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IDENTIFICATION OF AFRICANIZED HONEYBEES IN THE WESTERN HEMISPHERE BY DISCRIMINANT ANALYSIS

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Abstract.—Small collections and single individuals of Africanized honeybees are not reliably distinguished by conventional taxonomy from European bees. Discriminant analyses of up to 25 morphometric characters were performed on 101 collections of Africanized bees and 297 collections of European bees. When all characters were included, the analysis gave 100% separation of the samples. Individual bees can also be identified but with a higher risk of misclassification. An explanation of the method and discriminant coefficients for 5 sets of characters are provided.

The purpose of this paper is to describe a procedure for identifying individuals or small collections of dead, Africanized worker honeybees in the Western Hemisphere. The identity of such collections is often needed by agricultural inspection agents, beekeepers, or scientists (Anonymous, 1972b; Batra, 1976). Experienced persons frequently are able to recognize Africanized bees by their slightly smaller size, characteristics of their group behavior, and the location and features of their colonies (Anonymous, 1972c; Taylor and Williams, 1975). Individual worker bees or small collections isolated from their combs, however, are not reliably identified on sight or by conventional taxonomy (see, for example, Anonymous, 1972a). Easily recognizable, diagnostic features of anatomy are lacking. The key characters for the native African *Apis mellifera adansonii* as given by Maa (1953) require subjective judgements. The differences are much less clear when dealing with Africanized hybrids.

The circumstances that make this procedure necessary are as follows. In Europe and Africa, the subspecies of *Apis mellifera* are nearly alike in morphology and differ mainly in behavior and certain quantitative features (Ruttner, 1968). Prior to 1957 domestic honeybees in the Western Hemisphere were derived primarily from various European subspecies: *A. m. mellifera, A. m. ligustica, A. m. carnica,* and *A. m. caucasica.* Open mating among the subspecies, as well as selective breeding, have produced extensive genetic recombination. As a result, the distinctions among the subspecies have been obscured in the New World, and variability in color and behavior have probably increased. In 1957, 26 swarms of African honeybees, *A. m. adansonii,* escaped quarantine near Rio Claro, Brazil (see review by Michener, 1975). Mating between the African bees and the resident European bees gave offspring that perpetuated mostly African traits, hence the name "Africanized honeybees." Such hybrid bees are also variable in color and behavior. They have now replaced European bees over much of

South America east of the Andes and north of about 35°S latitude. As a consequence of these events in the Western Hemisphere, bees of European and African origins, both genetically heterogeneous and variable, are difficult to distinguish.

The most fruitful approaches in the taxonomy of honeybees have been quantitative. Shipman (1975) reported differences in the ratio of venom constituents in a single analysis of Africanized and European bees. Sylvester (1976) found differences in the relative frequencies of allozymes at one (malic dehydrogenase) out of 39 loci. Such biochemical methods are sensitive to genetic differences and would be the desired approach, but they have the disadvantage that material must be specially collected and preserved.

DuPraw (1964, 1965a, 1965b) first applied discriminant analysis to measurable features of wings. His success in correctly classifying samples of various subspecies led to other multivariate applications by Cornuet, et al. (1975), Louis and Lefebvre (1971), and Tomassone and Fresnaye (1971). All of these analyses have dealt with honeybees in the Old World. Morphometric methods can be applied to specimens that are easily preserved dry or in fluid. A disadvantage is that the phenotype is subject to environmentally induced variation. This might occasionally conceal the genetic ancestry.

In the present investigation, 25 morphometric characters were selected, mostly from those previously used by DuPraw (1965b) and Ruttner (1968). Discriminant scores computed for 398 collections from the New World that were adduced to be Africanized or European bees gave a correct classification for 100% of the samples.

Materials and Methods

Except as noted below, worker honeybees were collected from colonies. They were placed in alcohol or killed and stored dry. Ten bees were usually measured from each colony and constitute a "collection" in this study. For each specimen, the right fore and hind wings (dorsal side up), right hind leg (posterior side up), and third metasomal sternum (cleared and lightly stained; ventral side up) were mounted in Diaphane or Permount on a slide. After the mountant was firm, the slide was inverted and the image of the parts projected onto a desktop by a Bausch and Lomb Tri-simplex microprojector. Points defining the measurements were marked on a piece of paper and later measured. A mean for the ten bees was computed for each character and used to represent the collection in the subsequent analyses. Discriminant analysis was performed on a CDC-6400 computer, using the subprogram DISCRIMINANT of the "Statistical Package for the Social Sciences" (Nie et al., 1975).

