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Africanized Bees: Progress In Identification Procedures

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Through research done by Daly and the Baton Rouge Bee Lab, a quick Africanized honey bee identification method has been developed with 97% accuracy.

IN THE next few years, and most likely by the end of this decade, the Africanized bee will make its way to the United States. It will come in at ports and across the U.S. southern border from Mexico. One instance requiring the identification of large numbers of colonies has already occurred in California this summer.

It is vital to the United States to have a quick and low-technology way of identifying Africanized bees at sites where their presence is suspected. The U.S. Department of Agriculture (USDA) needs such an identification method in case the bees pose a health hazard, and in case regulatory action such as quarantines or eradication is called for. Scientists and regulatory personnel must be able to quickly set up makeshift laboratories at the site, and equipment must be fairly easily transported or purchased near the site.

Through research being done at the USDA, ARS, Honey-Bee Breeding, Genetics & Physiology Research Laboratory in Baton Rouge, Louisiana, and based on research done at the University of California at Berkeley by Dr. Howell V. Daly, a method of quick identification has been developed. The two-step identification system has been named FABIS by the USDA — FABIS standing for Fast Africanized Bee Identification System. (Rinderer et al. 1985).

Before FABIS is outlined, a look at the history of Africanized bee identification methods is needed. The first research was done by Dr. Daly at Berkeley (Daly and Balling 1978). His method required the use of rulers and protractors to measure projected images of bee parts mounted on microscope slides. The work was done manually and proved to be tedious and time-consuming. He continued his research, and published a revised version of his first method (Daly et al. 1982). This method was much improved through the addition of a computer-assisted measurement system. It uses a computer and a digitizer, which take the place of measurements by hand.

To take measurements, bee parts from a sample of 10 bees are mounted on microscope slides, and the slides are projected by a micro-projector onto a digitizer pad. The crosshairs on the digitizer cursor are held above specific points on the bee parts (for example, at one of the vein intersections on the bee's wing). When the cursor's button is pressed, the location of that point is entered into the computer. After several of these points are entered into the computer in a specific sequence, the computer analyzes them and estimates whether the individual bees and the sample of 10 bees are Africanized or European.

The measurements calculated by the computer include: the width and length of the fore-wing; a partial length and the width of the hind-wing; several angles and lengths determined by intersections of veins in the bee's fore-wing; the length of three different parts of a rear leg, and the width of one of those leg parts; five measurements on the bee's dissected sternite; and the number of hamuli — the hooks that hold the wings together when the bee is flying. A total of 25 measurements per bee are made, by marking 38 points with the digitizer and counting the number of hamuli.

While Daly's research was a major breakthrough in the field of Africanized bee identification, the method requires about 5 hours of work to make a determination, with most of this time spent dissecting the bees. This method, which we have come to call Step 4 (discussed later), is still the final one which is used to determine the identity of Africanized or questionable samples.

However, this method of identification of Africanized bees would take too much time in a situation that demanded immediate action on a large number of colonies. Another problem exists with Daly's method: it would be unsuitable for Third World countries, since it requires the use of computers, digitizers and other sophisticated equipment that would not be readily available. It became clear that a fast identification technique was needed — one that did not require high technology or sophisticated equipment, even if the method left a few samples unidentified.

In the meantime, Daly at Berkeley, supported by the USDA, was handling all the routine identifications by the Animal and Plant Health Inspection Service (APHIS) of samples from around the United States where the presence of Africanized bees was suspected. He found that he was spending too much time doing identifications instead of research.

At the time, it would have been logical for the Insect Identification and Beneficial Insect Introduction Institute (IIBIII) of the Agricultural Research Service (ARS), USDA to take over the routine bee identifications; however, the institute was not ready to take over this task. The Honey-Bee Breeding, Genetics, and Physiology Laboratory was therefore asked by the National Program Staff of the ARS, USDA in March of 1983 to temporarily take over routine bee identification, with financial support from APHIS; hence, we began to work on a quicker, low-technology method of identifying Africanized bees. Since Sylvester had done his doctoral work on the identification of Africanized bees, he took over the responsibility of establishing this identification laboratory.

After he spent some time with Daly in California, he began work on identification techniques in October 1983. The work began with experiments and studies with test colonies of European and Africanized bees in Venezuela, set up by this lab. We collected data in the spring and summer of 1984, and developed and tested FABIS in 1984 and 1985.

Using the method that Daly had developed with his 25-measurement system, and the bee data collected in Venezuela, Rinderer looked for the factors which are the most discriminating in bee identification and for factors which did not require tedious dissection. Rinderer determined that if only a few of Daly's 25 measurements were used, unknown samples would still be correctly identified 97% of the time and none were misidentified. The remaining 3% fell in the category of "unidentified."

