RESEARCH

Identification of Africanized and European Honey Bees: Effects of Nurse-bee Genotype and Comb Size

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ABSTRACT Africanized or European honey bees were nursed either by Africanized or European honey bees in combs built either by Africanized or European honey bees. The resulting eight groups of adult progeny types were then measured for weight and 25 morphometric characteristics. All of the morphometric characters were used to compute a discriminant score to test the morphometrically based identification methods for Africanized and European bees. Five of the characters were also compared separately. Africanized and European progeny differed strongly in all analyses. Type of nurse bee had small and nonsignificant effects on all characteristics except bee weight. Type of comb had significant effects: European comb resulted in larger progeny and Africanized comb resulted in smaller progeny. None of these independent variables interacted, although comb type and nurse bee type showed consistent additive effects. In spite of these findings, morphometrically based identification accurately discriminated Africanized and European bees.

DALY & BALLING (1978) developed a multivariate statistical analysis in which 25 morphometric characters distinguished honey bees (*Apis mellifera* L.) of European and Africanized ancestry from the American continents. This procedure was improved (Daly et al. 1982) by including computer-assisted measurements and data recording and is currently the preferred scientific and regulatory tool for identifying Africanized bees. The need for rapid and reliable identifications will increase as the Africanized bee population approaches the borders of the United States.

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Generally, an identification using this procedure involves comparing the discriminant score derived from multivariate analysis of a sample of bees having unknown origin with a range of discriminant scores that clearly fall into Africanized and European groupings. This range of scores was developed from measurements of bees known to be Africanized or European (Daly & Balling 1978).

One goal of United States regulatory agencies will be to determine the presence of Africanized bees in commercial apiaries. This presents potential obstacles to identification since the ancestry of nurse bees may not be the same as that of immature bees they rear. Also, the comb in colonies may have been produced by bees having different ancestry from that of bees currently in the colony. Perhaps this has an important bearing on the final morphology of the bees being reared since Africanized bees, compared with European bees, build combs having smaller workers cells (Michener 1975). All combinations of nurse bees, comb type, and brood are likely to occur in managed apiaries. Carlson & Bolten (1984) suggested that "morphometric analysis is limited despite computerization (Daly et al. 1982) because worker bee size (an integral component of morphometrics) is conditional, a result of the interaction of comb cell size, genotype, and nutrition."

A second regulatory goal is the development of an identification technique for field use. Highly typical bees might be identified accurately by bee weights alone since bees from Africanized and European swarms differ so strongly (Rinderer et al. 1982). However, the effects of consistently good colony conditions resulting from commercial colony management, nurse bee type, and comb type on the weights of Africanized and European bees are not known.

In this paper we evaluate the effects of nurse-bee geographical type[1] and comb size on the taxonomic value of bee weight, five chief characteristics used in discriminant analysis, and the complete discriminant analysis of Daly & Balling (1978).

Materials and Methods

Several experimental apiaries near Sarare, Venezuela, contained the 12 Africanized and 12 European colonies used in this study. All the worker bees of each colony were the progeny of the resident queen. Africanized queens were either collected from feral swarms or were the open-mated progeny of such queens. European queens were imported from the United States.

Four groups of worker progeny were reared from each queen. Two groups were reared from eggs laid in comb built by European bees. One of these groups was nursed by bees in the parent colony; the second group was nursed by bees in a colony of the alternate geographical type. Two additional groups were reared from eggs laid in comb built by Africanized bees. Again, the parent colony nursed one group and a colony of the alternate geographical type nursed the other group.

To obtain each group of eggs, a queen was confined in her colony to an area (10 by 10 cm) of comb for 24 h. The position of the area containing eggs was marked for later reference. The comb was then either left in the parent colony or was transferred to a colony of the other geographical type.

The day before the expected emergence of adult bees, combs were removed from colonies and placed in an incubator (35°C, 50% RH). Each comb was individually caged. If the rearing colony had produced additional brood on the comb, the original area (10 by 10 cm) was isolated with a fine-mesh cage. Adults that emerged from the original area (10 by 10 cm) during the expected 24-h period (Tribe & Fletcher 1977, Harbo et al. 1981) were collected.

[1] The European honey bees used in this study were from North America and have in their ancestry representatives of several subspecies. Africanized honey bees are descendants of *A. m. scutellata* imported from Africa and their hybrids with the various subspecies previously imported into South America. Neither of these honey-bee populations constitutes a subspecies nor a race, so they are called *geographical types* to indicate that they show major characteristics typical of temperately or tropically adapted honey bees.

Three groups of 10 bees were weighed on an electronic laboratory balance after honey-sac and rectal contents were removed (Gary & Lorenzen 1976). The bees were then placed in alcohol. One group of alcohol-preserved bees was later dissected and measured morphometrically with the procedures of Daly et al. (1982).

The weights of bees and six features of the morphometric analysis were chosen for analysis. Fore-wing lengths and widths, hind-wing lengths and widths, and femur lengths were selected because they are among the more important characteristics of the 25 used in the discriminant analysis procedure (Daly & Balling 1978). The discriminant analysis scores were also used, since they represent the complete discriminating capability of the procedure. Each of the seven measures was analysed with a three-way analysis of variance exhibiting the effects of geographical bee type, nurse-bee type, and comb type.

