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Original article

Morphometric identification of Africanized and European honey bees using large reference populations

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Summary — New discriminant analysis procedures have been presented to identify Africanized and European honey bees. These procedures are founded on data from 2 103 samples of honey bees collected from colonies at several locations in the western hemisphere and Kangaroo Island, Australia. Various univariate and multivariate analyses have been used to select the morphological characteristics and the groups of characteristics to be used in the analysis. The multivariate discriminant analysis correctly identified 565 (95.6%) of the 591 Africanized colonies and correctly identified all of the 1 512 European colonies.

***Apis mellifera* / Africanized honey bee / European honey bee / morphometric identification / multivariate discriminant analysis**

INTRODUCTION

The introduction of African honey bees *(Apis mellifera scutellata)* to Brazil (Kerr, 1957, 1967) has led to the spread of their Africanized progeny throughout most of the Americas (Rinderer, 1986). Soon after the introduction of African honey bees, there was a need to identify their Africanized progeny (Michener, 1975). Early efforts to provide identifications of Africanized and European bees based on morphology (Kerr, 1967, 1969; Rinaldi *et al,* 1971; Saramiento *et al,* 1974; Woyke, 1977) used univariate measurements that provided inconclusive results since measurements overlapped between the 2 groups of bees.

Vastly improved identifications of Africanized honey bees (AHB) were obtained when modern statistical methods of multivariate analysis were applied simultaneously to multiple measurements (Daly and Balling, 1978). Twenty-five morphological characters were selected from those studied by investigators of subspecific variation (Alpatov, 1929, 1948; Goetze, 1930; DuPraw, 1965a, 1965b; Ruttner, 1986, 1987). The discriminant functions for the identification procedures were based on 101 samples of feral Africanized colonies collected on the basis of behavior in South America and 297 Euporean colonies, primarily from commercial apiaries in the United States. Daly *et al* (1982) described data collection procedures that involved the projection of images of dissected morphological structures with a projecting microscope onto a digitizer connected to a computer. These procedures can be applied to data from either a single bee or to a group of bees from a colony. However, the ability of the procedure to accurately identify is greatly improved for group samples. The 1978 discriminant functions were estimated to misidentify only 0.5% of colony samples, using the limited data base to estimate population parameters.

The morphometric procedures of Daly and Balling (1978) have been used by scientists and regulators throughout the Americas to identify Africanized honey bees. Confidence in the procedures was enhanced when they produced accurate identifications when challenged with unusual bees. Africanized bees reared by European nurse bees on comb produced by Africanized bees were only slightly larger than Africanized bees from Africanized colonies and were easily identified as Africanized (Rinderer *et al,* 1986b). Africanized bees were larger still when they were reared by European nurse bees on commercial comb, but were still correctly identified (Rinderer *et al,* 1986b).

Severe nutritional deprivation of colonies was unable to produce European bees misclassified as Africanized, although some bees reared in the laboratory by hand were misclassified as Africanized (Herbert *et al,* 1988). Only experimentally produced worker bees from highly unnatural colonies having only drone comb produced small bees that were misidentified (Daly and Morse, 1991). A contributing factor to the value of morphometrics as an identification tool is the generally high heritability of morphological characters (Oldroyd *et al,* 1991).

As Africanized honey bees spread further into the United States, several laboratories are expected to be established to morphologically identify honey bees for regulatory purposes. To provide these laboratories with more accurate identification tools, we have developed new discriminant analysis procedures to identify Africanized and European honey bees. These procedures have been improved upon in several respects. Firstly, sample sizes of both Africanized and European bees are much larger and, hence, can be expected to include more of the variation of both groups. Secondly, feral European honey bees and commercially kept European honey bees are well represented, reducing the chance of misclassifying relatively small feral European honey bees as Africanized. Thirdly, 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. Fourthly, 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 probably led to only the most clearly

Africanized members of the sampled populations being collected as base-line material. Fifthly, the measurement of 2 wing angles used by Daly and Balling (1978), which have been difficult to standardize among laboratories, has been eliminated.

MATERIALS AND METHODS

The data from 2 103 colony samples of honey bees collected from colonies from the United States (970 colonies), Mexico (360), Costa Rica (75), Venezuela (390), Brazil (232), Argentina (52) and Kangaroo Island, Australia (24) 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 as random samples of clearly Africanized or European populations based on their location and date of collection. Some collections were specifically made from northern Mexico and several southwestern states in the US in order to increase the geographical and biological variability of the samples. The colony samples include 177 hived Africanized colonies which, with only an occasional exception, had bees reared on European comb foundations, 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).

Various univariate and multivariate analyses were used to select the morphological characteristics and the groups of characters to be used in a multivariate discriminant analysis. The accuracy of the multivariate discriminant analysis was determined by withholding and classifying 250 randomly selected colonies at a time as independent samples.

