

## Clinal geographic variation in feral honey bees in California, USA

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**Summary** — Feral honey bees in California are mongrel populations, partially differentiated in morphometrics from managed colonies as well as from European subspecies. Most morphometric variables had low but significant correlations with environmental factors and clinal patterns of spatial autocorrelation with distance. Some measurements of body size exhibited Bergmann's Rule, but the hind legs did not exhibit Allen's Rule. The geographic variation is presumably adaptive and has developed within 138 years in the presence of a large, mobile beekeeping industry. Colonies of small worker bees in the warmer and drier regions of the state may represent an ecotype adapted to desert conditions.

***Apis mellifera* / feral colony / morphometrics / geographic variation / Bergmann's Rule**

### INTRODUCTION

This paper examines the morphometric relationships of feral and hive bees in California and selected populations elsewhere. A search was made to identify European subspecies among feral bees in California by morphometric methods. Feral colonies were also studied to determine whether clinal geographic variation possibly could have developed during the past

138 years in the presence of a large, mobile beekeeping industry from which queens and drones must contribute annually to the gene pool of feral populations.

With certain exceptions, native populations of *Apis mellifera* L in Europe and Africa exhibit geographic variation. An overall decrease exists in body size from high to low geographic latitudes that corresponds to Bergmann's Rule. In higher latitudes of northern Europe and in central

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\* Correspondence and reprints

Russia, the proboscis and legs also are shorter relative to the body, thus corresponding to Allen's Rule (Alpatov, 1929; Ray, 1960; Ruttner, 1988). Alpatov (1929), failing to find the same rules in clinal variation among samples of bees from the eastern United States, commented: "It would be indeed unreasonable to expect such a rule if we remember how many queens are sent every year from the southern to the northern states."

Honey bees were successfully established in apiaries near San Jose, California, beginning in 1853 (Watkins, 1968a). The hives originated in New York and were presumably of *Apis mellifera mellifera* L. The Italian race, *A m ligustica* Spinola, was introduced to the eastern United States in 1859 (Sheppard, 1989a,b) and queens were brought to California in 1860 (Watkins, 1968b). Until 1922, when further importations were banned, as many as 8 subspecies had been introduced to North America, including some from Africa (Morse *et al*, 1979; Sheppard, 1989a,b). However, the Italian race soon predominated in managed apiaries.

Except for certain requirements that bees be certified free of disease, colonies have been transported freely among the states by migratory beekeepers and sold in interstate commerce. About 70% of the nation's commercial crop pollination occurs in California (Robinson *et al*, 1989). Mussen (1990) estimated that 650 000 managed colonies are moved each year to at least several locations within California for pollination of crops. Of these about 471 000 colonies are also moved to other states for about 6 months.

*A m mellifera* and *A m ligustica* are readily distinguished by morphometrics (Ruttner, 1988) and exhibit differences in allozymes of malate dehydrogenase. Sheppard (1988) found Mdh<sup>80</sup>, the allozyme characteristic of *A m mellifera*, still

extant among feral populations in the eastern United States. We searched here for morphometric evidence of these and other subspecies.

## MATERIALS AND METHODS

Honey bees were considered to be feral if the colony was established in a natural cavity such as a tree hole or in a man-made cavity such as the space between walls in a building (table 1, CAF). Other characteristics of feral nest sites from which we obtained samples have been discussed by Gambino *et al* (1990). Also classed as feral were swarms not in the immediate vicinity of an apiary (CAS) and samples taken from flowers, water or other food sources in wilderness areas remote from apiaries (CAC). Although samples of CAS and CAC could have been from managed colonies, only 3 colonies of CAS in subsequent morphometric analyses resembled samples of hive bees. A total of 278 samples of feral bees (CAF, CAS, CAC) were used for the analyses in which feral bees were compared to hive bees from California and other colonies elsewhere in the world. However, for comparisons among feral bees in California, only 240 samples at 169 sites (210 samples of CAF, 16 of CAS, 14 of CAC) were used because these had detailed locality information. Where more than one collection was made at a given site, one sample was arbitrarily selected according to the lowest accession number, giving 169 samples for spatial autocorrelation analysis.

During 1986 and 1987, samples of 20–30 worker honey bees each were taken from nest entrances or from swarms with a modified, battery-powered vacuum cleaner or an insect net. Samples were killed and preserved in 70% ethyl alcohol. Some additional samples of feral bees were collected in alcohol at about the same time by personnel of the California Department of Food and Agriculture.