The discriminating power of the seven univariate measurements was determined from both the data presented here and also from data of Daly & Balling (1978). The score is not a true univariate measure because it is the product of multivariate analysis. These determinations were calculated as the overall percentage of misclassifications, including both types of possible errors when the midpoint between the means of bee categories were used to separate groups. These determinations, derived from z values, are based on the assumption that each characteristic for both geographical types had a normal distribution.

Results and Discussion

For all seven characteristics, the overall means for Africanized bees are significantly different from the means for European bees ($P < 0.0001$) (Table 1). In all cases, the values for Africanized bees were smaller. The scale of score is inverted and larger numbers indicate smaller bees.

Differences in nurse bees had nonsignificant effects on the morphological measures incuded in discriminant analysis. They did affect bee weight ($P < 0.006$); bees reared by European bees were heavier (Table 1).

Bees reared in the smaller Africanized comb had smaller mean values than bees reared in European comb for all seven characteristics ($P < 0.01$–0.0001) (Table 1).

None of the major factors of bee type, nurse-bee type, or comb type interacted (Table 1). However, bee categories for each geographical bee type having bees raised by the other bee type in the other bee-type's comb were consistently most similar to bees of the alternate geographical type for all seven characteristics. This consistent trend in the data indicates that the small and nonsignificant effects of nurse-bee type and the large and significant effects of comb type are additive and, in combination, produce the most atypical bees within a geographical type.

Because of the additive effects of nurse-bee type and comb type, specific cate-

Table 1. Values of seven morphological characteristics ($\bar{x} \pm$ SE) for Africanized and European honey bees that developed from eggs laid in comb constructed by Africanized or European honey bees and nursed as larvae by Africanized or European honey bees, analyses of variance for these dependent and independent variables, and estimates of the power of the characteristics to discriminate between Africanized and European honey bees regardless of rearing conditions

	Wt of 10 bees (g) (n = 12, 3)[a]	Fore-wing length (n = 12, 10)	Fore-wing width (n = 12, 10)	Hind-wing length (n = 12, 10)	Hind-wing width (n = 12, 10)	Femur length (n = 12, 10)	Morphometric score (n = 12, 1)[b]
Bee category[c]							
A A A	0.96 + 0.02	8.72 ± 0.04	2.92 ± 0.02	4.16 ± 0.02	1.62 ± 0.02	2.52 ± 0.01	3.40 ± 0.22
A A E	0.97 ± 0.02	8.78 ± 0.03	2.93 ± 0.02	4.16 ± 0.02	1.63 ± 0.02	2.54 ± 0.01	3.42 ± 0.20
A E A	1.00 ± 0.03	8.65 ± 0.04	2.84 ± 0.03	4.11 ± 0.02	1.60 ± 0.02	2.51 ± 0.01	3.18 ± 0.24
A E E	1.06 ± 0.03	8.88 ± 0.04	2.98 ± 0.03	4.21 ± 0.02	1.66 ± 0.02	2.55 ± 0.01	2.80 ± 0.24
E A A	1.04 ± 0.03	9.00 ± 0.04	2.97 ± 0.03	4.23 ± 0.02	1.70 ± 0.02	2.59 ± 0.01	−1.04 ± 0.24
E A E	1.10 ± 0.02	9.23 ± 0.04	3.08 ± 0.02	4.33 ± 0.02	1.79 ± 0.02	2.65 ± 0.01	−1.94 ± 0.22
E E A	1.06 ± 0.03	9.08 ± 0.04	3.03 ± 0.03	4.29 ± 0.02	1.75 ± 0.02	2.62 ± 0.01	−1.45 ± 0.23
E E E	1.12 ± 0.02	9.21 ± 0.03	3.08 ± 0.02	4.34 ± 0.02	1.78 ± 0.02	2.66 ± 0.01	−1.82 ± 0.21

Analyses Source	df	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Bee type (B)	1	22.3	0.0001	220.8	0.0001	44.8	0.0001	83.0	0.0001	92.8	0.0001	148.5	0.0001	907.7	0.0001
Nurse (N)	1	8.0	0.006	1.1	0.30	0.1	0.73	1.0	0.32	1.0	0.33	1.04	0.31	3.3	0.07
Comb (C)	1	7.6	0.007	40.5	0.0001	19.3	0.0001	16.9	0.0001	13.0	0.001	23.1	0.0001	6.5	0.01
B × N	1	2.0	0.16	0.2	0.66	1.4	0.23	1.7	0.19	0.3	0.61	0.4	0.51	0.8	0.38
B × C	1	0.8	0.36	0.4	0.51	0.0	0.95	1.1	0.30	0.5	0.49	0.8	0.36	2.1	0.15
N × C	1	0.6	0.45	0.4	0.51	1.2	0.29	0.6	0.43	0.1	0.75	0.1	0.81	0.0	0.84

