

THE IDENTIFICATION OF AFRICANIZED HONEY BEES: AN ASSESSMENT OF MORPHOMETRIC, BIOCHEMICAL, AND MOLECULAR APPROACHES

T. E. RINDERER

USDA-ARS Honey-Bee Breeding, Genetics & Physiology Laboratory
1157 Ben Hur Road, Baton Rouge, Louisiana 70820, U.S.A.
E-mail: trindere@asrr.arsusda.gov

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African honey bees (*Apis mellifera scutellata*) were introduced into Brazil in 1955 with the intention of providing improved honey bee breeding stock for Brazilian apiculture. The spread of the descendants of the introduced African bees, known as Africanized bees, was a matter for scientific study and regulatory concern. This attention produced the need for an identification tool that could be employed in research, survey, and detection and regulation. In part due to a long history of study and in part due to its intrinsic value, the discriminant analysis of morphometric data has become the tool of choice for identifying Africanized honey bees. Cost of analysis led to the development of simple methods to screen large numbers of samples without sacrificing the overall quality of identifications. With these screening procedures, all colonies that are determined to be European at a $P \geq 0.99$ are considered European. All colonies that are not determined to be European are considered unidentified. Samples which remain unidentified after the initial screening are identified by a more complex morphometric procedure called USDA-ID. USDA-ID was developed primarily to provide several laboratories that were established to morphologically identify honey bees for regulatory purposes more accurate identification tools based on new discriminant analysis procedures. The characteristics of these procedures are discussed and their weaknesses and strengths are compared to those of several other identification tools.

Key words: *Apis mellifera scutellata*, Africanized bees, morphometrics, biochemical identification, molecular identification

INTRODUCTION

African honey bees (*Apis mellifera scutellata*) were introduced in 1955 with the intention of providing improved honey bee breeding stock for Brazilian apiculture (KERR 1957). In 1956, some of this stock was released. The released stock interbred with previously imported honey bee stocks from Europe, producing hybrid progeny known as Africanized honey bees. The success of Africanized honey bees in the New World was tremendous. The rapid and widespread colonization by Africanized bees of much of South America, all of Central America, and finally the southern portions of North America, including all of Mexico and portions of the southern United States, is one of the most remarkable ecological events of this century. Within three decades a small experimental im-

portation of honey bees became a population of many millions of colonies which now occupy about 25 million square kilometers.

Africanized honey bees are noted for several other traits beyond their remarkable potential for reproduction and range extension in the New World. Foremost among these traits is their intense level of stinging in defense of their nests (COLLINS & RINDERER 1991). Stinging, along with poor honey production (RINDERER *et al.* 1984) and generally poor apicultural value (DANKA *et al.* 1987) have generated scientific, regulatory and apicultural concerns about Africanized honey bees (RINDERER *et al.* 1993b). Proper study and regulation (survey, detection and control) efforts concerning Africanized honey bees require the ability to quickly and accurately identify Africanized honey bees. Scientific needs of identification will vary according to the specific study. For example, population genetic studies of the Africanization process benefit from having data from several measurement systems. However, for regulatory purposes, a useful method for identifying Africanized honey bees should depend upon one technique that can be applied uniformly by personnel from several laboratories having only brief training and limited equipment. Many countries and their states, provinces and even local jurisdictions have an interest in identifying Africanized honey bees. Ideally, all of them would use the same procedures and be able to reach quite similar identification conclusions. Many of these identification services would necessarily need to use relatively unskilled labor and could not afford elaborate or expensive equipment. At the same time, the technique should access a reasonably large portion of the genome in order to provide a clear identification of a variety of intermediate types arising from hybridization.

These identification needs arose in the context of a long tradition of honey bee taxonomy. The genus *Apis* is comprised of nine species which are easily distinguished by morphology and life history details. However, one species, *Apis mellifera*, has been the subject of intense taxonomic study. This study is motivated by *A. mellifera*'s economic value as a hive bee in combination with its wide range of subspecies diversity. The home range of *A. mellifera* includes all of Africa, most of Europe and most of the Arabian peninsula. The wide range of locally adapted ecotypes in this species has a correspondingly wide range of desirability for various economical characteristics. This variance in economic value has led to a long history of research efforts concerning the subspecific taxonomy of *A. mellifera*.

HISTORICAL ORIGINS

Research concerning the identification of Africanized honey bees was founded on the products of prior subspecific honey bee taxonomy. In many re-

gards, efforts to identify Africanized honey bees extended that work, especially in regard to choices and uses of statistical methods. However, the procedures developed to identify Africanized honey bees were produced in response to a practical need to have a fast, inexpensive and easily transferable identification technology.

Early interest in subspecific variation in *A. mellifera* led to the introduction of a tri-nomial classification system (VON BUTTEL-REPEN 1906). In the next half century, the application of this system in addition to other taxonomic work resulted in 600 different names in the literature by 1953 for honey bees. In a revision of the genus, MAA (1953) retained 146 names from his compilation of 600. RUTTNER (1987) extended this move toward recognizing fewer species and subspecies and in his revision used 37 names for the genus, with 24 of them used for subspecies of *A. mellifera*.

RUTTNER's 1987 work culminated efforts by himself and others to base a taxonomy of honey bees on morphometric data. The application of morphometric data to honey bee questions arose in Russia with the work of COCHLOV (1916), who compared groups using univariate measurements. Quite quickly, this work was extended to include multivariate measurements and studies of ecological correlates to geographical trends in morphology (ALPATOV 1929). GOETZE (1930) contributed to the development of honey bee morphometrics by studying ratios of lengths of wing veins as a way to include the geometry of wing venation and by suggesting that morphometric values could be used as breeding values indicating the presence of economically desirable characters which were correlated to morphometric characters. DUPRAW (1964), in an attempt to develop a numerically based non-Linnean taxonomy, overcame certain mathematical difficulties in using ratios of lengths of wing veins by using measurements of the angles formed by vein intersects and introducing more advanced forms of multivariate statistics. Hence, when African honey bees were released in Brazil in 1956-57, the morphometric study of subspecies variation in honey bees was already advanced and able to serve as a foundation for the development of identification procedures based on morphological evaluation.

