Fast Africanized Bee Identification System (FABIS) Manual[1]

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INTRODUCTION

THE MAIN characteristic of Africanized bees which must be considered in any discussion, but particularly a discussion of identification, is that they are hybrids. The extent and effect of this hybridization may vary in different locations and situations, but it is present. Electrophoretic analysis of enzymes (Sylvester 1986) and morphometric analysis (Buco, et al. 1986) of African, European and Africanized populations of bees have clearly shown that Africanized bees are hybrids. The result of this hybridization is that mixed or intermediate colonies exist. Also, since bees live in social colonies with multiply-mated queens, sister workers will make up subfamilies, each descended from a different drone, which may be Africanized, European, or intermediate. The workers in any one colony may also be descended from more than one queen because of queen replacement or drifting. Therefore, the most that can be expected of any method of identification is that it will correctly identify Africanized and European colonies and identify various intermediate colonies as intermediate.

Nevertheless, an accurate and reliable method of identifying Africanized bees in the field is needed. Research at the USDA, ARS, Honey-Bee Breeding, Genetics & Physiology Research Laboratory in Baton Rouge, Louisiana, has developed a method for quick identification of Africanized worker bees. This identification system was named FABIS — Fast Africanized Bee Identification System. This work extends and simplifies research done by Daly et al. (1982) within the context of the research of Ruttner et al. (1978), DuPraw (1965) and Alpatov (1929).

FABIS is a process for quick, simple, field screening of large numbers of bee colonies. FABIS has been tested with data from other Africanized and European colonies, and the accuracy of the method has been verified for European bees from the U.S. and Africanized bees from Venezuela.

FABIS is discussed in more detail in a scientific article (Rinderer, et al. 1986b). Fresh weight is called "wet weight" in that article. An earlier version of FABIS was discussed by Rinderer et al. 1986a) and by Sylvester and Rinderer (1986).

FABIS OUTLINE

This gives a basic "bare bones" FABIS procedure, with references to the appropriate methods. Sampling, alternate procedures, equipment, comb measurement, etc., are discussed under those headings. Containers of bees, as well as parts removed for measurement, should always be labeled for cataloging.

1. Measure forewing lengths (for method, see I).
2. Determine the probability of Africanization (PA) from Table 1 (for method, see V A).
3. If PA is less than 0.90, the sample is European and the procedure is over. If PA is 0.99 or greater, the sample is Africanized* and the procedure is over. If PA is between 0.90 and 0.99 (0.90≤PA<0.99), go to step 4.
4. Measure femur lengths (for method, see II).
5. Determine PA for forewing plus femur lengths from Table 1 (for method, see V B).
6. If PA is less than 0.90, the sample is European. If PA is 0.99 or greater, the sample is Africanized.* If 0.90≤PA<0.99, go to step 7.
7. Measure fresh weight (for method, see III) or dry weight (see IV).
8. Determine PA for forewing plus femur lengths plus fresh (dry) weight from Table 1 (for method, see V B).
9. If PA is less than 0.90, the sample is European. If PA is 0.99 or greater, the sample is Africanized.* If 0.90≤PA<0.99, go to step 10.
10. These samples are suspicious of Africanization or are Africanized.* For further assistance on Africanized samples contact your state apiary inspector.

PROCEDURE

Calibration

In order to make accurate measurements, the projection system must be calibrated so that the projected image is the proper size. An ocular micrometer is fastened to a 22x40 mm coverslip with tape so that the micrometer scale is in the same focal plane as will be the bee body parts. This coverslip is placed in a 35 mm slide mount and placed in the slide projector. A calibration image is projected on the wall and the projector or the "zoom" lens moved until the 10 mm scale on the slide-mounted ocular micrometer produces a 0.5-meter image on the wall. This is a 50-to-1 magnification, so that measurements can be made and divided by 50. Or, if a custom-made scale is used (Figure 2), the measurements are read directly from this scale by measuring the length of the projected image.

Calibration should be checked frequently to ensure the system remains accurate. We recommend that recalibration be done hourly. Before any other action is taken as a result of a sample scoring as Africanized, the calibration should be checked to confirm that the measurements are correct.