A fore wing, hind wing, hind leg, and the third abdominal sternum (counting from the petiole) were removed from each of 10 bees from a sample and mounted on a microscope slide. Using the method initially devised for discriminating Africanized bees from European bees (Daly and Balling, 1978; Daly *et al*, 1982), 25 measurements were made on parts of each bee as fol-

**Table 1.** OTUs used in this study, their abbreviated names and number of samples.

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<i>African group (AFR)</i>	
ADA	<i>A m adansoni</i> from West Africa; <i>N</i> = 12.
AFZ	"Africanized bees" from Central America, South America, and a few swarms intercepted north of Mexico; <i>N</i> = 273.
CAP	<i>A m capensis</i> from the Cape region of South Africa; <i>N</i> = 15.
SEA	<i>A m scutellata</i> from East Africa; <i>N</i> = 31.
SSA	<i>A m scutellata</i> from Southern Africa; <i>N</i> = 43.
<i>Central Mediterranean group (CME)</i>	
AMN	Mostly managed colonies with some feral colonies and swarms from North America excluding California; <i>N</i> = 212.
AMC	Mostly managed colonies from Central America; <i>N</i> = 135.
AMS	Managed colonies from South America; <i>N</i> = 118.
CAH	Established colonies in man-made hives in California; <i>N</i> = 49.
CAF	Established feral colonies in California from natural or artificial cavities or exposed; <i>N</i> = 244.
CAS	Swarms from California; <i>N</i> = 20.
CAC	Composite samples taken from traps, flowers, other food sources, or water in wilderness areas of California; <i>N</i> = 14.
CAR	<i>A m carnica</i> from inbred lines at Institut fur Bienenkunde, Oberursel, West Germany; <i>N</i> = 8.
LIG	<i>A m ligustica</i> from Kangaroo Island, Australia; <i>N</i> = 11.
<i>West Mediterranean group (WME)</i>	
MSA	Probably <i>Apis m iberica</i> , samples from Colombia (15) and Venezuela (13); <i>N</i> = 28.
MAU	<i>A m mellifera</i> from Sweden (1) and Tasmania (7); <i>N</i> = 8.

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Major geographic groups (AFR, CME, WME) are those of Ruttner (1988). *N* = number of samples.

lows: fore wing length (FWLN); fore wing width (FWWD); fore wing cubital vein "a" length (CUBA); fore wing cubital "b" vein length (CUBB); angles between veins of the fore wing (AN29 to AN36, AN38, AN39); hind wing length (HWLN); hind wing width (HWWD); count of ha-

muli on hind wing (HAMU); hind femur length (FELN); hind tibia length (TBLN); hind basitarsus length (TRLN); hind basitarsus width (TRWD); 3rd sternum length (STLN); 3rd sternum, wax mirror length (WXLN); 3rd sternum, wax mirror width (WXWD); and 3rd sternum,

width between mirrors (WXDS). Arithmetic means of measurements for the 10 bees were used for statistical analysis.

Latitude (LAT) and longitude (LON) to the nearest minute (converted to decimal degrees) and elevation (ELE) to the nearest 50 m relative to sea level (range -50 to 1 700 m) were determined from quadrangle maps for 169 collection sites (Anonymous, 1986a, b). Five environmental variables at each site were estimated by projecting maps with isoclines of various climatic factors (Leighly, 1938; Bennett, 1965; Donley *et al*, 1979) onto a map of collection sites. These projections were aided with enlarged photocopies of maps on transparent film, a Bausch and Lomb stereo zoom-transfer scope, and overhead projector. The following environmental variables were recorded:

**SUN:** Annual mean daily insolation in Langleys per day (1 Langley = 1 g-cal/cm<sup>2</sup>); coded as 1 : < 350; 2 : 350-400, 3 : 400-450, 4 : 450-500, and 5 : 500-550 Langleys/day (no collection sites with higher values). Annual insolation is lowest along the northern coast and highest in the Mojave Desert.

**TEMPR:** Annual range of temperature in °C, measured as the difference between the average temperature of the coldest month and the average temperature of the warmest month; coded as 1 : < 6°; 2 : 6-9°; 3 : 9-12°; 4 : 12-15°; 5 : 15-18°; and 6 : 18-21° (no collection sites with higher values). Annual temperature range is lowest along the northern coast and highest in southern interior deserts such as Death Valley.

**FRZFREE:** Mean annual length of freeze-free period in days (mean number of days per year with minimum temperature > 0 °C); coded as 2 = 120-180 (no collection sites with values lower than these), 3 = 180-240, 4 = 240-300, 5 = 300-330, and 6 = over 330 days. The lowest number of days are in higher elevations of northern California and the Sierra Nevada, higher values are at lower elevations of interior valleys and along coast, and the highest value is along the southern coast.

**RAIN:** Mean annual precipitation in cm. For the state, we estimated values for sites as the middle values of isoclines given by Donley *et al* (1979) and for the San Francisco Bay area, we used values given by Rantz (1971). Values ranged from 5 to 175 cm, with no collections at sites with values above or below this range.

**SIGRAIN:** Significant precipitation measured in number of days with precipitation in excess of 0.25 cm; coded as 1 = < 15 days per year, 2 = 15-30, 3 = 30-45, 4 = 45-60, 5 = 60-75, and 6 = > 75 days per year. Significant precipitation and mean annual precipitation are lowest in southern interior deserts and highest on the north coast and in the northern Sierra Nevada.

