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Honey bee morphometrics: linearity of variables with respect to body size and classification tested with European worker bees reared by varying ratios of nurse bees

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SUMMARY

The effects of nutritional stress on body size of worker honey bees and their morphometrics were examined, and morphometrics of stressed bees were compared with the morphometrics of large reference populations of European and Africanized honey bees. Workers from European queens (4 commercial queens from California plus 2 open stock queens and 2 feral queens from Louisiana) were reared with 4 ratios of nurse bees:eggs: 0.5:1, 1:1, 5:1, and 100:1. Measurements of 25 morphometric variables were taken from each of 32 samples of usually ten bees per sample. Raw measurements of stressed bees were analysed separately by analysis of variance and multiple range tests. The treatments resulted in different phenotypes irrespective of the queen or her geographic origin. Workers reared at ratios of 0.5:1, 1:1 and 5:1 were consistently smaller than workers reared at 100:1. Based on the size of the workers reared at 0.5:1, the initial ratio was apparently altered by the nurse bees, who probably destroyed eggs or larvae, to yield a ratio close to 5:1. This homeostatic behaviour under stress produced adult bees closer to normal size. Principal component analysis showed that 11 of 14 variables involving distance measurements were highly related to the first principal or general size component (PC1) in both stressed and normal bees. Such variables, when standardized, had consistent linear regressions on PC1. Evidence of curvilinear regression among size-related variables was inconsistent and limited to three instances, each involving less than 1% of variance. The correlations of size-related variables with PC1 for European, Africanized, and stressed bees had a similar pattern but the correlations often differed in magnitude. The slopes of linear regression for most size-related variables of European and Africanized bees were the same. Africanized bees, however, were not simply scaled reductions of the larger European bees, but exhibited differences among most size-related variables in Y intercepts. The induced variation in morphometrics of stressed European bees simulated some features of the covariation observed in normal European and Africanized bees, but differences existed in the magnitudes of correlations, and slopes and Y intercepts of most size-related variables with respect to PC1. Despite the small size of some stressed bees, classification of the abnormal phenotypes by current methods gave only 2 samples misidentified as Africanized.

INTRODUCTION

The purposes of this study were to examine the effects of nutritional stress on the morphometrics of European worker honey bees (*Apis mellifera*), to compare the stressed bees with normal European and Africanized bees with respect to correlation and regression of variables in relation to general body size, and to test a current identification method with the stressed bees.

Of several techniques used to distinguish European and Africanized bees, discriminant analysis of 23–25 morphometric variables has become the standard method for regulatory entomology (Daly, 1988, 1991). The general approach has been improved by increasing the number of samples in the reference populations, each sample with usually 10 worker bees from a colony, and increasing the genetic diversity of reference populations. Recently, using 23 variables, Rinderer *et al.* (1993) provided new statistical criteria for classification based on analysis of 1502 samples of European bees and 565 samples of Africanized bees.

In univariate analysis, differences between sample populations of Africanized and European bees can be shown for most of the morphometric variables. This statistical distinction includes the relatively smaller size of Africanized bees. In the multivariate technique of principal component analysis, the relationship of each morphometric variable to overall body size can be examined. In such studies, the first principal component is usually considered the general size component. A component score can be computed for each bee that is a standardized measure of general size relative to all other bees in the analysis. Correlation and linear and polynomial regression analyses can be performed on the bivariate distributions of the component scores versus the standardized distributions for each of the variables (see Pimentel & Smith, 1986).

Eleven of the 25 morphometric variables are known to be highly related to general body size and contribute to the rate of correct classification of normal bees (Daly, 1992). These variables have a high heritability so the phenotypic distinction in size between Africanized and European bees has a genetic basis (Oldroyd *et al.*, 1991). However, unusually small European bees can be produced by abnormal environmental conditions. The measurements of such bees, especially those related to size, may be in the range of Africanized bees and create a risk of misidentification (Daly, 1975). This risk is not simple to evaluate because the discriminant analysis includes other variables, not highly related to general size, so that European bees even in the general size range of Africanized bees might be correctly identified.

It has been of interest, therefore, to examine the effects of unusual circumstances during the rearing of larvae on the morphometrics of adult European and Africanized worker bees. Cross-fostering experiments, in which Africanized bees were reared by European nurse bees and in cells of different dimensions, and *vice versa*, resulted in abnormal sizes of bees of both types, but the progeny were correctly identified by the morphometric method (Rinderer *et al.*, 1986). European worker bees reared on nutritionally deficient diets were abnormal and some individual bees were misidentified, but the usual samples of ten bees were correctly identified (Herbert *et al.*, 1988). Examination of adult Africanized bees that had been parasitized during their development by 0–5 *Varroa jacobsoni* indicated that the inconsistent effects were detectable only at higher rates of infestation and mainly involved the wings (Daly *et al.*, 1988). A comparable study of parasitized European bees has not been made. Finally, European worker bees reared in drone cells in a colony with only drone comb were actually smaller than Africanized bees in a majority of size-related measurements and were misidentified (Daly & Morse, 1991).

In this study, we repeated the method of Eischen *et al.* (1982a, 1982b, 1984) that varied nurse bees:larva ratios and produced profound effects on the dry weight and lifespan of the resulting workers. Our stressed bees were compared with large reference populations of Africanized and European bees to answer the following questions: do Africanized and European bees, with their close genetic similarity, also share the same statistical relationships of morphometric variables with general size such that Africanized bees are scaled reductions of European bees? Do the size-related variables in the current morphometric method for classification of Africanized bees have an allometric or curvilinear relationship to general size (Houck, 1990)? If so, this might weaken the effectiveness of the method. Are the relationships of covariation between variables and general size induced in stressed bees the same as those of normal bees? Will abnormally small European bees be misidentified by current methods?

METHODS AND MATERIALS

General colony manipulations

Four levels of nutritional stress were achieved by varying the ratio of nurse bees (i.e. young adult workers) to larvae being reared; the procedure is generally that of Eischen *et al.* (1982a, 1982b). Rearing colonies were established with four treatments of nurse bee to larva ratios of 0.5:1 (200 nurses : 400 eggs), 1:1 (400 nurses : 400 eggs) 5:1 (1000 nurses : 200 eggs) and 100:1 (40 000 nurses : 400 eggs). The intention was that the 0.5:1 treatment would have an abnormally large number of larvae reared by few nurse bees and, at the other extreme, the 100:1 treatment would have an abnormally small number of larvae reared by many nurse bees. The worker bees reared in these colonies were the 'stressed' bees.

Origin of queens and collection of eggs

Eggs were obtained from eight queens. Four commercial queens from California were sent to Baton Rouge by Dr Eric Mussen, University of California at Davis. The other fout queens came from Louisiana: two queens were from feral nests near Baton Rouge and two were from open-mated, general stock of the Honey-Bee Breeding, Genetics and Physiology Laboratory. Batches of eggs were obtained by caging queens on empty comb for one to three days under queen excluder cages measuring 7 × 8 cm (for 200 eggs) or 13.5 × 8 cm (for 400 eggs). The empty combs previously had been used only once for honey storage. Each queen produced eggs for four rearing cycles; a queen's eggs received a different nurse bees:larva treatment in each cycle. The eight queens and four treatments produced 32 samples.

Rearing colonies

For the 0.5:1, 1:1, and 5:1 nurse bees:larva treatments, nurses were obtained by collecting eclosing workers from 10 stock colonies. Groups of 200 bees < 24 h old were held in laboratory test cages (Kulinčević & Rothenbuhler, 1973) in incubators (35°C and 50% RH) for four days. Caged bees were given 50% sucrose solution, bee-collected pollen and water *ad libitum*.

Rearing colonies were assembled when eggs were at a maximum of 65–70 h old. The comb containing eggs, a comb containing honey, and an empty comb were placed in 6-frame nucleus hives. To the hive were added an appropriate number of young workers (previously chilled), a queen in a hardware cloth holding cage, a division board feeder containing sucrose syrup, and a cake (*c.* 60 g) of bee-collected pollen moistened with syrup. Nurse colonies were moved into individual 3.0 × 2.5 × 2.5 m screen cages. The hives, closed during assembly, were opened about 5 h later at dusk.