RESULTS AND DISCUSSION

A subset of 87 Africanized and 48 European colonies was used to evaluate the potential of 47 morphological characteristics to contribute to the identification of Africanized and European honey bees. Morphological measurements of 47 characteristics (table I) were taken from the dissected body parts of 10 worker bees from each colony in the subset acording to the guidelines of Alpatov (1929), Daly and Balling (1978) and 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 proportion of the total variance included in the analysis using the characters which were 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 22 more characters from the sets studied by Alpatov (1929) and Ruttner (1987) increased the total variance assessed by the analysis to 95.9%, indicating that generally these characters collectively added 2.3% to the variance assessed by the analysis. The range of additional proportions of the total variance added to the analysis by each of the 22 characters was from 1.0% to 0.000001%. The strongest characters of this group were angle L13 and the length of the right distal segment of the proboscis (Ruttner, 1987). Unfortunately, these 2 characters were difficult to obtain from field samples collected by different persons using different methods or difficult to standardize among laboratories. They, along with the other characteristics in this group of 22, were not included in the final procedure. Of the 25 characters chosen by Daly and Balling (1978) as the most valuable contributors to the multivariate analysis, 2 measurements, Angles 38 and 39 (table I), contributed little to the power of the discriminant analysis. When their contribution to the analysis was evaluated with a stepwise discriminant analysis that used an early subset of 1 637 colonies (collected and measured prior to obtaining samples

Table I. Listing of the 47 morphological characteristics evaluated for their use in a multivariate discriminant analysis procedure to identify Africanized and European honey bees.

Characteristics retained for use in the multivariate discriminant analysis			
1	FWLN	Longitudinal distance of the forewing (F_L)	*, +
2	FWWD	Transversal distance of the forewing (F_B)	*, +
3	HWLN	Longitudinal distance of the hindwing	+
4	HWWD	Transversal distance of the hindwing	+
5	HAMU	Count of the hamuli of the hindwing	+
6	AN29	Measurement of angle *(A4)*	*, +
7	AN30	Measurement of angle *(B4)*	*, +
8	AN31	Measurement of angle *(D7)*	*, +
9	AN32	Measurement of angle *(E9)*	*, +
10	AN33	Measurement of angle *(G18)*	*, +
11	AN34	Measurement of angle *(I10)*	*, +
12	AN35	Measurement of angle 35	+
13	AN36	Measurement of angle 36	+
14	CUBA	Length of cubital distance *(a)*	*, +
15	CUBB	Length of cubital distance *(b)*	*, +
16	TBLN	Length of tibia *(Ti)*	*, +
17	FELN	Length of femur *(Fe)*	*, +
18	BTLN	Length of basitarsus (metatarsus) *(M_L)*	*, +
19	BTWD	Width of basitarsus (metatarsus) *(M_T)*	*, +
20	STLN	Longitudinal distance of sternite 3 *(S_3)*	*, +
21	WXLN	Longitudinal distance of wax mirror *(W_L)*	*, +
22	WXWDA	Transversal distance of wax mirror *(W_T)*	*, +
23	WXWDB	Distance between wax mirrors *(W_D)*	*, +
Characteristics not retained for use in the multivariate discriminant analysis			
24	AN38	Measurement of angle *(J16)*	*, +
25	AN39	Measurement of angle 39	+
26	AN40	Measurement of angle *(O26)*	*
27	AN42	Measurement of angle 42	This study only
28	AN43	Measurement of angle *(K19)*	*
29	AN20	Measurement of angle *(L13)*	*
30	POST	Length of the postmentum of the proboscis	❑
31	GLOS	Length of the glossa of the proboscis	❑
32	LPSEG	Length of the left proximal segment of the proboscis	❑
33	LDSEG	Length of the left distal segment of the proboscis	❑
34	RPSEG	Length of the right proximal segment of the proboscis	❑
35	RDSEG	Length of the right distal segment of the proboscis	❑
36	TER3	Longitudinal diameter of tergite 3 (T_3)	*
37	TER4	Longitudinal diameter of tergite 4 (T_4)	*
38	TOMA	Width of tomentum A on tergite 4 *(a)*	*
39	TOMB	Width of tomentum B on tergite 4 *(b)*	*
40	ST6L	Longitudinal distance of sternite 6 (L_6)	*
41	ST6T	Transversal distance of sternite 6 (T_6)	*
42	PIG2	Pigmentation of tergite 2	*
43	PIG3	Pigmentation of tergite 3	*
44	PIG4	Pigmentation of tergite 4	*
45	PIGS	Pigmentation of the cupolla of the scutellum *(Sc)*	*
46	PIGK	Pigmentation of the metanotum of the scutellum *(B)*	*
47	PIGB	Pigmentation of the mesonotum of the scutellum *(K)*	*

❑ Measurements following the procedures of Alpatov (1929); * Measurements following Ruttner (1987) including notations in parentheses; + measurements following Daly and Balling (1978).

from northwestern Mexico, Arizona and New Mexico that brought the total colony collection to 2 103), the proportion of the variance that these 2 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 2 angles were included. These angles are formed from the intersects of thick veins and their measurement requires visually estimation of the geometric center of the intersection. This skill is difficult to teach and 7 different persons made the measurement in consistently different ways. For these reasons, we eliminated these 2 wing venation angles as components of the final procedure, which uses the remaining 23 characteristics.

In an attempt to reduce the number of body parts dissected and measured, we also evaluated the importance of the groups of measurements made on various body parts to the accuracy of identification. Again, we used the data from the subset of 1 637 colonies. Measurements from all the body parts considered contributed to reductions in the rate of misclassifications (table II) and were necessarily included in the final procedure.

In developing the final discriminant functions, samples from the 2 103 colonies provided measurements of 23 characteristics from each of 10 worker bees. Sample means were calculated for measurements of each characteristic and were used to estimate population means and variances. Measurements of commercial and feral Africanized honey bees and commercial, feral, and rustic European honey bees are different for some univariate measurements within major groups (table III). However, these differences are minor when compared to the differences between the major groups of Africanized and European honey bees (table III).

