Morphometric Differences Among Africanized and European Honey Bees and Their F_1 Hybrids (Hymenoptera: Apidae)

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ABSTRACT Discriminant analysis procedures were applied to morphometric data from Africanized and European honey bees and their F_1 hybrids. Functions were developed to allow the correct classification of 10-bee samples from all three groups. Samples that are a mixture of bees can be evaluated using the classification of individual bees based on individual-bee discriminant functions, which are available.

KEY WORDS Insecta, *Apis mellifera*, morphometrics, discriminant analysis

THE INTRODUCTION OF AFRICAN BEES (*Apis mellifera scutellata*) to Brazil (Kerr 1957) has led to the subsequent spread of their progeny throughout most of South America and all of Central America (Rinderer 1986). Morphometric analyses of these progeny indicate clearly that they are somewhat hybridized with the European populations of bees previously imported to South America (Buco et al. 1987). Thus, the troublesome bees of South and Central America are correctly termed Africanized, and their population fits King's (1968) definition of a "hybrid swarm" at the subspecific level. That is, this population constitutes "a continuous series of morphologically distinct hybrids resulting from hybridization of two [sub]species followed by crossing and backcrossing of subsequent generations."

For scientific and regulatory purposes, there was a need to unequivocally identify Africanized bees (Michener 1972). As a consequence of this need, Daly & Balling (1978) developed a procedure to discriminate between Africanized and European honey bees in the Western Hemisphere. This procedure was based on the discriminant analysis of 25 morphometric characteristics. Later, Daly et al. (1982) improved the procedure by developing computer-assisted measurement. Simplification of the procedures and the addition of different morphological measures resulted in a "fast Africanized bee identification" protocol (FABIS) for use by persons in the field (Rinderer et al. 1986a,b). FABIS will correctly identify almost all samples of bees as Africanized or European. The few remaining ambiguous samples can be submitted to the much more elaborate and time-consuming procedures of Daly et al. (1982). Used together, the two procedures make it possible to conduct large-scale programs, such as the regulatory activities recently completed in California (Gary et al. 1985).

Heretofore, research on honey bee identification has focused on the need to distinguish members of the hybrid swarm of Africanized bees from the mixtures of European bees (probably also hybrid swarms of a different origin) common to the Americas. No attention was given to identifying F_1 progeny of Africanized and European bee types. Our paper reports the results of applying the procedures of multivariate discriminant analysis to the problem of identifying the F_1 progeny of Africanized and European matings.

Materials and Methods

Swarms of feral Africanized honey bees were collected and sampled in west central Venezuela near Acarigua. Samples of worker bees were taken either when a swarm was collected or after a swarm was established as a colony with natural comb in its brood nest. Thirty swarms were sampled; each sample consisted of 10 worker bees.

Worker bees from 30 colonies of European bees reared near Acarigua, which represented a variety of commercial stocks, were also sampled. These colonies had brood nest comb made from standard European-sized comb foundation.

Worker bees (F_1) were obtained from 30 colonies derived from instrumentally inseminated queens. Approximately equal numbers of Africanized and European queens were used. The sampled worker bees were reared by their sisters from comb having European bee cell sizes.