MORPHOMETRIC APPROACHES TO IDENTIFYING AFRICANIZED HONEY BEES

Early Procedures

The release of African honey bees resulted in a population of honey bees in the Americas called Africanized honey bees which to some degree were the products of hybridization between African honey bees and various European subspecies of honey bees. Thus, the problem of identifying them included two as-

pects new to the morphometric study of subspecies variation. First, the group requiring identification was not a subspecies. Second, the group requiring identification had some parentage from the contrasting group, a collection of European subspecies. Hence, the problem of identifying Africanized bees could not rely on previously established descriptions for subspecies.

Early attempts to find a univariate character which provided certain identification of Africanized honey bees were not successful (DALY 1991). This result led DALY and BALLING (1978) to develop a multivariate morphometric procedure to distinguish between the Africanized honey bees of South America and the European honey bees of North America. To do so, they built discriminant functions from base-line data derived from 101 colonies of Africanized honey bees from South America and 297 colonies of European honey bees from North America. Ten worker bees from each colony were dissected and 25 morphometric characters were measured for each bee. The measurements were made for forewing, hindwing, hindleg, and sternite characteristics which were mainly either linear measurements or angular measurements (Fig. 1), although the number of wing hooks was also included. Using classification at the highest group probability, the expected misclassification rate was 0.5%.

DALY *et al.* (1982) provided an innovative development to morphological studies generally by adapting computers to assist in the measurement and analysis of data. A projecting microscope projects an image of dissected body parts onto the surface of a digitizing pad. Points are digitized and linear or angular data are interpreted by a program designed to receive data input. The data can then be submitted to analysis programs structured for specific purposes.

Current procedures

Screening with Simple Techniques. Currently used morphometric systems are both simplifications and developments of the foundations supplied by DALY and BALLING (1978) and DALY *et al.* (1982). It is costly to provide a full morphometric analysis of every sample from survey and detection programs or other programs that produce a large number of samples. This cost led to the development of simple methods to screen large numbers of samples without sacrificing the overall quality of identifications. RINDERER *et al.* (1986a) developed two simplified techniques appropriate for field or laboratory use. The simplest approach used a single character (forewing length) and correctly identified 86% of 136 colony samples at $P > 0.90$. The second approach used four morphometric measurements (forewing length, partial hindwing length, femur length and "clean" weight) and correctly identified 91% of colony samples at $P > 0.90$.

The procedures of RINDERER *et al.* (1986a) were used in the regulatory quarantine when Africanized honey bees were found in California, USA (GARY *et al.* 1985). Approximately 25,000 colonies were identified before the quaran-

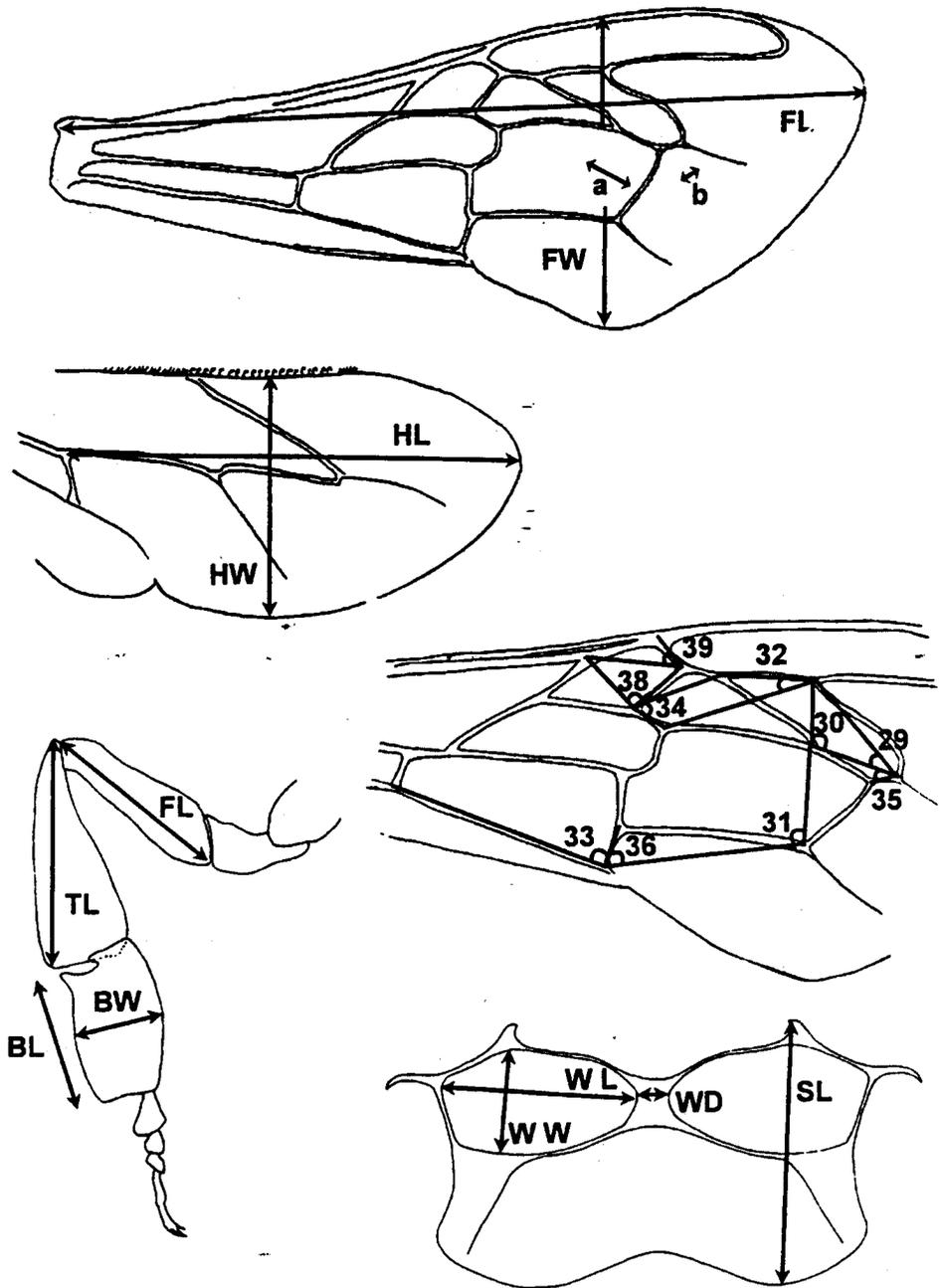


Fig. 1. Ten worker bees from each colony were dissected and 25 morphometric characters were measured for each bee. The measurements were made of forewing, hindwing, hindleg, and sternite characteristics which were mainly either linear measurements or angle measurements as illustrated, although the number of wing hooks was also included

tine was ended. These identifications were made during a 12-week period in a temporary laboratory using, for the most part, unskilled help. All colonies that were determined to be European at a $P \geq 0.99$ were considered European. All colonies that were not determined to be European were considered unidentified and were then identified using the procedures of (DALY *et al.* 1982). Periodically, (two to four a day) quality-control samples of Africanized honey bees were placed as "blind" samples among the unknown samples being processed. Every quality control sample was identified as Africanized.