Multivariate discriminant analyses with > 3 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, 3 groups were formed from the 2 103 total colonies studied for use in the final multivariate discriminant analysis: 591 colonies

Table II. The effect on misclassification rates based on the greatest probability of group membership when measurements from various body-parts are excluded from the discriminant analysis. For each case, 1 926 total colonies were classified.

Measurements excluded	*No of remaining measurements*	*No of misclassified colonies*	*Misclassified colonies (%)*
None	23	5	0.003
Forewing angles and internal lengths	13	17	0.009
Hindwing characteristics	21	13	0.007
Sternite characteristics	19	25	0.013
Leg characteristics	19	22	0.012

Table III. Means ($\bar{X}$), standard deviations (SD), and maximum value (max) and minimum value (min) of 23 morphometric characteristics of Africanized and European honey bees and results of univariate analyses comparing means.

Probability character	*Africanized honey bees*		*European honey bees*			
	Commercial	*Feral and rustic*	*Commercial*	*Feral and rustic*	*Unknown*	$\bar{X}A \neq \bar{X}E$
Forewing length (FWLN)						
($\bar{X}$)	8.727[a]	8.669[b]	9.151[c]	9.151[c]	9.023[d]	0.0001
(SD)	0.148	0.176	0.150	0.161	0.130	
(min)	8.311	8.114	8.770	8.579	8.702	
(max)	9.136	9.459	9.563	9.843	9.326	
Forewing width (FWWD)						
($\bar{X}$)	3.006[a]	2.981[b]	3.130[c]	3.121[c]	3.026[d]	0.0001
(SD)	0.073	0.066	0.067	0.066	0.072	
(min)	2.716	2.756	2.895	2.899	2.862	
(max)	3.198	3.160	3.336	3.367	3.237	
Hindwing length (HWLN)						
($\bar{X}$)	4.135[a]	4.118[b]	4.308[c]	4.328[d]	4.218[e]	0.0001
(SD)	0.071	0.086	0.088	0.088	0.080	
(min)	3.931	3.857	4.030	4.043	4.033	
(max)	4.305	4.397	4.553	4.584	4.408	
Hindwing width (HWWD)						
($\bar{X}$)	1.694[a]	1.676[b]	1.823[c]	1.815[c]	1.742[d]	0.0001
(SD)	0.052	0.046	0.049	0.051	0.065	
(min)	1.502	1.500	1.662	1.641	1.580	
(max)	1.866	1.797	1.970	2.008	1.917	
Hamuli number (HAMU)						
($\bar{X}$)	21.190[a]	21.261[a]	21.072[c]	20.829[d]	21.034[cd]	0.0001
(SD)	0.919	0.942	0.946	0.975	1.113	
(min)	18.100	18.300	18.500	18.000	19.000	
(max)	23.600	23.800	23.500	24.100	23.400	
Angle 29 (AN29)						
($\bar{X}$)	31.791[a]	32.105[b]	30.163[c]	30.342[c]	29.466[d]	0.0001
(SD)	1.414	1.433	1.606	1.456	1.046	
(min)	27.797	28.899	25.797	25.155	26.668	
(max)	35.855	38.339	36.447	35.651	31.531	
Angle 30 (AN30)						
($\bar{X}$)	106.159[a]	104.437[b]	108.492[c]	108.163[c]	108.517[c]	0.0001
(SD)	3.718	3.964	4.477	3.836	3.717	
(min)	97.038	81.701	92.530	92.514	99.382	
(max)	118.051	112.706	119.060	119.789	115.573	
Angle 31 (AN31)						
($\bar{X}$)	102.734[a]	102.000[b]	100.128[d]	99.777[c]	100.355[d]	0.0001
(SD)	1.977	1.802	1.895	2.366	2.071	
(min)	98.253	96.377	92.574	83.768	94.778	
(max)	109.667	108.674	105.588	109.428	104.432	

Table III continued

Probability character	*Africanized honey bees*		*European honey bees*			
	Commercial	*Feral and rustic*	*Commercial*	*Feral and rustic*	*Unknown*	$\bar{X}A = \bar{X}E$
Angle 32 (AN32)						
$(\bar{X})$	19.426[a]	19.398[a]	21.501[c]	21.104[d]	21.854[e]	0.0001
(SD)	0.838	0.888	1.160	1.316	0.912	
(min)	17.154	16.565	17.904	16.698	19.237	
(max)	21.496	21.609	24.937	25.100	23.637	
Angle 33 (AN33)						
$(\bar{X})$	95.462[a]	95.563[a]	94.061[c]	94.819[d]	92.419[e]	0.0001
(SD)	1.986	1.912	1.815	2.385	2.007	
(min)	90.453	91.057	89.623	86.992	87.098	
(max)	103.698	102.891	99.304	102.199	99.226	
Angle 34 (AN34)						
$(\bar{X})$	51.035[a]	51.069[a]	51.803[c]	50.836[d]	50.437[d]	Non-sig
(SD)	2.094	1.929	2.416	2.688	1.803	0.8629
(min)	45.481	46.755	45.376	42.252	45.951	
(max)	56.905	58.247	59.718	62.826	53.889	
Angle 35 (AN35)						
$(\bar{X})$	22.704[a]	23.188[b]	22.977[c]	22.920[c]	22.391[d]	0.0511
(SD)	1.272	1.490	1.396	1.525	1.507	
(min)	19.177	16.713	19.798	17.790	18.648	
(max)	25.736	28.679	27.650	28.997	25.501	
Angle 36 (AN36)						
$(\bar{X})$	60.851[a]	60.714[a]	62.169[c]	61.415[d]	63.841[e]	0.0001
(SD)	1.878	1.770	1.862	2.252	1.923	
(min)	55.029	55.502	56.950	54.307	58.858	
(max)	65.997	66.441	67.223	69.375	70.105	
Vein B cubital index (CUBB)						
$(\bar{X})$	0.230[a]	0.229[a]	0.236[c]	0.236[cd]	0.241[d]	0.0001
(SD)	0.014	0.016	0.019	0.021	0.016	
(min)	0.190	0.182	0.178	0.158	0.204	
(max)	0.266	0.275	0.286	0.298	0.304	
Vein A cubital index (CUBA)						
$(\bar{X})$	0.501[a]	0.506[a]	0.549[c]	0.545[cd]	0.539[d]	0.0001
(SD)	0.026	0.025	0.023	0.028	0.029	
(min)	0.408	0.443	0.458	0.424	0.486	
(max)	0.561	0.606	0.647	0.640	0.597	
Tibia length (TBLN)						
$(\bar{X})$	3.075[a]	3.051[b]	3.196[c]	3.209[c]	3.153[d]	0.0001
(SD)	0.069	0.073	0.058	0.064	0.054	
(min)	2.899	2.855	2.992	3.004	3.007	
(max)	3.233	3.274	3.336	3.394	3.301	
Femur length (FELN)						
$(\bar{X})$	2.494[a]	2.477[b]	2.633[c]	2.639[c]	2.593[d]	0.0001
(SD)	0.049	0.050	0.046	0.048	0.041	
(min)	2.372	2.343	2.513	2.491	2.503	
(max)	2.626	2.692	2.755	2.798	2.737	