Two rearing colonies for the 100:1 nurse bees:larva treatment were standard field colonies of about 40 000 adult bees given batches of 400 eggs. All brood had been removed from the colonies on the previous day and the resident queens were placed in holding cages. Sucrose syrup and pollen cake were fed *ad libitum*.

Samples of bees

Brood combs were removed to incubators when sufficient cells had been sealed. When workers eclosed they were placed in laboratory test cages and held for three days during sclerotization; sucrose syrup, pollen and water were fed *ad libitum*. Samples of workers were chilled and placed in 70% ethanol prior to dissection for morphometric analyses.

Preparation of samples

The preserved bees were sent to Berkeley where microscope slides were prepared of the forewing, hindwing, hindleg, and third abdominal sternum. Of the 32 samples, 28 had 10 bees, two had nine bees, and two had six bees, for a total of 310 bees.

Variables measured

Each bee was measured for 25 morphometric variables using a digitizer and computer as described by Daly *et al.* (1982). The variables include 14 distances (measurements between two points such as a length or width), 10 angles between veins in the forewing, and the number of hamuli on the hindwing (Daly and Balling, 1978). The distances were abbreviated as follows: FWLN, forewing length; FWWD, forewing width; HWLN, hindwing length; HWWD, hindwing width; TBLN, hind tibia length; FELN, hind femur length; TRLN, hind basitarsus length; TRWD, hind basitarus width; STLN, third sternum length; WXLN, third sternum, wax mirror length; WXWD, third sternum, wax mirror width; WXDS, third sternum, width between wax mirrors; CUBB, forewing cubital vein 'b' length; and CUBA, forewing cubital vein 'a' length. The angles were abbreviated AN29 to AN36, AN38, and AN39. The number of hamuli was HAMU.

Statistical analyses of treatments

Analysis of variance (ANOVA) of each of the 25 untransformed variables was used to test the significance of varying levels of nutritional stress (the treatment) on worker size. In the randomized block design (blocking on queen), 'treatment × block' was used as the error term to test treatment effects. Significant *F*-tests were followed by Student-Newman-Keuls (SNK) and Duncan's Multiple Range Tests to determine pairwise differences between stress levels.

Statistical analysis of morphometrics

For comparison of the stressed bees with reference populations of European and Africanized bees, data for 25 variables were organized in four matrices as follows: (1) 'TRT', the 32 samples of stressed ('treated') bees from European queens; (2) 'EUR', 271 samples of European bees (100% of samples with probability > 0.9 of being European by classification of Rinderer *et al.* (1993) including 32 samples from managed colonies in California, 12 samples of Wisconsin stock managed in Ohio, four samples of Colorado stock managed in Ohio, seven colonies of New Zealand stock via British Colombia managed in Ohio, and 216 samples of feral colonies from California (see Daly *et al.*, 1991); (3) 'AFZ', 273 samples of Africanized bees and presumed hybrids with European bees (23.4% of samples with probability of being Africanized (pA) = 0.990–1.0; 28.9% pA = 0.900–0.99; 35.9%

pA = 0.5–0.9; and 11.7% pA = 0.0–0.5.; as classified by Rinderer *et al.* (1993) from Central America, South America, and a few swarms intercepted in the USA; and (4) the data pooled from the three collections to make a matrix designated as 'ALL' with a total of 576 samples.

The discriminant analysis used by Daly and Balling (1978) and, most recently, by Rinderer *et al.* (1993), involved variables for which the original data were distances measured in millimeters, angles measured in degrees, and a count of hamuli. For this mixture of units of measurement, the most appropriate dispersion matrix was of correlation coefficients. During the computation, the data for each variable were, in effect, standardized and became independent of the original units. Therefore, to match the transformed data used in the classification procedures, the data of matrix ALL were standardized: each raw measurement of a variable was subtracted from the mean of that variable and the remainder divided by the standard deviation of that variable. As a result, the distribution of each variable had a mean of 0.0 and a variance of 1.0, but the relationships of Africanized, European, and stressed bees with respect to each other were retained.

The standardization of the data was independent of the question of whether the distributions were normal. In a study of similar data, Daly (1992) found that a higher number of distributions were not normal than would be expected by chance alone. Some transformations did not correct this condition and it was necessary to proceed with some non-normal distributions. However, when the results of multivariate analyses of the data were evaluated empirically by several methods, they were found to be consistent with the expected biological classification.

To obtain a measure of general body size for each sample, a principal components analysis (PCA) of matrix ALL was performed, the first principal component (PC1) extracted, and the component score on PC1 computed for each of the 576 samples. These scores from the analysis of matrix ALL were used in further analyses either in the one matrix ALL or divided into three submatrices according to the collection AFZ, EUR, or TRT. To determine whether a variable's regression with respect to sample scores on PC1 from matrix ALL (designated the independent variable) was linear or curvilinear, a linear and quadratic polynomial was fitted to each of the variables (first for matrix ALL and then separately for the other three submatrices). The orthogonal polynomial method was used (Sokal & Rohlf, 1981; program POLY of Rohlf, 1989). ANOVA of each case indicated the significance of the fit of linear and curvilinear equations. The percentages of variance explained by the significant terms of the fitted curve also were computed. Analysis of covariance was performed pairwise to determine if the linear slopes (coefficient b) and Y intercepts (a) for a given variable, regressed separately on the sample scores of PC1 from matrix ALL in each of the three submatrices, were the same. Tests for differences among and between correlation coefficients were made with the z transformation as described by Sokal and Rohlf (1981).

Computations

Univariate analysis of variance (ANOVA), Duncan's multiple range test (DMRT), and Student-Newman-Keuls test (SNK) were made with SAS programs (SAS Institute Inc., 1987). The morphometric computations

TABLE 1. Values of *F* ratios for analysis of variance of 25 morphometric variables (VAR). Model for each: treatments (TRT, 4 treatments) x queens (QUEEN, 8 queens), $n = 32$. Key: * = $0.05 > P > 0.01$; ** = $0.01 > P > 0.001$; *** = $P < 0.001$.

No.	VAR	TRT	QUEEN
1	FWLN	21.06***	0.38
2	FWWD	23.53***	0.96
3	HWLN	13.10***	0.39
4	HWWD	12.44***	0.62
5	TBLN	18.54***	0.29
6	FELN	18.07***	0.41
7	TRLN	14.60***	0.43
8	TRWD	9.46***	1.06
9	STLN	7.10**	0.60
10	WXLN	5.26**	1.04
11	WXWD	7.28**	0.42
12	WXDS	0.39	3.31*
13	CUBB	6.98**	5.86***
14	CUBA	7.00**	4.54**
15	AN29	4.30*	2.61*
16	AN30	5.06**	3.01*
17	AN31	5.40**	3.90**
18	AN32	3.40*	2.82*
19	AN33	1.25	2.87*
20	AN34	0.79	6.74***
21	AN35	3.80*	7.07***
22	AN36	1.53	3.38*
23	AN38	1.04	0.33
24	AN39	3.03	6.32***
25	HAMU	9.04***	7.07***