Finally, 15 geographic/climate areas were delimited on the basis of climatic zones and the proximity of collections in an area (fig 4). Ten regional climate zones are commonly used in daily weather reports for the state. Collections were made in 7 of these zones (south coast/mountain, desert, San Joaquin Valley, central coast, San Francisco Bay, Sacramento Valley, Northwest California), but no collections were made from Shasta-northeast California, Sierra Nevada/Lake Tahoe, or central coast inland climates. Where possible, some climate zones were further subdivided by eye such that an area had an average cluster of about 16 collection sites with a range of 4-44 collections. As an indication of the different habitats represented, native vegetation types (Kuchler, 1977) are given although many areas now are predominantly agricultural or urban. The geographic coordinates of the center of a geographic/climate area were determined for the midpoint between the maximum and minimum latitudes and longitudes, respectively, of collection sites within the area. The 15 areas and number of samples (*N*) were as follows.

**SAND:** coastal, coastal valleys and mountain valleys in San Diego County west of the coast range divide; south coast/ mountain climate; mostly southern oak forest, chaparral, and coastal sage; *N* = 19.

**AZBR:** western Colorado desert in Anza Borrego State Park (eastern San Diego County); desert climate; Sonoran creosote bush and juniper-pinon woodland; *N* = 4.

**INDS:** interior desert of Mojave and Sonoran deserts of southeastern California at middle and low elevations; desert climate; creosote bush with some dry woodlands at higher elevations; *N* = 44. Some collection sites located in forested San Bernardino mountains and coastal-influenced chaparral and sage communities of western Riverside County. Most sites were near the edge of the desert zone.

**LKIS:** A cluster of sites near Lake Isabella in a mountain basin at the confluence of the north

and south forks of the Kern River; near boundary between south coast/mountain and Sierra Nevada/Lake Tahoe climates; blue oak-digger pine, chaparral, and California prairie;  $N = 16$ .

*LAOR*: area with coastal climate influence west of coast range divide and mainly in Los Angeles and Orange Counties; south coast/mountain climate; southern oak forest and coastal sage;  $N = 18$ .

*STBA*: coastal and coastal valley areas, mainly in western Santa Barbara County; south coast/mountain climate; southern oak and oak savanna;  $N = 16$ .

*KERN*: southern end of San Joaquin Valley in Kern County; San Joaquin Valley climate; California prairie and San Joaquin saltbush;  $N = 32$ . Distinguished as a separate group because of the large number of samples taken here.

*SAJO*: central San Joaquin Valley north of Kern saltbush zone; Sacramento Valley climate; California prairie and tule marsh;  $N = 10$ .

*MONT*: coastal valley and interior valley of Monterey County; central coast climate; blue oak-digger pine and prairie;  $N = 5$ .

*SACL*: coastal valley of Santa Clara County; San Francisco Bay area climate; broadleaf and mixed broadleaf-conifer forest and oak savanna;  $N = 15$ .

*SMSF*: San Mateo and San Francisco Counties with direct ocean or coastal climate influence; San Francisco Bay area climate; mixed hardwood forest and northern coastal grass communities;  $N = 14$ .

*EBAY*: coastal valley and intermediate valley zones of coast ranges on east side of San Francisco Bay; San Francisco Bay area climate; mostly mixed hardwood forest;  $N = 15$ .

*MARN*: northern coastal and coastal valleys of Marin County; San Francisco Bay area climate; mixed hardwood and oak-pine forest;  $N = 6$ .

*SACV*: valley and adjacent foothills of Sacramento Valley; Sacramento Valley climate; blue oak-digger pine and riparian forest;  $N = 12$ .

*NWCA*: large area of coast and coast range of northwestern California; northwest California climate; mostly blue oak-digger pine forest;  $N = 14$ .

The morphometrics of 278 feral samples (grouped as OTUs CAF, CAS, CAC of table I) and 49 samples of hive bees in California (CAH)

were examined by discriminant function analysis (DFA) in relation to 894 samples from elsewhere in the world that were grouped in 12 OTUs (table I). This was followed by cluster analysis (unweighted pair-group method using arithmetic averages or UPGMA) of the centroids of the 16 OTUs. Geographic variation among 240 samples of feral honey bees in California was examined in five steps: 1), bivariate correlations of each morphometric variable with physical variables of geographic position and environmental factors; 2), bivariate correlations of the physical variables with overall measures of covariation among the variables by using sample component scores for components 1–4 obtained from principal component analysis (PCA) of the 25 morphometric variables; 3), spatial autocorrelation of 169 samples (one per collection site) for morphometric variables and sample component scores for components 1–4 over distances up to 1 244 km; 4), 3 cluster analyses of individual samples as OTUs based on 11, 18, and 25 standardized morphometric variables, using average taxonomic distances and UPGMA, together with correlations of taxonomic distances with great circle distances in nautical miles among the collection sites; (5) DFA based on 11, 18, and 25 morphometric variables with the samples grouped in 15 geographic/climate areas, followed by cluster analysis with UPGMA of Euclidean distances among the DFA centroids of the 15 geographic/climate groups, preparation of a phenogram, and correlation of morphometric distances among the 15 geographic/climate groups and the corresponding great circle distances among the areas.

