with samples ranging in the interval >0 but <50% Africanized. Implications are discussed of using these and alternative identification methods in regulatory programs.

KEY WORDS Africanized bees, colony defense, identification

AFRICANIZED HONEY BEES (*Apis mellifera* L.) have descended from African queens (*Apis mellifera scutellata* Lepeletier) that were introduced into Brazil in 1956 (Kerr 1967). African queens and drones presumably mated with the local population of honey bees introduced from Europe into Brazil, resulting in offspring that perpetuated mostly African traits. Since then, colonies of Africanized honey bees have spread throughout most of South and Central America and reached the United States in 1990 (Sugden & Williams 1991). Relative to commercial bees of European origin, Africanized honey bees are considered undesirable for apicultural practices. They are excessively defensive (Stort 1974, 1975a, b, c; Collins & Kubasek 1982; Collins et al. 1982; Villa 1988; Guzmán-Novoa & Page 1993, 1994), show a high tendency to swarm (Winston 1979, Otis 1980), and apparently are poor honey producers (Rinderer et al. 1985, Rinderer 1988).

It is necessary to monitor the introgression of African genes into European populations to judge the efficacy of programs designed to mitigate the effects that African traits have on commercial honey bee populations. Apiculturists and regulatory agencies need reliable and rapid laboratory procedures to identify large numbers of colony samples if quarantine or other regulatory measures are adopted. Identification methods used for regulatory operations must be sensitive enough to detect accurately intermediate and low levels of Africanization resulting from introgression of genes from the feral Africanized population into populations of commercial bees. This is necessary because at least one of the most objectionable characteristics of Africanized bees, their extreme defensive behavior, is inherited as a dominant trait (Stort 1974, 1975a; Guzmán-Novoa & Page 1993, 1994). This genetic dominance results in extreme defensive behavior of commercial colonies with relatively low degrees of Africanization.

Several methods have been tested to identify Africanized bees (Daly & Balling 1978, Daly et al. 1982, Sylvester 1982, Carlson & Bolten 1984, Rinderer et al. 1986a, Del Lama et al. 1988, Smith 1988, Smith et al. 1988, Brand et al. 1991, Hall & Smith 1991); however, only those based on morphometrics have been implemented in regulatory programs. USDA–ARS currently recognizes morphometric techniques as the only permissible identification methods (Sylvester et al. 1992). Morphometric techniques with multivariate discriminant function analyses provide

[1] CENIFMA-INIFAP-SARH, Paseo Jurica 634, Jurica Campestre, Queretaro, QRO 76030, Mexico.
[2] Department of Entomology, University of California, Davis, CA 95616.
[3] To whom correspondence should be addressed.

0013-8746/94/0507–0515$02.00/0 © 1994 Entomological Society of America

good separation between known colonies of Africanized and European honey bees, and even of some F_1 hybrids (Rinderer et al. 1990). However, these methods have not been tested for identification of independent collections of bees of various known degrees of Africanization. Researchers and regulatory agencies have justified the use of morphometric identification on the assumption that discriminant scores can predict defensive behavior. However, this relationship also has not been tested using bees of various known degrees of Africanization (Collins et al. 1994).

In this study, we determined the sensitivity of three different morphometric methods used by USDA–ARS to detect Africanized colonies. We also determined the correlative relationships between morphometric scores and defensive behavior.

Materials and Methods

Experiments were conducted between March 1991 and December 1992 at the facilities of Miel Vita-Real in Ixtapan de la Sal, Mexico, located ≈150 km southwest of Mexico City.

Source Colonies. The presumed European honey bee colonies were derived from stocks that had been imported previously from several queen breeders in the United States. The presumed Africanized colonies came from box traps that were used to capture swarms during the dry season (January–April). Because that is not the usual swarming time for European colonies in the area, the probability of the bees being Africanized was increased. Selected colonies were differentiated morphometrically as Africanized (probability of being Africanized = 1.00) or as European (probability of being European = 1.00) (Daly & Balling 1978, Sylvester & Rinderer 1987). In addition, workers in European parental colonies had the slow and medium alleles of malate dehydrogenase (Mdh-1), whereas Africanized colonies were fixed for the fast allele that is predominant in African and Africanized bees (Sylvester 1976, 1982; Contel et al. 1977; Nunamaker et al. 1984).

Experiment 1: Mixed Semen. Colonies were constructed that consisted of five different levels of Africanization: 0.0, 12.5, 25, 50, and 100% (Guzmán-Novoa & Page 1994). Eight source colonies of European and four of Africanized bees were selected. The selected European colonies provided queen and drone mothers for the study. The selected Africanized colonies provided queen mothers and also served as parental stock in the defensive behavioral assays. Africanized drones were obtained from six arbitrarily selected, morphometrically verified Africanized source colonies from the state of Guerrero, Mexico. These drones were placed in cages and maintained in a queenless nursery colony until needed for instrumental insemination.

At least 20 workers and 10 drones from each of the selected colonies were assayed by cellulose acetate gel electrophoresis to determine the Mdh genotypes of potential virgin queen and drone mothers. European queens were selected that produced a high proportion of workers homozygous for the slow or medium migrating alleles of Mdh-1 (Sylvester 1976, Contel et al. 1977). Selected Africanized queens produced a large proportion of progeny that were homozygous for the fast allele (Sylvester 1982).

Super-sister European queens (super sisters have the same queen mother and drone father [see Page & Laidlaw 1988]) were inseminated instrumentally (Laidlaw 1977) with ≈2 μl of semen sampled from homogenized pools of eight drones. Semen was collected from marked, mature drones and pooled in four different European/Africanized volume ratios as follows: 1.00:0.00, 0.75:0.25, 0.50:0.50, and 0.00:1.00.

To prepare the homogenized pools of semen, drones of each type were obtained from their respective source colonies and mixed together so that each of two cages contained a random representation of European or Africanized drones. Semen from each drone was collected into an instrumental insemination syringe tip with ≈1 μl of diluent (Williams & Harbo 1982) and was placed in a sterile, numbered, 1.5-ml microcentrifuge tube. After the semen of a batch of 40 drones of both types was collected, their thoraces were assayed by cellulose acetate gel electrophoresis to validate their genotype. Then, according to the treatment, the semen of eight drones was recollected (from eight different tubes) and placed in another sterile 1.5-ml tube that was microcentrifuged (Fisher Scientific, model 250 C) at 10,000 × *g* (Moritz 1983) for 1 min. Four queens were inseminated with each batch of pooled semen. This procedure was repeated until all queens were inseminated.

To produce "pure" Africanized colonies, three Africanized queens were reared from one of the previously selected Africanized colonies. These queens were inseminated with ≈2 μl of semen from the same Africanized drone source colonies that were used to inseminate the European queens. Three additional selected Africanized colonies, each of which had been captured in swarm traps in the states of Mexico, Morelos, and Guerrero, respectively, were used. This treatment was intended broadly to represent Africanized colonies, thereby showing average measurements of morphometric characters and an average defensive response equivalent to typical Africanized colonies.

Colored, numbered plastic tags (Graze KG, Weinstadt, Germany) were glued to the thoraces of all inseminated queens, and the right wing of each queen was clipped to prevent flight. Queens were placed temporarily into a queenless nursery colony, then removed 24 h after in-

semination and exposed to CO_2 for 8 min to stimulate egg laying (Mackensen 1947). Queens were introduced into nucleus colonies by confining them with wire push-in cages. The colonies, established in Dadant jumbo-size hives, contained ≈1 kg of worker bees and three combs with brood, pollen, and honey. These colonies were fed 50% (by volume) sucrose solution as needed. Initiation of egg laying by the queens was determined by daily observations. Three weeks after onset of oviposition, each colony was transferred into a full-sized Dadant jumbo hive.

Nine weeks after queen insemination, 30 colonies composed of progeny of the experimental queens (6 colonies per treatment) were relocated to 3 adjacent apiaries 1,425 m above sea level. Hives were positioned at least 5 m apart to minimize interhive drifting of workers. The three apiaries were ≈800 m apart.

Colony worker populations were equalized by removing bees and frames of brood from the most populous colonies. The resulting experimental colonies each contained ≈3,500 cm^2 (three to four frames) with capped brood and six frames with adult bees. Colony equalization manipulations were performed 16 d before the first behavioral tests were conducted on 5 August, 12 wk after the queens were inseminated.

For the defensive behavior assay, a black suede leather patch (5 by 6 cm) was attached to a piece of white wood (0.7 by 0.5 by 100 cm) and rhythmically waved (elevated ≈4 cm and lowered ≈4 cm) twice per second ≈5–10 cm in front of the entrance of each hive. The time the first bee stung the patch was recorded, and bees were permitted to sting the patch target for 60 s after the first sting. This test was performed by 14 people on 10 adjacent colonies to decrease the risk that bees from one hive would sting the patches presented to other colonies. Each target was used for only one assay, and tests were conducted blindly (i.e., the operators did not know the type of colony they were testing). Sting bioassays were performed on three occasions, twice on one day (morning and afternoon) and in the morning 2 d later. All colonies were tested on the same days. After each trial, the leather patches were packed and sealed in marked 20-ml plastic vials for subsequent analyses. Stings deposited in the leather patches were recorded, providing a count of the number of stings.

One hour after the third assay, a random sample of bees (controls) was taken from each of the 12 colonies that, according to the insemination mixtures, were expected to possess ≈25% and 50% Africanized × European hybrid workers. Each sample was obtained by shaking all bees off of combs into a cage where they were mixed before sampling. At least 100 bees were removed from the cage and placed into marked 150-ml plastic vials and stored in a freezer at −18°C until electrophoretic analysis.

Experiment 2: Backcrosses. Hybrid queens were backcrossed successively to generate colonies with various degrees of Africanization (Guzmán-Novoa & Page 1993). European and Africanized colonies were obtained and identified as in experiment 1. Ten queens were reared from one Africanized colony, and each was inseminated instrumentally (Laidlaw 1977) with the semen from a single, different European drone. Drones were obtained from two different unrelated European source colonies. Each queen was marked with white paint on the thorax, had her right wing clipped to prevent subsequent flight, and was placed in a Dadant jumbo-size nucleus hive containing ≈1 kg of worker bees and three combs with brood, pollen, and honey. One of these 10 queens arbitrarily was designated to be the queen mother for the first backcross generation. Eleven new queens were reared from this F_1 queen and inseminated in the same manner as described above. Finally, a third generation of 10 queens was produced from an arbitrarily selected queen of the first backcross generation and was treated similarly. Drones from two different European colonies were used to inseminate each generation of queens (six source colonies in total). This backcrossing procedure was performed with a 1-mo interval between generations. The F_1 and the two backcross generations constituted the experimental treatments. In addition, three source colonies of each type (European and Africanized) were used as representative standards (controls). The same colonies were used when comparing the defensiveness of F_1 and backcrossed bees.

Colonies containing the inseminated queens of each generation were established, manipulated, and tested as in experiment 1. Colonies were tested three times. Two trials were conducted in 1 d (morning and afternoon). The third trial took place in the morning, 2 d later. Successive generations were tested at 5-wk intervals.

Electrophoretic Analyses. Between 48 and 89 workers were analyzed by cellulose acetate gel electrophoresis to determine the proportions of hybrid (Africanized × European) and European workers from the 12 colonies containing mixed genotypes in experiment 1. The proportion of hybrid worker bees in those colonies was not different from expectations of 25 and 50% (0.22 ± 0.014 SE, $n = 6$, and 0.45 ± 0.054 SE, $n = 6$), respectively.

Morphometric Analyses. Worker honey bees were collected from 61 experimental colonies in vials with 95% ethanol and subjected to three morphometric discriminant analyses: forewing length (Sylvester & Rinderer 1987), Daly and Balling discriminant function analysis (Daly & Balling 1978, Daly et al. 1982), and USDA–ID version 2, a modification of the Daly and Balling technique (Rinderer et al. 1993).

Table 1. Correlation coefficients among morphometric scores, defensive behavior, and percentage Africanization of worker honey bee samples

Parameter	% Africanization	Forewing length (IX)	Daly and Balling (CV)	Daly and Balling (MC)	USDA–ID[a] version 2 (WES)	No. stings	Time to sting
% Africanization		0.36*	0.67**	0.62**	−0.52**	0.68**	−0.49*
Forewing length (IX)	−0.63***		−0.68**	−0.52**	−0.31*	0.34*	0.11ns
Daly and Balling (CV)	0.78***	−0.75***		0.76***	−0.54***	0.53***	0.33*
Daly and Balling (MC)	0.73***	−0.61***	0.81***		−0.62***	0.53***	0.25ns
USDA–ID version 2 (WES)	−0.61***	0.41**	−0.57***	−0.63***		0.52**	0.23ns
No. of stings	0.74***	−0.55***	0.65***	0.65***	−0.58***		−0.54**
Time to sting	−0.50**	0.27*	−0.35*	−0.35*	−0.27ns	−0.57**	

Data above the diagonal in the correlation matrix include only those colonies ranging from 12.5 to 50% (n = 49 for all methods except USDA–ID, where n = 47) Africanized. Data below the diagonal include all colonies ranging from 0 to 100% (n = 61 for all methods except USDA–ID, where n = 54) Africanized. *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***$P < 0.001$; ns = $P \geq 0.05$.

[a] USDA–ID values represent the probability that a given colony was Africanized.

For the first method used, we only considered one character, the length of the forewing. For the other two methods, we used the complete morphometric multivariate techniques, which measure 25 or 23 characters for the Daly and Balling and USDA–ID methods, respectively. Forewing length determinations were performed in our laboratory at Ixtapan, Mexico, whereas the Daly and Balling discriminant analyses were performed in two laboratories that are fully equipped for this purpose located in Cuernavaca, Mexico, and Mexico City. Both of these laboratories are operated by trained technicians who work for the Secretariat of Agriculture and Water Resources (SARH) African Bee Program. The software used in these facilities was installed by W. L. Rubink from the USDA–ARS Honey Bee Laboratory at Weslaco, TX. A blind dataset containing samples from 54 experimental colonies, digitized in Mexico City, was analyzed in the Weslaco laboratory by Rubink using new USDA–ID version 2 discriminant functions (Rinderer et al. 1993).

Statistical Analyses. Analysis of variance (ANOVA) and correlation analyses were performed on both the morphometric scores and the defensive behavior data (Sokal & Rohlf 1981). Estimated degree of Africanization of individual colonies, based on electrophoretic samples, was used for correlation analyses involving the 12.5 and 25% Africanized, mixed semen, treatment groups of experiment 1. However, these same colonies were lumped into discrete treatment groups of 12.5 and 25% for ANOVA.

Results

All morphometric analyses correctly identified all of the presumed pure European and Africanized colony samples. However, they failed to identify most of the colonies that were intermediate in degree of Africanization. Morphometric scores correlated significantly with degree of Africanization and defensive behavior.

Repeatability of Morphometric Analyses. Classifications of colonies were highly correlated for samples analyzed in the Cuernavaca and Mexico City laboratories (r = 0.81, $P < 0.001$ [Table 1]), although the actual scores based on the discriminant functions of Daly & Balling (1978) differed significantly (F = 14.49; df = 1, 54; $P < 0.001$).