Discrimination							
$\bar{x}$ separation probability[d]	A	0.05	0.03	—	0.03	0.01	0.02	0.0001
	E	—	0.03	0.01	0.001	0.002	0.02	0.0001
% misclassification								
Daly & Balling (1978)		—	7.6	19.2	10.4	15.0	7.8	0
These data[e] (worst case)		100.0	33.4	100.0	44.4	38.9	12.0	1.0

[a] n is for each bee category and presented as the number of colonies and then the number of subsamples per colony.
[b] Single subsample on score is derived from the multivariate analysis of 25 different morphometric measures from 10 bees.
[c] Bee categories are presented as geographical bee type, nurse type, comb type. A, Africanized; E, European.
[d] The probabilities are derived from paired t test comparisons. Certain means for bee categories of each geographical type are collected by vertical lines. Each identified mean differs significantly from all means of the alternate geographical type. The probability is for the difference between the single category mean collected by a line least different from the other geographical type compared with the most similar mean of the other geographical type. For weight of 10 bees, none of the E bees differed significantly from all of the A bees. For fore-wing width, none of the A bees differed significantly from all of the E bees.
[e] Comparing the bee categories of the two geographical types that are most similar.

gories of bee type have reduced probabilities associated with mean separation tests when compared with the analyses of variance where categories are combined (Table 1). For bee weight, fore-wing width, hind-wing length, and hind-wing width the means of some categories of Africanized bees are not significantly different from the means of some categories of European bees (Table 1). Where the means of specific categories are different from the means of all categories of the other geographical type, the probability of differences is reduced when compared with the probability of overall differences. The important exception is morphometric score, which retains the high probability of mean separation of 0.0001 even for the closest categories of Africanized and European bees.

The percentage of misclassification based on z values evaluates the effects of nurse-bee type and comb type on the diagnostic power of the seven morphological characteristics (Table 1). No single measurement is diagnostic with the procedures of Daly & Balling (1978). Nonetheless, the discriminant analysis of the entire 25-character set is completely diagnostic. In the worst case, comparing Africanized bees nursed by European bees in European comb with European bees nursed by Africanized bees in Africanized comb, the percentage of misclassification increases for the individual morphological measures. For two measures, bee weight and fore-wing width, the percentage of misclassification rises to 100 since the Africanized bees are more typically European than the European bees. Despite these effects, only 1% misclassification is predicted for comparisons of bees in these worst case categories using the complete morphometric analysis (score).

European bees nursed by Africanized bees in Africanized comb would only occur when a beekeeper has requeened an Africanized colony that was permitted to produce its own comb. This condition is likely to be a rare circumstance in commercial apiaries. Where it has happened, the apiary owner would know. If this category is ignored, the next worst case is Africanized bees nursed by European bees on European comb compared with European bees reared by European bees on Africanized comb. Here, the predicted percentage of misclassification is zero using the morphometric score.

An interesting feature of these data is the significant effect of nurse-bee type on bee weight compared with its weak and insignificant effect on the other morphometric measures. Bee weight is apparently increased by European nursing mostly by increasing biomass not strongly related to the linear measures of body parts. A likely way for this to happen is for fat reserves to be increased. Such effects diminish the potential usefulness of bee weight as a preliminary identification tool for field use.

Nonetheless, despite morphological changes induced primarily by comb type and to a much lesser degree by nurse bee type, the morphometric analysis procedure of Daly & Balling (1978) retains its power to discriminate Africanized and European bees accurately. It is sufficiently strong that more abbreviated forms of the analysis may be developed for laboratory and field use.

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References Cited

Carlson, A. D. & A. B. Bolten. 1984. Identification of Africanized and European honey bees, using extracted hydrocarbons. Bull. Entomol. Soc. Am. 30(2): 32–35.

Daly, H. V. & S. S. Balling. 1978. Identification of Africanized honeybees in the Western Hemisphere by discriminant analysis. J. Kans. Entomol. Soc. 51: 857–869.

Daly, H. V., K. Hoelmer, P. Norman & T. Allen. 1982. Computer-assisted measurement and identification of honey bees. Ann. Entomol. Soc. Am. 75: 591–594.

Gary, N. E. & K. Lorenzen. 1976. A method for collecting the honey-sac contents from honeybees. J. Apic. Res. 15: 73–79.

Harbo, J. R., A. B. Bolten, T. E. Rinderer & A. M. Collins. 1981. Development periods for eggs of Africanized and European honeybees. J. Apic. Res. 20: 156–159.

Michener, C. D. 1975. The Brazilian bee problem. Annu. Rev. Entomol. 20: 399–416.

Rinderer, T. E., K. W. Tucker & A. M. Collins. 1982. Nest cavity selection by swarms of European and Africanized honeybees. J. Apic. Res. 21: 98–103.

Tribe, G. D. & D. J. C. Fletcher. 1977. Rate of development of workers of *Apis mellifera adansonii* L., pp. 115–119. *In* D. J. C. Fletcher [ed.], African bees: taxonomy, biology and economic use. Apimondia, Bucharest.

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