Statistical analyses were performed on IBM 3090 and IBM PC/AT computers with several program packages: SPSSX 3.1 (Norusis, 1985) for mainframe computers; and BIostat II (Pimentel and Smith, 1986), NTSYS-pc 1.50 (Rohlf, 1988), SAAP 4.3 (Spatial Autocorrelation Analysis Program, D Wartenberg, Piscataway, NJ), and STATGRAPHICS 3.0 (Statistical Graphics Corporation, Rockville, MD) for IBM PC. Univariate and bivariate statistical procedures followed Sokal and Rohlf (1981). For additional information on DFA and PCA see Norusis (1985), for cluster analysis by UPGMA and phenograms see Sneath and Sokal (1973), and for spatial autocorrelation analysis see Sokal and Oden (1978a,b).

**RESULTS**

***Relation of feral samples to other populations***

In the classification phase of the DFA of 16 OTUs, each of the 1221 samples could be assigned to any one of the OTUs with a probability of 6.2% that the classification was correct by chance alone. Overall, 63.8% of the samples in the DFA were correctly assigned to their respective OTUs and 98.9% were correctly assigned to one of 2 major groups of European or African ancestry (table I, CME+WME or AFR).

Of the feral samples, 84.9% were classified in the 3 California feral OTUs (table II, CAF, CAS, CAC). Only 8.3% resembled samples of hive bees in California (CAH), making a total of 93.2 % assigned to California OTUs. Feral samples resembling other European OTUs from the Americas totaled 5.3% (AMN, AMC, AMS). In contrast, California hive bees were less similar among themselves with only 46.9% cor-

rectly classified as CAH. Of the remaining samples, 28.6% resembled the feral OTUs for a total of 75.5% assigned to California OTUs. The last 24.4% of samples were classified in other European OTUs of the Americas.

Only 1 sample of feral honey bees was classed as *A m mellifera* and one sample as *A m carnica* (table II). The former sample had a discriminant score for which the probability of group membership in MAU was 0.80, but assuming the sample was a member of MAU the conditional probability of membership was only 0.17. Similarly, the latter sample had a probability of group membership in CAR of 0.82 and a conditional probability of membership of 0.12. No feral samples from California were assigned to the African OTUs CAP, ADA, SEA, or SSA, but 2 samples of CAF were classified as AFZ. These samples, discussed further on, have low probabilities of group membership of 0.55 and 0.59 in AFZ and conditional probabilities of 0.11 and 0.32.

The DFA also supplied coordinates for centroids based on group means of 25 var-

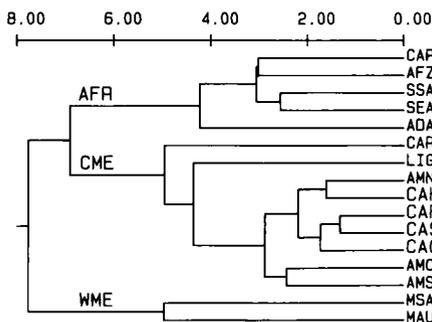
**Table II.** Partial results of DFA of 16 OTUs including California feral samples. Percentage of samples of feral colonies (CAF), swarms (CAS), composite collections (CAC), total (TOT) of all feral samples (CAF, CAS, CAC), and California hive colonies (CAH) classified as one of 10 OTUs (see table I for key to abbreviations). *N* = number of samples.

<i>N</i>		Percentage classified as:									
		CAF	CAS	CAC	CAH	AMN	AMC	AMS	AFZ	CAR	MAU
CAF	244	50.0	20.1	14.8	8.2	2.5	1.2	1.6	0.8	0.4	0.4
CAS	20	10.0	60.0	10.0	15.0	5.0					
CAC	14	21.4	7.1	64.3			7.1				
TOT	278	45.7	22.3	16.9	8.3	2.5	1.4	1.4	0.7	0.4	0.4
CAH	49	8.2	12.2	8.2	46.9	16.3	2.0	6.1			

tables for each OTU with reference to 15 orthogonal functions. Euclidean distances among centroids of 16 OTUs were computed, the OTUs clustered with UPGMA, and a phenogram prepared (fig 1). This phenogram is an approximate representation of relationships of the OTUs when maximally separated in a 15-dimensional space. The cophenetic correlation of the phenogram with the distance matrix was 0.9. Within CME, the American OTUs form a cluster separate from subspecies CAR and LIG, of which the latter joins the American stem. California OTUs join with AMN, but within this North American group only the California hive bees (CAH) directly join AMN while the California feral OTUs (CAF, CAS, CAC) form a separate cluster.