Less than 80% of the samples with intermediate degrees of Africanization were scored as Africanized (Table 2). Of 16 F_1 hybrid samples (50% Africanized), 13, 6, and 2 were scored Africanized by the forewing length, Daly and Balling, and USDA–ID methods, respectively. Two additional F_1 colonies were classified as Africanized with evidence of introgression of European genes by the USDA–ID method. Detection of Africanization of 12.5 and 25% was nearly impossible. Of 33 samples, 9 were scored as Africanized by the forewing length method (27.3%), 1 by the Daly and Balling method in Mexico City (3.0%), and 0 of 31 samples (0.0%) by the USDA–ID method (see Table 2). However, one sample (3.0%) was classified as Africanized with evidence of introgression of European genes by the USDA–ID method.

Classification scores differed among treatments. For example, when the forewing length method was used, all groups except for the 12.5 and 25% Africanized were significantly different from each other (F = 11.97; df = 4, 56, pairwise tests based on Fisher's protected least significant distance [PLSD]; 12.5 and 25% treatments for experiments 1 and 2 were combined for analyses). Workers from 100% Africanized colonies had an average forewing length of 8.78 mm, whereas pure European colonies averaged 9.19 mm. These values are close to the respective mean forewing length values presented by Rinderer et al. (1993). Hybrid colony averages ranged between 8.94 and 9.07 mm among all treatment groups of experiments 1 and 2 (F = 9.06; df = 6, 54; $P < 0.0001$).

Correlation of Africanization and Defensive Behavior. In all cases, defensive behavioral traits

Table 2. Number (and percentage) of colonies of known degrees of Africanization that were classified Africanized (A), European (E), unclassified (U), and, for the USDA–ID analyses, Africanized with evidence of the introgression of European genes [A(I)], and European with evidence of the introgression of Africanized genes [E(I)]

% Africanization[a]	Discriminant analysis procedures[a]											
	Forewing length (IX)			Daly and Balling (CV)		Daly and Balling (MC)		USDA–ID version 2 (WES)				
	A	E	U	A	E	A	E	A	A(I)	E	E(I)	
100 Am	6 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	6 (100.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	
50 Em	4 (66.7)	2 (33.3)	0 (0.0)	1 (66.7)	5 (83.3)	1 (16.7)	5 (83.3)	1 (16.7)	1 (16.7)	4 (66.7)	0 (0.0)	
50 Am	9 (90.0)	1 (10.0)	0 (0.0)	5 (50.0)	5 (50.0)	5 (50.0)	5 (50.0)	0 (0.0)	1 (10.0)	7 (70.0)	2 (20.0)	
25 Bm	2 (18.2)	8 (72.7)	1 (9.1)	0 (0.0)	11 (100.0)	1 (9.1)	10 (90.9)	0 (0.0)	1 (9.1)	10 (90.9)	0 (0.0)	
25 Em	2 (33.3)	2 (33.3)	2 (33.3)	0 (0.0)	6 (100.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)	
12.5 Bm	2 (20.0)	7 (70.0)	1 (10.0)	0 (0.0)	10 (100.0)	0 (0.0)	10 (100.0)	0 (0.0)	0 (0.0)	10 (100.0)	0 (0.0)	
12.5 Em	3 (50.0)	1 (16.7)	2 (33.3)	0 (0.0)	6 (100.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)	
0.00 Em	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	6 (100.0)	0 (0.0)	0 (0.0)	6 (100.0)	0 (0.0)	

Colonies contained either Africanized (Am), European (Em), or backcrossed (Bm) mother queens. The percentage Africanization was a result of either backcrossing (experiment 2) or inseminating European queens with mixtures of European and Africanized semen.

[a] Performed in four different laboratories: Ixtapan (IX), Cuernavaca (CV), Mexico City (MC), and Weslaco, TX (WES).

(number of stings and time to sting) were significantly correlated with colony levels of Africanization. The number of stings received in test targets demonstrated higher correlations than the time to receive the first sting (Tables 1, 3, and 4).

The numbers of stings received in the test targets correlated significantly with scores of all morphometric methods when all the samples were included in the analyses. Correlations were significant also when only colonies with intermediate (12.5–50% Africanized) genotypes were considered. These correlations were dependent on the extreme defensive behavioral responses of "pure" Africanized and F_1 colonies. No significant correlations between defensive traits and methods occurred when only colonies presumed to be between 0 and 50% (presumed 12.5 and 25% Africanized) were analyzed (Table 4).

Time to sting was significantly correlated with the morphometric methods when samples representing all treatments were analyzed, reflecting the influence of the extreme genotypic groups. When 100% Africanized and European samples were not included in the analyses, time to sting was only correlated with the Daly and Balling method performed in Cuernavaca ($r = 0.33$, $P < 0.05$ [Table 1]).

Table 3. Mean ± SE time to sting (seconds) and number of stings in 60 s

% Africanization[a]	No. colonies	Time to sting	No. stings
100 Am	6	7.2 ± 2.24	137.3 ± 11.15
50 Am	10	11.1 ± 1.37	92.8 ± 14.23
50 Em	6	10.2 ± 2.58	130.8 ± 9.87
25 Bm	11	20.9 ± 5.73	42.3 ± 7.81
25 Em	6	16.3 ± 3.01	84.3 ± 10.56
12.5 Bm	10	45.0 ± 10.47	19.4 ± 3.88
12.5 Em	6	32.2 ± 8.22	42.0 ± 8.11
0.00 Em	6	55.9 ± 10.06	24.7 ± 7.18

[a] Colonies of different treatments have either Africanized (Am), European (Em), or hybrid (Bm) mother queens.

Discussion

Differences in average classification scores and in the number of samples scored as Africanized or European in two different laboratories (Cuernavaca and Mexico City) suggest that discriminant analyses performed in different laboratories by different people may yield different results. It was evident that the Cuernavaca scores were consistently higher than those reported in Mexico City for the same treatments. This could be a result of differential adjustment of the projected images of anatomical characters and potentially may result in a higher number of samples scored as Africanized in Cuernavaca.

Forewing length was a more reliable method for the detection of Africanization in hybrid bee samples, with 43% of the samples scored as Africanized, 14% as unidentified, and 43% misidentified as Europeans. Only 14 and 9% of hybrid samples were scored Africanized by the Daly and Balling (Mexico City) and USDA–ID methods, respectively (the classifications Africanized and Africanized with evidence of introgression of European genes were combined for the USDA–ID method). These results are consistent with those of Rinderer et al. (1986a). They found that the single character that best discriminated between Africanized and European bees was forewing length.

Colonies with intermediate and low levels of Africanization were not included in databases used as standards for morphometric analyses. The morphometric tests appear to be conservative, with an increased likelihood that colonies of intermediate degree of Africanization will be classified as European. Colonies of various degrees of Africanization may be common in apiar-

Table 4. Correlation coefficients among morphometric scores, defensive behavior, and percentage Africanized of worker honey bee samples

Parameter	% Africanization	Forewing length (IX)	Daly and Balling (CV)	Daly and Balling (MC)	USDA–ID[a] version 2 (WES)	No. stings	Time to sting
% Africanization		−0.14ns	0.15ns	0.22ns	[b]	0.50*	−0.43*
Forewing length (IX)	−0.01ns		−0.67**	−0.62**	[b]	−0.13ns	−0.04ns
Daly and Balling (CV)	0.11ns	−0.69**		0.69**	[b]	0.15ns	0.11ns
Daly and Balling (MC)	0.16ns	−0.43**	0.50**		[b]	0.06ns	0.06ns
USDA–ID version 2 (WES)	0.38*	−0.06ns	−0.27ns	0.38*		[b]	[b]
No. stings	0.30*	−0.18ns	0.16ns	0.12ns	−0.03ns		−0.54**
Time to sting	−0.41*	0.04ns	−0.10ns	−0.02ns	−0.13ns	−0.52**	

Data above the diagonal in the correlation matrix include only the backcrossed colonies belonging to the 12.5 and 25% treatments (n = 21). Data below the diagonal include mixed genotypes and backcrossed colonies of the 12.5 and 25% treatments (n = 33 for all methods except UDSA–ID, where n = 31). *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$; ns = $P \geq 0.05$.

[a] USDA–ID values represent the probability that a given colony was Africanized.

[b] All probabilities of Africanized were 0; therefore, correlation analyses were not performed.

ies located in Africanized areas where they are periodically requeened with commercially produced European queens. European daughters will be raised in these colonies when the mother queen dies or swarms. These daughters will then take mating flights and mate with a large number of drones from the area (see Page [1986] for review of mating behavior), including feral and commercial Africanized males. The resulting commercial colonies will then be Africanized, and undetectable. The failure to detect them may lead to the spread of Africanization if they are transported to non-Africanized areas for honey production or pollination services.

It is possible that our results show low reliability of morphometric techniques for hybrid bees because we used parents that were not Africanized or because our European parents were unusually large (or both). However, this is unlikely because repeated, blind morphometric analyses demonstrated that our presumed Africanized parental colonies were highly Africanized, with short wing measurements (8.78 ± 0.07 mm) and Africanized discriminant scores (Daly and Balling score average = 2.92 ± 0.02; probability of being Africanized = 1.00). Forewing length and discriminant scores of our European parental colonies are similar to those reported in the literature for average European workers. Moreover, our Africanized parental colonies reacted over seven times faster and stung over five times more than our European colonies (Table 3), again suggesting that they were highly Africanized.

Our results could have been affected by raising all of our sample bees in combs built by European workers. European bees build larger cells than Africanized bees (reviewed in Michener [1975]). Oldroyd et al. (1991) demonstrated high heritabilities for most of the 25 characters used in the multivariate analyses. Rinderer et al. (1986b) also demonstrated an environmental effect when they raised Africanized and European workers in different-sized combs and found that cell size was an environmental factor that significantly affected morphometrics. The larger European comb resulted in larger Africanized bees, whereas the smaller Africanized comb resulted in smaller European bees. However, despite these influences, the progeny were identified correctly by the 25-character discriminant analysis. It should also be noted that Africanized bees raised in commercial hives probably come from European-sized cells. Therefore, if morphometrics are to be used to detect Africanization of commercial colonies, they must be robust with respect to brood cell sizes.

In Mexico, the Africanization of managed colonies may be higher than what morphometric studies have indicated, a consequence of the lack of sensitivity of current morphometric methods. In a study conducted 3 yr after the arrival of Africanized bees in the state of Yucatán (Millan 1990), only 16% of 337 colonies sampled across the state were determined to be Africanized according to the morphometric analyses applied. Despite this apparent low level of Africanization, there had been hundreds of reported stinging incidents caused by commercially managed colonies during that time (Jorge Gonzalez, Assistant Director, SARH African Bee Program, personal communication). This observation is consistent with the failure of morphometric techniques to detect low levels of hybridization and the genetic dominance of strong defensive behavior (Guzmán-Novoa & Page 1993, 1994).

Defensive traits were highly correlated with the level of Africanization. The number of stings demonstrated the highest correlations. Number of stings showed similar correlations with the complete morphometric discriminant analyses (Daly & Balling and USDA–ID). Consequently, both methods could probably serve as indicators of Africanization with similar degrees of sensitivity. Time to sting, however, was a poor indicator of Africanization at lower levels of Africanization.

All morphometric methods yielded scores that correlated significantly with the actual percentage of Africanization of samples. The complete discriminant analyses showed higher correlations than the forewing length method. Correlations were significant when extreme types (0, 50, and 100% Africanized) were considered in the analyses; however, only the USDA–ID method correlated significantly with samples ranging between (but not including) 0 and 50% Africanized.

Alternative Methods of Identification. Accurate and rapid methods that identify low levels of Africanization are necessary before implementation of commercial honey bee certification programs. Such programs have been proposed to regulate the transportation of commercial colonies and to regulate the queen production industry of the United States (Sylvester et al. 1992). Morphometric methods are slow and unreliable. The complete 23- or 25-character analysis requires ≈4–5 h for one person to process a sample of 10 bees. Alternative methods include assays for specific proteins (Hung 1990, Davidson et al. 1992, Freeman et al. 1992), isozymes (Sylvester 1982, Nunamaker et al. 1984, Del Lama et al. 1988, Spivak et al. 1988), cuticular hydrocarbons (Carlson & Bolton 1984, Smith 1988, Brand et al. 1991), and mitochondrial DNA analyses (Hall & Muralidharan 1989, Smith et al. 1989, Hall & Smith 1991). However, none of these methods has been tested independently for their efficacy in determining hybrid colonies.

It is not likely that any technique or set of techniques will lead to reliable determination of lower levels of Africanization for individual colonies. Page & Erickson (1985) demonstrated the difficulties of using allozyme analyses to distinguish Africanized and European colonies under conditions of interbreeding and gene introgression. Any technique based on single-gene information contained in the nuclear genome (such as specific protein analyses) will have the same difficulties. Mitochondrial DNA is accurate for establishing maternal lineage (Hall & Muralidharan 1989) but is of limited use for commercial colonies, where the queens are expected to be of European descent, but mated to Africanized drones. Techniques that use presumed polygenic traits, like cuticular hydrocarbons, will have the same difficulties as those based on morphometrics.

Therefore, a combination of simple techniques may be the most advisable procedure at the current time. Forewing length is easily measured (requires about 16 min per colony) and provides relatively reliable information about Africanization and defensive behavior, particularly when commercial bees are sampled in Africanized areas. Commercial European bees are significantly larger in size than both European feral and Africanized bees (Daly et al. 1991). Errors will be made on a colony-by-colony basis; however, average wing lengths for apiaries or commercial beekeeping establishments may yield useful information about the amount of genetic control of a particular beekeeping operation. As beekeepers lose control of their stocks, wing lengths should decrease.

Mitochondrial DNA analyses are fast and also may be reliable indicators of control of management practices. Beekeepers who requeen regularly, remove empty hive equipment, and do not collect feral swarms should be free of African-type mitochondria. A single laboratory technician can make mitochondrial determinations on ≈50 colonies per day. Defensive behavior tests could be applied routinely to test for the effects of Africanization. Regulation and certification of honey bee stocks should, therefore, emphasize colony behavior and beekeeping management, not the pedigrees of individual colonies.

Acknowledgments

Guillermo García, owner of Vita-Real, provided labor, colonies, transportation, and laboratory space. Esperanza Ochoa, Gonzalo Delabra, and Bill Rubink performed morphometric analyses of worker bees. Jorge J. González, Francisco Souza, and Enrique Estrada provided valuable assistance. This study was funded in part by INIFAP–SARH and by contracts from the California Department of Food and Agriculture.

References Cited

Brand, H. M., S. L. Puleo & E. E. Brand. 1991. Identification of the Africanized bee in South America by the composition of its wax. Bee Sci. 1(2): 106–111.

Carlson, D. A. & A. B. Bolten. 1984. Identification of Africanized and European honey bees using extracted hydrocarbons. Bull. Entomol. Soc. Am. 30: 32–35.

Collins, A. M. & K. J. Kubasek. 1982. Field test of honey bee (Hymenoptera: Apidae) colony defensive behavior. Ann. Entomol. Soc. Am. 75: 383–387.

Collins, A. M., H. V. Daly, T. E. Rinderer & J. R. Harbo. 1994. Correlations between morphology and colony defense in *Apis mellifera* L. J. Apic. Res. (in press).