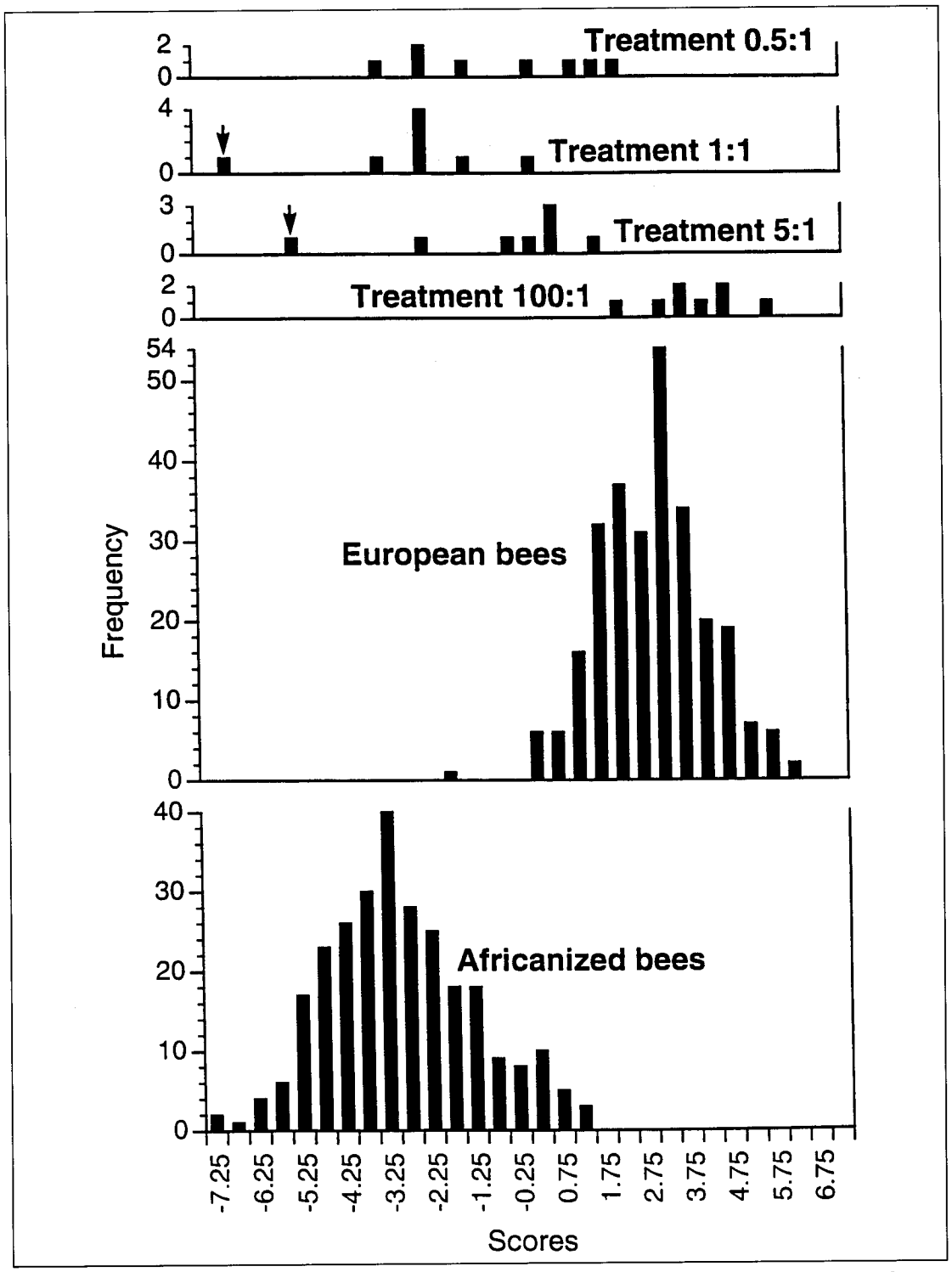

FIG. 1. Frequency distribution of PC1 scores for samples grouped according to European samples, Africanized samples, and stress treatments 0.5:1, 1:1, 5:1, and 100:1.
Note that the units of the frequency scales on left axis represent two samples for Africanized and European bees and one sample for the stressed bees. Arrows indicate two samples of stressed European bees that were misidentified as Africanized by the method of Rinderer *et al.* (1993).

were performed on an IBM AT personal computer. Most statistical procedures followed Sokal and Rohlf (1981). The program packages BIOM (Rohlf, 1989), Statgraphics 3.0 (STSC Inc., 1988) and BIOSTAT II (Pimentel & Smith, 1986) were used for orthogonal polynomial regression, correlation, sums of squares simultaneous test procedure, and principal components analyses. Other computations were made with programs written by HVD.

RESULTS

In the following sections on results and discussion, the variables were divided into four groups: 11 size-related distance variables (table 1, No. 1. FWLN to No. 11 WXWD); three size-unrelated distance variables (No. 12 WXDS to No. 14 CUBA); 10 angles of venation (No. 15 AN29 to No. 24 AN39); and the count of hamuli (No. 25 HAMU).

TABLE 2. Correlations (*r*) of variables with principal components. Numbered sequence of variables (No.), variables (VAR), component correlations with PC1-PC3 for matrix ALL, and, separately, correlations for each of AFZ, EUR, and TRT with PC1 (* = correlation different from 0.0 at probability < 0.05, i.e, for ALL, *r* exceeds critical value of 0.084 with d.f. = 574; for AFZ, *r* exceeds 0.120 with d.f. = 271; for EUR, *r* exceeds 0.121 with d.f. = 269; and for TRT, *r* exceeds 0.349 for d.f. = 30).

No.	VAR	ALL			AFZ	EUR	TRT
		PC1	PC2	PC3	PC1	PC1	PC1
1	FWLN	0.95*	–0.14*	–0.02	0.85*	0.73*	0.93*
2	FWWD	0.89*	–0.01	–0.10*	0.76*	0.60*	0.87*
3	HWLN	0.86*	–0.20*	–0.03	0.70*	0.60*	0.91*
4	HWWD	0.88*	0.06	–0.07	0.66*	0.53*	0.90*
5	TBLN	0.89*	–0.18*	–0.03	0.77*	0.75*	0.97*
6	FELN	0.96*	–0.12*	–0.04	0.85*	0.78*	0.97*
7	TRLN	0.91*	–0.13*	–0.03	0.80*	0.70*	0.97*
8	TRWD	0.85*	–0.08	–0.00	0.71*	0.65*	0.90*
9	STLN	0.92*	–0.12*	0.02	0.79*	0.62*	0.88*
10	WXLN	0.87*	–0.16*	–0.02	0.71*	0.48*	0.85*
11	WXWD	0.94*	–0.10*	–0.03	0.81*	0.55*	0.93*
12	WXDS	–0.54*	0.21*	0.11*	–0.14*	0.15*	–0.30
13	CUBB	0.35*	–0.61*	0.42*	0.24*	0.12	0.18
14	CUBA	0.53*	0.35*	–0.41*	0.32*	0.27*	0.30
15	AN29	–0.46*	–0.38*	–0.09*	–0.37*	–0.30*	–0.39*
16	AN30	0.48*	0.36*	0.20*	0.38*	0.29*	0.54*
17	AN31	–0.20*	–0.40*	0.24*	–0.07	–0.03	0.39*
18	AN32	0.72*	0.35*	0.01	0.49*	0.35*	0.60*
19	AN33	–0.31*	–0.55*	–0.66*	–0.31*	–0.31*	–0.18
20	AN34	0.23*	0.34*	–0.07	0.01	0.13*	–0.10
21	AN35	0.13*	0.58*	–0.53*	0.12*	0.07	0.28
22	AN36	0.28*	0.53*	0.68*	0.23*	0.26*	0.19
23	AN38	–0.08*	–0.22*	0.21*	–0.05	–0.15*	–0.17
24	AN39	0.34*	0.12*	0.13*	0.02	0.17*	0.19
25	HAMU	–0.06	0.11*	–0.12*	0.14*	–0.09	0.44*