The 101 collections of Africanized bees were collected by various persons in Argentina (6 collections), Brazil (54), Guyana (2), Surinam (12), and

French Guiana (27). Two collections included 7 bees each, and 1 had 9 bees. All others were based on 10 bees each. The collections were judged by the collectors to be Africanized on the basis of the beekeeper's knowledge, or firsthand observations of the bees' behavior, size, color, location, nature of the colony, etc. Twelve collections were of bees collected at food sources and the remainder were from colonies. Included are both wild colonies and swarms, as well as those in man-made hives. Excluded from the Africanized sample were bees from South America that were known to be definitely European in origin or were collected from food sources near hives of known European bees.

The 297 collections of European bees were taken in Brazil (2 collections), Canada (4), Colombia (15), Costa Rica (54), French Guiana (11), Guyana (11), Mexico (4), Surinam (71), United States including Hawaii (111), and Venezuela (14). The collections from Mexico were collected from food sources. All other European collections were from colonies. One collection was based on 7 bees, 1 had 8, and 1 had 9. All others had 10 bees each. Most of the collections from Central and South America were from man-made hives, but some from the United States were from swarms or wild colonies. Excluded from the analysis were recent experimental hybrids of Africanized bees with European bees.

Characters Measured

Most of the measurements are those that have proved useful by other investigators, e.g., the angles between wing veins (DuPraw, 1965b) or linear measurements of wings, leg segments, and sterna (Ruttner, 1968). The 25 characters selected for use in this analysis are listed below and illustrated in Figs. 1 and 2. The first two linear measurements (forewing length and width) were taken on projected images at 17×, and the remainder at 36.5×. The images were measured to the nearest 0.5 mm and then converted to the true metric distance. The angles between veins were measured by marking points (visually centered on projected images at 36.5×) at the junctures of veins and connecting the points with straight lines. Angles were measured to the nearest 1° with a protractor. Hamuli were recorded as a simple count.

A. Forewing (Figs. 1B and 2):
   1. Length (W_L), from maximum apical curvature to base of costal vein.
   2. Width (W_W), maximum taken at right angle to length.
   3. Vein M, proximal abscissa (a), length (otherwise known as the numerator of the "cubital index").
   4. Vein M, distal abscissa (b), length (otherwise known as the denominator of the "cubital index").
   5 to 14. Angles between veins (angles 29–36, 38, 39).

B. Hind Wing (Fig. 1B):
   15. Length (HWLN) from maximum apical curvature to junction of crossvein cu-v and vein M + Cu.
   16. Width (HWWD), maximum taken at right angle to length.
   17. Hamuli number (Ha).

C. Hind Leg (Fig. 1A):
   18. Femur length (Fe_L).
   19. Tibia length (Ti_L).
   20. Basitarsus length (Ta_L).
   21. Basitarsus width (Ta_W), maximum taken at right angle to length.

D. Third sternum, counting posterior to petiole (Fig. 1C):
   22. Length (St_L).
   23. Wax mirror width (Wm_W).
   24. Wax mirror length (Wm_L).
   25. Distance between wax mirrors (Wm_D).

Univariate Analysis

The simplest approach to the problem would be to find an easily measured character that gives a clear separation between Africanized and European bees. A separate analysis of variance for each character indicates that the means of 22 of the 25 characters are significantly different between the Africanized and European bees (see Table 1, F-ratio in right column; *** = significance probability < .001). Such pairwise comparisons are unsatisfactory from a statistical viewpoint because no account is taken of the interrelationships among the 25 characters.