Collins, A. M., T. E. Rinderer, J. R. Harbo & A. B. Bolten. 1982. Colony defense by Africanized and European honey bees. Science (Washington, DC) 218: 72–74.

Contel, E.P.B., M. A. Mestriner & E. Martins. 1977. Genetic control and developmental expression of malate dehydrogenase in *Apis mellifera*. Biochem. Genetics 15: 859–876.

Daly, H. V. & S. S. Balling. 1978. Identification of Africanized honey bees in the Western hemisphere by discriminant analysis. J. Kans. Entomol. Soc. 51: 857–869.

Daly, H. V., K. Hoelmer, P. Norman & T. Allen. 1982. Computer-assisted measurement and identification of honey bees (Hymenoptera: Apidae). Ann. Entomol. Soc. Am. 75: 591–594.

Daly, H. V., K. Hoelmer & P. Gambino. 1991. Clinal geographic variation in feral honey bees in California, USA. Apidologie 22: 591–609.

Davidson, F. I., R. Udagawa, E. Verdel & G. B. Kitto. 1992. Africanized honey bee specific proteins and their use for immunoassay development. Bee Sci. 2: 193–199.

Del Lama, M. A., R. A. Figueiredo, A.E.E. Soares & S. N. Del Lama. 1988. Hexoquinase polymorphism in *Apis mellifera* and its use for Africanized honeybee identification. Rev. Bras. Genet. 11: 287–297.

Freeman, G. W., C. A. Wagg & J. T. Schuler. 1992. A survey of New Jersey honey bees to determine the presence of proteins specific to Africanized honey bees. Am. Bee J. 132: 542–543.

Guzmán-Novoa, E. & R. E. Page. 1993. Backcrossing Africanized honey bee (*Apis mellifera* L.) queens to European drones reduces colony defensive behavior. Ann. Entomol. Soc. Am. 86: 352–355.

1994. Genetic dominance and worker interactions affect honey bee colony defense. Behav. Ecol. 5: 91–97.

Hall, H. G. & K. Muralidharan. 1989. Evidence from mitochondrial DNA that African honey bees spread as continuous maternal lineages. Nature (Lond.) 339: 211–213.

Hall, H. G. & D. R. Smith. 1991. Distinguishing African and European honeybee matrilines using amplified mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. 88: 4548–4552.

Hung, A.C.F. 1990. Preliminary evidence for a protein specific to an African honey bee race (*Apis mellifera scutellata*). Am. Bee J. 130: 49–50.

Kerr, W. E. 1967. The history of the introduction of Africanized bees to Brazil. S. Afr. Bee J. 39: 3–5.

Laidlaw, H. H. 1977. Instrumental insemination of honey bee queens. Dadant and Sons, Hamilton, IL.

Mackensen, O. 1947. Effect of carbon dioxide on initial oviposition of artificially inseminated and virgin queen bees. J. Econ. Entomol. 40: 344–349.

Michener, C. D. 1975. The Brazilian bee problem. Annu. Rev. Entomol. 20: 399–416.

Millan, M.A.G. 1990. Distribution of the Africanized honey bee in commercial apiaries in the state of Yucatán. Tésis, Médico Veterinario y Zootecnista, Universidad Autonoma de Yucatán, Mexico.

Moritz, R. F. 1983. Homogeneous mixing of honeybee semen by centrifugation. J. Apic. Res. 22: 249–255.

Nunamaker, R. A., W. T. Wilson & B. E. Haley. 1984. Electrophoretic detection of Africanized honey bees (*Apis mellifera scutellata*) in Guatemala and Mexico based on malate dehydrogenase allozyme patterns. J. Kans. Entomol. Soc. 57: 622–631.

Oldroyd, B., T. Rinderer & S. Buco. 1991. Heritability of morphological characters used to distinguish European and Africanized honeybees. Theor. Appl. Genet. 82: 499–504.

Otis, G. W. 1980. The swarming biology and population dynamics of the Africanized honey bee. Ph.D. dissertation, University of Kansas, Lawrence.

Page, R. E. 1986. Sperm utilization in social insects. Annu. Rev. Entomol. 31: 297–320.

Page, R. E. & E. H. Erickson. 1985. Identification and certification of Africanized honey bees. Ann. Entomol. Soc. Am. 78: 149–158.

Page, R. E. & H. H. Laidlaw. 1988. Full sisters and super sisters: a terminological paradigm. Anim. Behav. 36: 944–945.

Rinderer, T. E. 1988. Evolutionary aspects of the Africanization of honey-bee populations in the Americas, pp. 13–28. *In* G. R. Needham, R. E. Page, Jr., M. Delfinado-Baker & C. E. Bowman [eds.], Africanized honey bees and bee mites. Horwood, Chichester, U.K.

Rinderer, T. E., A. M. Collins & K. W. Tucker. 1985. Honey production and underlying nectar harvesting activities of Africanized and European honeybees. J. Apic. Res. 23: 161–167.

Rinderer, T. E., H. A. Sylvester, M. A. Brown, J. D. Villa, D. Pesante & A. M. Collins. 1986a. Field and simplified techniques for identifying Africanized and European honey bees. Apidologie 17: 33–48.

Rinderer, T. E., H. A. Sylvester, A. M. Collins & D. Pesante. 1986b. Effect of nurse-bee genotype and comb size on morphometrically based identification of Africanized and European honey bees. Bull. Entomol. Soc. Am. 32: 150–152.

Rinderer, T. E., S. M. Buco, W. L. Rubink, H. V. Daly, J. A. Stelzer, R. M. Riggio & F. C. Baptista. 1993. Morphometric identification of Africanized and European honey bees using large reference populations. Apidologie 24: 569–585.

Rinderer, T. E., H. V. Daly, H. A. Sylvester, A. M. Collins, S. M. Buco, R. L. Hellmich & R. G. Danka. 1990. Morphometric differences among Africanized and European honey bees and their F1 hybrids (Hymenoptera: Apidae). Ann. Entomol. Soc. Am. 83: 346–351.

Smith, D. R., O. R. Taylor & W. M. Brown. 1989. Neotropical Africanized honey bees have African mitochondrial DNA. Nature (Lond.) 339: 213–215.

Smith, R.-K. 1988. Identification of Africanization in honey bees based on extracted hydrocarbons assay, pp. 275–280. *In* G. R. Needham, R. E. Page, Jr., M. Delfinado-Baker & C. E. Bowman [eds.], Africanized honey bees and bee mites. Horwood, Chichester, U.K.

Sokal, R. R. & F. J. Rohlf. 1981. Biometry. Freeman, New York.

Spivak, M., T. Ranker, O. Taylor, W. Taylor & L. Davis. 1988. Discrimination of AHBs using behavior, cell size, morphometrics and a newly discovered isozyme polymorphism, pp. 313–324. *In* G. R. Needham, R. E. Page, Jr., M. Delfinado-Baker & C. E. Bowman [eds.], Africanized honey bees and bee mites. Horwood, Chichester, U.K.

Stort, A. C. 1974. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. I. Some tests to measure aggressiveness. J. Apic. Res. 13: 33–38.

1975a. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. II. Time at which the first sting reached the leather ball. J. Apic. Res. 14: 171–175.

1975b. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. IV. Number of stings in the gloves of the observer. Behav. Genet. 5: 269–274.

1975c. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. V. Number of stings in the leather ball. J. Kans. Entomol. Soc. 48: 381–387.

Sugden, E. A. & K. R. Williams. 1991. October 15: the day the bee arrived. Glean. Bee Cult. 119(1): 18–21.

Sylvester, H. A. 1976. Allozyme variation in honeybees (*Apis mellifera* L.). Ph.D. dissertation, University of California, Davis.

1982. Electrophoretic identification of Africanized honeybees. J. Apic. Res. 21: 93–97.

Sylvester, H. A. & T. E. Rinderer. 1987. Fast Africanized bee identification system (FABIS) manual. Am. Bee J. 127(7): 511–516.

Sylvester, H. A., T. E. Rinderer & H. Shimanuki. 1992. Certification options for dealing with Africanized bees. Am. Bee J. 132(3): 182–184.

Villa, J. D. 1988. Defensive behavior of Africanized and European honeybees at two elevations in Colombia. J. Apic. Res. 27: 141–145.

Williams, J. L. & J. R. Harbo. 1982. Bioassay for diluents of honey bee semen. Ann. Entomol. Soc. Am. 75: 457–459.

Winston, M. L. 1979. Intra-colony demography and reproductive rate of the Africanized honeybee in South America. Behav. Ecol. Sociobiol. 4: 279–292.

Received for publication 19 July 1993; accepted 4 March 1994.

The Impact of Africanized Bees on Mexican Beekeeping

by ERNESTO GUZMAN-NOVOA[a] and ROBERT E. PAGE, JR.[b,c]

INTRODUCTION

African bees (*Apis mellifera scutellata*) were introduced into Brazil in 1956 as part of a selective breeding program designed to produce a bee that was better adapted to tropical conditions (Kerr 1967). As a consequence of this breeding program, African bees became feral and spread over a major area of Southern Brazil by 1963 (Nogueira-Neto 1964). Feral colonies of African bees interbred to an unknown extent with the local populations of European honey bees, producing the now well-known africanized bee. Africanized bees have since spread through most of the Americas, and reached the United States in 1990 (Sugden and Williams 1991).

The objective of this paper is to give an overview of the current status of africanized bees and their effects on the Mexican beekeeping industry, as well as to describe how africanized bees have affected a particular commercial operation and what this operation is doing to cope with the problem. Beekeeping operations in Mexico are sophisticated and modern. The best businesses rival any in the world for methods of queen rearing, breeding, honey production, and pollination services. This was true before the invasion of africanized bees and is still true today. Several of the larger companies employ specialists to deal with disease problems and technicians skilled in instrumental insemination to produce controlled stocks for queen production. The impression that Mexican beekeeping is "Third World" and, therefore, defenseless against invasion by africanized bees is totally unfounded.

Distribution of Africanized Bees

Africanized bees were first detected in Chiapas, Mexico in late 1986 (Moffett *et. al.* 1987). Since then they have spread throughout all states in Mexico except the Baja California peninsula. It was hoped that africanized bees would not become established on the high, cold plateau of Mexico. However, they were detected in Mexico City in early 1990 and are now firmly established (Jorge J. González, Assistant Director of the Mexican African-Bee Program, pers. comm.).

Africanized bees were first reported in the United States in October, 1990 in Hidalgo Texas, near the border with Tamaulipas, Mexico (Sugden and Williams 1991). They have since spread through much of Texas and were detected in Arizona in the spring of 1993, New Mexico in the fall, and are currently on the western border of California (Arizona State Department of Agriculture). In July, 1993, SARH (Mexican Secretariat of Agriculture and Water Resources) officials confirmed the presence of africanized bees in Mexicali, Mexico, located approximately 25 Km from El Centro, California. It is believed that these bees are part of the migrating front spreading from southern Texas. If so, then it has taken less than three years for the africanized bees to spread from the coast of the Gulf of Mexico almost to the Pacific coast of California, a distance of more than 1,800 Km. This rate of spread is faster than that of 300-500 Km per year observed in South and Central America (Taylor 1977, 1985) and suggests that africanized bees will spread rapidly throughout the southwestern United States and throughout California.

IMPACT OF AFRICANIZED HONEY BEES ON THE MEXICAN BEEKEEPING INDUSTRY

Honey Production and Number of Colonies

As of 1992 the total production of honey in Mexico has not decreased. After the arrival of the first africanized bee swarms in Chiapas, national honey production figures were as follows: 62.9, 57.8, 61.8, 66.5, 69.5, and 64.0 thousand metric tons for the years 1987,1988, 1989, 1990, 1991, and 1992, respectively (source: SECOFI: Mexican Secretariat of Commerce and Industry). However, these data include reports from areas that have only recently become africanized. Honey production declined for the first 3 years following the invasion of africanized bees in Chiapas and the Yucatan Peninsula, the first states to be affected (Fig. 1). However, poor honey flow conditions could also have contributed to this decline, but difficulties associated with managing africanized bees are thought to be primarily responsible. Honey production has returned to nearly the same levels as before, probably a consequence in changes of management practices and increased number of colonies.

This pattern of decreasing honey production followed by

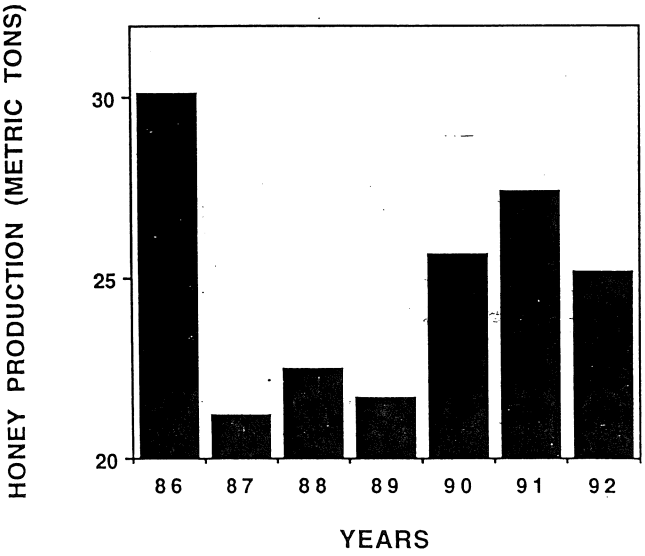

Fig. 1. Honey production for the period 1986-1992 in the states of Chiapas, Yucatan, Quintana Roo, and Campeche combined (source: SECOFI). These four states were the first areas where africanized bees became established in Mexico.

[a] CENIFMA-INIFAP-SARH, Paseo Jurica 634, Jurica Campestre, Queretaro, QRO. 76030, MEXICO
[b] Department of Entomology, University of California, Davis, CA 95616
[c] To whom reprint requests should be addressed

restored production to near previous levels is consistent with what occurred in areas of South America. A good example is Mr. W. Vogel, a Venezuelan beekeeper. He experienced decreasing honey yields and colony losses during the first years following africanization of his area. This resulted in dramatic changes in his management methods and, in time, his yields and number of colonies almost returned to previous levels.

Statistics obtained from the Mexican African-Bee Program (SARH) show that the annual national honey-yield per colony has decreased from 32.5 Kg in 1985-1986 to 26.8 Kg in 1991-1992. This information agrees with reports from individual beekeepers from southeastern Mexico who claim that honey yields per colony have decreased by 15-50%. Beekeepers attribute this decrease in yield to swarming, absconding, and competition from feral colonies. Decreases in honey yield per colony are not uniform throughout Mexico. Large beekeeping operations from Oaxaca on the Pacific coast used to average more than 100 Kg per colony, but they have gone out of business in the past two years after yields dropped to less than 50 Kg per hive. These beekeepers also blame the many problems associated with managing defensive bees for their failure. In the state of Guerrero (north of Oaxaca), however, beekeepers claim that honey yields are about the same as they were before africanization. It is likely that beekeepers in Guerrero have not yet felt the full impact of africanized bees.