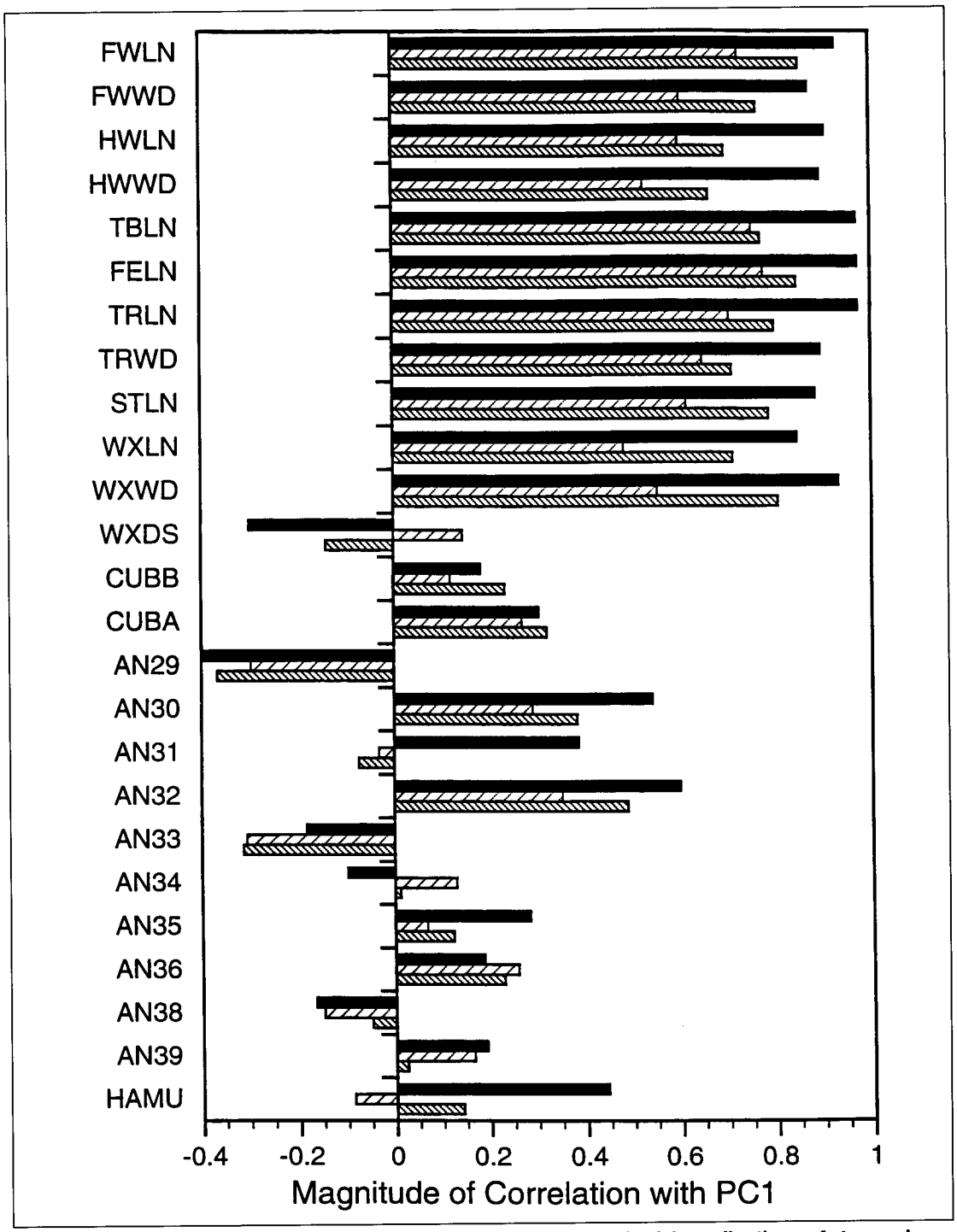

FIG. 2. Magnitudes of correlations of 25 variables with PC1 for each of the collections of stressed bees (black bars), European bees (sparse slash pattern), and Africanized bees (dense slash pattern). Explanation of the abbreviations for the variables is given in the text section on variables measured. Beginning at the top, the first eleven (FWLN to WXWD) were all highly related to PC1 as shown by high positive correlations, the next three (WXDS to CUBA) were distance measurements with lower or negative correlations with PC1, the next ten (AN29 to AN39) are the angles of venation with variable correlations, and at the bottom is the count of hamuli (HAMU) with variable correlations.

Univariate analysis of treatments

Among the treatments, ANOVA indicated significant differences for 13 of 14 distance variables, 5 of 10 angles, and the count of hamuli (table 1, significance probability < 0.05). For the size-related variables (table 1, variables 1–11), ANOVA of treatment x queens indicated differences among treatments (significance probability < 0.01), but not among queens (significance probability > 0.4). The ranked means of these variables consistently had treatment 100:1 with the largest bees and treatment 1:1 with the smallest bees; the means of the other two treatments were intermediate and varied from variable to variable in rank position. Multiple range tests (DMRT; SNK, d.f. = 3; SNK, d.f. = 21) of these variables generally indicated two groups: (1) 100:1 with the largest bees, and (2) the other three treatments of smaller bees combined. The variables of wing widths deviated from this generalization: FWWD in two of three tests exhibited a further division of the smaller stressed bees into two overlapping groups; and HWWD in one of three tests formed two overlapping groups with the intermediate treatments shared.

In ANOVA, among the 14 remaining variables (table 1, variables 12–25) all except AN38 had differences among queens (significance probability < 0.05).

TABLE 3. Tests for differences among and between correlations of variables with PC1 for Africanized, European, and stressed bees (for correlations, see table 2). Numbered sequence of variables (No.); variables (VAR); chi-square for test of homogeneity among the z-transformed correlations for each variable (Chi, * = chi-square exceeds 5.991, d.f. = 2, alpha = 0.05); pairwise *t*-tests between z-transformed correlations (AFZ:EUR, AFR:TRT, EUR:TRT, * = t > 1.96, alpha = 0.05).

No.	VAR	Chi	A : E	A : T	E : T
1	FWLN	23.72*	4.06*	1.73	3.52*
2	FWWD	19.31*	3.56*	1.73	3.30*
3	HWLN	18.48*	1.93	3.32*	4.17*
4	HWWD	21.97*	2.48*	3.34*	4.43*
5	TBLN	33.99*	0.53	5.55*	5.78*
6	FELN	32.30*	2.38*	4.51*	5.56*
7	TRLN	44.69*	2.57*	5.46*	6.59*
8	TRWD	12.29*	1.32	2.88*	3.46*
9	STLN	23.28*	4.03*	1.70	3.48*
10	WXLN	25.60*	4.21*	1.81	3.67*
11	WXWD	50.53*	5.73*	2.84*	5.37*
12	WXDS	13.99*	3.36*	0.86	2.34*
13	CUBB	1.90	1.38	0.27	0.34
14	CUBA	0.44	0.66	0.10	0.20
15	AN29	0.95	0.90	0.17	0.56
16	AN30	3.27	1.22	1.04	1.57
17	AN31	6.10*	0.49	2.47*	2.25*
18	AN32	5.35	1.94	0.80	1.66
19	AN33	0.50	0.09	0.70	0.66
20	AN34	2.72	1.39	0.56	1.18
21	AN35	1.48	0.65	0.86	1.14
22	AN36	0.22	0.36	0.22	0.38
23	AN38	1.50	1.17	0.61	0.09
24	AN39	3.04	1.66	0.87	0.14
25	HAMU	12.67*	2.67*	1.71	2.89*

Differences among treatments existed for CUBB, CUBA, AN29, AN30, AN31, AN32, AN35 and HAMU, but no consistent pattern was seen in the ranking of the treatment means or in the groups identified by multiple range tests. No differences were evident among treatments for WXDS, AN33, AN34, AN36, AN38, and AN39.

Plots of the frequency of scores for samples with reference to PC1 summarized the effects of the stress treatments on size in comparison to the reference populations of European and Africanized bees (fig. 1). The 100:1 treatment produced the samples of bees with the highest scores and as a group differed from a second group of bees reared under the other three treatments (sums of squares simultaneous test procedure, alpha = 0.05; Sokal & Rohlf, 1981).

Relationship of variables to general size

The first three components of the PCA of matrix ALL accounted for 44.3%, 9.6%, and 7.0% of the variance, respectively, for a total of 60.9% (table 2, ALL). When considered separately for each of the three collections, the correlations for the stressed bees were frequently the most extreme, either positive or negative, compared to the correlations for the two large reference populations (fig. 2; table 2, AFZ, EUR, and TRT). Heterogeneity, as shown by a chi-square test of homogeneity, existed among the correlations for the