Table III continued

Probability character	*Africanized honey bees*		*European honey bees*			
	Commercial	*Feral and rustic*	*Commercial*	*Feral and rustic*	*Unknown*	$\bar{X}A \neq \bar{X}E$
Basitarsus length (BTLN)						
$(\bar{X})$	1.916[a]	1.905[b]	2.016[c]	2.017[c]	1.972[d]	0.0001
(SD)	0.046	0.048	0.045	0.047	0.038	
(min)	1.790	1.756	1.882	1.838	1.882	
(max)	2.023	2.068	2.182	2.172	2.048	
Basitarsus width (BTWD)						
$(\bar{X})$	1.080[a]	1.073[b]	1.128[c]	1.127[c]	1.127[a]	0.0001
(SD)	0.032	0.033	0.032	0.033	0.023	
(min)	0.989	0.981	1.032	1.006	1.078	
(max)	1.158	1.154	1.270	1.222	1.171	
Sternum length (STLN)						
$(\bar{X})$	2.594[a]	2.581[b]	2.763[c]	2.774[c]	2.699[d]	0.0001
(SD)	0.067	0.069	0.068	0.066	0.074	
(min)	2.390	2.341	2.522	2.541	2.503	
(max)	2.780	2.786	2.957	2.992	2.870	
Wax mirror length (WXLN)						
$(\bar{X})$	1.218[a]	1.212[c]	1.336[c]	1.357[d]	1.330[c]	0.0001
(SD)	0.045	0.050	0.043	0.048	0.037	
(min)	1.127	1.063	1.218	1.197	1.259	
(max)	1.334	1.376	1.474	1.518	1.468	
Wax mirror width A (WXWDA)						
$(\bar{X})$	2.197[a]	2.182[c]	2.401[c]	2.410[b]	2.367[d]	0.0001
(SD)	0.056	0.063	0.054	0.059	0.046	
(min)	2.045	1.971	2.242	2.213	2.241	
(max)	2.353	2.466	2.549	2.612	2.463	
Wax mirror width B (WXWDB)						
$(\bar{X})$	0.310[a]	0.313[a]	0.270[c]	0.257[d]	0.266[c]	0.0001
(SD)	0.036	0.045	0.036	0.034	0.026	
(min)	0.224	0.170	0.188	0.128	0.205	
(max)	0.403	0.598	0.568	0.370	0.316	

Means for the same characteristics followed by different letters within Africanized or European groups are significantly different ($P < 0.05$) according to analysis of variance followed by Duncan's Studentized multiple range test. The probability of means of Africanized groups being different from means of European groups was determined using ANOVA.

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 colonies). 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 measurements of the 23 characteristics showed that significant differences existed among the 3 groups (Wilks' λ = 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 2 groups.

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 IV) according to our recommended regulatory standards (table VI; fig 1). In 19 (3.21%) cases, 'Africanized' colonies were basically European with morphological evidence of the introgression of Africanized genes and in 7 (1.19%) cases were morphologically European. Perhaps they were, since the sole criterion for inclusion in the 'Africanized' group was to have been collected in an area considered to be generally Africanized. If an imported European colony was invoved in a sample's parentage, it would, nonetheless, have been initially