Officials of SARH report that africanization has caused some large beekeeping operations to reduce their numbers of hives due to difficulties associated with managing africanized bees. Offsetting the down scaling of large operations is an increase in new beekeepers that maintain small numbers of colonies. This is due to the increased availability of swarms of africanized bees. A net increase in the total numbers of hives partially explains the recent increase in honey production following the decline in southeastern Mexico and the overall small change in total production in Mexico. Honey yields have been reduced by about 18% per hive, but numbers of managed hives have increased from 2.2 million in 1986 to 2.6 million in 1992 (Dirección General de Estadística-SARH).

The opinions of beekeepers vary about the productivity of africanized bees. Some believe that they are superior honey producers compared with European bees, while others think they are about the same or slightly worse. Some colonies produce amounts of honey "as they had never produced", while others produce "no honey at all." Nearly all beekeepers agree that there is much more variation in honey yield per colony within apiaries after africanization.

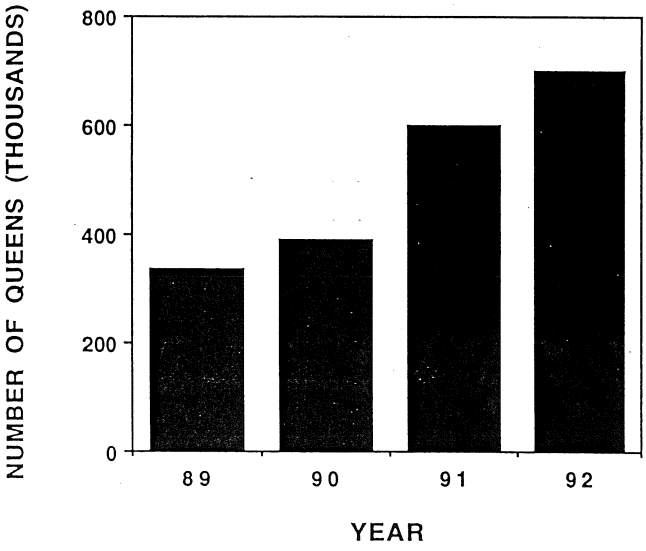

Fig. 2. Number of queen honey bees produced in Mexico between 1989 and 1992 (source: Dirección General de Estadística - SARH).

Queen Production

The production of queen honey bees has more than doubled during the past three years (Fig. 2). This increase is a response to increasing demand for gentle stock to replace africanized bees in managed colonies. The largest single purchaser of queens is the National African-Bee Program. They purchase nearly 100,000 queens annually and distribute them to beekeepers at subsidized prices. Unfortunately, an additional 2 million queens are needed to annually requeen all of the managed colonies.

Stinging Incidents

Beekeepers are able to take precautions in the apiary and avoid serious stinging incidents, but it is impossible to prevent interactions between the public and managed africanized bees. The number of human deaths per year due to bee stings continues to increase as Mexico becomes more africanized (Fig. 3). SARH officials estimate that at least 30% of the fatal incidents go unreported because they occur in areas with poor health and communication infrastructure. SARH officials predict that the death rate will rise to about 60 deaths per year (one death per 1.4 million people per year) once all areas of Mexico become highly africanized. Of the total official deaths spanning a period of six years (192), 71% have been people 51 years of age or older.

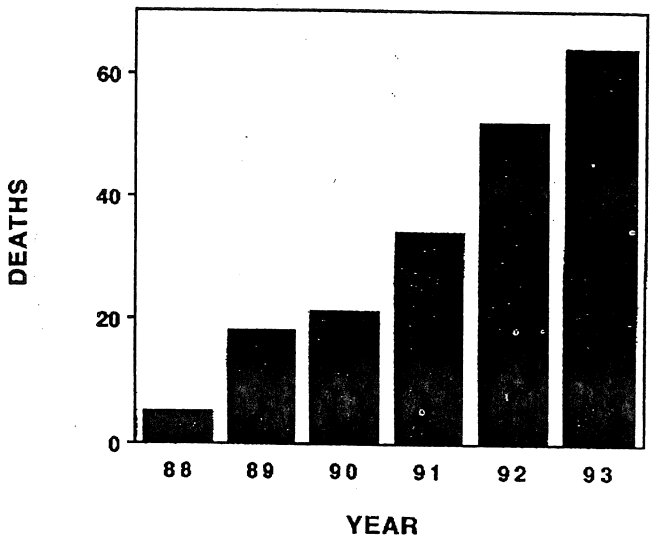

Fig. 3. Number of people killed by africanized bees in Mexico for the period 1988-1993 (source: Dirección General de Estadística - SARH).

The National African-Bee Program captured and eliminated an average of 117,000 swarms each year during 1990-1992. Most of these swarms were captured in swarm traps located in urban areas. They believe that this swarm capture-elimination program has helped to minimize the number of stinging incidents in urban areas like Mexico City and Guadalajara.

There are no official figures on animal deaths due to stinging incidents. Yet, it is common to hear stories of cows, horses, and chickens being killed by severe stinging attacks. Cows and horses are often killed because their owners tether them near apiaries or feral colonies. Chickens are typically killed while confined in cages where they cannot escape. Incidents often occur when animals (including humans) pass by apiaries that have recently been manipulated by beekeepers.

Beekeepers usually pay for the medical expenses and cover the costs of lost animals when it can be proven that their bees were responsible for an attack. Normally, the injured party contacts the beekeeper directly and they settle "out of court" with-

out incurring additional legal costs. The beekeeper typically does not pay for damages other than those that actually occur.

Changes in Management Practices

The presence of africanized bees in Mexico has forced many changes in management practices that have increased the production costs of commercially managed colonies by about 30% - 50%. These increased costs are due primarily to the following factors:

1. Relocation of apiaries. Apiaries have been relocated in order to diminish stinging risks of humans and domesticated animals. In States such as Veracruz, Mexico, Morelos, and Puebla, where population density is high, beekeepers report having relocated over 35% of their apiaries. However, in states such as Yucatan and Oaxaca, where population density is lower, fewer than 15% of apiaries have been relocated. Many beekeepers, such as Mr. A. Acevedo from Matais Romero, Oaxaca, have reduced their number of colonies per apiary. Mr. Acevedo maintains 1,800 hives and has reduced the average number of colonies from 25 to 18 in each of his apiaries. It is his opinion, as well as that of many other beekeepers in his area, that fewer colonies reduces competition for floral resources that are already heavily exploited by feral colonies. In addition, having fewer colonies reduces the amount of time spent in an apiary and decreases colony defensiveness and robbing behavior (A. Acevedo, pers. comm.). Beekeepers also move their more defensive colonies to areas of the apiary several meters away from the rest of the hives. Beekeepers manipulate the more defensive colonies in an apiary last.

Relocation of apiaries to more isolated areas has resulted in higher maintenance costs per colony. Apiaries are spread farther apart resulting in higher fuel costs and wear and tear on vehicles. Labor costs are also increased because individual laborers can work fewer colonies per day because they spend more time traveling between apiaries.

2. Cost of labor. Labor costs have increased for several reasons: 1. Each laborer works fewer hives per day because apiaries are spread over greater distances and are in less accessible areas. Therefore, it takes more laborers to provide the same amount of colony work. 2. Laborers demand higher wages because the bees are highly defensive. 3. Colonies require more management, requiring more visits, to control for swarming and to harvest honey. Honey is harvested three or four times per season compared with one or two times per season before africanization.

3. Queen replacement costs. Prior to africanization fewer than 10% of Mexican beekeepers requeened regularly. Today, most beekeepers as a minimum replace the queens of the most defensive colonies, many requeen all of their colonies regularly. Even beekeepers with minimal financial resources try to produce new queens by replacing their old queens with queen cells produced from larvae grafted from their least defensive colonies. These practices, of course, have increased costs.

4. Cost of protective equipment. Beekeepers must purchase better protective equipment to work with highly defensive colonies. Only seven years ago few beekeepers wore coveralls and almost none wore gloves. Today, almost all beekeepers wear coveralls and many wear gloves.

5. Cost of feeding bees. Africanized bees respond to nectar dearth by absconding. Therefore, beekeepers continuously feed sugar during these periods in order to decrease colony loses. Absconding is a major problem in some areas such as Sinaloa where beekeepers routinely rent their colonies for pollination services. J. Pompa is one of the largest beekeepers in the area and maintained about 9,000 colonies in 1992 for migratory pollination services. In 1993, he lost almost 7,000 of them due to absconding (J. Pompa, pers. comm.). Movement of africanized bees to exploit honey resources does not seem to pose a similar problem. As in past years, beekeepers still move over 35,000 colonies from the state of Veracruz to Puebla and Tlaxcala during the summer and return them the following spring - so far, without serious difficulties.

Increased production costs are affecting larger beekeeping operations more adversely than smaller ones. Beekeepers with large numbers of colonies usually have more difficulties finding suitable places for bee yards, have to travel greater distances, and have to spend more money on labor. Moreover, larger operations are not able to check their colonies as often as smaller ones. As a result, large beekeeping establishments are reducing their numbers of colonies, and many have even quit keeping bees altogether. As mentioned above, small beekeeping operations are on the increase and this trend toward smaller businesses is expected to continue.

In summary, beekeeping in Mexico today is much more expensive than it was before africanization. This increase in cost is the result of difficulties that arise from managing bees that are defensive and prone to swarm and abscond. Beekeepers who have remained in business have reduced their numbers of colonies, spread them out in more remote apiaries, feed their bees regularly, and requeen more frequently.

Vita Real, S.A. : A Case Study

So far, we have discussed beekeeping in Mexico in general. Now we will present details of a single beekeeping operation that we have been working with for the past three years. The company, Vita-Real, S.A., is owned by Mr. Guillermo García and is located in Ixtapan de la Sal, approximately 150 Km southwest of Mexico City.

Vita Real currently maintains around 3,800 colonies. Apiaries are located at elevations from 1,300 to 2,600m above sea level, spanning climates ranging from subtropical to temperate. This beekeeping operation annually produces about 136 metric tons of honey, 11,000 queens, and 90 Kg of royal jelly. This production is obtained, packed, and marketed with the help of 45 employees, of which 17 are beekeepers.

Africanized bees were first detected by SARH officials in the Ixtapan vicinity in the spring of 1990 (Jorge J. González, Assistant Director of the Mexican African-Bee Program, pers. comm.). However, they probably had been in the area before 1990. Beekeepers at Vita Real noticed that defensive behavior began to increase in managed hives in 1989, before any africanized colonies were detected in the area. In addition to this observation, samples of bees taken from managed hives in 1990 showed that some of them already had African type mitochondrial DNA (Hall and Muralidharan 1989, Hall and Smith 1991), an absolute indicator that africanization was already in progress. However, these same samples were classified European based on the standard morphometric identification methods. Samples taken in early 1991 showed more African mitochondrial DNA, africanized morphometric scores, as well as pronounced strong defensive behavior in some of the colonies. We emphasize here that Vita Real requeens regularly, all hives at least annually, and inspects all colonies at least once every three weeks. In spite of this effort, africanization still progresses.

Difficulties for Vita Real

At present, the main problems posed by africanized bees to Vita Real, are similar to those of Mexico in general. Below we list specific difficulties in order of importance.

1. Apiary location. Finding suitable apiary locations is the main problem, not only for Vita Real, but for most commercial beekeeping operations. News media sensationalism, as well as frequent stinging incidents throughout Mexico, have caused landowners to be reluctant to allow managed bees on their property, even when they get paid for the location.

2. Hiring beekeepers. Hiring beekeepers, or personnel to be trained as beekeepers, is difficult. Most would rather work at lower paying field labor than to work with highly defensive africanized bees.

3. Stinging incidents. In 1991, Vita Real colonies were involved in 19 confirmed, serious stinging incidents involving people and animals. In 1992, the first year after initiating a selective breeding program at Vita Real (see details below), the number of confirmed incidents was only five. So far, during 1993 (up to November), only one stinging incident has been confirmed.

Feral colonies, and managed colonies that belong to less conscientious beekeepers, however, still present difficulties for Vita Real. People blame Vita Real for their animal losses, even when their colonies were not responsible. This occurs because Vita Real is the largest beekeeping operation in the area. In 1992, angry residents burned one apiary and sprayed three others with pesticides. Two more apiaries were sprayed in 1993 and dozens of colonies have been overturned.

4. Increased costs of production. Costs for Vita Real have gone up to U.S. $42.00 per hive per year. This amount is about 40% higher than before africanization for all of the same reasons listed above.

5. Other minor problems. Vita Real beekeepers believe that queens are now superseded more often than before africanization. In addition, it is difficult to establish and maintain mating nuclei because the bees don't remain in the nucs. Queens often emerge into empty nuclei without workers to care for them. About 45% of mating nuclei produce successfully mated queens, compared with about 55% in 1989, a reduction of about 20%.

Management for Honey Production

Apiaries have been relocated away from people and animals, but they are still near roads. In Vita Real, honey is harvested in Spring and Autumn. Approximately 85% of the crop is obtained from late September to late November. Therefore, management for honey production is focused on the three to four months prior to the initiation of the main honey flow. Management activities include the following:

Mating yard near Ixtapan de la Sal, Mexico.

1. Requeening. Locating queens in africanized colonies is very difficult because the workers and queens run all over the combs. Vita Real's bees, however, are not yet highly africanized thus, queen finding is about the same as with European colonies. Colonies are checked three weeks after requeening to verify that queens were accepted. (We introduced more than 800 marked queens and determined that on average about 70% of them get accepted.) Colonies that did not accept a queen the first time are given a second queen; colonies with poor brood patterns are requeened again. All highly defensive colonies are requeened when they are identified. Vita Real introduces an average of about 1.5 queens per colony per year.

2. Routine examinations. All 3,800 colonies are examined every three weeks, or less. When an apiary is checked, all colonies are heavily smoked before any are opened for inspection. After inspection, all colonies are smoked again before leaving the apiary. Colonies are examined for diseases, queen cells, and food reserves.

3. Feeding. About 25 Kg of sugar are fed to each colony between late June and late August or early September, depending on the length of the rainy season.

4. Movement of bees. Approximately 1,200 colonies are moved from lower elevations (1,300 - 1,800 m) to higher elevations (2,200-2,600m) in January of each year. These colonies, together with others permanently located at higher elevations, produce the Spring crop that is harvested in April. Colonies are relocated back to lower elevations between June and August to prepare them for the main Autumn nectar flow. Colonies are transported on trucks with entrances opened, without nets, and without many problems.

5. Honey harvest. Honey is harvested once per colony during the spring and an average of three times, spaced two or three weeks apart, during the Fall honey flow. This is a marked change in management as a consequence of africanization. Prior to africanization Vita Real harvested honey from each colony once, or at most two times, during the Fall flow. However, our preliminary experimental results have shown that by harvesting three times, compared to two, it is possible to increase the yield per colony by about 30% in this area.

Queen Breeding and Production

Vita Real queens are raised using techniques that are common throughout the United States and Mexico. Queen and drone mothers, however, are selected based on extensive evaluations of honey production and defensive behavior.

Honey production is measured for each of the approximately 3,800 colonies by counting the number of combs of honey harvested. Data for each colony are entered into a computer file and evaluated by Statview®, a statistical analysis program. Each colony is evaluated against the average production of its own apiary and assigned a Z score, which is a statistical measurement of how much greater or smaller that colony's honey yield is from the apiary average (see Rinderer 1986 for a more detailed explanation on how to use Z scores in selective breeding). Colonies are then listed in descending order according to their Z scores. The top 7% of the colonies (approx. 250) are selected for defensive behavior assays.