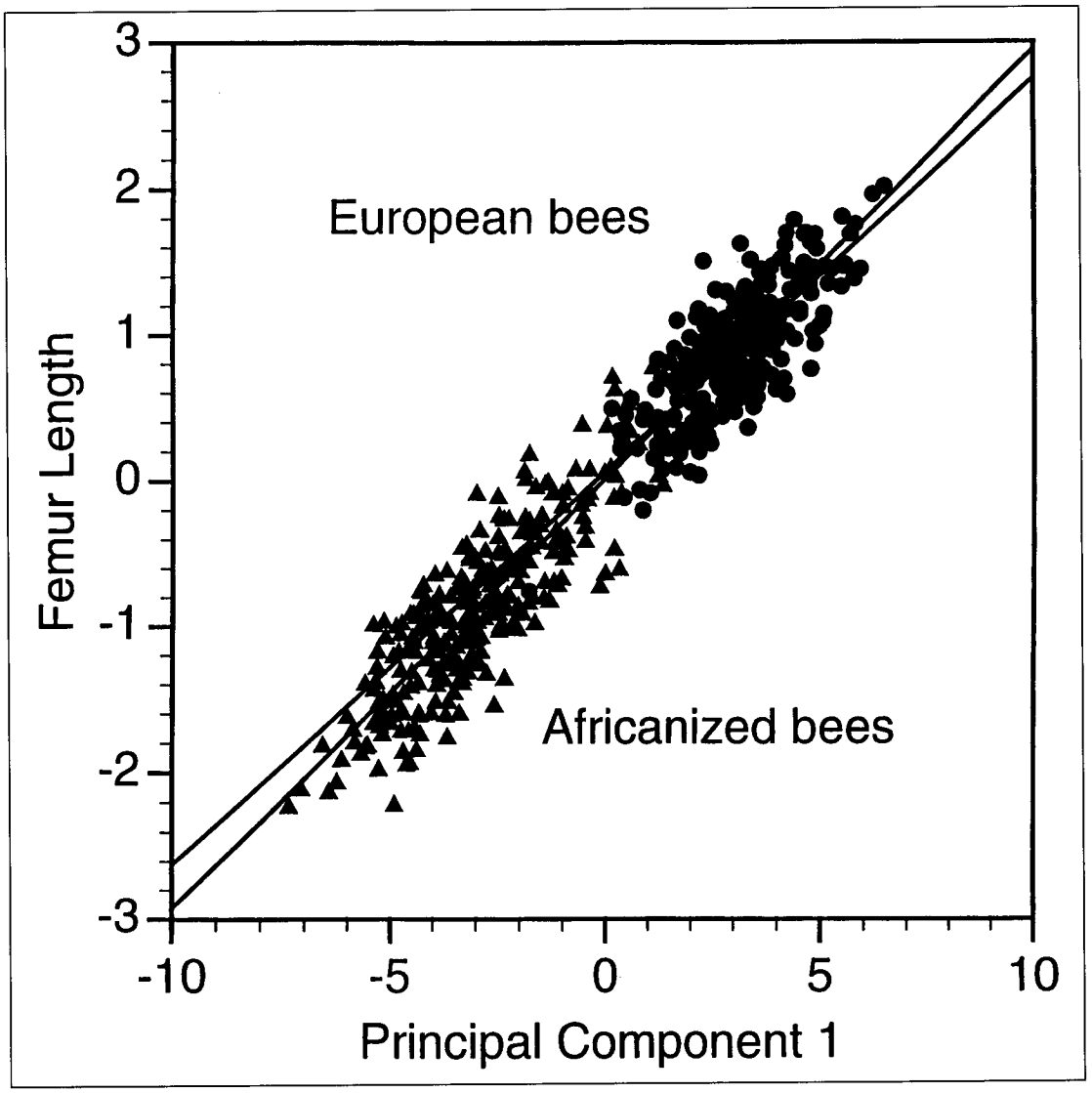

FIG. 3. Standardized femur length (FELN) in relation to sample scores on PC1 for European and Africanized bees. Plotted from PCA of matrix ALL.

size-related variables and WXDS (table 3, variables 1–12). Pairwise *t*-tests indicated that the correlations for stressed bees differed from either Africanized or European bees or both. Among the remaining 13 variables, some of which had no significant correlation with PC1, only AN31 and HAMU had differences in correlations among the collections (table 3, variables 13–25).

The first component (PC1) had the highest positive correlations with the first 11 variables that we have designated as size-related ($r \geq 0.85$; table 2, FWLN to WXWD for matrix ALL). The size-related variables were consistent in several characteristics. All had a strong linear regression on PC1 (table 4). Slopes of the regressions for FWLN, FWWD, HWLN, HWWD, FELN (figs 3 and 4), STLN, and WXLN were the same for Africanized, European, and the stressed bees (table 5). Differences, however, also were evident: Africanized and European bees differed in the slopes of WXWD (fig. 5). Stressed bees differed from both large reference populations in their slope for TRLN, and with one or the other of the large reference populations in their slopes for TBLN, TRWD, and WXWD (fig. 6). More importantly, except for FELN, the Y intercepts of the slopes had two or three pairwise differences for each variable when compared among the three collections (table 5, a). Alone among the size-related variables, the relation of FELN to general size

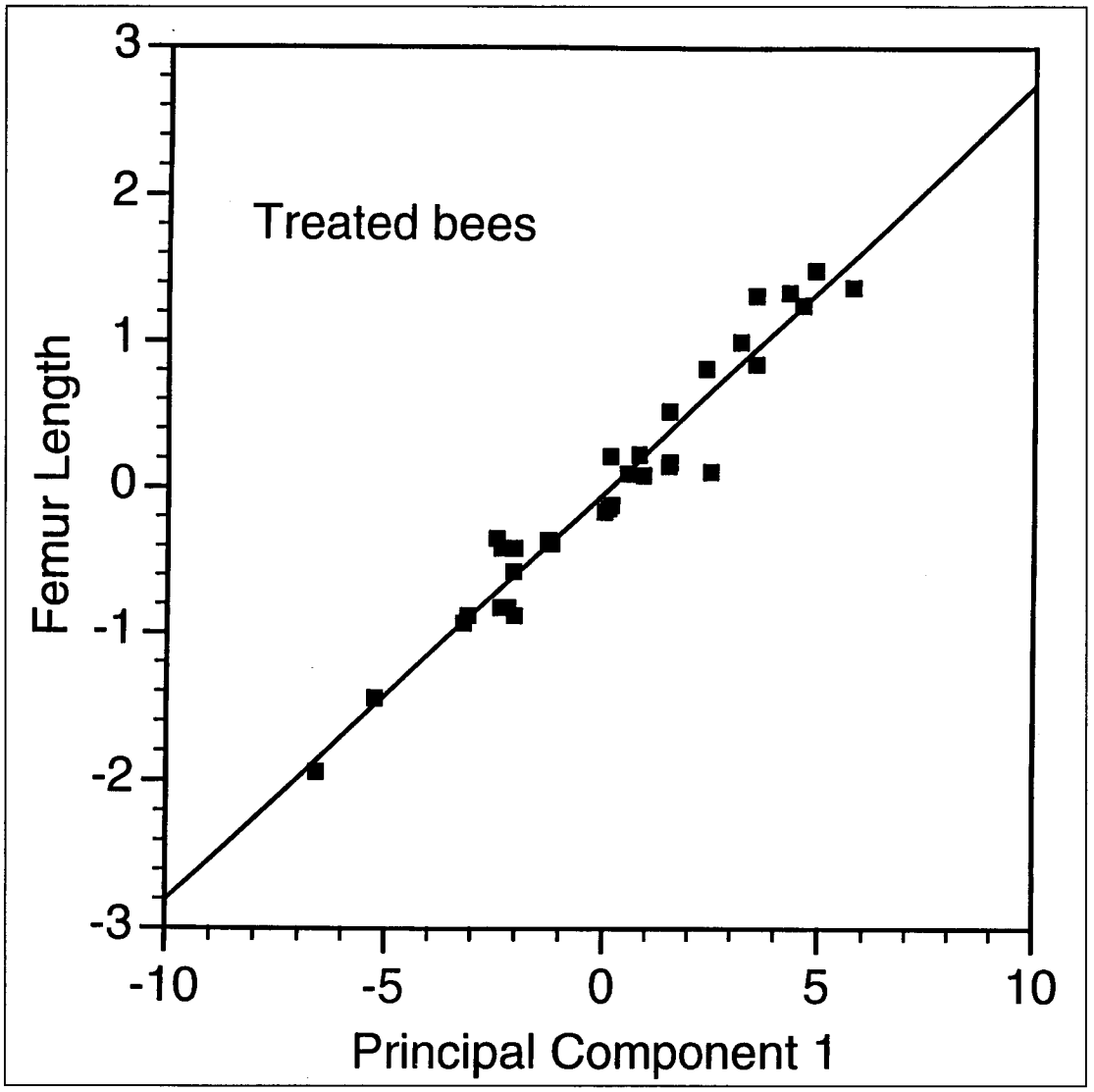

FIG. 4. Standardized femur length (FELN) in relation to samples scores on PC1 for stressed bees. Plotted from PCA of matrix ALL. The regressions for femur length of European, Africanized, and stressed bees are linear and do not differ in slopes or Y intercepts.