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*Where significant costs result from identification as Africanized see PROBABILITIES.

I. Forewings

Prepare the 22x40 mm coverslips by fastening 2 together on the short side with a small piece of clear tape acting as a hinge. Carefully remove one forewing from each bee, so that the entire forewing is obtained. If care is not taken, the wing often breaks ("break point" in Figure 1), leaving the base on the bee, and the correct length cannot then be measured. On fresh samples, the forewings can usually be simply pulled from the bees, which have been killed in an insect killing jar or by being briefly frozen. Lay 5 forewings between 2 coverslips. Two coverslip mounts will be needed to mount the 10 forewings per sample. A skilled person may be able to place 10 wings between a pair of coverslips. Then close the pair of coverslips so that the wings are separated and their ends are visible, and fasten the other ends together with a small piece of tape. Label the coverslips with the sample number. Forewings are not fastened directly to single coverslips with transparent tape because the adhesive on the tape blurs the wingtip and it cannot be seen clearly.

Fit the 22x40 mm coverslips into standard, 35 mm slide mounts (Figure 3). Project these slides onto the wall, measure the images to obtain 10 forewing lengths (Figure 4) and calculate the average for the sample.

II. Femur

One of the hindlegs from each of 10 bees is removed, prepared, mounted between coverslips, and labeled in the same way as forewings. Preparation of the hindleg has two main steps: 1) the trochanter must be removed from the femur so that the length to the tip of the femur, which is covered by the trochanter, may be measured (Figure 1); 2) the leg must be bent as shown in Figure 1 so that both ends of the femur can be clearly seen. The end of the femur which is covered by the trochanter is fragile and often obscured if pieces of the trochanter remain. Therefore, it is easier to properly remove the trochanter while using a dissecting microscope.

III. Fresh Weight

The fresh weight is determined by removing the abdomens (gasters) only, by carefully pulling them off with fingers or fine-pointed forceps. Also, any pollen pellets are removed from the legs, the weight of 30 bees is measured on a balance to the nearest 0.01 g, and this weight is divided by 3 to give the average weight per 10 bees. In order to obtain accurate weights, the bees should be kept alive, frozen with dry ice in portable coolers or killed with cyanide and transported to the lab. The weights should be measured as soon as possible.

IV. Dry Weight

In some cases, the bees cannot be collected, transported and processed

Table 1 — Formulae for calculating the functions for 2 or 3 characteristics and probability table for 8 measurements and functions.

Measurement[a]	Bee Type	Mean[b] (Average)	Range[b]	Critical Value of Measurement	Probability Sample is Africanized PA[c]
Forewing Length				8.968	0.99
	E	9.281	9.098-9.460	8.991	0.95
	A	8.715	8.412-8.950	9.001	0.90
Fresh Weight				0.4639	0.99
	E	0.535	0.488-0.576	0.4707	0.95
	A	0.424	0.365-0.477	0.4737	0.90
Dry Weight				0.1503	0.99
	E	0.171	0.152-0.186	.0.1532	0.95
	A	0.145	0.124-0.165	0.1545	0.90
Femur Length[d]					
	E	2.708	2.464-2.770		
	A	2.531	2.440-2.614		

Function[a]	Critical Value of Function	PA
-104.422 +	-0.560	0.99
(8.541 x Forewing Length) +	-0.320	0.95
(10.413 x Femur Length)	-0.212	0.90
-84.298 +	-0.576	0.99
(8.012 x Forewing Length) +	-0.354	0.95
(24.78413 x Fresh Weight)	-0.254	0.90
-94.050 +	-0.580	0.99
(9.744 x Forewing Length) +	-0.334	0.95
(38.670 x Dry Weight)	-0.226	0.90
-89.415 +	-0.532	0.99
(6.601 x Forewing Length) +	-0.316	0.95
(7.235 x Femur Length) +	-0.220	0.90
(22.381 x Fresh Weight)		
-99.41 +	-0.521	0.99
(7.571 x Forewing Length) +	-0.299	0.95
(9.6833 x Femur Length) +	-0.195	0.90
(34.047 x Dry Weight)		