Defensive behavior is measured with a behavioral assay similar to the one used by Villa (1988, see also Guzmán-Novoa and Page 1993 and in press). A black suede leather patch (10 x 10

cm) is suspended on a piece of white wood (0.7 x 0.5 x 100 cm) and rhythmically elevated (~ 4 cm) and lowered (~ 4 cm; two cycles per second) approximately 5-10 cm in front of the entrance of each hive. The bees are permitted to sting the patches for 60 seconds. The number of stings are counted and recorded after each of two trials. All colonies in an apiary are tested simultaneously in order to decrease the likelihood that bees from a single defensive hive sting the patches presented to others.

The brood pattern is checked for the 100 least defensive colonies. Ten workers from each of the 60 colonies with the most uniform brood patterns are collected and subjected to forewing length measurements (FABIS I, see Sylvester and Rinderer 1987 and Rinderer et. al. 1986). Approximately 35-45 queens from superior performing colonies with average forewing lengths of at least 9.1 mm are finally selected as queen mothers. We use forewing length as a measure of how successful the program is. Feral africanized bees are smaller than managed, commercial bees. Therefore, if the managed bees in the breeding program become smaller over time, then it demonstrates a lack of sufficient control over selection and that the commercial queens are mating with too many feral drones. Forewing length is also a good measure of the success of the breeding program because it is significantly correlated with defensive behavior, at least during the early stages of africanization of commercial colonies (rho= -0.74, n=80, P<0.0001, Spearman rank correlation; see Fig. 4). Previous studies

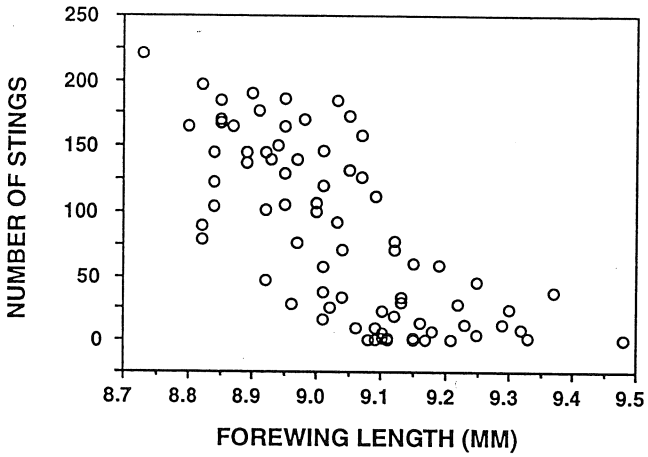

Fig. 4. Number of stings deposited by bees in a leather patch in a 60 s interval as a function of average forewing length from managed Vita Real colonies.

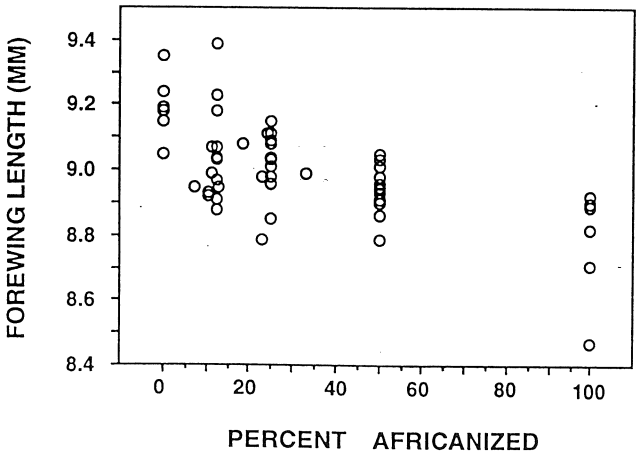

Fig. 5. Average forewing length for bees belonging to colonies with different degrees of africanization. Colonies were from Guzmán-Novoa and Page (1993, in press).

(Guzmán-Novoa et. al., unpublished data) have also shown a strong, significant correlation between forewing length and defensive behavior (r= -0.55, n= 61, P < 0.001), and between forewing length and the degree of africanization (r= -0.63, n=61, P<0.001; see Fig. 5).

Virgin queens produced from the selected queen mothers are mated in four mating yards. These four yards contain a total of about 1,700 nuclei. About 50 drone mother colonies are located within a 3 Km radius of each mating yard. Each drone mother colony contains two drone combs and are fed weekly to ensure an adequate supply of desirable drones. The first queens produced are used to requeen the drone mother colonies. The rest of the colonies (ca. 3,800) are requeened between May and August.

Current Status of Vita Real's Stock

Honey production has declined since the arrival of africanized bees. The average yield for Vita Real was 44 Kg per colony for the 10 year period, 1981-1990. For the last two years, the average yield per colony has been only 35 Kg, a 20% decrease. Despite this decrease, Vita Real is doing better than other honey producers operating in the same general area. One large beekeeping establishment that maintains about 12,000 hives near Vita Real, produced only 12 Kg per colony last year. They have experienced more beekeeping and production problems than Vita Real because they maintain larger numbers of colonies, with less intensive management, and they do not have a selective breeding program.

The degree of africanization of Vita Real bees does not seem to have increased during the last two years since the implementation of our breeding program. In fact, it appears to have declined. Colonies now have lower defensive scores, larger average forewing length measurements, and there has been a substantial decrease in the number of stinging incidents involving Vita Real colonies. These results, along with the fact that all swarms captured in the area during the last two years have been shown to be africanized, suggest that Vita Real's breeding program has been successful. However, it is still too early to predict long-term success. We expect the bees will become progressively more africanized in time, however, we also expect to be able to improve our stocks through strong selection and mating control.

Conclusion

Vita Real's management and breeding programs are sophisticated and require much more effort than those demonstrated by most U.S. beekeeping operations. Their programs require better record keeping and more money invested per colony than before africanization. The only available alternative is to keep africanized bees, have serious stinging problems, and lose more honey production. These probably represent the same options available to U.S. beekeepers who are unfortunate enough to operate in africanized areas.

Most Mexican beekeepers agree that problems associated with africanized bees are very difficult problems, but that they can be technically improved. However, they think that the low honey prices that have prevailed for 15 years without substantial increases, may become a much worse problem than africanized bees. With increased costs and lower productivity per hive, beekeepers need higher honey prices to survive.

Acknowledgements

We thank Enrique Castañeda and Jorge J. González of the Mexican African-Bee Program, SARH for valuable information provided. We also thank the various beekeepers who expressed their opinions contained in this manuscript. This research was funded in part by INIFAP-SARH, and by contracts from the California Department of Food and Agriculture. Kim Fondrk provided technical support and greatly improved an earlier draft of the manuscript.

References Cited

Guzmán-Novoa, E. and R.E. Page, Jr. 1993. Back crossing africanized honey bee queens to European drones reduces colony defensive behavior. *Ann. Entomol. Soc. Am.* 86(3): 352-355.

Guzmán-Novoa, E. and R.E. Page, Jr. Genetic dominance and worker interactions affect honey bee colony defense. *Beh. Ecol.* (in press).

Hall, H.G. and K. Muralidharan. 1989. Evidence from mitochondrial DNA that African honey bees spread as continuous maternal lineages. *Nature* 339: 211-213.

Hall, H.G. and D.R. Smith. 1991. Distinguishing African and European honeybee matrilines using amplified mitochondrial DNA.Proc. Nat. Acad. Sci. USA. 88: 4548-4552.

Kerr, W.E. 1967. The history of the introduction of African Bees to Brazil. *S. Afr. Bee J.* 39: 3-5.

Moffett, J.O., D.L. Maki, T. Andre and M.M. Fierro. 1987. The Africanized bee in Chiapas, Mexico. *Am. Bee J.* 127: 517-519, 525.

Nogueira-Neto, P. 1964. The spread of a fierce African bee in Brazil. *Bee World.* 45: 119-121.

Rinderer, T.E. 1986. Selection. In: Bee Genetics and Breeding. Ed. T.E. Rinderer. Academic Press Inc. pp. 305-321. Orlando, Fla.

Rinderer, T.E., H.A. Sylvester, M.A. Brown, J.D. Villa, D. Pesante and A.M. Collins. 1986. Field and simplified techniques for identifying Africanized and European honey bees. *Apidologie* 17: 33-48.

Sugden, E.A. and K.R. Williams. 1991. October 15:the day the bee arrived. *Gle. Bee Cult.* 119(1): 18-21.

Sylvester, H.A. and T.E. Rinderer. 1987. Fast africanized bee identification system (FABIS) manual. *Am. Bee J.* 127(7): 511-516.

Taylor, O.R. 1977. Past and possible future spread of Africanized honey bees in the Americas. *Bee World* 58: 19-30.

Taylor, O.R. 1985. African bees: potential impact in the United States. *Bull. Entomol. Soc. Am.* 31: 15-24.

Villa, J.D. 1988. Defensive behaviour of Africanized and European honeybees at two elevations in Columbia. *J. Apic. Res.* 27: 141-145.

* Reprinted from Volume 134, No. 2,
February 1994 American Bee Journal

Backcrossing Africanized Honey Bee Queens to European Drones Reduces Colony Defensive Behavior

ERNESTO GUZMÁN-NOVOA[1] AND ROBERT E. PAGE, JR.[2]

Ann. Entomol. Soc. Am. 86(3): 352–355 (1993)

ABSTRACT The defensive behavior of honey bee, *Apis mellifera* L., colonies of different genotypic composition was studied. Workers within colonies varied with respect to the proportion of their genotypes that were of African origin. Two components of defensive behavior were measured: the time it took for the first worker in a colony to respond and sting a moving leather patch, and the total number of stings deposited by workers in the patch during a 60-s interval following the first sting. Hybrid (F_1) colonies were not different for either variable from feral-captured Africanized colonies. However, colonies of the two backcrossed generations had fewer stings in patches than Africanized colonies and did not differ from European colonies. Results suggest that the intense defensive behavior of Africanized bees is dominant over the less defensive behavior of the Europeans we tested. Results also suggest that it is possible to reduce the defensiveness of Africanized colonies to levels not different from those of European colonies after only two generations of crossing Africanized queens to European drones.

KEY WORDS Africanized bees, behavioral genetics, colony defense

THE HIGHLY DEFENSIVE BEHAVIOR of Africanized honey bees, *Apis mellifera* L., has caused much concern among beekeepers, scientists, and the general public. Apicultural management practices have changed dramatically wherever Africanized honey bees have become established because of their extreme defensiveness and other objectionable characteristics (Cobey & Locke 1986). One of the greatest problems is the increased time and expense required for managing bee colonies. Operating commercial colonies of Africanized bees, compared with operating European honey bee colonies, requires more management to reduce their high swarming tendency, to provide them with food during periods of scarce resources, and to harvest honey more frequently.

Colonies used for pollination and honey production are frequently transported. Africanized bees become defensive and frequently abscond as a result of the disturbance, resulting in a substantial loss of workers; colonies of European bees are relatively unaffected (Danka & Rinderer 1986, Danka et al. 1987). Africanized bees are considered too defensive to be managed for pollination services (Loper 1991). Colonies used in the pollination of crops are distributed in high densities in fields and orchards where the defensive behavior of colonies may present a hazard to humans and farm animals.

Quantitative studies of defensive behavior have shown that Africanized bees respond faster and with more stings than bees of European descent (Collins & Kubasek 1982; Collins et al. 1982; Stort 1974, 1975a, b, c; Villa 1988; Guzmán-Novoa & Page 1993). Collins et al. (1984) estimated the heritabilities for two traits believed to be important components of defensive behavior: the time it takes the first bee to reach a leather patch presented to a colony, and the number of stings in the patch in a 30-s interval following presentation. Estimates of heritability were reported in their study as 0.59 and 0.57 for components 1 and 2, respectively. On the basis of those high indices, Collins (1986) conducted a bidirectional selection program for more defensive and less defensive Africanized colonies. After two generations, there were significant differences between the two lines for time to react and number of stings. However, less success was achieved in the less defensive direction than in the high defensive direction. Collins suggested that these results may be caused by the low frequency of alleles for the less defensive phenotype in the Africanized population. If true, then the infusion of European genes into Africanized populations will be necessary if we hope to be successful at reducing the defensive behavior of Africanized bees.

The objective of this study was to determine the number of successive backcrosses of Africanized and hybrid queens to drones of European descent that are needed to restore defensive be-

[1] Department of Entomology, University of California, Davis, CA 95616.
[2] To whom correspondence should be addressed.

0013-8746/93/0352–0355$02.00/0 © 1993 Entomological Society of America

Table 1. Mean ± SE time (s) to sting

Generation	Treatment			F^a	df
	Hybrid experimental	Africanized control	European control		
F_1 hybrids	11.1 ± 1.37b (n = 10)	9.3 ± 0.99b (n = 3)	58.7 ± 3.74a (n = 3)	161.36	2, 20
Backcross 1	20.9 ± 5.73b (n = 11)	8.3 ± 0.70c (n = 3)	52.0 ± 4.55a (n = 3)	50.79	2, 22
Backcross 2	45.0 ± 10.47a (n = 10)	11.7 ± 1.72b (n = 3)	51.3 ± 9.19a (n = 3)	17.55	2, 20

Different letters indicate significant differences of means based on one-way ANOVA and protected LSD tests. Comparisons are valid only within the same row. Statistical tests (F and LSD) are based on ln (x) transformed data because the data were not homoscedatic. Means and SE are actual nontransformed values.

[a] $P < 0.001$.

havior to European levels. Hybrid F_1 and subsequent colonies derived from backcrosses were evaluated for defensiveness each generation and compared with defensive responses of European and Africanized control colonies.

Materials and Methods

Three colonies of Africanized honey bees were obtained from box traps used to monitor swarms near Tonatico, Mexico, during the spring of 1991. In addition, six colonies of European bees were derived from descendants of queens that had been previously imported from the United States by a local beekeeper. Morphometric analyses (Daly & Balling 1978) of the progeny confirmed the origins of these colonies. Ten queens were reared from one colony of Africanized bees, and each was instrumentally inseminated (Laidlaw 1977) with the semen from a single, different European drone. Drones were obtained from two different unrelated European source colonies. Each queen was marked with white paint on the thorax, had her right wing clipped to prevent flight, and was placed in a Dadant jumbo-sized nucleus hive containing ≈1 kg of worker bees and three combs with brood, pollen, and honey. One of these 10 queens was arbitrarily designated to be the queen mother for the first backcross generation. Eleven new queens were reared from this F_1 queen and inseminated in the same manner as described above. Finally, a third generation of 10 queens was produced from an arbitrarily selected queen of the first backcross generation and was treated similarly. Drones from two different European colonies were used to inseminate each generation of queens (six source colonies in total). This backcrossing procedure was performed with a 1-mo interval between generations.

The F_1 and the two backcross generations constituted the experimental treatments. Additionally, three source colonies of each type (European and Africanized) were used as representative standards (controls). The same three European and three Africanized colonies were used as controls for all comparative defensive tests.