### **Correlation of morphometrics with physical variables**

Based on 240 samples of feral bees, no significant correlation ( $P > 0.05$ ) was found between the physical variables and TRWD, AN39, CUBA, and HAMU (table III). However, a group of 6 variables,



**Fig 1.** Phenogram of 16 OTUs showing morphometric relations of feral and hive honey bees in California (large letters) in relation to other populations (see table I for key to abbreviations for OTUs).

FWLN, HWLN, AN31, CUBB, STLN, and WXLN had significant positive correlations ( $P < 0.05$ ) with LAT, LON, RAIN, and SIGRAIN and significant negative correlations with SUN and TEMPR. WXWD was similar to this group but lacked positive correlations with rainfall; AN29 and AN38 were likewise but lacked negative correlations with sun/temperature. In contrast, a second group of 4 variables, AN30, AN32, AN35, and WXDS, had negative correlations with LAT, LON, RAIN, and SIGRAIN and positive correlations with SUN and TEMPR. Of the remaining variables, FWWD and HWWD had unique low, positive correlations with ELE. The 3 length variables from the hind legs each had only 1–2 weak correlations, including LON, in common. Similarly, AN33, AN34, and AN36 had only 1–2 correlations, including TEMPR in common. FRZFREE had correlations only with AN38 and WXDS.

Overall measures of covariation among the morphometric variables were obtained by PCA performed on the 25 morphometric variables. The first 4 components, PC1–PC4, accounted for 55.9% of the variance and are reported here (table IV). PC1 had positive correlations ( $>$  arbitrary value of 0.5) with FWLN, FWWD, HWLN, HWWD, TBLN, FELN, TRLN, TRWD, STLN, WXLN, and WXWD. PC1 evidently was the general size component correlated with the major body distance measurements. PC2 had correlations of both signs  $>$  0.5 with AN29, AN30, AN32, AN33, AN35, AN36, CUBB and WXDS. Similarly, PC3 had correlations of both signs  $>$  0.5 with AN30, AN31, AN35, and CUBA; PC4 had correlations of both signs  $>$  0.5 with AN33 and AN36. PC2, PC3, and PC4 were therefore related to the vein pattern in the fore wing, the veins CUBB and CUBA, and WXDS on the sternum.

Correlations of the sample scores for these components with the physical vari-

**Table III.** Correlations of morphometric variables with physical variables in California.

	<i>Geographic position</i>			<i>Rainfall</i>		<i>Sun/temperature</i>		
	<i>LAT</i>	<i>LON</i>	<i>ELE</i>	<i>RAIN</i>	<i>SIGRAIN</i>	<i>SUN</i>	<i>TEMPR</i>	<i>FRZFREE</i>
<i>Wings:</i>								
FWLN	0.28	0.34	–	0.23	0.21	–0.22	–0.24	–
FWWD	–	–	0.16	–	–	–	–	–
HWLN	0.23	0.30	–	0.26	0.23	–0.27	–0.31	–
HWWD	–	–	0.18	–	–	–	–	–
AN29	0.17	0.18	–	0.16	0.19	–	–	–
AN30	–0.24	–0.27	–	–0.26	–0.26	0.22	0.14	–
AN31	0.24	0.25	–	0.15	0.23	–0.19	–0.18	–
AN32	–0.32	–0.37	–	–0.41	–0.36	0.34	0.35	–
AN33	–	–	–	–	–	–	–0.19	–
AN34	–	–	–	–0.19	–	–	0.21	–
AN35	–0.28	–0.31	–	–0.35	–0.32	0.27	0.29	–
AN36	–	–	–	–	–	–	0.18	–
AN38	0.19	0.14	–	0.15	0.16	–	–	–0.22
AN39	–	–	–	–	–	–	–	–
CUBB	0.27	0.32	–	0.35	0.31	–0.24	–0.32	–
CUBA	–	–	–	–	–	–	–	–
HAMU	–	–	–	–	–	–	–	–
<i>Legs:</i>								
TBLN	–	0.16	–	–	–	–	–0.18	–
FELN	–	0.14	–	–	–	–	–	–
TRLN	–	0.15	–	–	–	–	–	–
TRWD	–	–	–	–	–	–	–	–
<i>Sternum:</i>								
STLN	0.17	0.24	–	0.20	0.18	–0.19	–0.25	–
WXLN	0.19	0.24	–	0.25	0.21	–0.17	–0.28	–
WXWD	0.18	0.27	–	–	–	–0.21	–0.25	–
WXDS	–0.26	–0.26	–	–0.27	–0.27	0.18	0.18	0.15
<i>PCA:</i>								
PC1	0.17	0.24	–	0.15	–	–0.17	–0.21	–
PC2	–0.37	–0.41	–	–0.44	–0.42	0.35	0.38	–
PC3	–	–	–	–	–	–	–	–
PC4	0.15	0.15	–	0.16	0.14	–	–	–0.14

Rows 1-25 are correlations with variables measured on bees (key to abbreviations for variables in *Materials and Methods* section of text); rows labeled PC1-PC4 are correlations with scores for individual samples on principal components 1-4 based on the 25 variables. Figures given are correlations greater than the critical values of 0.138 ( $P = 0.05$ ) and 0.181 ( $P = 0.01$ ) for  $N = 240$ .

ables were computed (table III). The correlations of PC1 were broadly similar to those of the first group of 6 morphometric

variables above with positive correlations with LAT, LON and RAIN and negative correlations with SUN and TEMPR. Note,

**Table IV.** Component correlations for first 4 principal components for PCA of 25 morphometric variables (key to abbreviations of variables in *Materials and Methods*). Percentage of variance explained by each component in parentheses.