TABLE 4. Coefficients of determination (R^2, read as percentage of variance explained) for orthogonal polynomial fits of variables standardized and regressed on the common PC1. In the first row for each variable the coefficient is for the linear fit and in the second row beneath is the additional increment for the quadratic fit. Significance probabilities are indicated by asterisks based on F ratios. Key: * = 0.05 > P > 0.01; ** = 0. 01 > P > 0.001; *** = P < 0.001.

No.	VAR	ALL		AFZ		EUR		TRT	
		R^2	F	R^2	F	R^2	F	R^2	F
1.	FWLN	90.86	5718.42***	72.99	739.75***	52.65	298.26***	86.41	186.80***
		0.04	2.44	0.37	3.76	0.03	0.18	0.18	0.39
2.	FWWD	79.93	2291.50***	58.55	381.80***	36.60	156.96***	76.26	96.17***
		0.09	2.50	0.04	0.29	0.92	3.96*	0.74	0.93
3.	HWLN	73.45	1589.05***	48.65	256.26***	36.14	152.76***	82.20	136.18***
		0.06	1.34	0.10	0.53	0.45	1.90	0.29	0.48
4.	HWWD	77.77	2014.01***	44.07	213.60***	27.76	103.18***	80.38	118.82***
		0.10	2.55	0.22	1.06	0.13	0.47	0.00	0.00
5.	TBLN	79.06	2164.45***	59.58	398.19***	56.76	353.14***	94.31	488.79***
		0.01	0.14	0.02	0.12	0.16	1.00	0.09	0.48
6.	FELN	91.70	6351.23***	71.81	687.96***	60.60	412.48***	94.65	514.26***
		0.02	1.50	0.00	0.02	0.03	0.20	0.02	0.10
7.	TRLN	83.57	2914.67***	64.08	484.30***	49.73	267.40***	94.95	546.16***
		0.00	0.00	0.19	1.47	0.42	2.28	0.00	0.02
8.	TRWD	73.14	1563.48***	50.52	275.81***	42.22	198.78***	80.34	122.70***
		0.06	1.23	0.02	0.09	0.87	4.09*	0.67	1.03
9.	STLN	85.06	3280.23***	62.18	446.63***	37.92	163.76***	78.25	105.88***
		0.08	2.92	0.24	1.71	0.01	0.06	0.31	0.42
10.	WXLN	75.95	1823.54***	50.64	280.93***	23.44	82.04***	71.71	77.30***
		0.18	4.28*	0.69	3.82	0.00	0.00	1.39	1.50
11.	WXWD	89.23	4754.64***	65.07	505.80***	30.66	118.50***	86.82	191.04***
		0.02	1.08	0.20	1.55	0.00	0.00	0.00	0.00
12.	WXDS	28.81	233.82***	2.00	5.54*	2.16	5.93*	8.97	2.96
		0.58	4.72*	0.58	1.61	0.15	0.41	3.16	1.04
13.	CUBB	12.35	80.77***	5.55	15.88***	1.44	3.93*	3.40	1.05
		0.00	0.02	0.13	0.38	0.22	0.60	2.92	0.90
14.	CUBA	28.05	223.49***	10.38	31.29***	7.28	21.46***	9.30	3.15
		0.02	0.13	0.00	0.00	1.82	5.37*	5.13	1.74
15.	AN29	21.65	158.47***	13.39	41.79***	8.83	25.99***	15.51	5.33*
		0.07	0.53	0.13	0.42	0.11	0.34	0.12	0.04
16.	AN30	22.77	168.96***	14.75	46.85***	8.46	24.88***	29.38	12.06***
		0.02	0.12	0.24	0.75	0.40	1.17	0.00	0.00
17.	AN31	3.98	23.75***	0.54	1.47	0.10	0.28	14.95	5.12*
		0.00	0.01	0.07	0.20	2.48	6.82**	0.28	0.10
18.	AN32	52.15	626.67***	24.05	86.57***	12.48	38.22***	35.99	16.53***
		0.17	2.00	0.92	3.32	0.00	0.01	0.87	0.40
19.	AN33	9.83	62.51***	9.79	29.36***	9.38	27.75***	3.39	1.02
		0.04	0.23	0.20	0.61	0.06	0.17	0.04	0.01
20.	AN34	5.17	31.34***	0.01	0.04	1.73	4.75*	0.97	0.39
		0.33	2.03	0.32	0.86	0.83	2.27	26.94	10.84***

TABLE 4. Continued.

No.	VAR	ALL		AFZ		EUR		TRT	
		R^2	F	R^2	F	R^2	F	R^2	F
21.	AN35	1.78	10.39**	1.55	4.26*	0.46	1.23	8.07	2.65
		0.07	0.39	0.33	0.92	0.32	0.86	3.76	1.24
22.	AN36	7.90	49.37***	5.30	15.12***	6.70	19.34***	3.56	1.07
		0.34	2.14	0.00	0.00	0.40	1.16	0.16	0.05
23.	AN38	0.70	4.07*	0.24	0.66	2.23	6.15*	2.74	0.85
		0.14	0.83	0.18	0.49	0.54	1.49	3.26	1.01
24.	AN39	11.44	74.33***	0.06	0.16	2.77	7.65**	3.68	1.14
		0.40	2.58	0.53	1.43	0.37	1.04	2.91	0.90
25.	HAMU	0.35	2.02	2.03	5.60*	0.77	2.12	19.67	7.10**
		1.23	7.13**	0.18	0.51	2.60	7.22**	0.01	0.00

was essentially identical for Africanized, European, and the stressed bees (figs 3 and 4).

The next three distance variables, WXDS, CUBB, and CUBA, had lower or negative correlations with PC1 (table 2; $r \leq \pm 0.54$). They were designated 'size-unrelated' distances for brevity although each variable had a significant correlation and linear regression with PC1 in matrix ALL and when examined separately for Africanized and European bees. The correlations, slopes, and Y intercepts of CUBB and CUBA with respect to PC1 were the same for European and Africanized bees, but WXDS differed in these statistics between the collections. Stressed bees had no significant correlation or regression for these variables.

The angles exhibited significant correlations and a linear relation to PC1 in matrix ALL (table 2; $r = -0.46$ to 0.72) , but when examined separately according to collection, only AN29, AN30, and AN32, had consistent correlations and regressions (table 4). The correlations and slopes for these 3 variables also were the same among the three collections, but had differences in Y intercepts (table 3, 5). AN31 was the only angle variable to exhibit heterogeneity among the correlations according to collection (table 3). The remaining angles had no pattern that could be briefly summarized.

The count of hamuli had no significant correlation with PC1 in matrix ALL and among European bees, but a significant correlation existed for Africanized and stressed bees, resulting in significant heterogeneity among the collections for the correlations, slopes, and Y intercepts (tables 2–5).

Tests of linearity

Polynomial regression analysis of each variable in matrix ALL in relation to PC1 indicated linear regression, either positive or negative, for all variables except HAMU where no linear regression existed. Evidence of significant curvilinearity was found in matrix ALL for one of the size-related variables, WXLN, and for WXDS and HAMU, where the additional percentages of variance explained by the quadratic terms were 0.18%, 0.58%, and 1.23%, respectively (table 4). Taken separately, matrices AFZ and EUR were similar, with linear regression in most variables except AN31(AFZ and EUR), AN34(AFZ), AN35(EUR), AN38(AFZ), AN39(AFZ), and HAMU (EUR) where no linear regression was found. Evidence of curvilinearity was found among the size-related variables for FWWD(EUR, additional variance explained 0.92%) and TRWD(EUR, 0.87%). Curvilinearity among the other variables was found in CUBA(EUR, 1.82%), AN31(EUR,2.48%), and HAMU(EUR, 2.6%). The stressed bees exhibited linear regression in the 11 size-related distance variables plus AN29 to AN32, and HAMU. Curvilinearity was found in AN34 for the stressed bees, but the linear term was not significant (table 4). No significant linear relationship to PC1 was shown for HAMU in matrix ALL and the European bees, but the quadratic term was significant in both (Table 4). The opposite was true for Africanized bees and the stressed bees.