Although the procedures of RINDERER *et al.* (1986a) were successful in the California regulatory action, possible ways to improve the procedures became apparent. In response to this need, RINDERER *et al.* (1987) provided eight simple identification techniques to identify Africanized and European honey bees. These techniques are based on measurements of dry weights, wet weights, forewing lengths, femur lengths and various groups of two or three of these characters (Fig. 2). Two of these techniques have seen regulatory and research use. Forewing length is a convenient measure which can be made on most samples of honey bees regardless of the way in which they have been collected or preserved. Hence, most programs interested in identifying Africanized honey bees have relied on forewing length as their first identification screen. Some regulatory and research programs having access to fresh samples and skilled technicians to identify the samples have used measurements of wet weight for this purpose.

Full morphometric procedures. In the last few years, spreading populations of Africanized honey bees have entered the states of Texas, New Mexico, Arizona, and California in the United States. As Africanized honey bees spread further into the United States, several laboratories were established to morphologically identify honey bees for regulatory purposes. To provide these laboratories with more accurate identification tools, new discriminant analysis procedures were developed to identify Africanized and European honey bees (RINDERER *et al.* 1991a, 1993a). These procedures are improved over those of DALY and BALLING (1978) in several respects. First, sample sizes of both Africanized and European bees are much larger than those previously used and hence can be expected to include more of the variation of both groups. Second, feral European honey bees and commercially kept European honey bees are well represented, reducing the chance of misclassifying feral European honey bees as Africanized. Third, Africanized honey bees reared in hives having European comb foundation and feral Africanized honey bees are well represented, reducing the chance of Africanized honey bees being misclassified as European. Fourth, the classification of base-line colonies as Africanized or European is determined by whether or not the general population was considered to be Africanized at the time of collection and the specific history of the queen that produced the colony. This eliminates the bias inherent in making decisions based on field behavior which likely led to

only the most clearly Africanized members of the sampled populations being collected as base-line material. Fifth, the measurement of two wing angles used by DALY and BALLING (1978) which have been difficult to standardize between laboratories has been eliminated.

The data from 2,500 colony samples of honey bees collected from colonies from several locations in the Western Hemisphere and Kangaroo Island, Australia, were reviewed to assign them as feral colonies, rustic colonies (hived without comb foundation or requeening) or commercial (hived with comb foundation or requeening) and to assure that they could be considered random samples of clearly Africanized or European populations based on their date of collection and other information. Additional samples from colonies were collected from several locations, including northern Mexico and several southwestern states in the U.S. in order to increase the geographical and biological variability of the samples. Overall, data from 2,103 colonies were used in the final analysis. This total includes 177 hived Africanized colonies which, with only occasional exception, had bees reared on European comb foundation, 414 feral and rustic (hived without comb foundation or requeening) Africanized colonies, 331 commercial European colonies, 1,111 feral and rustic European colonies, and 70 European colonies from unknown hives (swarms and other unknowns).

A sub-set of 87 Africanized and 48 European colonies was used to evaluate the potential of 40 morphological measurements to contribute to the identification of Africanized and European honey bees. Morphological measurements of 40 characteristics were taken from the dissected body parts of ten worker bees from each colony in the subset according to the guidelines of RUTTNER (1987). These data were analyzed using a step-wise discriminant analysis which permitted the average squared canonical correlation to be used to estimate the additional variance contribution of the characters not used by DALY and BALLING (1978). According to the analysis, the 25 characteristics selected by DALY and BALLING (1978) provided an analysis that was based on 93.6% of the total variance. The addition of the other 15 characters increased the total variance assessed by the analysis to 95.9%, indicating that generally these characters collectively added 2.3% to the variance accessed by the analysis. The range of additional proportions of the total variance added to the analysis by each of the 15 characters was from 1.0% to 0.000001%. The strongest characters of this group were angle L13 (RUTTNER 1987) and the length of the right distal segment of the proboscis. These characters were judged either to be difficult to obtain from field samples collected by different persons using different methods or difficult to standardize among laboratories. Of the 25 characters chosen by DALY and BALLING (1978) as the most valuable contributors to the multivariate analysis, two measurements, Angles 38 and 39, contributed little to the power of the discriminant analysis. When their contribution to the analysis was evaluated with a step-wise discrimi-