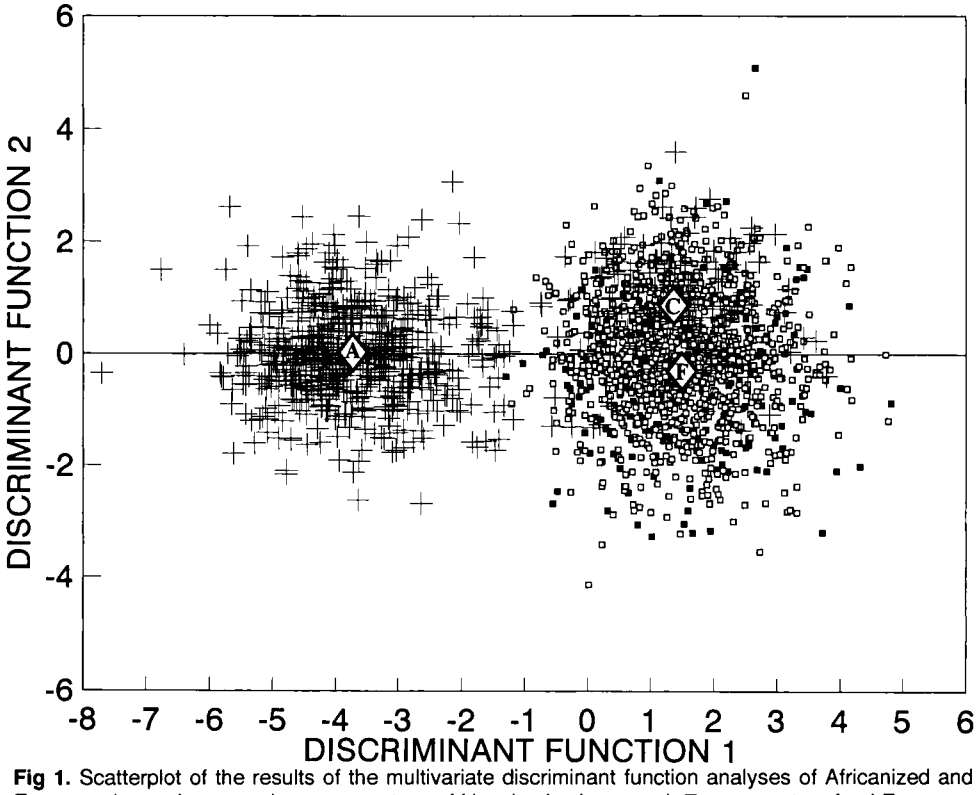

Fig 1. Scatterplot of the results of the multivariate discriminant function analyses of Africanized and European honey bees: each + represents an Africanized colony, each ❑ represents a feral European colony and each ■ represents a commercial European colony. Some colony indicators are not visible due to the large number of colonies represented in the figure.The centroid for each group is marked by a ◊ : 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.

Table IV. Classification results (numbers and (percentages)) of the multivariate discriminant 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 Africanized 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 (0.20)	1 108 (99.73)

The accuracy of the multivariate discriminant 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 VI.

considered as 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. Three (0.20%) colonies from areas thought to only have European honey bees were suspected to have some Africanized genes. This may cause, as a consequence, the early and undetected intrusion of Africanized bees into some of the collection sites. Alternatively, these colonies may be extreme samples from the European population. When the colonies were classified based strictly on their greatest probability of group membership, 3 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 Ilanos 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 a *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 ≈ 71% of the time. The analysis of each 10-bee sample for a colony required a minimum of 2 h for dissection, preparation, measurement and calculation.

Table V presents the unstandardized function coefficients and constants necessary to apply our discriminant analysis results to the identification of unknown samples. Mean body part measurements from an unknown sample of 10 worker bees are multiplied by the corresponding coefficients for each of the 2 functions. The 2 sums of these products are added to the appropriate constants to calculate the 2 function values required to determine the probability of group membership.

With *a* as Africanized, *e* as commercial European and *f* as feral European, a first step in calculating exact probabilities of

Table V. Unstandardized coefficients and constants for calculation of the canonical discriminant functions in the discriminant analysis of Africanized and European honey bees derived from the analysis of 10 bees from each of 2 103 colonies.

Character [a]	*Coefficients*	
	Function 1	*Function 2*
FWLN	2.284004	3.886434
FWWD	–7.733152	–4.732451
HWLN	–0.695236	–7.549505
HWWD	9.651663	2.702524
HAMU	–0.113852	0.194252
AN29	–0.230897	–0.136877
AN30	–0.061456	–0.056467
AN31	–0.073426	0.272091
AN32	0.261013	0.362003
AN33	0.085631	0.046098
AN34	0.004550	0.039860
AN35	–0.099473	–0.121173
AN36	0.099225	0.241756
CUBB	–4.369450	10.497011
CUBA	–0.065479	13.959551
TBLN	–9.210478	–6.098357
FELN	14.004288	–0.035503
BTLN	–3.141886	1.250275
BTWD	4.182923	7.423959
STLN	–0.278394	0.028753
WXLN	4.074946	–8.936000
WXWDA	7.581698	4.726269
WXWDB	–5.774247	6.301744
Constant	–30.35230	–42.63713

[a] A list of characters with acronyms appears in table III in the same order as the acronyms in this table.

group membership (SAS Institute, 1982) is to determine 3 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 are calculated as :

$$D_a^2 = (\text{Function 1} + 3.74565)^2 / 1.06029 + (\text{Function 2} - 0.011983)^2 / 0.85387;$$

$$D_e^2 = (\text{Function 1} - 1.38471)^2 / 0.91774 + (\text{Function 2} - 0.85650)^2 / 0.99387;$$

and

$$D_f^2 = (\text{Function 1} - 1.49272)^2 / 0.99540 + (\text{Function 2} + 0.33146)^2 / 1.06926$$

Each of the 3 posterior probabilities of group membership of a sample is then given by:

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

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; Lobo *et al,* 1989; Del Lama *et al,* 1990; Hall, 1990; Rinderer *et al,* 1991, 1992; Sheppard *et al,* 1991a, 1991b; Moritz and Meusel, 1992), and especially as Africanized bees continue their range expansion into areas with 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 that a colony may have resulted from extensive hybridization. Intermediate scores may arise for individual colonies as a 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 F_1 hy-