Comparison of the two distributions of the sample measurements for each character indicates an overlap in every character (see Table 1, "range" columns). For example, length of the forewing (W_L) is easily measured and the means are well separated, yet the identification of bees with intermediate measurements would be uncertain (Fig. 3B). If the midpoint between the means, 8.885 mm, is used to separate the groups, then the probability of misclassifying a collection of Africanized bees as European and *vice versa* on the basis of forewing length is computed to be 7.5% (based on the assumption that the character has a normal distribution). In our study 32 or 8.0% of the collections would have been misclassified if 8.885 mm was used as the classification point. Of all characters, wax mirror width (Wm_W) has the largest F-value and consequently gives the best separation (Fig. 3A), but it is only slightly better than length of forewing. Furthermore, it is more time-consuming to measure and also exhibits an overlap in intermediate measurements (theoretical probable misclassification of 6.8%; in this study 26 collections or 6.5% were misclassified). When several characters of a collection are examined conflicting identifica-

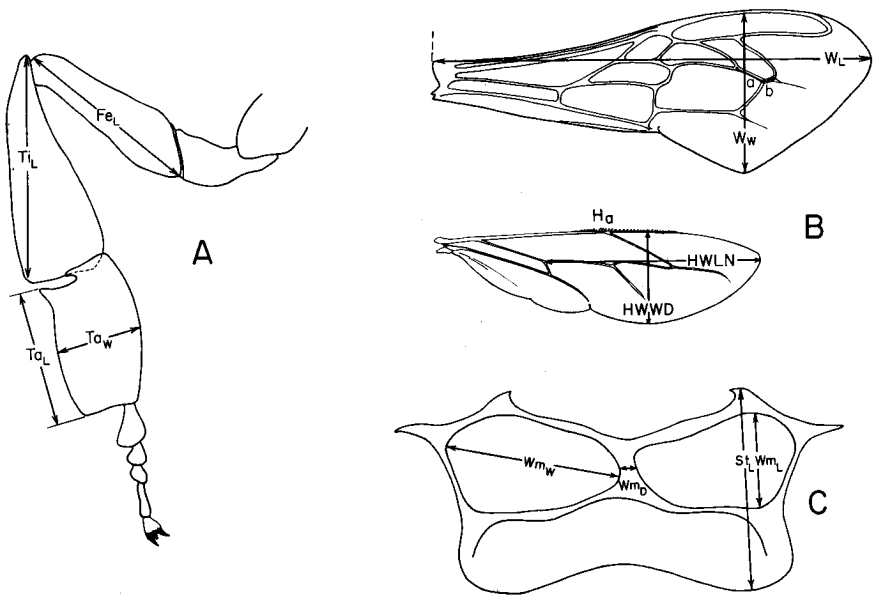

Fig. 1. Structures of the worker honeybee, showing characters: A, right hind leg; B, right forewing and hindwing; C, third metasomal sternum.

tions are sometimes indicated. Thus, of the 25 characters none provides a totally satisfactory guide to identification when used individually.

Discriminant Analysis

Discriminant analysis is a technique in which measurements of two or more characters are weighted and combined linearly to give maximal separation of two or more groups (for explanation of the method see Blackith and Reyment, 1971 or Van de Geer, 1971). Each analysis begins with groups of known identity. The multivariate method uses the non-overlapping or independent information contributed by each character to produce a linear function that will classify the known collections with a minimum probability of misclassification. Different combinations of characters may be used to achieve statistical distinction between the groups. In the simplest case of two groups the analysis yields a single weight or discriminant function coefficient for each character. Each measurement of the specimen or the collection mean is multiplied by the corresponding coefficient and the products summed, then corrected by a single constant to give a discriminant score for the specimen or collection.

The scores for each group form distributions, each with a mean score. Half the distance (D) between the mean scores, i.e., the midpoint, is used to separate the groups because this point gives the minimum misclassifica-