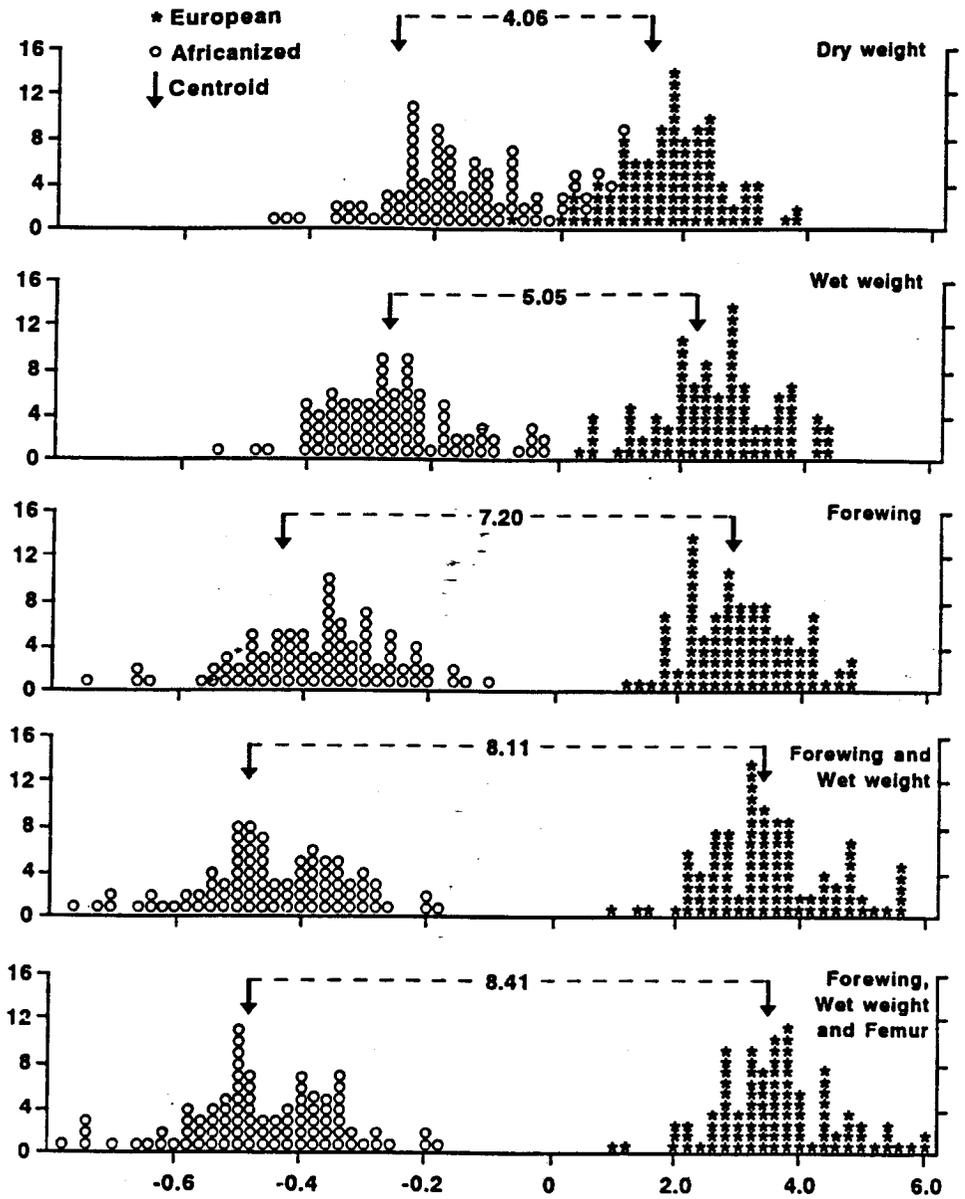


Fig. 2. Histograms of the placement of individual observations on a common discriminant axis for dry weights, wet weights, forewing lengths, the combination of forewing lengths and wet weights and the combination of forewing lengths, wet weights, and femur lengths. Numbers between arrows indicate Mahalanobis distances between group centroids

nate analysis that used an early subset of 1,637 colonies, the proportion of the variance that these two characters added to the overall analysis was only 0.22%. A cross validation analysis in which each of the 1,637 colonies was individually held from the data set and analyzed according to the measurements made on the other colonies produced the same results whether or not these two angles were included. These angles are formed from the intersections of thick veins and their measurement requires judging the geometric center of the intersection. This skill is difficult to teach and different persons will make the measurement in consistently different ways. For these reasons, these two wing venation angles were eliminated as components of the final procedure.

The importance of the groups of measurements made on various body parts to the accuracy of identification was also evaluated using an early subset of 1,926 colonies. All body parts included in the final set contributed reductions in the rate of misclassifications and were considered important to include in the final procedure.

Colony samples provided twenty-three measurements from each of ten worker bees. Sample means were calculated for each measurement and were used to estimate population means and variances. Measurements of commercial and feral Africanized honey bees, commercial, feral, and rustic European honey bees are different for some univariate measurements within major groups. However, these differences are minor when compared to the differences between the major groups of Africanized and European honey bees.

Multivariate discriminant analyses with more than three groups showed considerable overlap of the different Africanized and the different European groups. Based on the results of these preliminary multivariate discriminant analyses and the univariate analyses, three groups were chosen to be represented in the final multivariate discriminant analysis: 591 colonies of Africanized honey bees (combining feral, rustic, and managed colonies), 401 colonies of European bees (combining managed colonies and colonies of unknown origin), and 1,111 colonies of feral European bees (combining rustic and feral honey bees). Multivariate analyses of the colonies in these groups produced discriminant functions and coefficients that can be used to identify Africanized honey bees and commercial and feral European honey bees.

A multivariate analysis of variance (MANOVA) of the 23 characteristics showed that significant differences existed among the three groups (Wilks' Lambda = 0.1295, $p = 0.0001$). A post-MANOVA analysis of the Mahalanobis distances among the centroids of the groups revealed that each group was significantly different from the other two groups (Fig. 3).

The multivariate discriminant analysis correctly identified 565 (95.60%) of the 591 Africanized colonies and correctly identified all of the 1,512 European colonies (Table 1) according to our recommended regulatory standards (Table 2;

Fig. 3). In 19 (3.21%) cases, Africanized colonies were declared to be European with evidence of the introgression of Africanized genes and in 7 (1.19%) cases were declared to be European. Perhaps they were, since the sole criterion for inclusion in the Africanized group was to be found in an area considered to be generally Africanized. If an imported European colony somehow was involved in the sample's parentage it would nonetheless have been considered to be Africanized. This rate of misclassification is low and not likely to trouble commercial beekeeping, since in sensitive situations such as breeding programs, those colonies that are declared to be European with evidence of the introgression of Africanized genes would be culled from use. Three (0.14%) European colonies were