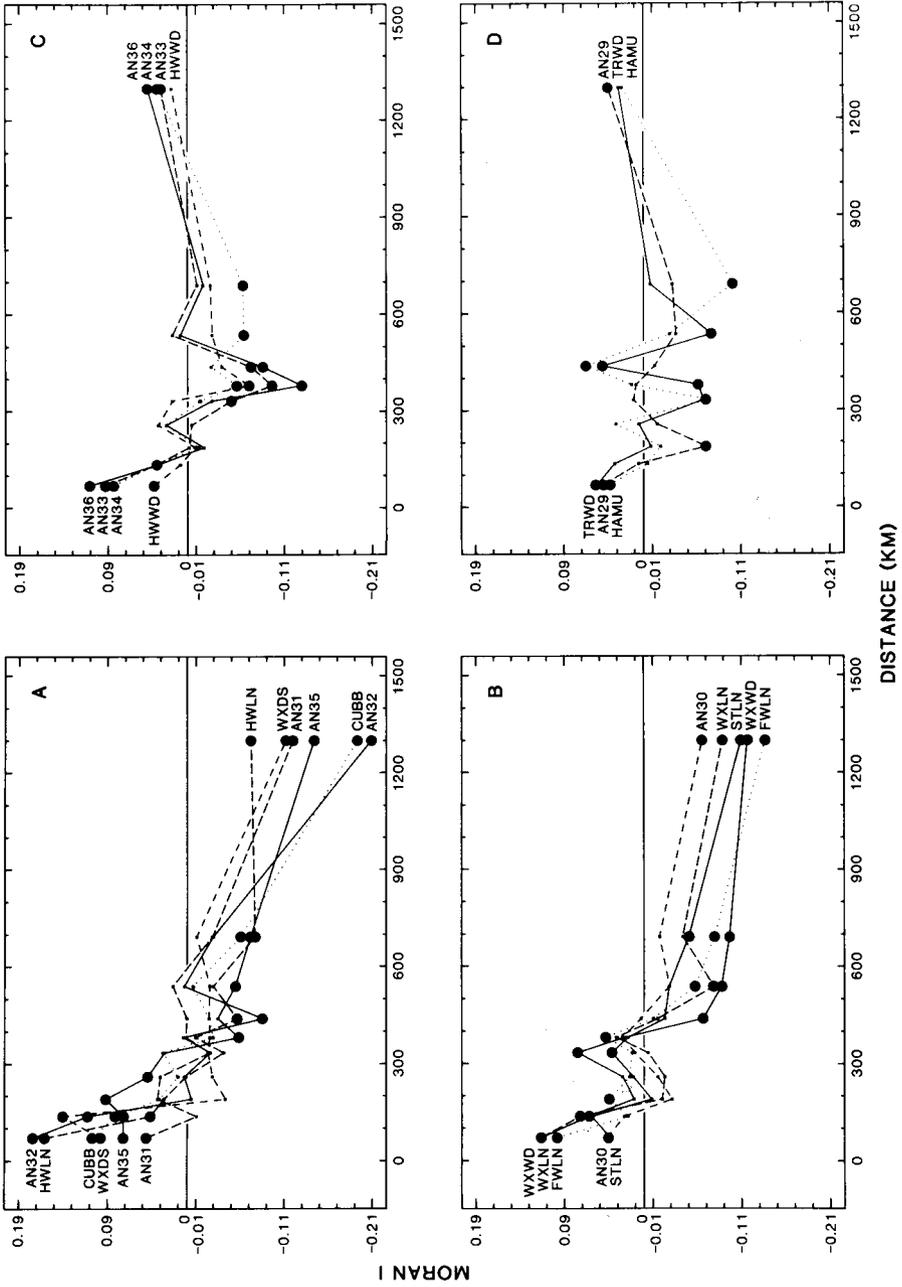
	PC1 (26.12%)	PC2 (14.88%)	PC3 (8.03%)	PC4 (6.84%)
FWLN	0.878	-0.075	0.031	0.128
FWWD	0.757	0.260	0.074	0.007
HWLN	0.793	-0.091	-0.054	0.104
HWWD	0.618	0.413	0.059	0.128
HAMU	0.052	0.120	0.177	0.120
AN29	-0.065	-0.535	0.289	0.418
AN30	0.088	0.568	-0.616	-0.397
AN31	0.218	-0.393	-0.527	-0.099
AN32	0.090	0.733	-0.176	-0.282
AN33	-0.135	-0.552	0.224	-0.740
AN34	0.005	0.422	0.030	0.023
AN35	-0.177	0.495	0.538	-0.062
AN36	0.045	0.496	-0.232	0.759
AN38	-0.039	-0.412	-0.011	0.150
AN39	-0.010	0.119	-0.086	-0.041
CUBB	0.360	-0.521	-0.321	0.161
CUBA	0.069	0.320	0.802	-0.057
TBLN	0.862	-0.034	0.063	-0.085
FELN	0.858	0.017	0.088	-0.102
TRLN	0.799	0.115	0.071	-0.099
TRWD	0.672	0.235	0.010	-0.122
STLN	0.730	-0.260	0.034	0.005
WXLN	0.592	-0.383	0.134	-0.001
WXWD	0.680	-0.142	0.028	-0.074
WXDS	0.058	0.561	-0.081	0.003