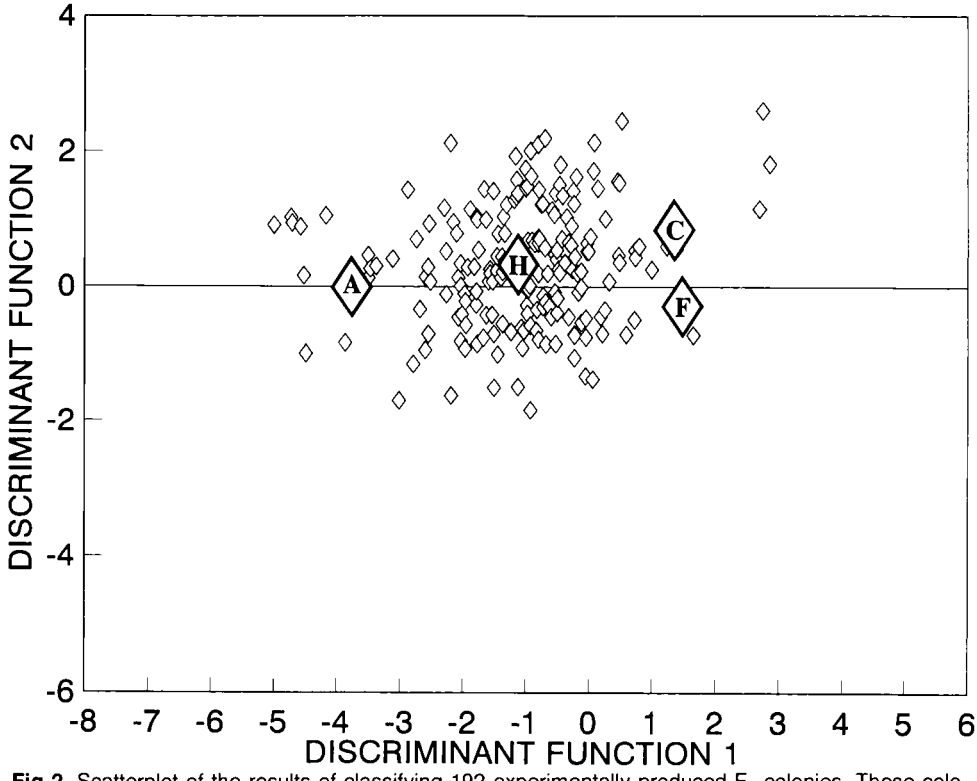

Fig 2. Scatterplot of the results of classifying 192 experimentally produced F_1 colonies. These colonies were generally intermediate between Africanized and European colonies. Each ◊ represents 1 of these colonies. The centroid for the group is marked by a ◊ containing an H. The centroids of the groups used to derive the discriminant functions are each marked by a ◊: 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.

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

Probabilities	*Determination*
$0.990 \leq pA \leq 1.00$	Africanized
$0.900 \leq pA < 0.99$	Africanized with evidence of the introgression of European genes
$0.500 \leq pA < 0.900$	European with evidence of the introgression of Africanized genes, recommend measurement of 10 additional bees
$0.000 \leq pA < 0.500$	European

*p*A: probability of belonging to the Africanized group.

brid colonies. These colonies were generally intermediate between Africanized and European colonies (fig 2). Table VI provides the guidelines used by the USDA for interpreting posterior probabilities. These guidelines will permit the identification of clearly Africanized honey bee colonies. Remaining colonies are either clearly European or insufficiently Africanized to consider them to be objectionable as production colonies. We recommend that colonies which produce intermediate scores be evaluated using 10 additional bees.

More rigorous guidelines would be appropriate for selecting breeder colonies of European stock or progeny testing queens for international trade in queen honey bees. Regardless of the location of the production of queen honey bees, instrumental insemination or careful open mating procedures (Hellmich *et al,* 1986, 1988; Hellmich and Waller, 1990) will produce European queens mated to European drones. The lack of significant introgression of Africanized genes into European stock can be certified by the use of these and other techniques (Rinderer *et al,* 1986a, 1987).

Data collection procedures, data management procedures, and data analyses were carried out *via* the IBM PC compatible computer program "USDA-ID" which is available from TER or SMB on the requestor's 5 1/4" or 3 1/2" diskette.

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Résumé — Identification morphométrique des abeilles africanisées et européennes à partir de grandes populations de référence. Nous avons mis au point des procédures d'analyse discriminante pour identifier les abeilles africanisées et européennes, qui présentent des améliorations sur plusieurs points. Tout d'abord, la taille des échantillons d'abeilles africanisées et européennes est beaucoup plus grande et on peut s'attendre à ce qu'ils renferment une plus grande part de la variation des 2 groupes. Ensuite, les abeilles européennes sauvages et les abeilles européennes élevées commercialement sont bien représentées, réduisant ainsi la probabilité de classer en abeilles africanisées des abeilles européennes relativement petites. Troisièmement, les abeilles africanisées élevées dans des ruches avec des feuilles de cire gaufrée européenne et les abeilles africanisées sauvages sont bien représentées, réduisant ainsi la probabilité de classer des abeilles africanisées en abeilles européennes. Quatrièmement, la classification en africanisées ou en européennes des colonies (CO) de départ est déterminée par le fait que la population générale était ou non considérée comme africanisée au moment du prélèvement des échantillons, ainsi que par l'histoire spécifique de la reine qui a produit la CO. Cela élimine le biais inhérent aux décisions basées sur le comportement en plein air, qui conduisait vraisemblablement à ne récolter comme matériel de départ que les membres les plus nettement africanisés des populations échantillonnées. Cinquièmement, la mesure des 2 angles alaires utilisée par Daly et Balling (1978), qui avait été difficile à standardiser parmi les différents laboratoires, a été éliminée. Les données de 2 103 échantillons d'abeilles prélevés dans des CO des États-Unis (970 CO), du Mexique (360), du Costa-Rica (75), du Venezuela (390), du Brésil (232), de l'Argentine (52) et de l'île Kangourou, Australie (24) ont été classées en CO sauvages, CO rustiques (ruches sans cire gaufrée ni remérage) ou CO