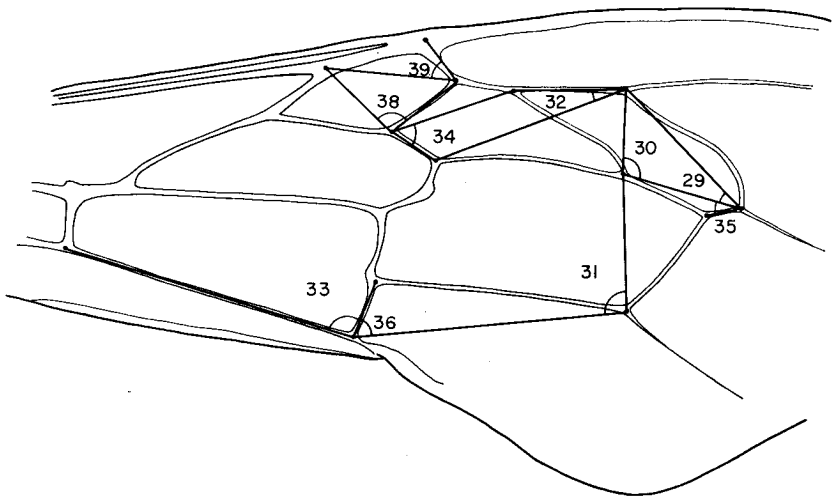

Fig. 2. Angular measurements of the fore wing.

tion. Unless the separation of known groups is complete, the scores for some specimens or collections will fall within the range of scores delimited for the other group. According to their score such specimens would be misclassified. The actual rate of misclassification of known specimens, or better, the theoretical probability of misclassification, provides a guide to the future effectiveness of a set of characters in classifying new specimens that are derived from populations similar to the initial, known groups.

In any given analysis, the coefficients, constant, mean scores, and rate of misclassification depend not only on the choice of characters, but also on the choice of specimens that are included in the known groups. We have attempted to make the analyses generally useful by including bees from different geographic regions and a large total number of collections.

Using the stepwise methods of the computer program, the combination that gives the best discrimination with a minimum of two characters is wax mirror width (Wm_W) and angle 39 (Fig. 3C; set 1, Table 2). As an example of the identification procedure, the score for a new collection is computed as follows:

Set 1	Mean	Coefficient	Product
Wm_W	2.413 mm	13.8708	33.470
A39	42.2°	0.1847	7.794
		Sum	41.264
		Constant	−40.038
		Score	1.23

Table 1. Means, standard deviations, and ranges for 25 characters of Africanized and European honeybees. N is the number of collections of usually 10 bees each.

Char.		Africanized (N = 101)			European (N = 297)			F-ratio
		Mean	S. dev.	Range	Mean	S. dev.	Range	
1	W_L	8.65	0.152	8.33 – 9.05	9.12	0.167	8.59 – 9.62	626.2***
2	W_W	2.94	0.0677	2.81 – 3.14	3.08	0.0702	2.82 – 3.32	276.9***
3	a	0.508	0.0262	0.437– 0.584	0.548	0.0391	0.420– 0.678	94.3***
4	b	0.225	0.0157	0.184– 0.264	0.234	0.0230	0.175– 0.306	14.2***
5	A29	31.5	1.46	27.8 – 34.7	30.0	1.50	25.5 – 35.2	81.1***
6	A30	105.2	3.68	97.1 –113.7	108.2	3.82	91.1 –117.7	47.4***
7	A31	102.0	1.98	97.6 –106.5	99.5	3.32	87.4 –111.2	48.5***
8	A32	19.4	0.84	17.4 – 21.6	21.5	1.60	15.2 – 25.9	152.2***
9	A33	96.2	1.76	90.8 –100.5	94.8	2.56	87.6 –103.3	22.7***
10	A34	50.8	1.98	46.4 – 56.8	51.3	2.55	44.1 – 63.8	3.2 *ns*
11	A35	23.1	1.28	19.3 – 26.3	22.8	1.66	17.7 – 27.7	3.1 *ns*
12	A36	60.3	1.71	55.7 – 66.3	62.2	2.50	54.6 – 70.9	48.1***
13	A38	91.2	1.96	85.3 – 96.1	93.8	3.01	83.6 –100.8	69.7***
14	A39	40.1	1.97	36.2 – 46.3	43.9	2.81	33.9 – 52.2	153.6***
15	HWLN	4.17	0.0778	3.97 – 4.38	4.38	0.0847	4.12 – 4.60	473.4***
16	HWWD	1.65	0.0512	1.53 – 1.83	1.77	0.0633	1.56 – 1.97	309.8***
17	Ha	21.1	0.94	19.1 – 23.3	21.3	1.17	18.1 – 24.9	1.2 *ns*
18	Fe_L	2.51	0.0548	2.40 – 2.65	2.66	0.0524	2.53 – 2.82	530.9***
19	Ti_L	3.14	0.0603	2.98 – 3.28	3.25	0.0705	3.07 – 3.45	198.8***
20	Ta_L	1.94	0.0470	1.84 – 2.05	2.03	0.0510	1.90 – 2.20	273.0***
21	Ta_W	1.10	0.0252	1.04 – 1.17	1.14	0.0287	1.06 – 1.23	156.7***
22	St_L	2.55	0.0607	2.40 – 2.67	2.72	0.0663	2.51 – 2.91	498.0***
23	Wm_W	2.17	0.0724	2.01 – 2.38	2.36	0.0631	2.21 – 2.56	667.7***
24	Wm_L	1.27	0.0468	1.17 – 1.38	1.38	0.0437	1.25 – 1.51	542.2***
25	Wm_D	0.318	0.0392	0.237– 0.427	0.270	0.0420	0.13 – 0.49	101.5***