Tests of classification

Each sample of stressed bees was classified by the original method of Daly and Balling (1978) and by the most recent method of Rinderer *et al.* (1993). For the 1978 method: 22 samples were identified as European with a probability > 0.9, three samples as unidentified with a probability < 0.9 for either group, and seven samples misidentified as Africanized with a probability > 0.9. For the newer method: 30 samples had a probability of being Africanized (pA) = 0.00–0.06 and were identified as European (combined commercial and feral groups), and two samples, one each from treatments 1:1 and 5:1 with pA = 0.94 and 0.98, respectively, were misidentified as

'Africanized with evidence of introgression of European genes'.

DISCUSSION

As expected, a wide range of body sizes was produced in bees that were reared by different numbers of nurse bees. Differences in 19 of the 25 morphometric variables were observed among the treatments (table 1). The frequencies of sample scores for the first principal component (PC1) illustrated the overall effects of treatments in comparison to Africanized and European bees (fig. 1). The largest bees were reared under treatment 100:1. They formed a distinct group based on sample scores and overlapped the scores of normal European bees. The other three treatments produced smaller bees that formed a second group and overlapped the distribution of sample scores for Africanized bees. The range in sizes among treatments was considerable: mean forewing length under treatment 1:1 was 8.62 mm versus 9.24 mm for treatment 100:1. In this regard, the experimental bees exceeded the range of mean forewing sizes of feral Africanized and feral European bees at 8.67 and 9.15 mm, respectively, as reported by Rinderer *et al.* (1993).

Bees reared from the most severe treatment of 0.5:1 were relatively larger than would be expected based on the trend toward smaller size indicated by the increasing stress of treatments 5:1 and 1:1 (fig. 1).

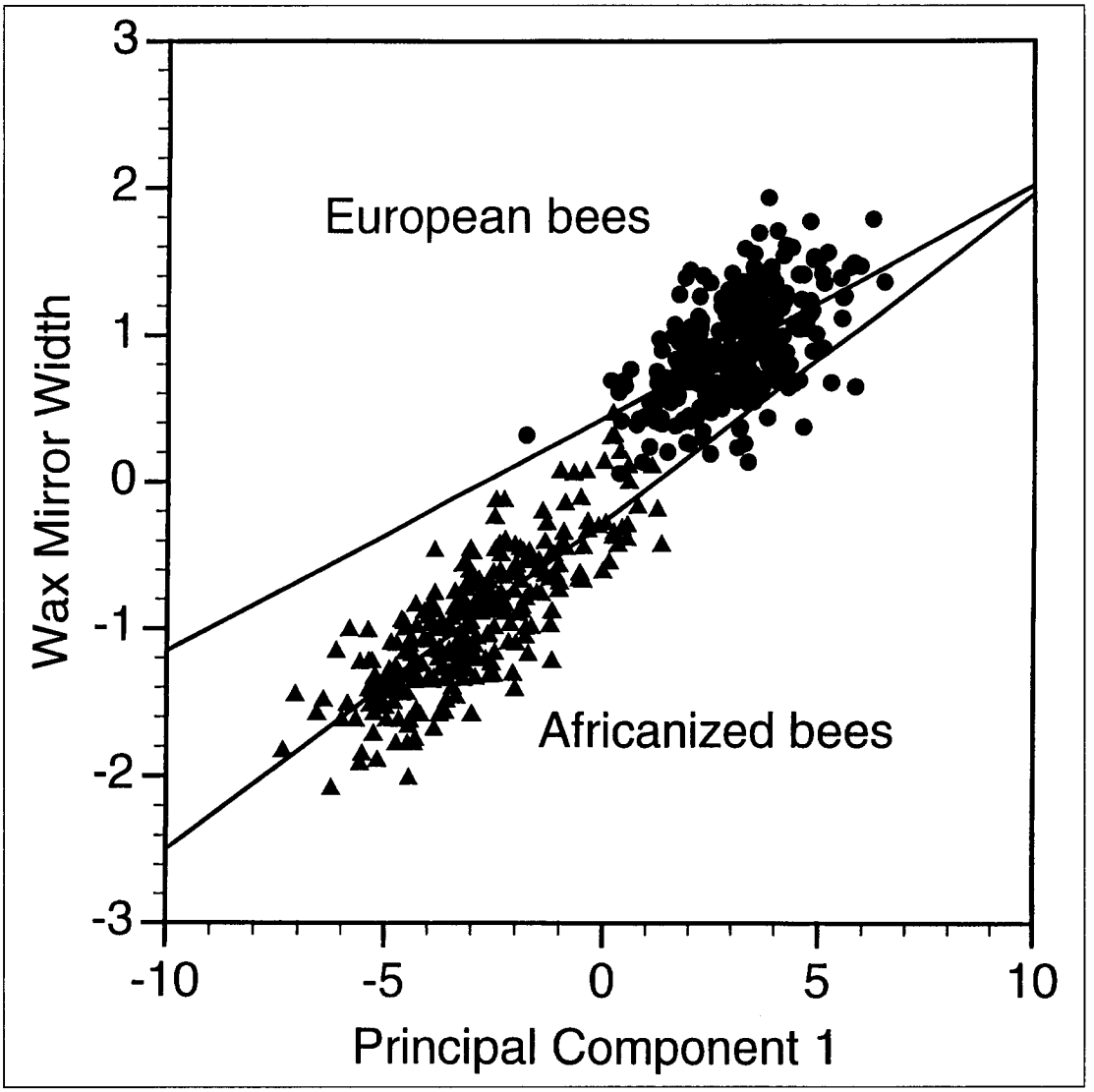

FIG. 5. Standardized width of wax mirror (WXWD) in relation to sample scores on PC1 for European and Africanized bees. Plotted from PCA of matrix ALL.

TABLE 5. Pairwise analysis of covariance for linear regressions of variables with PC1 for Africanized, European, and stressed bees (for coefficients of determination see table 2). Numbered sequence of variables (No.); variables (VAR); *F* tests for differences between slopes (b) and Y intercepts (a) for AFZ:EUR (d.f. (b) = 540, d.f. (a) = 541), AFR:TRT (d.f. = 301, 302), EUR:TRT (d.f. = 299, 300). Key: * = 0.05 > *P* > 0.01; ** = 0.01 > *P* > 0.001; * = *P* < 0.001.**

No.	VAR	A:E		A:T		E:T	
		b	a	b	a	b	a
1	FWLN	0.02	12.14***	1.06	13.27***	0.86	29.96***
2	FWWD	2.09	5.87*	0.70	2.98	0.13	0.01
3	HWLN	0.10	3.17	1.36	95.41***	0.67	53.83***
4	HWWD	1.88	5.97*	1.57	44.96***	0.01	17.47***
5	TBLN	1.26	39.63***	5.28*	107.03***	2.07	16.05***
6	FELN	2.02	0.23	0.57	0.33	0.20	2.22
7	TRLN	0.02	15.35***	12.19***	38.45***	9.26**	0.14
8	TRWD	1.92	14.30***	6.88**	74.31***	1.72	13.57***
9	STLN	1.69	5.50*	0.58	55.58***	0.11	20.03***
10	WXLN	1.58	1.37	1.23	42.27***	0.00	16.49***
11	WXWD	13.58***	131.56***	0.21	74.71***	10.16**	2.31
12	WXDS	11.21***	54.23***	0.02	17.74***	7.84**	4.32*
13	CUBB	0.13	0.04	1.10	0.57	0.28	0.07
14	CUBA	0.35	0.34	1.08	0.42	1.78	0.16
15	AN29	0.82	7.15**	0.27	18.37***	1.11	3.42
16	AN30	0.51	7.91**	0.00	17.92***	0.22	1.49
17	AN31	0.05	1.90	7.07**	44.16***	3.82	9.77**
18	AN32	2.66	2.21	1.39	97.28***	3.35	21.88***
19	AN33	3.09	17.70***	2.66	0.59	6.22*	3.02
20	AN34	3.26	1.44	0.29	62.26***	2.99	24.38***
21	AN35	0.04	1.08	0.23	3.50	0.28	0.20
22	AN36	4.07*	7.72**	0.52	0.16	3.63	3.75
23	AN38	2.11	1.93	0.01	3.04	1.25	0.35
24	AN39	3.59	4.63*	0.54	0.95	0.66	0.48
25	HAMU	6.51*	4.57*	3.87*	57.42***	12.25***	13.09***

Nurse bees in some nuclei receiving the severe treatment apparently destroyed or did not rear some of the larvae. This behavior had a homeostatic effect by shifting the treatment toward a more normal ratio. As a result, bees from this treatment could not be distinguished from treatments 1:1 and 5:1 in sample scores or in multiple range tests of the morphometric variables.