however, that some other variables highly correlated with PC1, such as the wing widths and leg distances, had low or no correlations with these physical factors. PC2 had higher values that paralleled the second group of 4 variables above with correlations of opposite sign with the physical variables. Again, some variables correlated with PC2 had no or low correlations with the physical variables. PC3 had no significant correlations with the physical variables. PC4 had weak correlations similar to those of PC1 except that correlations

with SUN and TEMPR were not significant, but the unique correlation with FRZFREE was significant. No component had correlations with ELE.

#### ***Repeat sampling of colonies with small workers***

The average fore wing length  $\pm$  standard error for samples of California feral bees was 9.15 mm  $\pm$  0.0096 ( $N = 240$ ). To determine whether the unusual small size of



**Fig 2.** Correlograms for morphometric variables (key to abbreviations of variables in *Materials and Methods*). Maximum limits of distance classes with significant autocorrelation coefficients ( $P < 0.05$ ) are marked by solid circles; solid and broken lines to aid reading graph. See text for explanation.

workers in some colonies was temporary or was characteristic of the colony, we were able to take samples ( $N = 10$ ) a second time from colonies at 3 sites, including the sites where 2 samples were classified as Africanized: 1), at San Diego, sampled again after 21 months, average wing lengths were 8.65 mm (first collection) and 8.82 mm (second collection); 2), at 8 miles west of Poway, San Diego County, 4 colonies, sampled again after 8 months, average wing lengths were 8.92 mm and 9.08 mm, 8.98 mm and 9.28 mm, 9.02 mm and 9.04 mm, and 9.07 mm and 9.10 mm, respectively; and 3), at 20 miles north Winterhaven, Imperial County, 2 colonies, sampled again after 17 months, average wing lengths were 8.58 mm and 8.73 mm, and 8.64 mm and 8.91 mm, respectively.

Bees from these colonies were also compared to European and Africanized bees in analyses by OR Taylor for hexokinase and malate dehydrogenase enzymes (Spivak *et al*, 1988) and by RK Smith for cuticular hydrocarbons (Smith, 1988). The Poway and Winterhaven colonies had hydrocarbons and allozyme frequencies similar to European bees. The San Diego colony had less definitive characteristics: the patterns of hexokinase and cuticular hydrocarbons were similar to European bees, but the malate dehydrogenase was homozygous fast, which has a low frequency among American bees, a high frequency among Africanized bees, and also increases in frequency at lower latitudes (Rinderer and Sylvester, 1981; Badino *et al*, 1984).

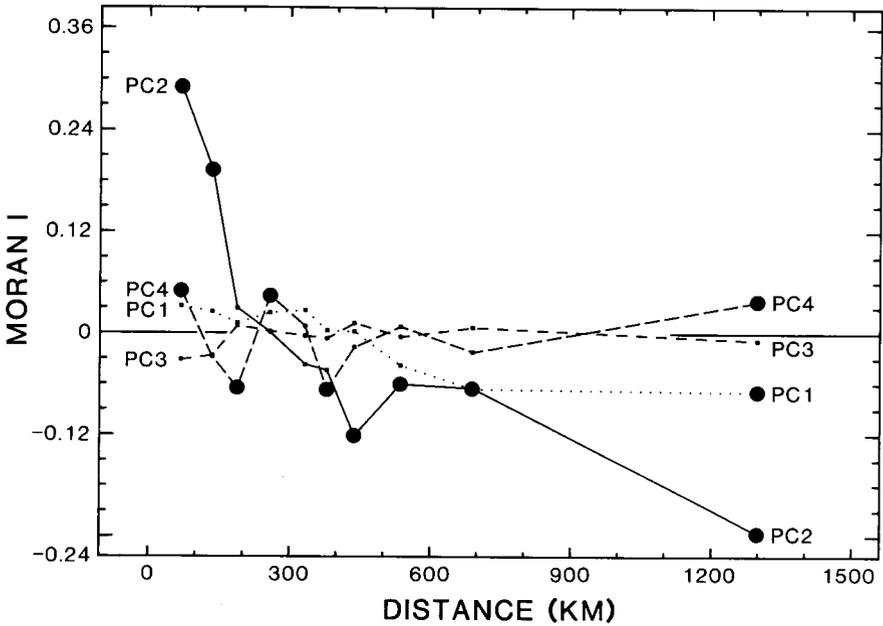
### **Spatial autocorrelation analysis**