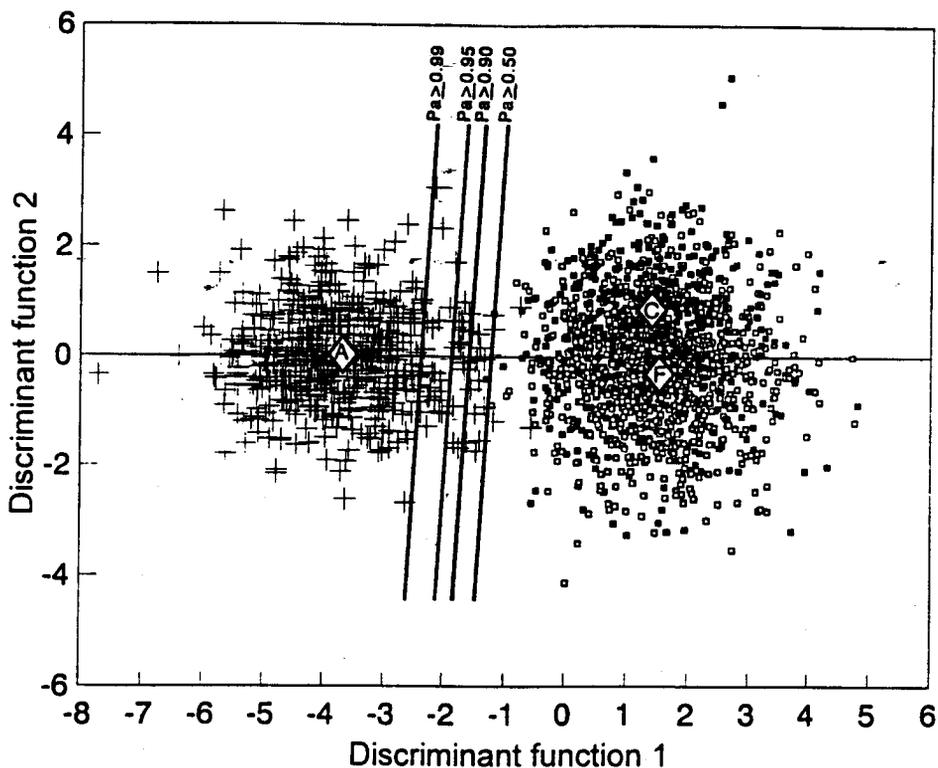


Fig. 3. Scatterplot of the results of the multivariate discriminant function analyses of Africanized and European honey bees: each + represents an Africanized colony, each □ represents a feral European colony and each ■ represents a commercial European colony. Some colony indicators are not visible due to the large number of colonies represented in the figure. The centroid for each group is marked by a \diamond : A = Africanized; F = feral European; C = commercial European. Using a pooled covariance matrix, the Mahalanobis distances between centroids are: Africanized to commercial European = 27.075; Africanized to feral European = 27.525; and commercial European to feral European = 1.356. Solid lines indicate a domain to the left of the line which includes colonies with a probability of being Africanized of ≥ 0.99 , ≥ 0.95 , ≥ 0.90 , and ≥ 0.50 .

Table 1. Classification results (numbers and (percentages)) of the multivariate discriminate analysis

From the known group	To the classified group			
	Africanized	Africanized with evidence of the introgression of European genes	European with evidence of the introgression of African genes	European
Africanized	545 (92.22)	20 (3.38)	19 (3.21)	7 (1.19)
European (Commercial)	0	0	0	401 (100.00)
European (Feral)	0	0	3 (0.2)	1,108 (99.73)

The accuracy of the multivariate discriminate analysis was determined by withholding and classifying 250 randomly selected colonies at a time as independent samples. Classification is based on probabilities of group membership as shown in Table 2.

sufficiently Africanized-like to be suspected of having some Africanized genes. They may have as a consequence of early and undetected intrusion of Africanized bees into some of the collection sites (GARY *et al.* 1985). Alternately, they may be extreme samples from the European population. When the colonies were classified based strictly on their greatest probability of group membership, three colonies from the Africanized group were declared European with probabilities of being Africanized (p_A) of 0.00004, 0.036, and 0.066 for colonies from the llanos of Venezuela, the Andes mountains of Venezuela, and the suburbs of Rio de Janeiro, Brazil. Two colonies from the European group were declared Africanized with p_A of 0.544 and 0.668 for a colony from Mexico and for a feral colony from the desert of California. Additionally, the analysis was able to differentiate between commercial European and feral European colonies about 71% of the time.

Unstandardized function coefficients and constants are necessary to apply the discriminant analysis results to the identification of unknown samples. Mean body part measurements from an unknown sample of ten worker bees are multi-

Table 2. Guidelines for evaluating posterior probabilities of group membership in the identification of unknown samples

Probabilities	Determination
$0.990 \leq p_A \leq 1.00$	Africanized
$0.900 \leq p_A \leq 0.990$	Africanized with evidence of the introgression of European genes
$0.500 \leq p_A \leq 0.900$	European with evidence of the introgression of African genes
$0.000 \leq p_A \leq 0.500$	European

p_A : probability of belonging to the Africanized group.

plied by the corresponding coefficients for each of the two functions. The two sums of these products are added to the appropriate constants to calculate the two function values required to determine the probability of group membership (RINDERER *et al.* 1993a).

With *a* as Africanized, *e* as commercial European and *f* as feral European, a first step in calculating exact probabilities of group membership (SAS Institute, 1982) is to determine three generalized square distances according to the general formula (in matrix notation),

$$D_i^2 = (X - \bar{X}_i) (\Sigma^{-1})^{1/2} (X - \bar{X}_i)'$$

where *i* is *a*, *e*, *f*.

Individual generalized square distances for unknown samples are based on derived functions. Character set measurements are multiplied by coefficients produced by the analysis of the baseline population data and the sums of these products are added to a function constant. Two derived functions are used to calculate exact probabilities of group membership:

$$D_i^2 = (\text{Function 1} + \text{Constant}_{i1})^2 / \text{Constant}_{i2} + (\text{Function 2} - \text{Constant}_{i3})^2 / \text{Constant}_{i4}$$

Each of the three posterior probabilities of group membership of a sample is then given by:

$$P_i = \frac{\exp(-0.5 D_i^2)}{\sum_{i=a,e,f} \exp(-0.5 D_i^2)}$$

The determination of group membership for unknown samples is based on an interpretation of posterior probabilities of group membership. As hybridization of Africanized and European honey bees continues (BUCO *et al.* 1987, DEL LAMA *et al.* 1990, LOBO *et al.* 1989, HALL 1990, RINDERER *et al.* 1991b, 1992, SHEPPARD *et al.* 1991a, b, MORITZ & MEUSEL 1992, MCMICHAEL 1994), especially as Africanized bees continue their range expansion into areas having more temperate climates (SHEPPARD *et al.* 1991a), some samples will have probabilities of group membership which are intermediate between classification as clearly Africanized or clearly European. Intermediate probabilities are an indication of a colony possibly resulting from extensive hybridization. Intermediate scores may arise for individual colonies as the result of hybridization or simply because the colonies are rare cases sampled from the extremes of variation of one or another group. We evaluated 192 experimentally produced F₁ colonies. These colonies were generally intermediate between Africanized and European colonies (Fig. 4).