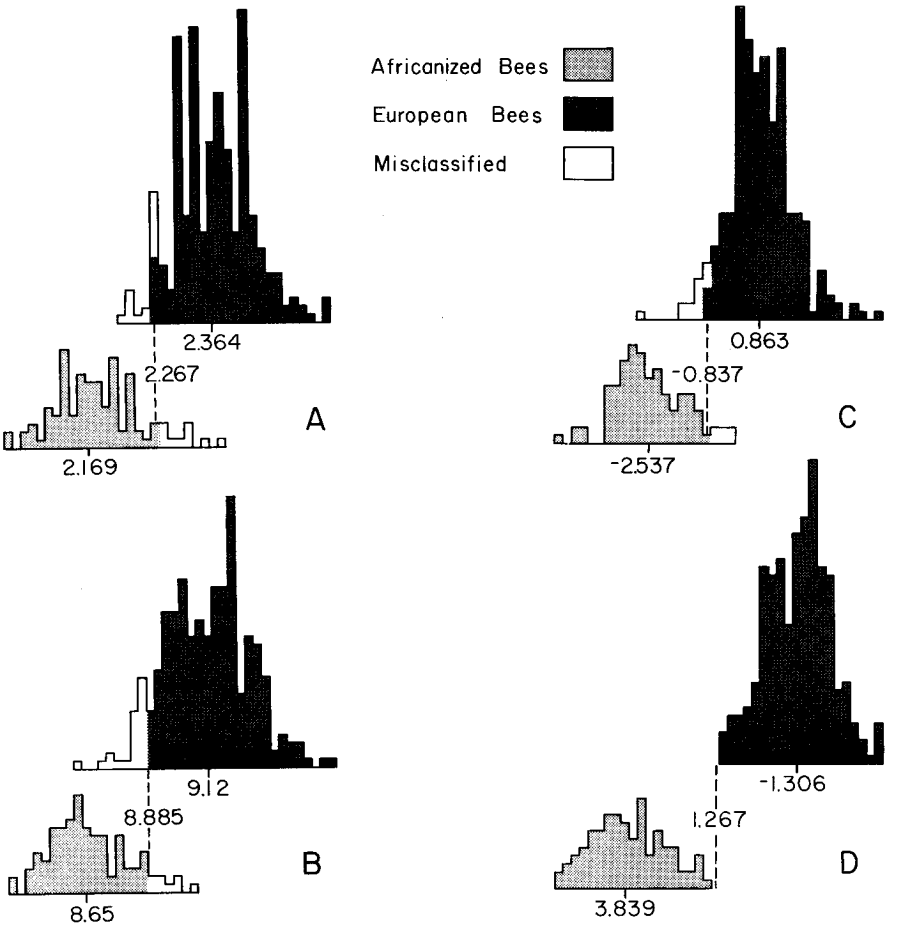

Fig. 3. Frequency distributions comparing Africanized and European bees: A, measurements of the wax mirror width (Wm_W); B, measurements of fore wing length (W_L); C, discriminant scores based on character set 1; D, discriminant scores based on character set 6 (note scale is reversed). The means of the distributions and midpoints are indicated for each pair of graphs.