The principal component analysis of the matrix ALL, in which all samples were pooled, provided a first component (PC1) with the properties appropriate for a general size component. PC1 involved the highest percentage of variance; it had high positive correlations with a majority of variables that measure size such as lengths and widths; and it had lower or no correlations with other variables, such as angles and counts, that are usually not considered measures of size (table 2). The correlations of the 25 variables with PC1 for the Africanized, European and stressed bees, when plotted separately, were similar in magnitude relative to each other (fig. 2).

All variables in matrix ALL, except the count of hamuli (HAMU), exhibited a linear regression on PC1. Evidence of curvilinear relations between a variable and

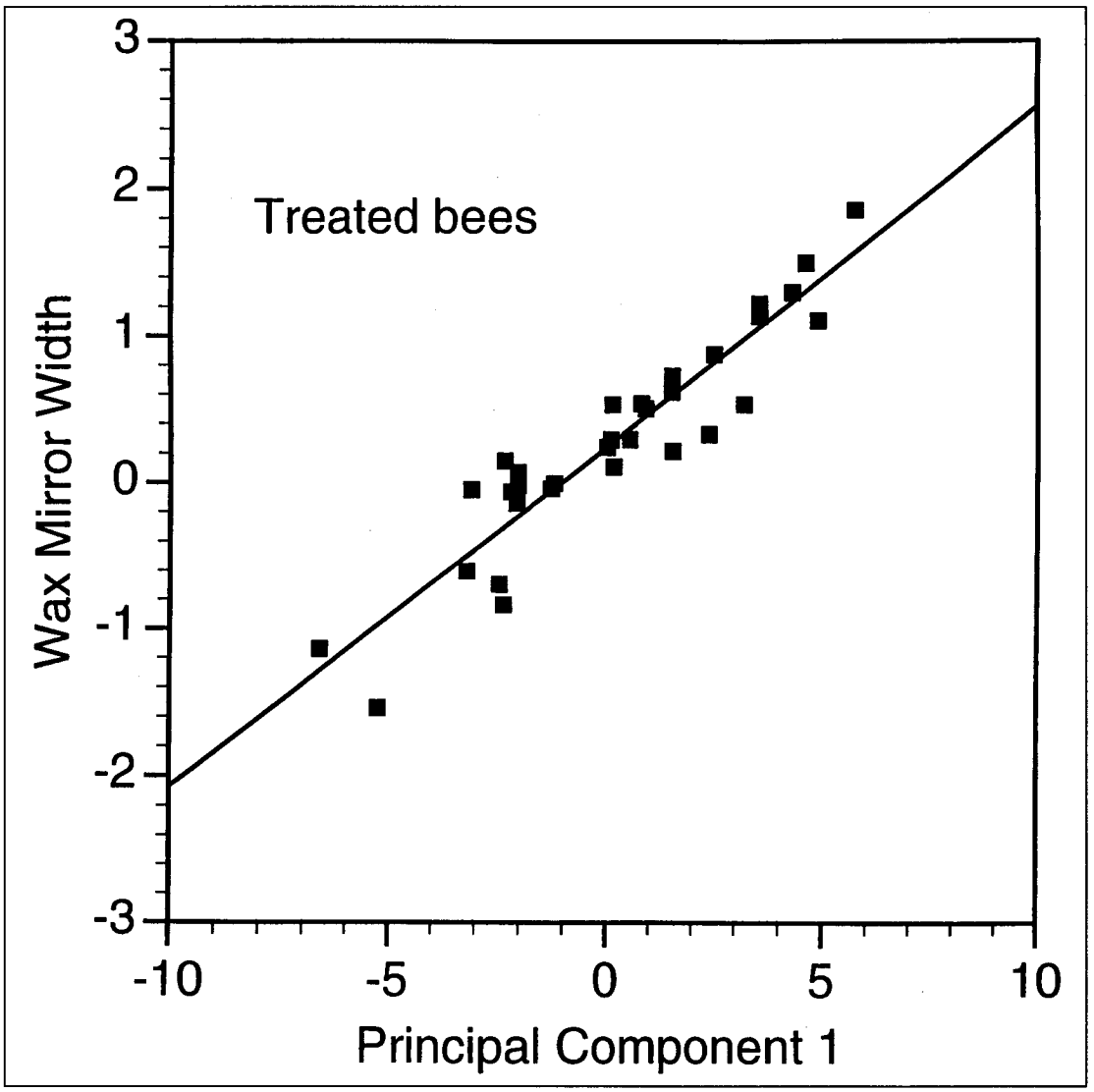

FIG. 6. Standardized width of wax mirror (WXWD) in relation to sample scores on PC1 for stressed bees. Plotted from PCA of matrix ALL. The regressions for width of wax mirror of European, Africanized, and stressed bees are linear, but some slopes and Y intercepts differ (table 5).

PC1 in matrix ALL was found for wax mirror length (WXLN) and distance between wax mirrors (WXDS), with the quadratic term for each accounting for an additional 0.18% and 0.58% of variance, respectively. Evidence for curvilinearity in the regressions of size-related variables on PC1 in Africanized and European bees, when considered separately, was inconsistent and involved two instances, each with less than 1% of additional variance explained by the quadratic term (table 5). Curvilinear relations with PC1 among the other 14 variables in matrix ALL and across the reference populations was also inconsistent. Of 6 instances, 4 had no significant linear regression.

Africanized and European bees exhibited the same slopes for linear regression of 10 of the 11 size-related variables with PC1. Some, but not all, of the corresponding slopes for variables of the stressed bees were the same. These similarities point to common morphogenetic mechanisms in Africanized, European, and the stressed bees. However, only one variable, femur length (FELN), had consistent linear regressions with the same slopes and Y intercepts across all three collections. In this respect, the stressed bees were phenocopies of the same relationship of femur length to general size that was exhibited by Africanized and European bees (figs 3 and 4).

Conversely, numerous differences existed in the magnitudes of correlations, slopes, and Y intercepts of variables in relation to general size when compared pairwise between the Africanized and European bees and between these and the stressed bees. Noteworthy was the width of the wax mirror (WXWD), the single size-related variable to differ both in slope and Y intercept between Africanized and European bees (fig. 5). The induced phenotypes of stressed bees differed from both of the large reference populations in the relationship of WXWD to PC1 (fig. 6). Distance between wax mirrors (WXDS), was similar in this regard. As a distance measurement, WXDS is unique because it usually has a negative relationship to general size: the mean distance is larger in Africanized bees than European bees (Daly & Balling, 1978). In our analysis, WXDS for European bees had a slight positive correlation with the common PC1, but it was negative in matrix ALL and for Africanized bees (table 2). WXDS in the stressed bees was the only distance measurement to exhibit no response to the treatments and its negative correlation with PC1 was not different from zero.

The range in sizes that we obtained was suitable to test whether abnormal bees could be correctly identified by morphometric procedures. The original method of Daly and Balling (1978) classified three of 32 samples of stressed bees as unidentified and seven samples as Africanized. The method of Rinderer *et al.* (1993) gave only two samples misidentified as Africanized. The new method is clearly superior in correctly classifying abnormal European bees, in part because the European bees in the new, larger reference population contained a wider range of sizes. The two samples misidentified were one each in treatments 1:1 and 5:1; they were unusually small with mean forewing lengths of 8.22 and 8.48 mm, respectively.

In conclusion, when European bee larvae were not given adequate food in our experiment, the exoskeletons of the adults were miniaturized in a pattern that duplicated, in part, the statistical relationships of morphometric variables to general size shared by normal European and Africanized bees. However, Africanized bees and stressed bees were not scaled reductions of European bees in all aspects, which doubtless contributes to the success of classification by discriminant analysis. The stressed bees retained enough features of the parent race to be correctly classified in all but two samples of the smallest bees.

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