These morphometric procedures are used by the national governments of the United States, Mexico, Canada, Australia, New Zealand, and the United Kingdom as well as regional governments within these countries as the "offi-

cially recognized" means to identify Africanized honey bees. Scientists and regulators in other countries also rely on the procedures without them being designated as "officially recognized". The Agricultural Research Service of the United States Department of Agriculture has made the procedures accessible by providing training, a detailed manual for dissections, sample preparation, data entry and analysis (RINDERER *et al.* 1991a) and software programs (called USDA-ID) which guide data entry and provide analysis and identification.

The weaknesses and strengths of morphometric identification are often one and the same attribute. Since morphology is an old pursuit, it is sometimes considered outdated, but its long tradition makes it well established. It has a less perfect link to genes than DNA-based analyses but most of the characteristics have high heritabilities (OLDROYD & RINDERER 1991) and collectively the characteristics measured represent a far wider array of genes than could be reasonably ac-

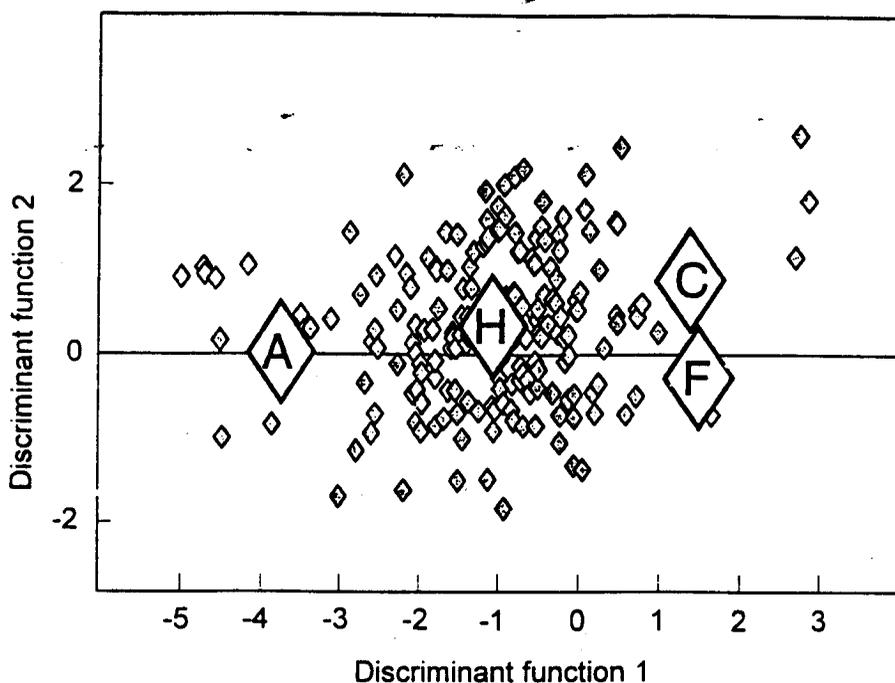


Fig. 4. Scatterplot of the results of classifying 192 experimentally produced F_1 colonies. These colonies were generally intermediate between Africanized and European colonies. Each \diamond represents one of these colonies. The centroid for the group is marked by a \diamond containing an H. The centroids of the groups used to derive the discriminant functions are each marked by a \diamond : A = Africanized, F = feral European, C = commercial European. The Mahalanobis distances between centroids are: hybrid to Africanized = 8.070; hybrid to feral European = 8.143; and hybrid to commercial European = 7.423.

cessed through DNA-based analyses. It is not suited to detect minor gene flow and hence not a fine-grained tool to study population genetics. However, because of this characteristic, it will only declare a sample to be Africanized or European with evidence of Africanized gene intrusion on very strong evidence. In the same regard, USDA-ID is structured to provide clear Africanized or European identifications. This is necessary for regulators to have unambiguous identifications. However, it also fosters a misinterpretation that samples with intermediate morphology are not found. Because of the clear identification produced by the program, it is not transparently easy to understand that samples that have intermediate morphology are interpreted by the software and assigned to either the Africanized or the European classifications.

OTHER TECHNOLOGIES

Many other approaches to identifying Africanized honey bees have been explored. For varied reasons they have proved less desirable than morphology, although several of them have value for scientific studies of various types.

Isozymes. Honey bees have far less isozyme variation than most organisms (SYLVESTER 1982). Only two enzymes, malate dehydrogenase (SYLVESTER 1982) and hexokinase (SPIVAK *et al.* 1988) show variation between Africanized and European honey bees. Using a known group of 12 Africanized and 19 European colonies, SPIVAK *et al.* (1988) used variation in these two enzymes to classify colonies. Thirteen percent of the Africanized group were misclassified as were 16% of the European colonies. Thus, the technique has little use for identification. It does have some application for detecting changes through time in population genetic studies.

DNA analyses. A variety of techniques fall within the general category of DNA analysis. The banding patterns produced by electrophoretically separating fragments of mitochondrial DNA produced by restriction enzymes (differential restriction fragment length patterns (mt-RFLP)) have been used in a variety of studies (ARIASD *et al.* 1990, HALL 1990, HALL & MURALIDHARAN 1989, RINDERER *et al.* 1991b, 1992, SHEPPARD *et al.* 1991a, SMITH *et al.* 1989, MCMICHAEL 1994, MORITZ & MEUSEL 1992). A serious weakness to using mt-RFLPs (or other ways of analyzing mt-DNA) as an identification tool is that mitochondria are only inherited from the maternal parent. Hence, backcrossing of an F₁ queen to the paternal parental type may produce colonies of honey bees that have a nuclear DNA subspecies constitution that differs substantially from that of the mt-DNA. Hence, in a recent survey, 17% of the Africanized honey bees of south Texas had European mitochondria (W. S. SHEPPARD, pers. comm.).