These analyses used in FABIS are statistical procedures which compare body part measurements of unknown samples to chosen groups of samples (base populations) and give probability estimates that the unknown sample has body part measurements similar to those of a sample taken from the base population. For FABIS, the samples in the 2 base populations (Africanized and European) were identified based upon their known history and field behavior and this identification was confirmed by Daly's 1982 method. In developing FABIS, "correct identifications" were those in which FABIS classified a sample as belonging to its known base population with a probability of 90% or more. "Misidentifications" would be those where a sample was classified as belonging to the wrong base population with a probability of 90% or more. "Unidentified" samples were those few which were not identified as belonging to either group with a probability of 90% or more.

Fore-wing length is still the one most important measurement in identifying an Africanized bee. With that measurement alone, Africanized and European bees are correctly identified in 85 out of 100 cases. Based upon this knowledge, fore-wing length alone became Step 1 in FABIS.

Where fore-wing length alone would not correctly identify a sample, a group of three measurements and one weight proved to be the best. They were: the measurements of the lengths of the fore-wing, partial hind-wing and femur (Figure 1), and the "clean weight" of

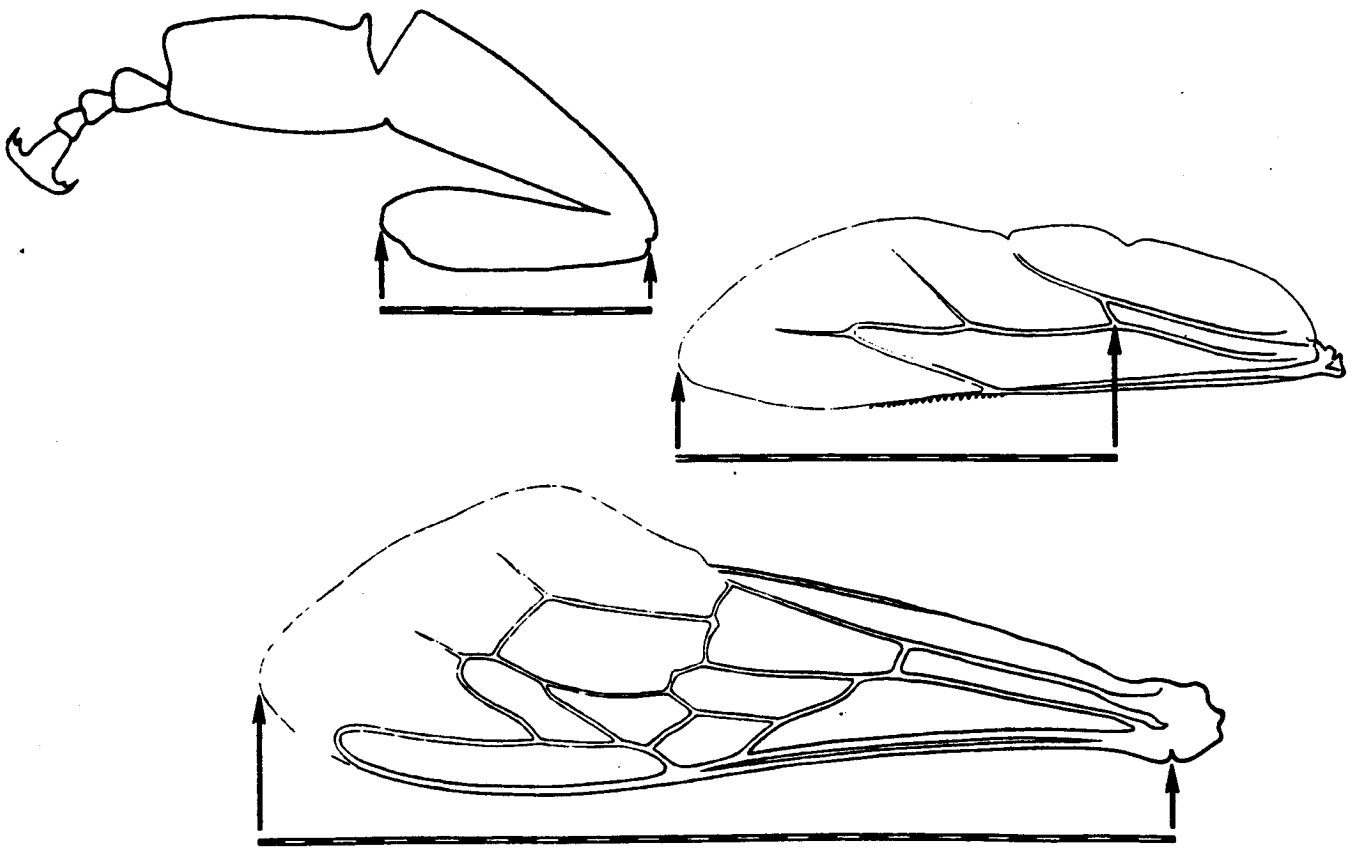

Figure 1. The distance measured on fore-wing, hind-wing and femur.