[a] Forewing length is the average of measurements of 10 bees, in mm;
 Femur length is the average of measurements of 10 bees, in mm;
 Fresh weight is the average weight of 10 bees (30 ÷ 3), in grams;
 Dry weight is the average weight of 10 bees (30 ÷ 3), in grams.
[b] Means and ranges for 10-bee samples, n_E = 100 and n_A = 84.
[c] The probability a sample is European (PE) = 1 - PA.
[d] Femur length is not useful as a single measurement, so no PA is given.
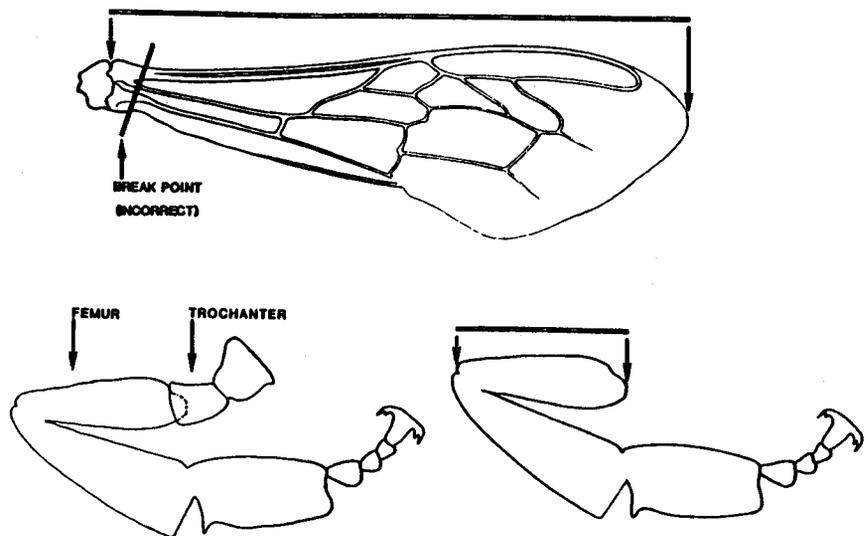

Fig. 1 Body parts measured.

carefully enough to yield an accurate fresh weight. If so, they can be prepared as for fresh weight, dried in a convection drying oven for 24 hours at 60°C (140°F), and then weighed to

yield an average dry weight per 10 bees. A drying oven with a fan should be avoided since it probably will blow the dried bees out of the drying containers.

V. Analysis of Measurements

A. After the average lengths and weights have been calculated, they will be compared to values in Table 1. For single measurements, the calculated average value is used directly to determine the probability the sample is Africanized (PA). If the calculated value is less than or equal to the table value for 0.99, the sample has a PA of 0.99 or more (PA ≥ 0.99) (e.g., a mean forewing length of 8.968 mm gives a PA of 0.99 or 99%). If the value is larger than 8.968 but equal to or less than 8.991, the PA is less than 0.99 but greater than or equal to 0.95 (0.99 > PA ≥ 0.95); and similarly for 0.90. If the calculated value is greater than the table value for 0.90, the sample is clearly European in any case. In most cases, the cutoff point to use is PA = 0.99.

B. Where 2 or 3 measurements are made, the average values interact and cannot be used directly. Simple formulas are given where the calculated averages are substituted in the proper formula, a result (function) is calculated, and the result is compared to the given table values in the same way as for single measurements.

PROBABILITIES

The probability level chosen for identifying a sample as Africanized or European may be changed depending on such factors as how much it costs to identify a sample, how much it costs to requeen or destroy a colony, how well the identifiers are trained, and how similar the population being examined is to the FABIS base populations. Choosing a lower PA cutoff point is appropriate when the identification cost is low compared to the cost of requeening or destroying a colony, when the identifiers are not well-trained or when the normal range of measurements for the population is unknown. This is especially true for the first identification in an area or where a quarantine or other expensive reaction would result from an identification as Africanized.