As with mt-DNA RFLP analysis, nuclear DNA RFLP analysis has been used to study the process of Africanization. One study (MCMICHAEL 1994) showed that as many as 50% of the alleles at certain loci in Africanized populations came from European parentage, indicating extensive hybridization in many Africanized populations. Thus, hybridization events negate the value of a limited number of loci as identification tools. In theory, direct examination of nuclear DNA using RFLP or other techniques will produce quality identifications. However, such analyses must be based on several loci.

The same conditions seem to hold when honey bee nuclear DNA is analyzed as variation in randomly amplified DNA segments (RAPD) (R. Page, pers. comm.). A large number of RAPD markers may produce information useful for identifications. However, the ability to identify unknowns is about the same as that of morphometric analysis and the costs of doing so are much higher. No identification procedures based on RAPD analysis of DNA have been published.

Much the same can be said of the analysis of the size variation of tandem repeat hypervariable microsatellite and minisatellite DNA regions. Although variation exists between parental populations, Africanized honey bees themselves do not have diagnostic nuclear DNA characteristics yet found which are qualitatively different (ESTOUP 1995). Frequency differences which are usually minor are common, and many of them could be combined to produce good identification systems. However, the costs, compared to the costs of morphometric analysis, are prohibitive.

Other Techniques. Behavioral characteristics such as flight behavior and stinging behavior, cuticular hydrocarbon components, the spectral analysis of pyrolysis products, etc., have all been evaluated as identification tools. All of them have proved unsuccessful because of cost efficiency, identification ability, or the lack of potential for many laboratories to conduct the techniques required for identification.

CONCLUSION

The pioneers of honey bee taxonomy who chose the study of numerical morphology as the way to describe subspecies chose well. Studies related to identifying Africanized honey bees have shown that numerical morphological characteristics are ideal in several ways. First, environmental circumstances, even extreme ones, have minimal effects on morphological characteristics of honey bees (DALY *et al.* 1995, OLDROYD & RINDERER 1991, RINDERER *et al.* 1986*b*). The effects of environment on morphology may be buffered since adults must care for honey bee brood. Second, the linkage between morphology and the honey bee genome is evidently close and broadly based. Most of the measured

characteristics have a very high heritability (OLDROYD & RINDERER 1991). Third, known genetically intermediate colonies overall have intermediate morphology with specific morphological characteristics having inheritance patterns that are often independent and indicative of polygenic origins. Hence, the entire pattern of morphology studied is regulated by a wide array of genes which mostly have additive rather than non-additive genetic effects (ROBERTS 1961, OLDROYD & MORAN 1987, RINDERER *et al.* 1990).

Morphological characters are therefore very suitable and currently superior to many other methods for distinguishing Africanized and European honey bees. The rapidity with which such determinations may be made (RINDERER *et al.* 1987) and the fact that morphology reflects many interacting aspects of the genome suggest that morphology may remain the method of choice for identifying honey bees subspecies and for studying dynamic genetic changes within and among populations of honey bees.

* * *

Acknowledgements – In cooperation with the Louisiana Agricultural Experiment Station.

REFERENCES

- ALPATOV, W. W. (1929) Biometrical studies on variation and races of the honey bee *Apis mellifera* L. *Rev. Biol.* **4**: 1–57.
- ARIASD, M. C., SOARES, A. E. E. & NOBREGA, F. G. (1990) Improvements to the mitochondrial restriction maps for Italian and Africanized honey bees. *Brazilian Journal of Genetics* **13**: 501–507.
- BUCO, S. M., RINDERER, T. E., SYLVESTER, H. A., COLLINS, A. M., LANCASTER, V. A. & CREWE, R. (1987) Morphometric differences between South American Africanized and South African (*Apis mellifera* scutellata L.) honey bees. *Apidologie* **18**(3): 217–222.
- BUTTEL-REEPEN, H. VON (1906) *Apiacta. Mit. Zool. Mus. Berlin* **3**: 117–201.
- COCHLOV, B. P. (1916) Investigations on the length of the bee tongue. *Min. Agric. Petrograd*, pp. 17–41. [in Russian]
- COLLINS, A. M. & RINDERER, T. E. (1991) Genetics of defensive behavior I. Pp. 309–328. In SPIVAK *et al.* (eds): The "African" honey bee. Westview Press, Boulder, Colorado, U.S.A.
- DALY, H. V. (1991) Systematics and identification of Africanized honey bees. Pp. 13–44. In SPIVAK *et al.* (eds): The "African" Honey Bee. Westview Press, Boulder, Colorado, U.S.A.
- DALY, H. V. & BALLING, S. S. (1978) Identification of Africanized honey bees in the Western Hemisphere by discriminant analysis. *J. Kansas Entomol. Soc.* **51**: 857–869.
- DALY, H. V., HOELMER, K., NORMAN, P. & ALLEN, T. (1982) Computer-assisted measurement and identification of honey bees (Hymenoptera: Apidae). *Ann. Entomol. Soc. Am.* **75**: 591–594.
- DALY, H. V., DANKA, R. G., HOELMER, K., RINDERER, T. E. & BUCO, S. M. (1995) Honey bee morphometrics: Isometry and classification tested with European worker bees reared by varying ratios of nurse bees. *J. Apic. Res.* **34**: 129–145.