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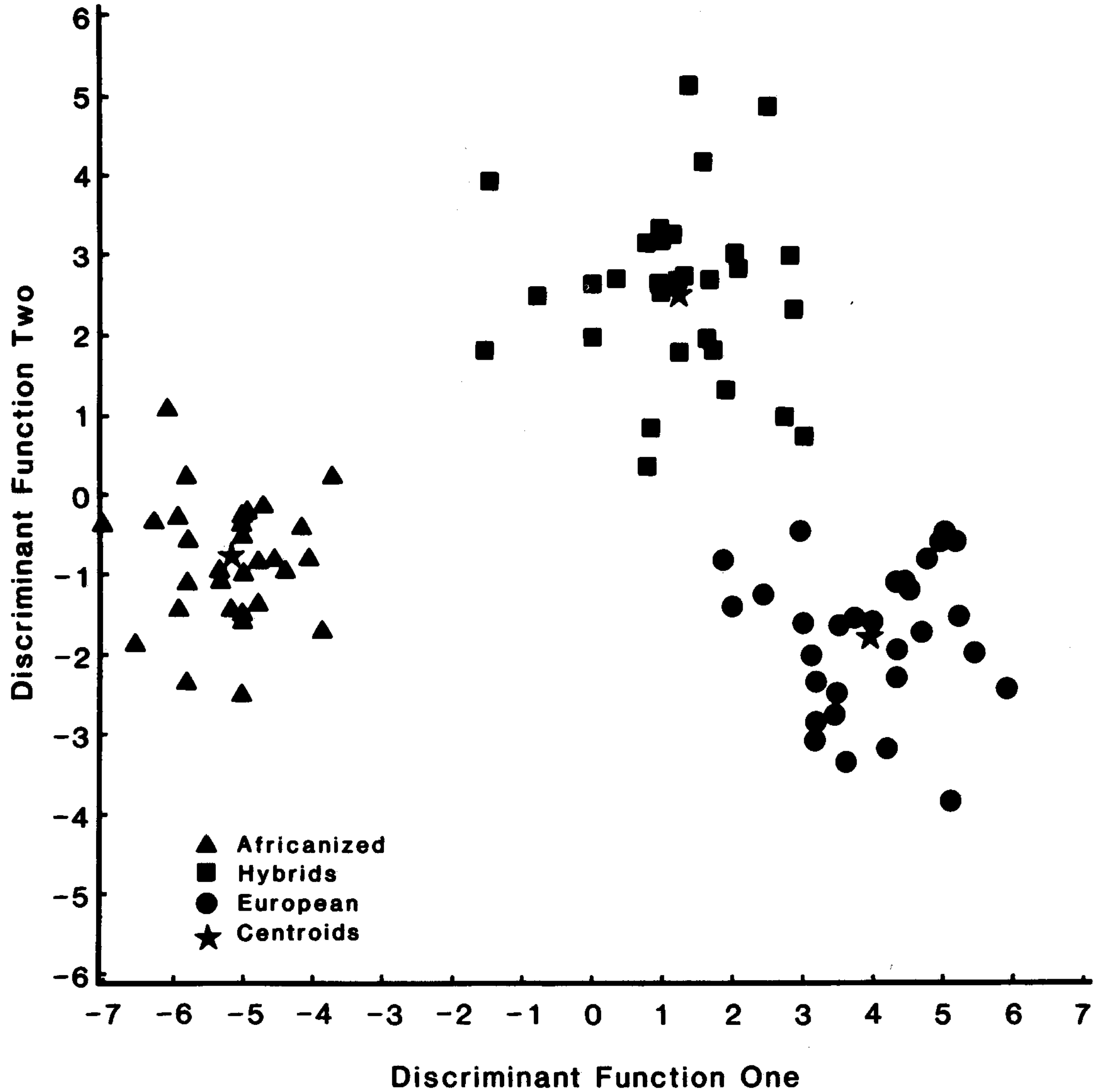

Fig. 1. Scatterplot of the results of the multivariate discriminant function analyses of Africanized and European bees and their F_1 hybrids. The centroid for each group is marked by a star. The Mahalanobis distance between centroids is as follows: Africanized to European = 8.868, Africanized to hybrid = 6.978, European to hybrid = 5.240.

All samples were measured morphometrically according to the computer-assisted procedures outlined by Daly et al. (1982). Twenty-five measurements were made (Table 1) on each of 10 bees for each sample. Sample means were calculated for each measurement. Sample means were used to estimate population means and variances.

The 25 measures were then used to compare the three groups of honey bee samples using multivariate discriminant analysis (SPSS 1983). Multivariate and univariate analyses of variance and least significant difference tests were conducted for measurements of each character to determine similarities of means.

Results and Discussion

The measurements of Africanized and European bees are remarkably similar to those reported by Daly & Balling (1978). The values for six of the characteristics of the F_1 samples were clearly intermediate; 12 were very similar or identical to the values for European bees; one was more extreme than the value for European bees; one was more extreme than the value for Africanized bees; four were very similar or identical to the values for Africanized bees; and for one characteristic, none of the values were different (Table 1). A multivariate analysis of variance (MANOVA) of the 25

Table 1. Descriptive statistics of 25 morphometric characteristics of Africanized and European honey bees and their F_1 hybrids[a,b]