commerciales (avec cire gaufrée et remérage) et l'on s'est assuré qu'elles pouvaient être considérées comme des échantillons aléatoires de populations nettement africanisées ou européennes d'après leur localisation et la date du prélèvement. Certains prélèvements ont été faits dans le nord du Mexique et dans plusieurs États du sud-ouest des États-Unis afin d'augmenter la variabilité géographique et biologique des échantillons. Les échantillons provenaient de 177 CO africanisées installées dans des ruches et qui, à de rares exceptions, avaient été élevées sur des cires gaufrées européennes, 414 CO africanisées sauvages et rustiques, 331 CO commerciales européennes, 1 111 CO européennes sauvages et rustiques et 70 CO européennes dont le type de ruche était inconnu. Plusieurs analyses à une variable et multivariables ont été faites pour choisir les caractéristiques morphologiques et les groupes de caractères à utiliser dans une analyse multivariable discriminante (amd). La précision de l'amd a été déterminée en retenant 250 CO prises au hasard et en les classant comme échantillons indépendants. L'amd a identifié correctement 565 (95,6%) des 591 CO africanisées ainsi que l'ensemble des 1 512 CO européennes (tableaux IV, VI; fig 1). Dans 19 cas (3,21%) des CO «africanisées» étaient fondamentalement européennes avec une preuve morphologique de l'introgression de gènes africanisés et dans 7 cas (1,19%) elles étaient morphologiquement européennes. Peut-être l'étaient-elles réellement, puisque le seul critère pour être inclus dans le groupe «africanisées» était d'avoir été prélevé dans une région considérée dans l'ensemble comme africanisée. Si une CO européenne importante était impliquée dans une origine d'échantillon, elle aurait cependant été considérée initialement comme africanisée. Ce taux de classification erronée est faible et n'est pas susceptible d'inquiéter l'apiculture commerciale, puisque dans des situations critiques, telles que des programmes de sélection, ces CO déclarées européennes avec preuve de l'introgression de gènes africanisés auraient été éliminées. Trois (0,20%) CO de régions, que l'on pensait peuplées uniquement d'abeilles européennes, sont suspectées d'avoir quelques gènes africanisés. Cela pourrait être la conséquence d'une intrusion précoce et non détectée d'abeilles africanisées dans certains des lieux de prélèvement. Ou bien ces CO peuvent être des échantillons extrêmes de la population européenne. Lorsque les CO ont été classées uniquement d'après leur plus forte probabilité d'appartenir à un groupe donné, 3 CO du groupe africanisé ont été déclarées européennes avec des probabilités d'être africanisées (PA) de 0,00004, 0,036 et 0,066 (CO du Llanos et des Andes, Venezuela et des faubourgs de Rio de Janeiro, Brésil). Deux CO du groupe européen ont été déclarées africanisées avec un PA de 0,544 et 0,668 (CO du Mexique et CO sauvage du désert de Californie). En outre l'analyse a pu différentier les CO européennes commerciales des CO européennes sauvages dans environ 71% des cas. L'analyse d'un échantillon de 10 abeilles par CO nécessite un minimum de 2 h (dissection, préparation, mesures et calculs). Les procédures de collecte, de traitement et d'analyse des données sont réalisées par le programme «USDA-ID» qui fonctionne sur compatible IBM-PC; on peut l'obtenir sur disquette 5 1/"4 ou 3 1/3" en s'adressant à TER ou SMB.

***Apis mellifera* / abeille africanisée / abeille européenne / identification / morphométrie / analyse discriminante multivariable**

Zusammenfassung — Morphometrische Identifizierung von Afrikanisierten und

Europäischen Honigbienen unter Verwendung sehr großer Referenz-Populationen. Wir haben neue Verfahren der Diskriminanzanalyse zur Identifizierung Afrikanisierter und Europäischer Honigbienen entwickelt. Diese Verfahren wurden in mehrfacher Hinsicht verbessert. Erstens ist das Probenvolumen sowohl für Afrikanisierte wie für Europäische Bienen viel größer und man kann deshalb erwarten, daß sie mehr von der Variation in beiden Gruppen erfassen. Zweitens sind von den Europäischen Bienen sowohl Wildvölker wie auch kommerziell gehaltene gut repräsentiert; dadurch wird die Möglichkeit verringert, relativ kleine wildlebende Europäische Bienen als Afrikanisiert zu klassifizieren. Drittens sind Afrikanisierte Bienen, die in Kästen ausgestattet mit Mittelwänden in europäischem Maß aufgezogen wurden, und Afrikanisierte Wildvölker gut vertreten, wodurch die Gefahr einer Fehlbestimmung, Afrikanisierte Bienen als Europäisch zu betrachten, verringert wird. Viertens wurde ein Grundstock von Völkern als Afrikanisiert oder Europäisch klassifiziert, je nach dem, ob die gesamte Population des Gebietes zur Zeit der Probenentnahme Afrikanisiert war oder nicht; dazu wurde die Geschichte der Königin, welche die Bienen des Volkes hervorgebracht hatte, berücksichtigt. Dies schließt Fehlbeurteilungen auf Grund des Verhaltens der Bienen im Freien aus, so daß wahrscheinlich nur eindeutig Afrikanisierte Völker aus der geprüften Population in den Grundstock des Materials einbezogen wurden. Fünftens wurde die Messung von zwei Flügelwinkeln, die von Daly und Ball (1975) benutzt wurden, aufgegeben, weil die Messung bei den verschiedenen Laboratorien nur schwer standardisiert werden konnte.