The simplest case (see FABIS OUTLINE) is for samples from a population which is known to fit the FABIS baseline (i.e., where the range of forewing lengths in the sampled population is within the range of lengths of the FABIS base population) and where the total cost due to destroying or requeening the sampled colony would be small. In such a case, forewing length or fresh weight alone at a probability of Africanization of 0.99 or greater (PA ≥ 0.99) would be
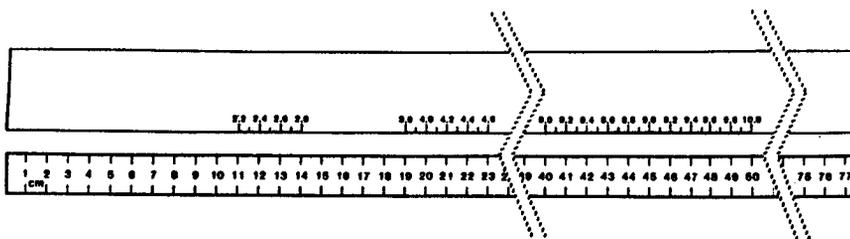

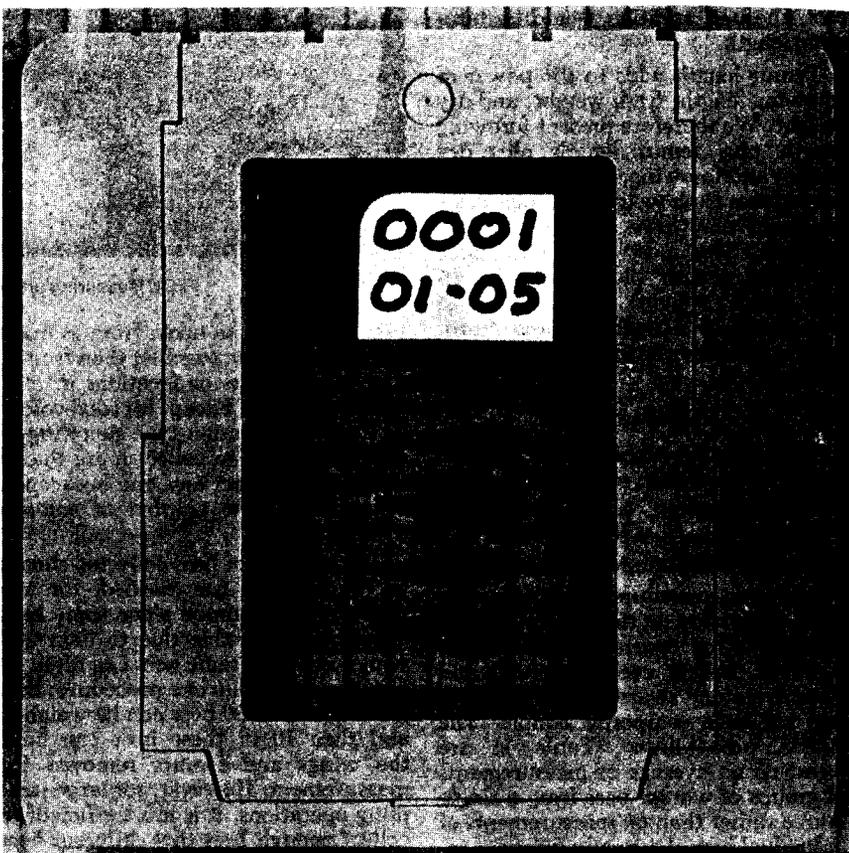Fig. 2 Custom-made, 0.5-meter scale marked in mm (above) compared to meter stick marked in cm (below).

Fig. 3 Forewings placed between coverslips, labeled, and mounted in a 35 mm slide mount.

sufficient to identify a sample as Africanized. Where the population baseline is unknown (especially for early samples), any colonies suspected of being Africanized based on the first test should be analyzed by additional FABIS measures or other methods for confirmation. Where there would be significant costs as a result of identifying a sample as Africanized, such analysis should include two or three FABIS measures and be confirmed by comb measurements, field behavior or Daly's full morphometric analysis, before a colony is declared Africanized.