Character	Africanized			European			F_1 hybrids			Type of similarity to parents[b]
	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	
Forewing length (FWLN)	8.696	0.131	8.48–9.01	9.118	0.100	8.92–9.29	9.031	0.138	8.80–9.35	A F_1 E Intermediate
Forewing width (FWWD)	2.927	0.071	2.71–3.03	3.068	0.046	3.00–3.18	3.052	0.068	2.93–3.19	A F_1 E European-like
Cubital vein distance B (CUB B)	0.221	0.015	0.19–0.25	0.219	0.018	0.18–0.25	0.239	0.017	0.21–0.28	E A F_1 more extreme than Africanized
Cubital vein distance A (CUB A)	0.503	0.030	0.44–0.56	0.554	0.026	0.49–0.60	0.552	0.023	0.51–0.61	A F_1 E European-like
Angle 29	31.22	1.30	28.2–33.5	28.82	1.14	26.2–31.0	29.77	1.88	26.0–36.0	E F_1 A Intermediate
Angle 30	105.77	3.70	99.3–112.7	111.40	3.38	104.7–120.1	107.48	3.95	100.1–115.9	A F_1 E Africanized-like
Angle 31	103.05	2.06	98.8–107.8	99.11	1.89	94.8–103.7	99.75	2.21	94.5–103.1	E F_1 A European-like
Angle 32	19.37	0.81	17.9–20.9	22.46	1.20	20.9–26.4	20.96	0.89	19.2–23.1	A F_1 E Intermediate
Angle 33	95.36	1.54	92.9–98.6	93.49	1.61	90.3–97.3	91.93	1.87	87.3–95.1	F_1 E A More extreme than European
Angle 34	50.56	1.97	46.2–56.3	52.57	1.83	49.3–57.1	51.41	1.87	47.4–56.4	A F_1 E Africanized-like
Angle 35	21.96	1.36	19.3–25.0	23.34	1.60	19.8–26.1	23.85	1.24	21.0–26.8	A E F_1 European-like
Angle 36	61.10	1.68	58.4–65.1	63.26	1.46	59.5–66.1	63.94	1.98	59.8–68.1	A E F_1 European-like
Angle 38	89.91	2.44	84.7–94.6	92.51	2.32	87.6–97.0	90.75	2.44	86.6–95.0	A F_1 E Africanized-like
Angle 39	41.15	1.29	38.4–43.2	43.74	2.09	40.0–48.6	41.75	1.72	38.5–46.6	A F_1 E Africanized-like
Hindwing length (HWLN)	4.126	0.056	4.02–4.24	4.288	0.071	4.15–4.41	4.238	0.074	4.08–4.38	A F_1 E Intermediate
Hindwing width (HWWD)	1.612	0.045	1.52–1.68	1.748	0.055	1.66–1.85	1.737	0.062	1.62–1.87	A F_1 E European-like

Table 1. Continued

Character	Africanized			European			F_1 hybrids			Type of similarity to parents[b]
	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	
Hamuli number (HAMU)	21.01	0.805	19.0–22.0	20.89	0.937	18.9–22.6	21.40	1.37	19.5–24.4	E A F_1 No differences
Femur length (FELN)	2.512	0.044	2.44–2.63	2.625	0.038	2.56–2.72	2.587	0.038	2.53–2.67	A F_1 E Intermediate
Tibia length (TBLN)	3.121	0.047	3.05–3.23	3.194	0.045	3.12–3.31	3.188	0.040	3.11–3.28	A F_1 E European-like
Basitarsus length (TRLN)	1.908	0.041	1.81–2.02	1.987	0.037	1.91–2.08	1.975	0.042	1.87–2.05	A F_1 E European-like
Basitarsus width (TRWD)	1.091	0.024	1.05–1.15	1.132	0.026	1.06–1.18	1.128	0.037	1.07–1.25	A F_1 E European-like
Third sternum length (STLN)	2.567	0.048	2.46–2.67	2.701	0.050	2.61–2.79	2.723	0.059	2.57–2.83	A E F_1 European-like
Wax mirror width (WXWDA)	2.200	0.050	2.19–2.29	2.414	0.050	2.31–2.49	2.395	0.050	2.29–2.52	A F_1 E European-like
Wax mirror length (WXLN)	1.238	0.035	1.16–1.32	1.333	0.030	1.27–1.40	1.329	0.041	1.26–1.43	A F_1 E European-like
Distance between wax mirrors (WXWDB)	0.332	0.032	0.27–0.40	0.251	0.035	0.19–0.34	0.296	0.048	0.22–0.40	E F_1 A Intermediate

[a] For each group, $n = 30$ collections of 10 bees each.

[b] Determinations based on analyses of variances followed by Duncan's multiple range tests ($P = 0.05$). A, Africanized mean; E, European mean; F_1, first filial generation mean. Symbols further right indicate larger values. Common underlines indicate no significant differences.

Table 2. Classification results of the multivariate discriminant analysis showing the numbers of samples of each group correctly classified at $P = 1.0$ to $P \geq 0.90$

Group	Probability of group membership			
	$P = 1.0$	$P \geq 0.99$	$P \geq 0.95$	$P \geq 0.90$
Africanized	30	30	30	30
European	25	29	30	30
F_1	20	27	28	29

characteristics indicated that differences existed among the three groups ($\Lambda = 0.01394$, $P < 0.0001$). A post-MANOVA analysis of the Mahalanobis distances between the centroids of the groups revealed that each group was significantly different from the other two groups.

Intermediate values for F_1 measures suggest the hypothesis that the differences between the parental types for such characters are regulated primarily by additive genes. The existence of F_1 values similar to those of the parental types or more extreme than values of both the parental types, suggests the hypothesis that the differences between the parental types are regulated by dominant or epistatic genes.