The score is greater than the midpoint value of –0.837, hence the sample is identified as European.

Although serving to illustrate the method, character set 1 is not the simplest to prepare and measure. Better success with less effort can be obtained by using set 2 (Table 2). A slide preparation of wings and legs can be measured with an ocular micrometer. The metric equivalents of the measurements are then combined, using the information in Table 3, to yield a score for each new collection in question. If a projection ap-

Table 2. Examples of sets of characters and success in separating collections of Africanized and European bees.

Character sets	D	Misclassification			
		Expected %	Known %	African-ized collec-tions	Euro-pean collec-tions
1. A39,Wm_W	3.400	4.46%	4.8%	6	13
2. W_L, HWLN, Fe_L, Ti_L	3.625	3.50%	3.3%	5	8
3. W_L, A32, A38, A39	3.844	2.73%	2.5%	1	9
4. all wing characters except b	4.346	1.49%	1.5%	0	6
5. all characters except Ta_W, Ta_L, A30, A31	5.140	0.509%	0.0%	0	0
6. all 25 characters	5.145	0.505%	0.0%	0	0

paratus is available, set 3 can be readily measured on the fore wings alone and scores computed (Table 3).

Character sets 2 and 3 are relatively easy to measure. They provide identifications that can be expected to be correct in 96.5% and 97.3% of new collections, respectively. One may, however, wish to reduce further the risk of misclassification. Each additional character added to the set increases the distance (D) between the mean discriminant scores and thereby reduces the rate of misclassification. The amount contributed by each additional character depends on the statistical properties of the characters already included. For example, when 12 other wing characters are added to those in set 3 to make set 4 (Table 2), the probable rate of misclassification is reduced by 1.24%. The remaining wing character, vein M abscissa b, did not contribute significantly to the set and was omitted. Because wings are easily slidemounted, the information needed to compute scores on the basis of wing characters is given in Table 4.

In our study, 21 characters (set 5, Table 2) are needed to reduce the rate of misclassification to less than 1%. Even the characters that are not significant in the univariate analysis do, in the context of others, contribute to the correct identification of a few collections. The remaining 4 characters contribute only slightly, but are readily measured once the slide is prepared. Table 4 provides the required information for all characters (set 6), and Fig. 3D illustrates the separation achieved.

Identification of Individual Bees

The univariate and discriminant analyses described above were based on the means of measurements taken from collections of bees, each usually the

Table 3. Discriminant function coefficients, based on collections of bees, for two sets of easily measured characters.

Set 2		Set 3	
Characters	Coefficients	Characters	Coefficients
1. W_L	2.7535	1. W_L	–5.1713
15. HWLN	2.6834	8. A32	–0.2909
18. Fe_L	27.9216	13. A38	–0.1456
19. Ti_L	–19.5551	14. A39	–0.1116
Constant	–46.5884	Constant	70.9936
Mean European	0.9200	Mean European	–0.9754
Midpoint	–0.8927	Midpoint	0.9464
Mean Africanized	–2.7054	Mean Africanized	2.8681

progeny of a single queen. The same analyses were repeated on individuals that comprised the collections: 2964 European and 1003 Africanized bees. The univariate F-ratios for individuals were parallel to the analyses of collection means except that only the hamuli have a significance probability > .001. If the midpoint of 8.885 mm for forewing length is applied to the individual European bees, 435 or 14.67% are misclassified. Sim-

Table 4. Discriminant function coefficients, based on collections of bees, for two sets of characters: set 4, all wing characters except b, and set 6, all 25 characters in study.