- DANKA, R. G., RINDERER, T. E., COLLINS, A. M. & HELLMICH, R. L. (1987) Responses of Africanized honey bees (Hymenoptera: Apidae) to pollination management stress. *J. Econ. Entomol.* **80**(3): 621–624.
- DEL LAMA, M. A., LOBO, J. A., SOARES, A. E. E. & DEL LAMA, S. N. (1990) Genetic differentiation estimated by isozymic analysis of Africanized honeybee populations from Brazil and from Central America. *Apidologie* **21**: 271–280.
- DUPRAW, E. (1964) Non-Linnean taxonomy. *Nature* **202**: 849–852.
- ESTOUP, A., GARNERY, I., SOLIGNAC, M. & CORNUET, J.-M. (1995) Microsatellite variation in honey bee (*Apis mellifera* L.) populations: Hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics* **140**: 679–695.
- GARY, N. E., DALY, H. V., LOCHE, S. & RACE M. (1985) The Africanized honey bee: Ahead of schedule. *California Agric.* **39**: 4–7.
- GOETZE, G. (1930) Variabilitäts- und Züchtungsstudien an der Honigbiene mit besonderer Berücksichtigung der Langrüsseligkeit. *Arch. Bienenkunde* **11**: 135–274.
- HALL, H. G. (1990) Parental analysis of introgressive hybridization between African and European honeybees using nuclear DNA RFLPs. *Genetics* **125**: 611–621.
- HALL, H. G. & MURALIDHARAN, K. (1989) Evidence from mitochondrial DNA that African honey bees spread as continuous maternal lineages. *Nature* **339**: 211–213.
- KERR, W. E. (1957) Introdução de abelhas africanas no Brasil. *Brasil Apícola* **3**: 211–213.
- LOBO, J. A., DEL LAMA, M. A. & MESTRINER, M. A. (1989) Population differentiation and racial admixture in the Africanized honeybee (*Apis mellifera* L.). *Evolution* **43**: 794–804.
- MAA, T. (1953) An inquiry into the systematics of the tribus Apidini or honey bees (Hym.). *Treubia* **21**: 525–640.
- MCMICHAEL, M. A. (1994) *Genomic DNA restriction fragment length polymorphisms at a highly polymorphic locus distinguish Old and New World subspecies of the honey bee*. PhD Dissertation. Univ. Florida, Gainesville, Florida, U.S.A.
- MORITZ, R. F. A. & MEUSEL, M. S. (1992) Mitochondrial gene frequencies in Africanized honeybees (*Apis mellifera* L.): Theoretical model and empirical evidence. *J. Evol. Biol.* **5**: 72–81.
- OLDROYD, B. P. & MORAN, C. (1983) Additive and heterotic genetic effects in the haplo-diploid honey bee, *Apis mellifera*. *Aust. J. Biol. Sci.* **36**: 323–332.
- OLDROYD, B. P. & RINDERER, T. E. (1991) Heritability of morphological characters used to distinguish European and Africanized honey bees. *Theor. & Appl. Genetics* **82**: 499–504.
- RINDERER, T. E., BOLTON, A. B., COLLINS, A. M. & HARBO, J. R. (1984) Nectar-foraging characteristics of Africanized and European honeybees in the neotropics. *J. Apic. Res.* **23**(2): 70–79.
- RINDERER, T. E., SYLVESTER, H. A., BROWN, M. A., VILLA, J. D., PESANTE, D. & COLLINS, A. M. (1986a) Field and simplified techniques for identifying Africanized and European honey bees. *Apidologie* **17**: 33–48.
- RINDERER, T. E., SYLVESTER, H. A., COLLINS, A. M. & PESANTE, D. G. (1986b) Identification of Africanized and European honeybees: Effects of nurse-bee genotype and comb size. *Bull. Entomol. Soc. Am.* **32**: 150–152.
- RINDERER, T. E., SYLVESTER, H. A., BUCO, S. M., LANCASTER, V. A., HERBERT, E. W., COLLINS, A. M. & HELLMICH, R. L. (1987) Improved simple techniques for identifying Africanized and European honey bees. *Apidologie* **18**: 179–197.
- RINDERER, T. E., DALY, H. V., SYLVESTER, H. A., COLLINS, A. M., BUCO, S., HELLMICH, R. L. & DANKA, R. G. (1990) Morphometric differences among Africanized and European honey bees and their F1 hybrids (Hymenoptera: Apidae). *Ann. Entomol. Soc. Am.* **83**: 346–351.
- RINDERER, T. E., BUCO, S. M., SYLVESTER, H. A. & STELZER, J. A. (1991a) *Manual for the laboratory preparation of honey bee samples and their analysis with the Universal System for the Detection of Africanized Bees*. USDA-ARS, Baton Rouge, Louisiana, U.S.A.

- RINDERER, T. E., STELZER, J. A., OLDROYD, B. P., BUCO, S. M. & RUBINK, W. L. (1991b) Hybridization between European and Africanized honey bees in the neotropical Yucatan Peninsula. *Science* **253**: 309–311.
- RINDERER, T. E., STELZER, J. A., OLDROYD, B. P., BUCO, S. M. & RUBINK, W. L. (1992) Out of Africa: Hybrid bees. *Science* **256**: 720–721.
- RINDERER, T. E., BUCO, S. M., RUBINK, W. L., DALY, H. V., STELZER, J. A., RIGGIO, R. M. & BAPTISTA, F. C. (1993a) Morphometric identification of Africanized and European honey bees using large reference populations. *Apidologie* **24**: 569–585.
- RINDERER, T. E., OLDROYD, B. P. & SHEPPARD, W. S. (1993b) Africanized honey bees in the Americas. *Scientific American* **269**: 84–90.
- ROBERTS, W. G. (1961) Heterosis in the honey bee as shown by morphological characters of inbred and hybrid bees. *Ann. Entomol. Soc. Amer.* **59**: 878–882.
- RUITNER, F. (1987) *Biogeography and taxonomy of honey bees*. Springer-Verlag, Berlin, 284 pp.
- SAS Institute (1982) *SAS User's Guide: Statistics*. SAS Institute, Cary, N. C., U.S.A.
- SHEPPARD, W. S., RINDERER, T. E., MAZZOLI, J. A., STELZER, J. A. & SHIMANUKI, H. (1991a) Gene flow occurs between Africanized and European-derived honey bee populations in Argentina. *Nature* **349**: 782–784.
- SHEPPARD, W. S., SOARES, A. E. E., DEJONG, D. & SHIMANUKI, H. (1991b) Hybrid status of honey bee populations near the historic origin of Africanization in Brazil. *Apidologie* **22**: 643–652.
- SMITH, D. R., TAYLOR, O. R. & BROWN, W. M. (1989) Neotropical Africanized honey bees have African mitochondrial DNA. *Nature* **339**: 213–215.
- SPIVAK, M., RANKER, T., TAYLOR, O. Jr., TAYLOR, W. & DAVIS, L. (1988) Discrimination of Africanized honey bees using behavior, cell size, morphometrics, and a newly discovered isozyme polymorphism. Pp. 311–324. In NEEDHAM *et al.* (eds): *Africanized Honey Bees and Bee Mites*. Ellis Horwood, Chichester, United Kingdom.
- SYLVESTER, H. A. (1982) Electrophoretic identification of Africanized honey bees. *J. Apic. Res.* **21**: 93–97.