the bee. These four measurements became Step 2 in FABIS.

The clean weight is the weight of the bee with its feces and nectar squeezed out. It is a measurement that Daly had not utilized, and it proved to be the second most important measurement in the FABIS identification method.

The equipment needed for Steps 1 and 2 includes bee dissecting tools; 22-by-40 millimeter (mm) microscope slide coverslips; 22-by-22 mm coverslips; transparent adhesive tape; 35 mm, plastic, slide mounts; a 35 mm slide projector; and a metric balance that is accurate to 0.01 gram.

A calibration image is projected on a wall, so that its projected image is in clear focus and of the correct size. The actual length of the bee parts can then be accurately calculated.

For Step 1, mounts are prepared by sandwiching fore-wings between coverslips to obtain the 10 fore-wing measurements per sample. These 22-by-40 mm coverslips fit into standard, 35 mm slide mounts. The slides are then projected onto the wall, the images are measured to obtain fore-wing length, and the average of 10 fore-wing lengths for the sample is calculated. The average fore-wing length is then compared to table values. Beside each possible measurement listed in the table, there are 2 probabilities: the probability of the sample being Africanized or European.

In general, an average wing length of 9.070 mm or longer discriminates the sample as European. Bees with an average fore-wing length between 9.069 and 8.946 mm must be tested in Step 2. Averages shorter than 8.945 mm are suspected of being Africanized, but are tested in Step 4 to make sure.

For Step 2 of FABIS, fore-wings, hind-wings, and femurs are mounted on coverslips (Figure 2) and the lengths are measured (Figure 1). The clean weight is determined by carefully squeezing out any nectar and feces, weighing 3 groups of 10 bees and taking the average of the 3 weights. Laboratory personnel found it easier to collect enough bees so that different bees can be used for each step. (In other words, in FABIS Step 2, use different bees to make the clean weight sample and to obtain the hind-wing, femur and