ALTERNATE PROCEDURES

Because of the improved accuracy of these techniques, there are several choices of identification procedures. Depending upon needs, capabilities, and equipment availability, any of three single measurements can be

selected for preliminary identification. Forewing length is the most powerful and fresh weight is second best. Because freezing and transport reduce the value of fresh weight, when fresh weights are used only freshly killed bees can be expected to give satisfactory results. When frozen and transported, all samples become heavier, apparently because of the freezing, thawing, and associated water condensation. These conditions can lead to misidentifications. Dry weight overcomes these technical difficulties in maintaining accurate weight. Thus, in spite of its lower discriminatory power, dry weight may be better than fresh weight in large programs or where transportation of samples is difficult. However, a reasonable extra effort to obtain an accurate fresh weight will be well repaid by the increased discriminating ability of fresh weight.

For all single characters, at least a

few samples may remain unidentified. Any of the combinations can be used to identify such samples. The best pair combines forewing length and fresh weight. Its major disadvantage is that it requires equipment to measure both length and weight, but it is the simplest and fastest. Forewing and femur lengths may be desirable where only length can be measured. This is the second best approach. Where dry weight was used as an initial screening tool, the best addition would be forewing length.

Femur length adds to the power of forewing length, fresh weight, and dry weight. We therefore present forewing length plus femur length plus dry weight and forewing length plus femur length plus fresh weight, because large programs may have a need for these analyses. The most precise of these (forewing length plus femur length plus fresh weight) gives the best separation of Africanized and European populations using these simple methods.

SAMPLING

1. Each sample should be collected from one swarm, colony, or hive.
2. When possible, collect only adult worker bees from brood comb.
3. Collect a minimum of 50 bees.

Sampling procedures will vary somewhat depending on the purposes of the collector. One constant requirement, however, is that each sample should come from one swarm, colony or hive and not be a composite sample. The FABIS probabilities (Table 1) are based on an average of measurements of groups of worker bees from a single source rather than on measurements of individual bees. Distinctly different individual bees in a sample will not be detected by this method. Bees of unknown origin (e.g. bees from a sweep-net sample or a feeding station) may be analyzed by these procedures but the results must be examined at the individual bee level. If all bees from such samples are Africanized, one or more colonies of Africanized bees are in the area. If all the bees from such samples are European, no Africanized bees are likely to be in the area. If some of the bees appear Africanized, then one or more Africanized colonies may be in the area. In this last case, positive confirmation can only come from direct sampling of the managed and feral colonies within flight range of the sample collection point.

For baseline or survey sampling, particular attention should be paid to swarms, feral colonies, and unmanaged colonies. They are the most likely to be of unique local types or be newly arrived Africanized colonies. Generally, it will be desirable to have as broad a survey as possible. That is,
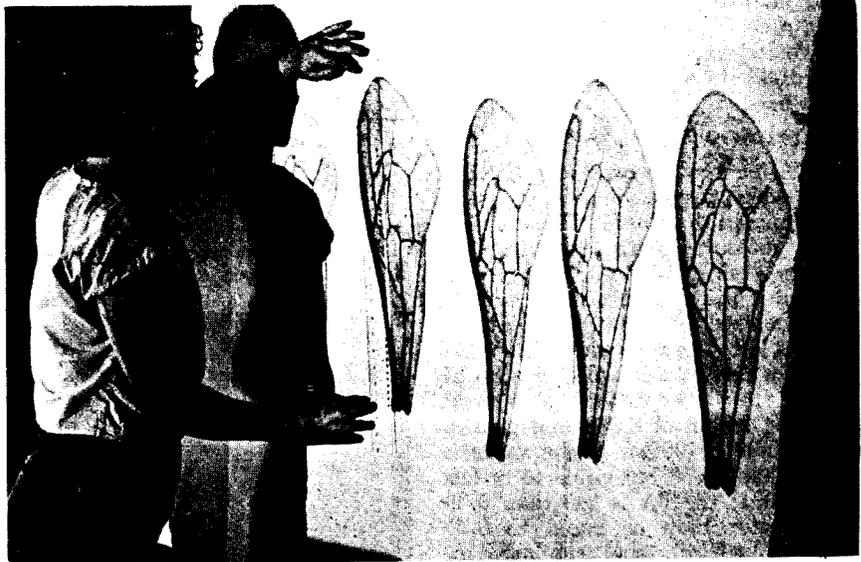

Fig.4 Measuring projected images of forewings.

samples should be taken from as many different areas as possible, then from as many beekeepers or locations in each area as possible. Except for total colony or stock certification-type sampling, it is not desirable to collect more than a few samples from any one beekeeper, regardless of the number of colonies owned.