Es wurden Daten von 2.103 Bienenproben aus Völkern verwendet, gesammelt in den Vereinigten Statten (970 Völker), Mexico (360), Costa Rica (75), Venezuela (390), Brasilien (232), Argentinien (52), und Kangaroo Island Australien (24), um sie entweder als 'Landvölker' (Kästen ohne Mittelwände und ohne Umweiselung) oder als 'kommerziell' (Völker auf Mittelwänden, mit Umweiselung) einzustufen und zu überprüfen, ob sie auf Grund ihrer Fundstelle und Sammeldatum als zufällig entnommene Proben von eindeutig Afrikanisierten oder Europäischen Populationen eingestuft werden können. Bestimmte Sammlungen wurden in Nordmexiko und in den SW Staaten der USA eigens zu dem Zweck durchgeführt, um die geographische und biologische Variabilität der Proben zu erhöhen. 177 Proben der Sammlung stammen von Afrikanisierten Völkern in Kästen, die, mit wenigen Ausnahmen, auf europäischen Mittelwänden aufgezogen worden waren, 414 aus Wildvölker oder Landvölkern mit Afrikanisierten Bienen, 331 aus kommerzielle Europäische Völker, 1.111 aus Europäischen Wild- oder Landvölkern und 70 aus Europäischen Völkern mit unbekannten Kastentypen.

Mehrere uni- und multivariate Analysen wurden durchgeführt, um die morphologischen Merkmale und die Merkmalsgruppen für die multivariate Diskriminanzanalyse zu selektieren. Die Genauigkeit der multivariaten Diskriminanzanalyse wurde dadurch überprüft, daß 250 zufällig ausgewählte Proben zurückgehalten und als unabhängige Proben klassifiziert wurden.

Die multivariate DA bestimmte korrekt nach unseren empfohlenen Standards 565 der 591 Afrikanisierten Proben (95.6%) und klassifizierte alle 1.512 Europäischen Proben richtig (Tab IV, VI; Abb 1). Bei 19 (3.21%) Fällen waren 'Afrikanisierte' Proben im Grunde Europäisch mit morphologischem Hinweis auf Ingression von Afrikanisierten Genen und in 7 (1.19%) Fällen waren sie morphologisch Europäisch; vielleicht waren sie es wirklich, denn das einzige Kriterium, um sie in die 'Afrikanisierte'

Gruppe einzuordnen, bestand in ihrer Herkunft aus einem Gebiet, das allgemein als Afrikanisiert galt. Falls sich unter den Vorfahren einer Probe ein importiertes Europäisches Volk befand, wäre es trotzdem anfangs als Afrikanisiert bezeichnet worden. Die Rate von falschen Klassifizierungen ist niedrig, sie wird die kommerzielle Bienenhaltung kaum stören, da diese Völker in kritischen Situationen wie zB Zuchtprogrammen leicht ausgeschieden werden können. Drei (0.20%) Völker aus Gebieten, die als Europäisch angenommen wurden, standen unter dem Verdacht, Afrikanisierte Gene zu enthalten. Das mag zutreffend sein, als Folge einer früheren unerkannten Einschleppung Afrikanisierten Völker in das Sammelgebiet. Andererseits könnten diese Völker extreme Proben der Europäischen Population sein. Wurden die Proben strikt nach der größten Wahrscheinlichkeit ihrer Gruppenzugehörigkeit klassifiziert, so wurden drei Proben der Afrikanisierten Gruppe für 'Europäisch'» erklärt, mit einer Wahrscheinlichkeit, Afrikanisiert zu sein (pA) von 0.00004. 0.036 und 0.066 (Völker von den Llanos von Venezuela, dem Andengebirge von Venezuela und den Vorstädten von Rio de Janeiro, Brasilien). Zwei Völker der Europäischen Gruppe wurden für Afrikanisiert erklärt mit pA von 0.544 und 0.668, für ein Volk aus Mexiko und ein Wildvolk aus der Wüste von Kalifornien. Außerdem war die Analyse in 71% der Fälle in der Lage zwischen Europäischen kommerziellen und Wildvölkern zu unterscheiden. Die Analyse der 10 Bienen einer Probe beanspruchte für Präparierung, Messung und Berechnung ein Minimum von zwei Stunden. Die Prozeduren der Datensammlung und des Datenmanagements sowie deren Analyse wurden mit dem IBM PC-kompatiblen Computerprogramm 'USDA-ID' ausgeführt, das auf Verlangen von TER oder SMB auf 5 1/4" oder 3 1/2" Diskette erhältlich ist.

***Apis mellifera* / Afrikanisierte Honigbiene / Europäische Honigbiene / morphometrische Identifizierung / multivariate Diskriminanzanalyse**

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