Colonies containing the inseminated queens of each generation were transferred to Dadant jumbo full-size hives 3 wk after the queens began egg-laying and were allowed to grow in worker population for 12 wk before the tests. Two wk before being tested, all colonies were equalized by removing bees and capped brood frames from the most populous colonies. The average population per colony, after adjustment, was four frames with capped brood (≈3,600 cm^2) and six frames covered with adult bees. Hives containing the experimental and control colonies were separated by at least 5 m to minimize intercolonial exchange of workers.

Colony defensiveness for each generation was determined by presenting a black suede leather patch (6 by 5 cm) suspended on a piece of white wood (0.7 by 0.5 by 100 cm) to each colony. This leather patch was rhythmically waved (elevated ≈4 cm) twice per s ≈5–10 cm in front of the entrance of each hive. The time for the first bee to sting the patch was recorded. Bees were permitted to sting for 60 s following the first sting. All colonies were tested simultaneously with the aid of 14 assistants. Tests were conducted blindly; i.e., the operators did not know the type of colony they were testing. After completing each test, the leather patch was placed inside a 20-ml plastic vial. Stings were counted later. Colonies were tested three times, twice on the same day (1000 and 1600 hours MST) and the third trial 2 d later at 1000 hours. Successive generations were tested at 5-wk intervals.

Data from the three individual trials of each colony per generation were summed. Analysis of variance (ANOVA), correlation, and regression analyses were performed on the data (Sokal & Rohlf 1981).

Results

Africanized and European control colonies differed significantly in both the elapsed time to receive the first sting in the target and the total number of stings in 1 min. Hybrid (F_1) colonies did not differ with respect to either variable from Africanized control colonies (Tables 1 and 2).

Table 2. Number of stings in 60 s (mean ± SE)

Generation	Treatment			F^a	Degrees of freedom df
	Hybrid experimental	Africanized control	European control		
F_1 hybrids	92.8 ± 14.23a (n = 10)	110.3 ± 4.49a (n = 3)	23.3 ± 1.39b (n = 3)	37.65	2, 20
Backcross 1	42.3 ± 7.81b (n = 11)	129.7 ± 5.89a (n = 3)	27.0 ± 3.01b (n = 3)	50.16	2, 22
Backcross 2	19.4 ± 3.88b (n = 10)	146.7 ± 7.60a (n = 3)	22.3 ± 3.27b (n = 3)	103.63	2, 20

Different letters indicate significant differences of means based on one-way ANOVA and protected LSD tests. Comparisons are valid only within the same row. Statistical tests (F and LSD) are based on ln (x) transformed data because the data were not homoscedatic. Means and SE are actual nontransformed values.
[a] $P < 0.001$.

Backcross 1 colonies differed from both Africanized and European colonies in the time to first sting (Table 1). However, colonies of backcrosses 1 and 2 had fewer stings in target patches than control colonies of Africanized bees and were not different from European (Table 2). Increased defensive responses were related to greater degrees of Africanization. The correlation between time to first sting and number of stings was strongly negative, and significant ($r = -0.53$, $n = 31$; $P < 0.001$; $ln\ (x)$ transformed data).

Discussion

The results suggest that it is possible to reduce the defensiveness of Africanized colonies to levels not different from those of Europeans after only two or three generations of crossing Africanized queens to European drones. Results also confirm our previous findings suggesting nonadditive genetic effects for speed of reaction and number of stings (Stort 1974, 1975a; Guzmán-Novoa & Page 1993). Genetic dominance effects for time to sting and for number of stings are suggested from the responses of F_1 colonies compared with the Africanized control colonies. In this case, F_1 colonies contained Africanized queens and hybrid workers, and the results are consistent with our previous study where queens of F_1 colonies were European (Guzmán-Novoa & Page 1993). These results suggest little or no direct effect of queen genotype on colony defensive responses.

Colonies of the first backcross generation (25% Africanized) were different from Europeans in the time to first sting. Stort (1975a) also showed that the distribution of colonies with F_1 queens backcrossed to the European parents was strongly shifted toward shorter times of reaction, suggesting nonadditive inheritance. The reduced backcross response of time to sting, compared with number of stings, may be a consequence of behavioral dominance (Page & Robinson 1991). Behavioral dominance may occur when an individual that has a low threshold of response to task-related stimuli performs a task (e.g., being the first to sting the patches) and causes a decrease in the likelihood that less sensitive individuals will perform that task. If genetic variability exists for thresholds of response to the experimental stimuli, then behavioral dominance may be expected to occur, because as soon as one bee stings the patch, she effectively eliminates that possibility for any others. In effect, we are always measuring just the most sensitive worker; therefore, the colony phenotype will always be that of the fastest responder.

The strong negative correlation between time to first sting and the number of stings validates previous results (Guzmán-Novoa & Page 1993). However, bees from the first backcross generation responded faster than Europeans but did not sting more. Therefore, when breeding for gentleness, the number of stings received is probably a more reliable trait to measure than the response time.

Studies assaying various cross-combinations between European races and Africanized bees could help to determine the most favorable strains of European bees to use in genetic programs. Different results may be achieved with different crosses. Kerr (1967), for example, reported that hybrids of African and Italian honey bees behaved more like the European parent. Kurletto (1975) found that, after three generations of backcrossing Africanized to Carniolan bees, the progeny showed reduced colony defense. Collins et al. (1988) reported an intermediate stinging response of F_1 colonies produced from naturally mated Italian and Caucasian queen bees in an Africanized area.

F_1 hybrid bees, like Africanized, are too defensive for the management practices commonly used. Colonies of backcrossed bees may, however, be acceptable. Matings must be controlled to produce generations of bees that are successively backcrossed to European drones. This is difficult to accomplish because queens normally mate while in flight away from the hive. Instrumental insemination could be used, but the method is laborious and not well suited for com-

mercial application. Instead, controlled natural mating is needed.

Cornejo et al. (1973) allowed queens to mate naturally and obtained gentle and almost "pure" populations of Italian honey bees (as ascertained by taxonomic analyses) in an Africanized area of Brazil. They eliminated feral colonies of honey bees and maintained a high population density of Italian drones. Mating control of 83–93% was obtained by Hellmich & Waller (1990) by saturating mating apiaries with drone-producing colonies. Similar results were found by Loper & Fierro (1990). In addition to saturating mating areas with drones, they also used aerial net traps to capture and eliminate Africanized drones before releasing virgin European queens.

If sufficiently high levels of mating control can be obtained, then the defensive behavior of colonies may be tolerable. Our previous data demonstrated that colonies having European queens inseminated with a 3:1 ratio of European/Africanized drone semen did not sting significantly more than pure European colonies (Guzmán-Novoa & Page 1993). Mating control of 75% would be needed for similar results.

Acknowledgments

Norman E. Gary and James R. Carey critically reviewed this manuscript. M. Kim Fondrk assisted with the insemination of queen bees. Guillermo García, owner of Vita-Real, provided labor force, colonies, transportation, and laboratory space. Jorge J. González assisted with morphometric analyses of worker bees. Marcelo Velazco, Hector Morales, Francisco Souza, and Enrique Estrada provided valuable assistance in various ways. This study was funded by California Department of Food and Agriculture contract 90-0346.

References Cited

Cobey, S. & S. Locke. 1986. The Africanized bee: a tour of Central America. Am. Bee J. 126: 434–440.

Collins, A. M. 1986. Bidirectional selection for colony defense in Africanized honey bees. Am. Bee J. 126: 827–828.

Collins, A. M. & K. J. Kubasek. 1982. Field test of honey bee (Hymenoptera: Apidae) colony defensive behavior. Ann. Entomol. Soc. Am. 75: 383–387.

Collins, A. M., T. E. Rinderer, J. R. Harbo & A. B. Bolten. 1982. Colony defense by Africanized and European honey bees. Science 218: 72–74.

Collins, A. M., T. E. Rinderer, J. R. Harbo & M. A. Brown. 1984. Heritabilities and correlations for several characters in the honey bee. J. Hered. 75: 135–140.

Collins, A. M., T. E. Rinderer & K. W. Tucker. 1988. Colony defence of two honeybee types and their hybrids. I. Naturally mated queens. J. Apic. Res. 27: 137–140.

Cornejo, L. G., L. de Santis, J. A. Vidal-Sarmiento & V. A. Muller. 1973. Results of work for Italianization of an Africanized zone with *Apis mellifera adansonii* in Rio Grande do Sul State (Brazil). Apiacta 8: 117–120.

Daly, H. V. & S. S. Balling. 1978. Identification of Africanized honey bees in the Western hemisphere by discriminant analysis. J. Kans. Entomol. Soc. 51: 857–869.

Danka, R. G. & T. E. Rinderer. 1986. Africanized bees and pollination. Am. Bee J. 126: 680–692.

Danka, R. G., T. E. Rinderer, A. M. Collins & R. L. Hellmich. 1987. Responses of Africanized honey bees (Hymenoptera: Apidae) to pollination-management stress. J. Econ. Entomol. 80: 621–624.

Guzmán-Novoa, E. & R. E. Page. 1993. Genetic dominance and worker interactions affect honey bee colony defense. Beh. Ecol. (in press).

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

Kerr, W. E. 1967. The history of the introduction of African bees to Brazil. S. Afr. Bee J. 39: 3–5.

Kurletto, S. 1975. Cruzamento das abelhas Africanizadas com as Carnicas. Congr. Bras. Apic. 3: 161–164.

Laidlaw, H. H. 1977. Instrumental insemination of honey bee queens. Dadant, Hamilton, IL.

Loper, G. M. 1991. Pollination tests with Africanized honey bees in southern Mexico, 1986–88. Am. Bee J. 131: 191–193.

Loper, G. M. & M. M. Fierro. 1990. Use of drone trapping and drone releases to influence matings of European queens in an Africanized honey bee area; Tapachula, Chiapas, Mexico. Am. Bee J. 130: 803–804.

Page, R. E. & G. E. Robinson. 1991. The genetics of the division of labour in honey bee colonies. Adv. Insect Physiol. 23: 117–169.

Sokal, R. R. & F. J. Rohlf. 1981. Biometry. Freeman, New York.

Stort, A. C. 1974. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. I. Some tests to measure aggressiveness. J. Apic. Res. 13: 33–38.

1975a. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. II. Time at which the first sting reached the leather ball. J. Apic. Res. 14: 171–175.

1975b. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. IV. Number of stings in the gloves of the observer. Behav. Genet. 5: 269–274.

1975c. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. V. Number of stings in the leather ball. J. Kans. Entomol. Soc. 48: 381–387.

Villa, J. D. 1988. Defensive behaviour of Africanized and European honeybees at two elevations in Colombia. J. Apic. Res. 27: 141–145.

Received for publication 31 July 1992; accepted 15 December 1992.

Genetic dominance and worker interactions affect honeybee colony defense

Ernesto Guzmán-Novoa
Robert E. Page, Jr.
Department of Entomology,
University of California,
Davis, CA 95616, USA

Colonies of honeybees (*Apis mellifera* L.) were established that varied in the proportions of their workers that were of European and hybrid (Africanized × European) descent. Colony defensive responses increased with higher proportions of hybrid workers. Colonies consisting exclusively of hybrid workers did not differ in their response from "pure" Africanized colonies, suggesting that the strong defensive behavior of Africanized workers is genetically dominant. European workers became more defensive in colonies that also contained hybrid workers, whereas hybrid workers became less defensive in the same mixed colonies. In mixed colonies hybrid workers were individually more likely than Europeans to sting a leather target but not more likely to guard the entrance. *Key words: Apis mellifera,* colony defense, defensive behavior, honeybees, worker interaction. *[Behav Ecol 5:91–97 (1994)]*

African honeybee queens (*Apis mellifera scutellata*) were introduced into Brazil in 1956 as part of a selective breeding program designed to produce a bee that was better adapted to tropical conditions. Progeny of these African queens presumably interbred with the local bee population and produced Africanized honeybee colonies. Feral populations of Africanized bees spread rapidly and by 1963 occupied a large area of southern Brazil (Nogueira-Neto, 1964). Africanized bees have spread throughout most of the Americas and reached the United States in 1990 (Sugden and Williams, 1991). The degree to which these bees represent African × European hybrids or "pure" African is controversial (Hall and Muralidharan, 1989; Lobo et al., 1989; Rinderer et al. 1991; Smith et al., 1989).

Several studies have demonstrated that Africanized honeybee colonies are more defensive than those of European honeybees and respond faster and in greater numbers to defensive stimuli (Collins et al., 1982; Michener, 1972; Stort, 1974, 1975a, b,c; Villa, 1988). These differences seem to be genetically determined (Collins, 1986; Collins et al., 1984; Stort, 1975a), but researchers differ in their conclusions about whether the differences demonstrate additive or nonadditive genetic variance. Stort (1974, 1975a) hypothesized that the extreme defensiveness of Africanized bees was genetically dominant, while Collins et al. (1988) proposed an additive mode of inheritance.

The defensive behavior of a colony can be partitioned into at least two behavioral categories: guarding the entrance (performed by guard bees) and stinging (performed by soldiers). Guard bees typically inspect, antennate, bite, and/or raise their forelegs to returning foragers at the hive entrance (Moore et al., 1987). Soldier bees (Breed et al., 1990) fly, pursue, and sting target stimuli upon colony disturbance. Guards and soldiers are at least partially independent behavioral groups. Breed et al. (1990) provided evidence that samples of guards and soldiers differed with respect to their subfamily composition. (Queen honeybees are polyandrous. Members of the same subfamily share a common father; see Page, 1986.) Observed differences in defensive behavior of honeybee colonies may be influenced by both genetic variability among colonies and the genotypic variability within them resulting from polyandry. Genetic variance results in variable mean genotypes among colonies, whereas genotypic variance within colonies may generate nonadditive interaction effects based on the specific genotypic mix of workers. This interaction may lead to increased or decreased defensive responses of colonies relative to the average genotype of workers. Genotypic variance within colonies has been found to influence several behavioral characteristics (see Page and Robinson, 1991, for review).

Africanized bees are undesirable for commercial beekeeping. However, the production of "pure" European colonies is problematic in areas with an Africanized feral population because honeybee queens mate with about 17 males (Adams et al., 1977) while in flight away from the nest. Therefore, it is likely that commercially produced virgin European queens mate with males of African and European descent in areas where both are present. These matings would result in colonies composed of two genotypic classifications of workers, European and Africanized × European hybrids. It is not known how genotypic variability within colonies affects defensive behavior of colonies. However, it is important to understand these effects in order to better understand the evolution of colony defense and to design breeding programs to reduce the defensive behavior of commercial honeybee colonies after Africanization of queen production areas of North America.

We tested the relative defensive behavior of honeybee colonies with differing degrees of "African-

Address reprint requests to R. E. Page, Jr.

Received 26 May 1992
Revised 18 January 1993
Accepted 25 January 1993

1045-2249/94/$5.00

© 1994 International Society for Behavioral Ecology

ization." We compared colonies of European, Africanized, and F_1 hybrid workers to determine the patterns of inheritance of defensive behavior. We compared those results with colonies consisting of mixtures of European and hybrid workers to determine if the genotypic composition of a colony differentially affects the defensive characteristics of the individual resident workers of different genotypic origins (European or hybrid).