Because 19 of the 25 characteristics in F_1 bees are similar to or greater than one or the other of the two parental types, the differences between the parental types are probably regulated mostly by dominant or epistatic genes. Each parental type appears to be dominant for many of the measured characteristics. This gives the overall description of F_1's a markedly distinct composition and makes them easily identified.

The multivariate discriminant analysis correctly identified all 90 samples as Africanized, European, or F_1 hybrids (Fig. 1; Table 2). Most classifications were at $P = 1.00$ and all but one were at $P > 0.90$. The remaining sample, an F_1 colony, was correctly classified at $P = 0.55$. As expected, the discriminant analysis procedure selected the F_1 category as the second most likely classification for all Africanized and European colonies. Interestingly, the second most likely category for the F_1 samples was European for all but three F_1 samples. Thus, the procedure is conservative in its classification of F_1's in ways appropriate to regulatory needs. Only clearly Africanized or F_1 colonies will be so classified.

Table 3 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 two functions. The two sums of these products are added to the appropriate constants to calculate the two function values required to calculate the probabilities of group membership.

With a as Africanized, e as European, and h as F_1 hybrid, a first step in calculating exact probabilities (SAS Institute 1982) of group membership

Table 3. Unstandardized coefficients and constants for calculating the canonical discriminant functions in the discriminant analysis of Africanized and European honey bees and their F_1 hybrids

Character[a]	Coefficients	
	Function 1	Function 2
FWLN	6.553025	−4.704781
FWWD	−3.433558	4.123205
CUB B	1.275416	66.48794
CUB A	2.102194	1.705759
AN29	0.2802932	−0.2902689
AN30	0.008252593	−0.06326601
AN31	−0.1692561	0.08344479
AN32	0.6772359	−0.1623292
AN33	−0.2313948	−0.1948230
AN34	0.1737859	−0.1307091
AN35	−0.06032329	0.7288357
AN36	0.08439073	0.6994936
AN38	0.3706354	−0.02048285
AN39	0.02372366	−0.190694
HWLN	−3.292304	−1.365968
HWWD	−0.8262137	−3.941143
HAMU	0.05754872	−0.1383035
FELN	17.41537	−10.86704
TBLN	−33.11546	17.64643
TRLN	12.92046	−9.06967
TRWD	12.16769	−1.282477
STLN	−8.214446	7.132527
WXWDA	8.823032	12.49315
WXLN	16.14653	−7.210118
WXWDB	−0.8005740	15.28092
Constant	−64.52793	1.286616

[a] A list of characters with acronyms appears in Table 1 in the same order as the acronyms in this Table.

is to determine three generalized square distances according to the general formula (in matrix notation),

$$D_i^2 = (X - \bar{X}_i)'(X - \bar{X}_i),$$

where i is a, e, h.

Individual generalized square distances are calculated as:

$$D_a^2 = (\text{Function 1} + 5.19365)^2 + (\text{Function 2} + 0.78136)^2,$$

$$D_e^2 = (\text{Function 1} - 3.98774)^2 + (\text{Function 2} - 1.79751)^2,$$

and

$$D_h^2 = (\text{Function 1} - 1.20591)^2 + (\text{Function 2} - 2.57888)^2.$$

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

$$P_i = \frac{\exp(-0.5D_i^2)}{\sum_{aeh} \exp(-0.5D_i^2)}$$

The identification of unknown samples can be expected to classify correctly samples of Africanized and European worker bees and samples that are composed of worker bees completely or mostly from F_1 hybrid subfamilies. According to established practice, such identifications would be made with posterior probabilities of group membership of $P > 0.90$. Samples that are a mixture of bees would probably be classified with a low probability of group membership ($P < 0.90$), or the range for the majority of measurements would span two or three of the group ranges presented in Table 1. Such samples would warrant close inspection. The classification of individual bees may provide evidence of mixed samples. Data supporting the classification of individual bees have been analyzed, and discriminant functions to identify individual bees have been developed. Group and individual bee analyses are carried out by the IBM PC-compatible computer program "PHYBRID," which is available from T. E. Rinderer on the requestor's 5-¼" or 3-½" diskette.

The power of discriminant analysis to identify F_1's of Africanized and European bees suggests the limits of this tool are not yet reached. Perhaps other types of crosses also can be identified with certainty. However, new identification capabilities need to be employed with care. Newly identifiable categories of Africanized bees should not be considered as objectionably Africanized for regulatory purposes until behavioral evaluations confirm that the bees are objectionable. The objectionable nature of F_1 colonies is clear from an examination of their defensive behavior (Collins & Rinderer, in press).

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