The number of bees collected should be more than are needed for the procedure(s) planned since some bees are often unsuitable due to damaged wings, etc. The same bees can often be used for more than one procedure; that is one group of 30 bees can be weighed and then 10 of these bees can have the wings and femurs removed for measurement. However, for large sampling operations, it is more efficient to collect enough bees that different bees can be used for each procedure.

EQUIPMENT NEEDED

Required
Fine-pointed forceps (tweezers)
Microscope slide coverslips #1, 22x40 millimeter (mm)
35 mm slide mounts (e.g., Polaroid®)[2]
35 mm slide projector
Ocular micrometer (e.g., Edmund Scientific #E30, 323 — $19.00)[2]
Straight edge, clear plastic, 0.5 meter length (see Fig. 2 & 4) (e.g., C-THRU® #M-111)[2]
Metric balance +/-0.01 gram
Calculator

Optional
Dissecting microscope

A dissecting microscope is very helpful when learning the proper dissection procedures. If available, it will also be helpful later to examine dissected parts to see if they are ready to mount. The slide mounts should be a brand that is open and then snaps shut

for mounting, because the coverslips are very thin glass that is easily broken. They cannot be bent for mounting. For measuring the projected images, a solid surface is needed so the images stay in focus during measuring and a light-colored, reasonably smooth surface is needed for ease in focusing. A simple way to meet these requirements is to fasten white paper onto a wall (Figure 4). Depending on its lens, the projector may need to be up to 20 feet from the wall. The projector should be on as secure a support as possible to minimize measurement errors due to movement of the projector. The lens on most projectors is curved to match the curvature of 35 mm slides and so produce an image entirely in focus. The coverslips are flat, which produces an image which is not entirely in focus. Then focus adjustments are necessary as measurements are taken across the image. A flat lens is available which produces a focused image from edge to edge and reduces time spent in adjustments.

To make the measurements, a custom-made, 0.5-meter-long scale is very helpful. This is simply a clear plastic straightedge, 0.5 meter in length, with 1 mm graduations, and with the 1 cm units replaced by numbers ⅕ as large (e.g., 20 becomes 4.0). This scale is then read directly to give actual body part lengths in hundredths of millimeters. In practice, the only points which need to be numbered are 0.0, 8.0 to 10.0 for forewings, and 2.2 to 2.8 for femurs. A simple way to produce such a scale is to purchase a standard 0.5 meter, clear plastic ruler and renumber the above points (Figure 2).

COMB MEASUREMENT

1. Measure the distance spanned by 10 worker-brood-size cells.

2. Do so 3 times across the 3 linear directions of the comb hexagonals and average the results.

3. Averages of 5.2 cm or more are European.

4. Averages of 4.9 cm or less are Africanized.

5. Averages between 4.9 and 5.2 cm are unidentified but accepted to be Africanized to some extent.

When identifications are desired for feral or other colonies which have produced worker comb, most colonies can be easily identified. This method is usable only for colonies where the present queen produced the workers drawing comb and where the comb was produced without comb foundation.

Rinderer *et al.* (1982) found that single measurements of the distance spanned by 10 European worker brood cells ranged from 5.0 to 5.5 cm. Averages of 3 measures ranged from 5.2 ± 0.02 (X ± SE) to 5.3 ± 0.02 cm. In contrast, single measurements of the distance spanned by 10 Africanized cells ranged from 4.5 to 5.0 cm. Averages of 3 measures ranged from 4.8 ±

0.02 to 4.9 ± 0.02 cm. Only fully formed worker-size cells in the central portion of a comb should be measured. For this procedure, each cell is interpreted to have one cell wall. The outside of the wall of the first cell is included in the measure. The last cell is measured up to the inner edge of the eleventh wall. Other points may be chosen instead as long as they correspond, so that 10 cell openings and 10 cell walls are measured; i.e., not outside to outside (11 cell walls) or inside to inside (9 cell walls).

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