METHODS

We conducted experiments between January and September 1991 at the facilities of Miel Vita-Real in Ixtapan de la Sal, Mexico (19° N, 99° W), approximately 150 km southwest of Mexico City. Apparently, the Africanized population expanded into the area during the spring of 1990. The first swarms of Africanized bees in the Ixtapan vicinity were reported by the Secretariat of Agriculture and Water Resources in March 1990 (González JJ, personal communication).

Treatment colonies

Super-sister European queen bees were instrumentally inseminated (Laidlaw, 1977) with the semen of European and Africanized drones mixed in different proportions. Super sisters have the same mother and father (see Page and Laidlaw, 1988). Africanized and European bee sources carried distinct enzyme markers that allowed blind data collection before subfamily identification of individuals that were engaged in stinging behavior and in guarding the entrance of mixed European/hybrid colonies (see Page and Robinson, 1991, for a review of the use of these enzyme markers in behavioral studies).

We selected Africanized and European colonies for experiments after morphometrically screening more than 100 local colonies (Daly and Balling, 1978). The presumed European colonies were derived from stocks that had been imported previously from several queen breeders in the United States. Selected colonies were well differentiated morphometrically as Africanized or as European (Sylvester and Rinderer, 1987).

We made crosses among European sources to obtain a single European queen mother that produced super-sister daughters that were homozygous for the slow allele of *Mdh*-1. Six more, presumably unrelated, European source colonies provided drones for inseminations of queens of treatment colonies. Drones from these queens carried slow or medium *Mdh*-1 alleles (see Contel et al., 1977; Sylvester, 1976). Six Africanized source colonies collected in the state of Guerrero, Mexico, provided drones with the fast *Mdh*-1 allele (see Sylvester, 1982) for treatment inseminations.

Each of 32 super-sister European queens was instrumentally inseminated with approximately 2 μl of semen taken from a single, homogenized pool composed of the semen of eight drones. We collected semen from marked, mature drones, and pooled the semen in four different European : Africanized ratios as follows: 1.00 : 0.00 (treatment 1), 0.75 : 0.25 (treatment 2), 0.50 : 0.50 (treatment 3), and 0.00 : 1.00 (treatment 4).

To prepare the homogenized pools of semen, we obtained drones of each type from the six respective source colonies and housed them together such that each of two cages contained a random representation of European or Africanized drones. Semen from each drone was collected (Williams and Harbo, 1982) and placed in a tube. Once we collected the semen of a batch of 40 drones of both types, we assayed their thoraces by cellulose acetate gel electrophoresis (Hebert and Beaton, 1989) to validate their genotype. Then, according to treatment, we recollected the semen of eight drones (from eight different tubes) and placed it in another tube that was microcentrifuged (Fisher Scientific, model 250 C) at 10,000 *g* (Moritz, 1983) for 1 min. Four queens were inseminated with each batch of pooled semen. We repeated this procedure until all queens were inseminated.

Treatment 5 colonies (all Africanized) were of two types. One consisted of three colonies that contained Africanized queens reared from one Africanized colony collected in a box swarm-trap near Ixtapan. We inseminated these queens with approximately 2 μl of semen from the same six Africanized drone source colonies that were used to inseminate the European queens. The second type consisted of three additional Africanized colonies that had been captured in the States of Mexico, Morelos, and Guerrero, respectively. Treatment 5 was intended to broadly represent the defensive response of typical Africanized colonies.

We were unable to test the actual drone mother colonies because of their distance from our test apiary and government restrictions on transporting Africanized bees. Therefore, we used treatment 5 colonies to represent the parental group based on the assumption that their mean defensive responses would be equivalent to the six parental colonies that provided the Africanized drones used for insemination. Our assumption is supported by the following points: colonies of treatment 5 contained queens from diverse origins (i.e., three different locations) and were chosen arbitrarily and the drone mother sources used to inseminate three of the queens of this treatment were the same used to inseminate queens of treatments 2, 3, and 4.

We glued colored, numbered plastic tags (Graze KG, Weinstadt, Germany) to the thoraces of inseminated queens and clipped the right wing of each queen to prevent flight. Queens were placed temporarily into a queenless nursery colony, then removed 24 h after insemination and exposed to CO_2 for 8 min to stimulate egg laying (Mackensen, 1947). We introduced queens into nucleus colonies by confining them with wire push-in cages. The colonies, established in Dadant jumbo-size nucleus hives, contained approximately 5000 worker bees and three combs with brood, pollen, and honey. We fed these colonies 50% (by volume) sucrose solution as needed. Initiation of egg laying by the queens was determined by daily observations.

Behavioral assays

Three weeks after onset of oviposition, each colony was transferred into a Dadant jumbo full-size hive. On 18 July, 9 weeks after queen insemination, we relocated 30 colonies composed of progeny of the experimental queens (six colonies per treatment) to 3 apiaries approximately 800 m apart, 1425 m

above sea level. We positioned hives at least 5 m apart to minimize interhive drifting of workers. Sixteen days before the first test was conducted, colony worker populations were equalized to contain approximately 3500 cm^2 (three to four combs) of capped brood and six combs with adult bees. Colony equalization was necessary to control for differences in colony population that may affect defensive behavior tests. We began on 5 August, 12 weeks after queens were inseminated.

The behavioral assay was similar to the one used by Villa (1988). We suspended a black suede leather patch (6 × 5 cm) on a piece of white wood (0.7 × 0.5 × 100 cm) and waved it (~4 cm up and down) twice per second approximately 5–10 cm in front of the entrance of each hive. The time for the first bee to sting the patch was recorded. We terminated individual colony tests when workers failed to sting within 120 s. Bees were permitted to sting during 60 s after the first sting. Three blind tests were performed: twice on 1 day (0930 h and 1730 h) and at 0930 h 2 days later. Fourteen operators (10 patch operators and 4 timekeepers) simultaneously collected data. The weather was sunny, with temperatures of 25°, 23°, and 26°C, respectively. After each trial, we packed and sealed the leather patches in marked vials and placed them in a freezer at −18°C. We counted stings deposited in the leather patches before electrophoretic analyses.

One hour after the third assay, random samples of bees ("controls") were taken from each of the 12 colonies that, according to the insemination mixtures, were expected to possess approximately 25% and 50% hybrid workers (treatments 2 and 3). We obtained each sample by shaking all bees off the combs into a cage where they were mixed before sampling at least 100 bees. We took samples as rapidly as possible to minimize loss of older bees that fly when disturbed and we placed the samples into marked vials and stored them in a freezer at −18°C. The next day, samples of at least 48 guard bees were collected with forceps from each of the 12 colonies containing mixed honeybee genotypes. We placed samples in marked vials and froze them until subsequent electrophoretic analysis.

Between 48 and 89 workers were analyzed to determine the proportions of hybrid and European workers for both the controls and the samples of guard bees from the 12 colonies. Additionally, we analyzed between 23 and 94 stings from test patches of each of the 12 colonies by electrophoresis to obtain data to estimate the proportion of hybrid and European bees that stung the leather patches. All the stings were assayed for colonies where bees stung the leather patches less than 70 times in the three trials summed. Otherwise, we randomly picked approximately one-third of the stings and assayed them from each of the three leather patches. Control samples from treatments 2 and 3 demonstrated that colonies did not deviate significantly from our target proportions of 0.25 and 0.50 hybrid workers (0.22 ± 0.014 SE, $n = 6$, and 0.45 ± 0.054 SE, $n = 6$, respectively).

Genetic models

We calculated expected values for time to sting and number of stings using two models. The first model assumes genetic dominance for the Africanized trait of high defensiveness but genotypic additivity among workers within colonies. By genotypic additivity we mean that genotypes of individuals are additive in their effects on colony-level behavior through their effects on individual worker behavior. Individual European workers in mixed-genotype colonies are expected to behave like European workers in "pure" European colonies, whereas hybrid workers are expected to act like "pure" Africanized (see Equation 1). The second model assumes both genetic and genotypic additivity where hybrids are intermediate between European and Africanized workers (see Equation 2). The expected values for the time to sting, $E(t)$, and stings in patches, $E(n)$, were obtained as follows:

$$E(t \text{ or } n) = P_H R_A + P_E R_E \quad (1)$$

$$E(t \text{ or } n) = D_A R_A + D_E R_E, \quad (2)$$

where P_H is the proportion of hybrid bees in the progeny; R_A is the mean Africanized response; P_E is the proportion of European bees in the progeny; R_E is the mean European response; D_A is the average degree of Africanization in the progeny; and D_E is the average degree of Europeanization in the progeny.

RESULTS

A repeated-measures ANOVA (Sokal and Rohlf, 1981) revealed no significant changes in time to sting ($F_{2,50} = 1.84$; $p > .05$) with repeated testing. The number of stings did decrease significantly with repeated trials ($F_{2,50} = 6.233$; $p < .01$). The mean number of stings per colony for all treatments combined was 98, 88, and 65 for trials 1–3, respectively. The time it took for the first bee to sting the leather patch varied significantly between treatments [$F_{4,25} = 10.12$, $p < .001$; test based on ln(x) transformed data; see Figure 1A]. Colonies of treatments 1–5 decreased in response time from a mean of 55.9 s for treatment 1 to 7.2 s for treatment 5.

Results were compared to expected values derived from Equations 1 and 2. The responses do not fit the additive model, as demonstrated by a significant quadratic regression coefficient ($x^2 = 106$, $p = .010$). In addition, the expected values of the F_1 colonies (50% Africanized) and the 25% Africanized colonies clearly lie outside of the 95% confidence intervals ($p < .001$ for each, t test with 5 df). The results appear to more closely fit the nonlinear dominance model. A significant quadratic regression coefficient ($x^2 = 363$, $p < .05$) was obtained when treatment 5 was excluded from the analysis, demonstrating that genetic dominance is not entirely responsible for the observed nonlinear relationship between proportion of hybrids and time to sting. One point, 25% Africanized, deviates significantly from its expected value ($p < .01$, t test with 5 df), suggesting that this model also fails to describe the results.

The number of stings recorded in 1 min varied significantly among treatments [$F_{4,25} = 14.81$, $p < .001$; test based on ln(x) transformed data; see Figure 1B], from an average of 24.7 in treatment 1 colonies to 137.3 in treatment 5 colonies. There were no significant differences between F_1 hybrid (treatment 4) and Africanized colonies. However, the average number of stings for colonies contain-

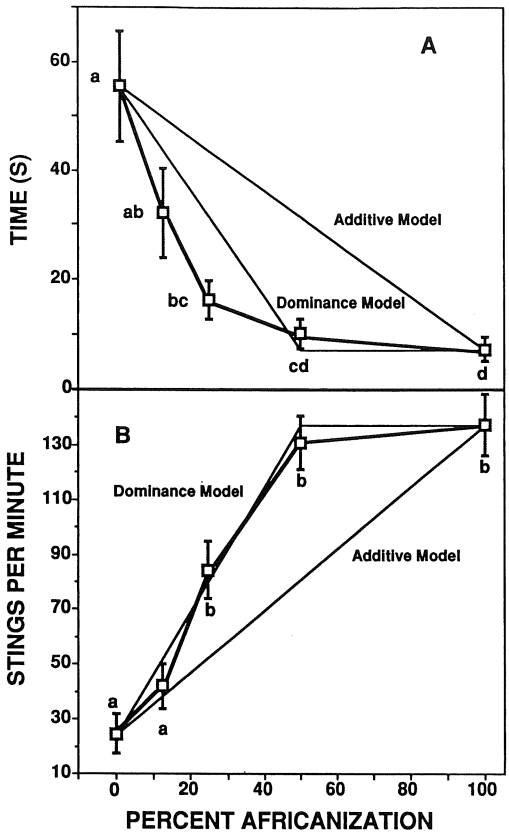

Figure 1
(A) The time to first sting as a function of percent Africanization and (B) the number of stings per minute as a function of percent Africanization. Expected values for the genetic additivity and genetic dominance with genotypic additivity models are presented as thin lines. Actual values for different treatments are represented by open boxes and SE bars and are connected by thick lines. Treatments with different letter designations are significantly different based on protected LSD tests of ln(x) transformed data.

ing both genotypes of workers (treatments 2 and 3) lie on the line segment bounded by treatments 1 and 4, suggesting that the defensive response increased linearly as the proportion of hybrid workers increased. In colonies that contained 25% hybrid bees there were 42.0 stings/min, whereas in colonies that contained 50% hybrids there were 84.3 stings/min.

We compared responses generated by the different treatments with the expected values of the two proposed models. Results were not different from a model of genetic dominance with genotypic additivity (Equation 1; Figure 1A), but did differ from the linear, additive model ($\chi^2 = -149$, $p < .001$). Additionally, the time to first sting and number of stings in the leather patches were highly correlated [$r = -.79$, $n = 30$; $p < .001$; correlation was obtained from ln(x) transformed data].

No difference was observed between the mean responses of the two types of colonies that composed treatment 5 ($t = 0.96$, ns, for time to first sting; $t = 0.32$, ns, for number of stings, df = 5), supporting our assumption that these represent the parental Africanized behavioral type. Furthermore, a third group of three different, randomly picked Africanized colonies was tested 14 days after the trials were performed, and their defensive responses (time to first sting = 10.4 s ± 2.43 SE, number of stings = 153.0 ± 9.91 SE) were not different from those of treatment 5 colonies ($t = 0.53$, ns, for time to first sting; $t = 1.08$, ns, for number of stings; df = 8).

Significant differences were found in 8 of 12 colonies (treatments 2 and 3; Table 1) between the proportion of hybrid bees in control samples and the proportion of hybrids stinging the leather patches. Hybrid workers in these treatments were overrepresented in number of stings relative to their frequency in colonies. However, hybrids in mixed colonies were not as likely to sting as bees from F_1 colonies composed entirely of hybrid workers (treatment 4). In contrast, European workers in mixed colonies were more likely to sting than those in pure European colonies (Figure 2). Stinging likelihoods (SL) were calculated relative to the defensive response of treatment 1 colonies. The likelihood of stinging in F_1 colonies was obtained by dividing the average number of stings for this treatment by 24.7, the average response for European

Table 1
Number of European (E) and hybrid (H) workers from treatment 2 and 3 colonies belonging to control samples (C), stings (S), and guards (G)

Colony no.	Controls		Stings		Guards		χ^2			
	E	H	E	H	E	H	C/G	C/S	G/S	df
Treatment 2										
40	41	11	35	40	40	8	0.33 (ns)	13.23***	16.54***	1
36	37	11	39	33	39	9	0.25 (ns)	6.51*	9.29**	1
43	40	12	41	19	44	10	0.34 (ns)	1.03 (ns)	2.59 (ns)	1
38	46	8	15	8	41	7	0.00 (ns)	3.91*	3.81*	1
42	39	13	42	34	46	14	0.04 (ns)	5.18*	6.73**	1
44	47	13	37	38	49	17	0.29 (ns)	11.93***	9.16**	1
Total							1.28 (ns)	41.79***	48.12***	6
Treatment 3										
21	30	26	36	36	24	24	0.13 (ns)	0.16 (ns)	0.00 (ns)	1
23	27	23	38	37	52	29	1.34 (ns)	0.13 (ns)	2.92 (ns)	1
27	46	16	31	34	45	9	1.43 (ns)	9.34**	16.24***	1
29	31	29	31	53	23	37	2.16 (ns)	3.11 (ns)	0.03 (ns)	1
24	30	59	15	63	12	40	1.77 (ns)	4.43*	0.28 (ns)	1
30	45	27	43	51	30	22	0.29 (ns)	4.60*	1.91 (ns)	1
Total							7.12 (ns)	21.77**	21.38**	6

Results of 2 × 2 contingency table analyses are presented for each group comparison. ns, $p > .05$; * $.01 < p < .05$; ** $.001 < p < .01$; *** $p < .001$.

colonies, whereas the stinging probabilities for treatment 2 and 3 colonies were estimated by

$$SL = \sum_{i=1}^{n} [(\bar{S}_i S_{G_i})/f_{G_i}]/24.7 \qquad (3)$$

where $\bar{S}_i$ is the mean number of stings for each individual colony for the three trials; S_{G_i} is the proportion of stings of genotype (European or hybrid) in the leather patch determined by allozyme analyses; f_{G_i} is the expected proportion of genotype (European or hybrid) in the colony (0.25, 0.50, or 0.75); and n is the number of colonies (six) for each treatment.