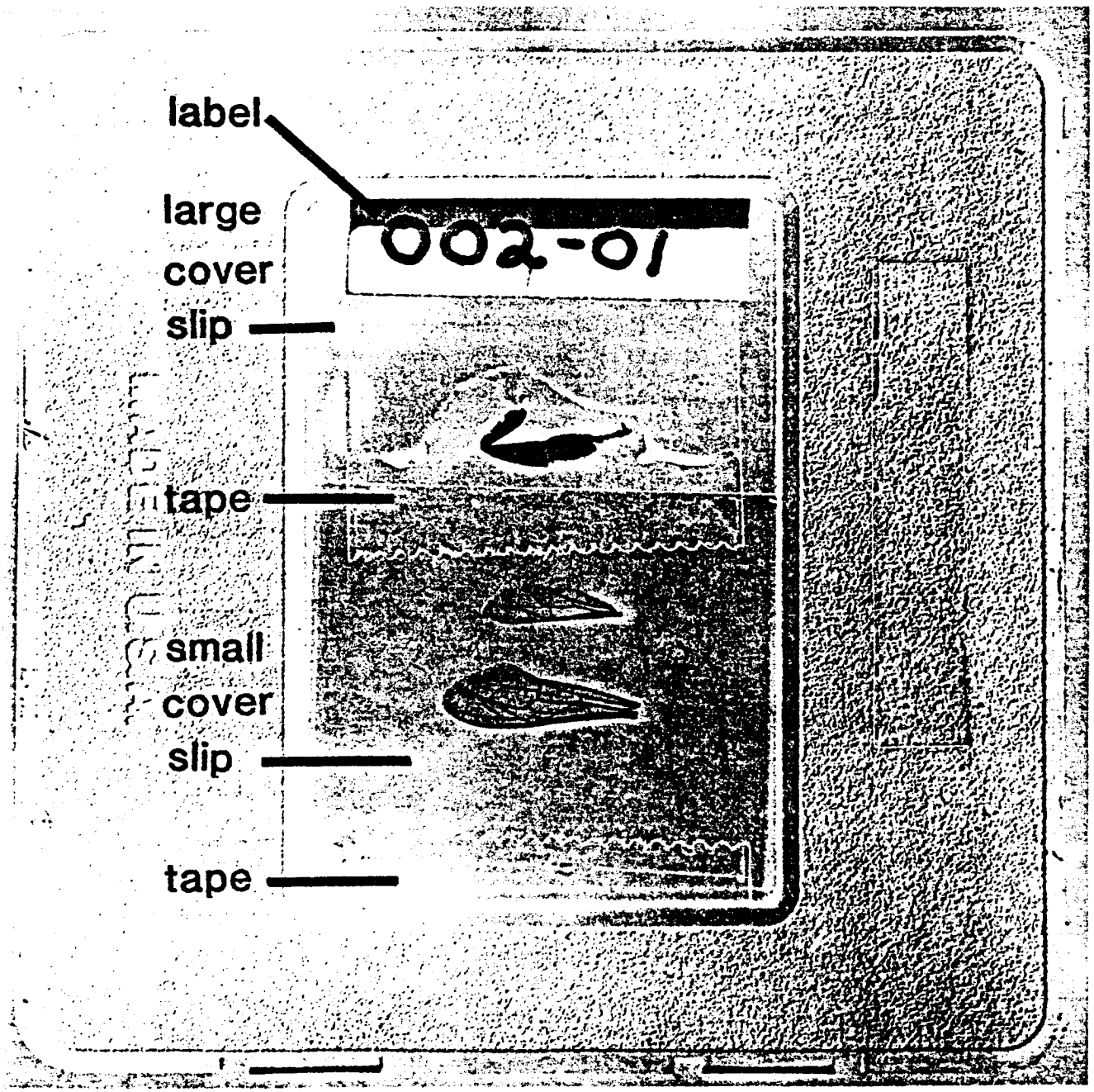

Figure 2. The assembly of body parts, cover slips, tape and label in a slide mount ready for projection.

fore-wing measurements.)

The average measurements of the fore-wing, partial hind-wing, femur, and the clean weight data, are entered into a small computer. The computer is programmed to evaluate the data and give a probability of the sample being either European or Africanized. Because these measurements interact, the results cannot be determined by simply looking at a table, as in Step 1.

FABIS is thus a 2-step process for quick and simple field screening of large numbers of bee colonies. After FABIS was developed, it was tested with data from other Africanized and European colonies, and the accuracy of the method was verified.

Work is continuing to improve Step 2. In particular, squeezing all of the nectar and feces out of a bee requires skill and practice, thus some error remains. Replacement measurements are being evaluated.

In the meantime, Daly developed a computer-assisted technique which included several measurements of the fore-wing only (Daly and Hoelmer 1986). Using a digitizer, 16 points on each projected fore-wing are entered in a computer to compare 47 lengths and angles of wing veins with a known data base and compute the probability of the sample's being Africanized or European. This eventually became Step 3 in bee identification, and Daly's 25 measurements became Step 4. Both Steps 3 and 4 are laboratory procedures which are not well adapted to field use.

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If a sample of bees is not conclusively identified as European after Step 1 of FABIS, the sample of bees should be tested in Step 2. Any sample that is identified as European at any level is officially declared European, but if a sample is suspected to be Africanized at any of the first three levels, laboratory personnel proceed to the fourth step for final confirmation of Africanization.

In July 1985, a colony of Africanized bees was found near Bakersfield, California. It is suspected that the bees came in with oil-drilling equipment that had been used in different locations around the world. There were about 20,000 colonies of bees within a 20-mile radius of the original California Africanized colony. At an organizational meeting of scientists near the site in California, representatives from UC Berkeley, the California Department of Food and Agriculture, and USDA personnel from this laboratory and APHIS decided upon a four-part identification procedure. The four steps used for identifying worker bees are as follows:

STEP 1: Take the length of the fore-wing (85 per cent unambiguous identification). This step takes about 20 minutes per sample of 10 bees.

STEP 2: Take the group of four measurements: the fore-wing length, the partial hind-wing length, the femur length and the clean weight (97 per cent unambiguous identification). This takes about 1 hour per sample of 10 bees.

STEP 3: Make computer-assisted measurements of fore-wings only (99 per cent unambiguous identification). This takes about 1 hour per sample of 10 bees.

STEP 4: Take 25, computer-assisted measurements (100 percent unambiguous identification). This requires about 5 hours per sample of 10 bees.

While awaiting the arrival of Africanized bees in the United States, work will continue on identification techniques.

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