Characters		Coefficients		Char. cont.		Coefficients cont.	
		Set 4	Set 6			Set 4	Set 6
1	W_L	–4.3169	–1.6672	14	A39	–0.1203	–0.09794
2	W_W	7.3939	6.5436	15	HWLN	–3.5363	–1.6948
3	a	–4.6612	–4.6348	16	HWWD	–6.1802	–6.6517
4	b		8.6729	17	Ha	0.06910	0.09415
5	A29	–0.04389	0.02846	18	Fe_L		–15.1122
6	A30	–0.03762	–0.006395	19	Ti_L		6.6036
7	A31	0.05111	0.02593	20	Ta_L		0.6594
8	A32	–0.2493	–0.1883	21	Ta_W		–0.2038
9	A33	–0.07315	–0.07260	22	St_L		2.8746
10	A34	–0.04799	–0.05546	23	Wm_W		–4.9703
11	A35	0.2280	0.2896	24	Wm_L		–4.9189
12	A36	–0.07554	–0.1081	25	Wm_D		2.5428
13	A38	–0.1305	–0.1196				
				Constant		75.1384	65.8162
				Mean Eur.		–1.1028	–1.3057
				Midpoint		1.0700	1.2669
				Mean Afr.		3.2429	3.8394

Table 5. Examples of sets of characters and success in separating individual Africanized and European bees.

Character sets	D	Misclassification			
		Expected %	Known %	Africanized individuals	European individuals
1. A39, Wm_W	2.39	11.60%	11.5%	135	322
2. W_L, HWLN, Fe_L, Ti_L	2.57	9.94%	9.9%	116	275
3. W_L, A32, A38, A39	2.74	8.53%	8.3%	75	255
6. all 25 characters	3.50	4.00%	4.4%	47	126

ilarly, 139 or 13.86% of Africanized bees are misclassified. The overall rate is 14.46%.

The success in classifying individual bees by discriminant analysis of various character sets is given in Table 5. Comparison with Table 2 shows that the identification of individuals involves a much higher risk of misclassification. The coefficients needed to identify individual bees, using character sets 2, 3, and 6 are given in Tables 6 and 7.

Conclusion

Individual or small collections of Africanized honey bees, when dead and isolated from their hives, are not reliably distinguished from European bees on sight or by microscopic qualitative features. If colony collections of known ancestry are compared quantitatively, then a significant difference can be demonstrated in 22 of 25 morphometric characters. Such characters compared one at a time will separate at best 93.5% of the known col-

Table 6. Discriminant function coefficients, based on individual bees, for two sets of characters.

Set 2		Set 3	
Character	Coefficients	Character	Coefficients
1. W_L	2.5164	1. W_L	–3.7687
15. HWLN	1.2159	8. A32	–0.2028
18. Fe_L	16.3439	13. A38	–0.08744
19. Ti_L	–10.6356	14. A39	–0.08279
Constant	–36.4909	Constant	49.8723
Mean European	0.6489	Mean European	–0.6919
Midpoint	–0.6344	Midpoint	0.6764
Mean Africanized	–1.9176	Mean Africanized	2.0448

Table 7. Discriminant function coefficients, based on individual bees, for character set 6, all 25 characters in study.

Characters	Coefficients	Char. cont.	Coeff. cont.	Char. cont.	Coeff. cont.
1. W_L	−1.6138	11. A35	0.09704	21. Ta_W	−0.03108
2. W_W	2.2029	12. A36	−0.05092	22. St_L	0.3507
3. a	−1.1526	13. A38	−0.06900	23. Wm_W	−3.1319
4. b	2.1995	14. A39	−0.06290	24. Ww_L	−2.2482
5. A29	−0.008612	15. HWLN	−0.7034	25. Wm_D	2.5006
6. A30	−0.01326	16. HWWD	−2.6979	Constant	44.8551
7. A31	0.04529	17. Ha	0.02474		
8. A32	−0.1136	18. Fe_L	−9.9623	Mean Eur.	−0.8861
9. A33	−0.03647	19. Ti_L	5.5413	Midpoint	0.8663
10. A34	−0.02437	20. Ta_L	0.7725	Mean afr.	2.6187

lections. When the characters are variously combined in discriminant analyses, the separations are improved. Two selections of easily measured characters yield correct identifications of 96.7% and 97.5% of known collections. All 25 characters will give 100% separation. The same procedures can be applied to individual bees, but with a higher risk of misclassification. The risk is minimized by carefully measuring all 25 characters on a collection of bees from the same colony and computing a discriminant score for the collection. In this paper we provide the needed coefficients based on collections from throughout the Americas and Hawaii.

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