The proportion of guard bees that were hybrids was not different from the relative frequency of hybrids in control samples for any of the 12 colonies where mixed genotypes were present (Table 1). Guards were also not different from stinging bees in five of six colonies with 50% hybrids. However, the difference between guards and workers stinging the suede patches was significant ($p < .05$) in five of the six colonies containing 25% hybrids.

DISCUSSION

Variation among treatments in the time taken to deliver the first sting cannot be satisfactorily explained by either of the proposed models. The observed nonlinear relationship between the degree of Africanization of colonies and response time is probably due to both genetic and behavioral dominance (Page and Robinson, 1991). Genetic dominance is demonstrated by the equivalent responses of hybrid and Africanized colonies. Behavioral dominance is suggested by the deviation of treatment 3 from the expected dominance model (see Equation 1 and Figure 1A). Behavioral dominance may occur when an individual that has a low threshold of response to specific behavior-related stimuli (e.g., a hybrid worker) performs the behavior (e.g., being the first to sting the patch), thereby causing a decrease in the probability that less sensitive individuals (e.g., European workers) would perform that specific behavior. If genetic variability exists for thresholds of response to the experimental stimuli, behavioral dominance may be expected to occur because stinging of the patch by one bee would effectively eliminate the possibility of being first for others. Consequently, the colony phenotype will always be that of the fastest responder. Variation between treatments may reflect differences in the relative numbers of European and hybrid workers present at the entrance that are primed to respond to the target stimulus.

The observed variation in behavior of colonies of the different treatments also suggests genetic nonadditivity for the number of stings. The greater defensive response of Africanized workers appears to be genetically dominant over the less defensive behavior of European workers. In addition, the responses of individual workers are influenced by the genotypes of other colony residents.

Genetic nonadditivity is inferred from the nearly identical responses of Africanized and F_1 colonies. If we assume that the defensive response of treatment 5 colonies is a valid estimate of the defensiveness of the Africanized drone parents, then the observation that there was no difference between

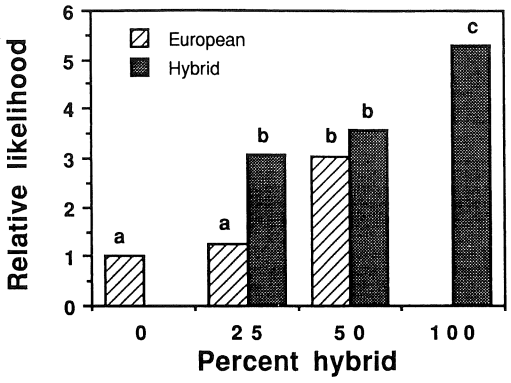

Figure 2
Relative likelihood of stinging for European and hybrid bees in colonies of treatments 1–4 (see text for how likelihoods were calculated). Significant differences were found between the two genotypes over treatments ($F_{3,20} = 12.5$; $p < .001$). Different letters indicate significant differences of means based on protected LSD tests.

the defensive responses of treatment colonies in this study from those expected under the conditions of Equation 1 supports the genetic dominance hypothesis. Other studies of extreme defensive behavior also support the hypothesis of genetic dominance for increased stinging behavior (Stort, 1974, 1975a). Contrary to the hypothesis of genetic dominance, Collins et al. (1988) suggested that defensive behavior was additive in inheritance, based on their observations of intermediate response of colonies with European queens and F_1 workers. The inconsistency of their results with those we report may be caused by lack of control over mating, or it may reflect real differences in sources of European or Africanized bees used.

Time to first sting and number of stings were negatively correlated in our study ($r = -.79$; $p < .001$). A correlated response between number of stings and speed of response to target ($r = -.48$) also was reported previously by Collins et al. (1984). However, because Collins et al. did not allow a fixed and equal independent period of time for the bees to sting the patches after the first reaction to target, the number of stings in their targets depended on the time to reaction, and the variables were not independent. Collins et al. (1984) proposed that the correlation between time to reaction and number of stings is genetic. However, their methods, like ours, were insufficient to demonstrate pleiotropy because the drones they used as sires came from behaviorally and genetically distinct populations with all traits effectively linked to the population of origin. It is possible that time of reaction to target and number of stings do covary genetically, but it is also possible that both traits have been selected independently and thus are affected by different genes (see Falconer, 1981).

Genotypic nonadditivity is demonstrated by the significant differences in relative likelihood of stinging of European and hybrid bees in colonies of mixed populations (Figure 2). Increasing genotypic diversity results in increasing interaction effects. As the frequency of hybrid bees increases in a colony, the relative defensiveness of individual European workers increases, whereas the defensiveness of individual hybrid bees decreases with the presence of European bees. The increase in the defensiveness of European bees, as well as the decrease of stinging response in hybrid bees, demonstrates that the apparent genotypic additivity inferred from Figure 1B was fortuitous and resulted from Europeans and hybrids covarying behaviorally. The behavioral covariance was probably a con-

sequence of behavioral dominance with stimulus feedback.

The observed environment × genotype interaction might be due to an increase in intensity of the stinging stimuli due to the increasing representation of hybrid workers. Stimulus levels may increase as a consequence of alarm pheromone released by stinging or nonstinging hybrid workers that are more responsive and may recruit the European workers with relatively higher thresholds of response. Collins et al. (1989) showed that Africanized workers have greater quantities of several of the chemical components of alarm pheromone. Also, responses to movement and to alarm pheromone at the hive entrance have been demonstrated to be significantly greater in Africanized than in European colonies (Collins et al., 1987), suggesting that Africanized bees have a lower threshold of response and/or a greater positive feedback effect on the stimulus environment. A low visual stimulus may be sufficient to release a fast and vigorous response from the hybrids, whereas higher levels of stimulation are needed for European bees to exceed their higher thresholds of response. These levels may be reached after the initial response by the hybrids.

The decrease in individual hybrid response with increasing proportions of European workers is also enigmatic. European workers recruited to sting the patch may in some way interfere with the hybrid recruitment and stinging process by taking longer to sting (occupying patch space), by releasing less alarm pheromone per sting, and/or by producing a less stimulating pheromone. However, the mechanisms resulting in these genotype × environment interactions remain unknown.

Winston and Katz (1982) demonstrated a similar genotype × environment interaction phenomenon for temporal polyethism when they cross-fostered Africanized and European workers. Africanized workers raised in their own colonies initiated foraging activities at earlier ages than European workers raised in their own colonies. Cross-fostered European workers, however, began foraging at a significantly earlier age than their Africanized nestmates, whereas cross-fostered Africanized workers foraged at the same time as their European nestmates. These asymmetries of response when cross fostered have also been shown for pollen-foraging behavior between selected honeybee strains (Calderone and Page, 1992).

Individual hybrids were more likely than European workers to sting patches (Table 1 and Figure 2). The relative genotypic frequencies of guards and stinging bees were also different. However, the relative genotypic frequencies of guards and controls did not differ significantly in any of the mixed colonies. This result was unexpected because guarding behavior has been demonstrated to be related to defensive behavior (Breed and Rogers, 1991; Breed et al., 1990; Moore et al., 1987). We therefore expected a disproportionate number of hybrid bees among the guards, as we found among the soldiers.

One explanation may be that our repeated testing resulted in a decrease in thresholds of response to guard-inducing stimuli to a point below the prevailing stimulus levels. If so, such a decrease would eliminate any genotypic differences and result in the guards resembling random samples of workers. If this is the case, then we would expect a significant increase in defensive response over the three trials we conducted. However, as discussed in the Results, we did not observe an increase in response with repeated tests.

Perhaps guarding and stinging are separate traits with different selective agents. For example, mass stinging, like that of Africanized bees, may be selected by vertebrate intrusion, whereas guarding behavior is selected by invertebrate intrusion, particularly conspecifics (Breed et al., 1990). Bees of European and African descent may not have differed much historically with respect to invertebrate intruders, but African populations may have had more vertebrate enemies.

The defensive behavior of honeybee colonies is a complex trait influenced by the genotypes of individuals, the environment, and the interactions of individuals with each other and with their environment. This is probably true for all colony traits, which demonstrates that we should not ignore the genotypic structure of colonies when we study their behavior.

We thank Norman E. Gary, James R. Carey, Gene Robinson, Jennifer Fewell, Mark Winston, and Anita Collins for their comments on this manuscript. M. Kim Fondrk assisted with the insemination of queen bees. Guillermo García, owner of Vita-Real, provided personnel, colonies, transportation, and laboratory space. Jorge J. González assisted with morphometric analyses of worker bees. Marcelo Velazco, Hector Morales, Francisco Souza, and Enrique Estrada provided valuable assistance in various ways. This study was funded by the California Department of Food and Agriculture (contract 90-0346).

REFERENCES

Adams J, Rothman ED, Kerr WE, Paulino ZL, 1977. Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera.* Genetics 86:583–596.

Breed MD, Robinson GE, Page RE, 1990. Division of labor during honey bee colony defense. Behav Ecol Sociobiol 27:395–401.

Breed MD, Rogers KB, 1991. The behavioral genetics of colony defense in honeybees: genetic variability for guarding behavior. Behav Genet 21(3):295–303.

Calderone NW, Page RE, 1992. Effects of interactions among genotypically diverse nestmates on task specialization by foraging honey bees (*Apis mellifera*). Behav Ecol Sociobiol 30:219–226.

Collins AM, 1986. Bidirectional selection for colony defense in Africanized honey bees. Am Bee J 126:827–828.

Collins AM, Rinderer TE, Daly HV, Harbo JR, Pesante DG, 1989. Alarm pheromone production by two honeybee (*Apis mellifera* L.) types. J Chem Ecol 15:1747–1756.

Collins AM, Rinderer TE, Harbo JR, Bolten AB, 1982. Colony defense by Africanized and European honey bees. Science 218:72–74.

Collins AM, Rinderer TE, Harbo JR, Brown MA, 1984. Heritabilities and correlations for several characters in the honey bee. J Hered 75:135–140.

Collins AM, Rinderer TE, Tucker KW, 1988. Colony defence of two honeybee types and their hybrids: I. Naturally mated queens. J Apic Res 27:137–140.

Collins AM, Rinderer TE, Tucker KW, Pesante DG, 1987. Response to alarm pheromone by European and Africanized honeybees. J Apic Res 24:217–223.

Contel EPB, Mestriner MA, Martins E, 1977. Genetic control and developmental expression of malate dehydrogenase in *Apis mellifera*. Biochem Genet 15:859–876.

Daly HV, Balling SS, 1978. Identification of Africanized honey bees in the western hemisphere by discriminant analysis. J Kans Entomol Soc 51:857–869.

Falconer DS, 1981. Introduction to quantitative genetics. London: Longman Group.

Hall HG, Muralidharan K, 1989. Evidence from mitochondrial DNA that African honey bees spread as continous maternal lineages. Nature 339:211–213.

Hebert PDN, Beaton MJ, 1989. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Beaumont, Texas: Helena Laboratories.

Laidlaw HH, 1977. Instrumental insemination of honey bee queens. Hamilton, Illinois: Dadant and Sons.

Lobo JA, Del Lama MA, Mestriner MA, 1989. Population differentiation and racial admixture in the Africanized honeybee (*Apis mellifera* L.). Evolution 43:794–802.

Mackensen O, 1947. Effect of carbon dioxide on initial oviposition of artificially inseminated and virgin queen bees. J Econ Entomol 40:344–349.

Michener CD (ed), 1972. Final report of the Committee on the African honey bee. Washington, DC: National Academy Press.

Moore AJ, Breed MD, Moor MJ, 1987. The guard honey bee: ontogeny and behavioral variability of workers performing a specialized task. Anim Behav 35:1159–1167.

Moritz RF, 1983. Homogeneous mixing of honeybee semen by centrifugation. J Apic Res 22:249–255.

Nogueira-Neto P, 1964. The spread of a fierce African bee in Brazil. Bee World 45(3):119–121.

Page RE, 1986. Sperm utilization in social insects. Annu Rev Entomol 31:297–320.

Page RE, Laidlaw HH, 1988. Full sisters and super sisters: a terminological paradigm. Anim Behav 36:944–945.

Page RE, Robinson GE, 1991. The genetics of the division or labour in honey bee colonies. Adv Insect Physiol 23: 117–169.

Rinderer TE, Steltzer JA, Oldroyd BP, Buco SM, Rubink WL, 1991. Hybridization between European and Africanized honey bees in the neotropical Yucatan Peninsula. Science 253:309–312.

Smith DR, Taylor OR, Brown WM, 1989. Neotropical Africanized honey bees have African mitochondrial DNA. Nature 339:213–215.

Sokal RR, Rohlf FJ, 1981. Biometry. New York: WH Freeman.

Stort AC, 1974. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil: I. Some tests to measure aggressiveness. J Apic Res 13:33–38.

Stort AC, 1975a. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil: II. Time at which the first sting reached the leather ball. J Apic Res 14:171–175.

Stort AC, 1975b. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. IV. Number of stings in the gloves of the observer. Behav Genet 5:269–274.

Stort AC, 1975c. Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. V. Number of stings in the leather ball. J Kans Entomol Soc 48: 381–387.

Sugden EA, Williams KR, 1991. October 15: the day the bee arrived. Glean Bee Cult 119:18–21.

Sylvester HA, 1976. Allozyme variation in honeybees (*Apis mellifera* L.) (PhD dissertation). Davis: University of California.

Sylvester HA, 1982. Electrophoretic identification of Africanized honeybees. J Apic Res 21:93–97.

Sylvester HA, Rinderer TE, 1987. Fast Africanized bee identification system (FABIS) manual. Am Bee J 127(7): 511–516.

Villa JD, 1988. Defensive behaviour of Africanized and European honeybees at two elevations in Colombia. J Apic Res 27:141–145.

Williams JL, Harbo JR, 1982. Bioassay for diluents of honey bee semen. Ann Entomol Soc Am 75:457–459.

Winston ML, Katz SJ, 1982. Foraging differences between cross-fostered honeybee workers (*Apis mellifera*) of European and Africanized races. Behav Ecol Sociobiol 10:125–129.