Spatial autocorrelation analysis of the 25 morphometric variables and component scores of PC1-PC4 for each of 169 samples was performed for 10 distance classes with equal frequencies of point pairs

(1419–1420 pairs in each class). The Moran I statistic is reported here. The maximum limits for the distance classes were: class 1, 69.3 km; 2, 135.3 km; 3, 188.4 km; 4, 258.4 km; 5, 332.7 km; 6, 379.6 km; 7, 437.6 km; 8, 537.1 km; 9, 690.8 km; and 10, 1 299.4 km. Correlograms for FWWD, AN38, AN39, CUBA, TBLN, FELN, TRLN (these are not illustrated) and sample component scores for PC3 (fig 3) each had a Bonferroni approximation for overall correlogram significance not different from a null hypothesis ( $P > 0.05$ ), while the remaining 18 variables and sample component scores for PC1, PC2, and PC4 had these statistics significantly different ( $P < 0.05$ ).

The 18 morphometric variables could be roughly sorted into 3 groups of variables with similar configurations. Correlograms of the first group exhibited a cline with significant positive autocorrelation coefficients ( $P < 0.05$ ) in near distance classes and significant negative coefficients in the far distance classes. The clines for HWLN, AN31, AN32, AN35, CUBB, and WXDS were simple slopes with positive values as far as class 4 in AN35 and negative values in distant classes, beginning with class 6 for AN35 (fig 2a). Also in the first group were the clines for FWLN, AN30, STLN, WXWD, and WXLN, but each had a drop in value near zero followed by a discrete positive peak in classes 5 or 6 that was significantly different ( $P < 0.05$ ) for FWLN, STLN, and WXWD (fig 2b).

Correlograms for variables in the second group were more complex (fig 2c): HWWD, AN33, AN34, and AN36 exhibited significant positive coefficients ( $P < 0.05$ ) in classes 1 or 2; a positive peak in class 4 for HWWD, AN34, and AN36; followed by significant negative values ( $P < 0.05$ ) as close as classes 5 or 6; and finally positive values of which those of AN36 and AN34 in class 10 were significant ( $P < 0.05$ ).



**Fig 3.** Correlograms for sample component scores for PC1–PC4. See figure 2 and text for explanation. Note scale for vertical axis is different from that in figure 2.

The remaining correlograms were assigned to a heterogeneous third group: TRWD, AN29, and HAMU were most similar to the second group, but the intermediate positive and negative oscillations in values did not closely parallel those of the second group or each other (fig 2d).

The correspondence between the correlograms for sample component scores and the groups of variables above was best for PC4 which matched the correlograms of the second group in general configuration and had its highest component correlations with AN33 and AN36 (fig 3). Autocorrelation coefficients for PC1, the general size component, were significant and negative ( $P < 0.05$ ) only for the far distance classes 9 and 10. Coefficients for PC2 exhibited a strong, simple cline like those of

the first group of variables and exhibited the highest coefficients of all correlograms: 0.29 in class 1 and  $-0.24$  in class 10.

#### ***Individual samples in morphometric and geographic space***

Average taxonomic distances were computed among the 240 samples based on 3 data sets: the 11 variables of group one as discussed above in the analysis of autocorrelation, the 18 variables that had significant autocorrelation, and all 25 variables. Correlations of average taxonomic distances based on these sets of standardized variables with great circle distances among the collection sites were 0.20, 0.18, and 0.17, respectively. All are significant values

( $P < 0.01$ ;  $N = 28\ 680$ ) and show slightly higher values with the sets of 11 and 18 variables that exhibited clines. However, except for relatively few clusters, visual inspection of the phenograms of the 240 samples based on 11, 18 and 25 variables did not exhibit recognizable geographic patterns (phenograms not illustrated here).

In other words, samples in the terminal clusters were often from diverse, sometimes distant, sites rather than contiguous areas.

### ***Grouped samples in morphometric and geographic space***

In contrast to the foregoing analysis of individual samples, geographic patterns emerged from DFAs based on the sets of 11, 18 and 25 variables when the samples were grouped into 15 geographic/climate areas. Correlations were computed between all Euclidean distances among the 15 geographic/climate group centroids and the great circle distances among the geographic centers of the 15 areas. These correlations based on the sets of 11, 18, and 25 variables were 0.26, 0.31, and 0.34, respectively (critical value at  $\alpha 0.01$  is 0.254;  $N = 105$ ). In this case, morphometric distances based on larger numbers of variables gave higher correlations with geographic distance irrespective of the variables' autocorrelation properties.

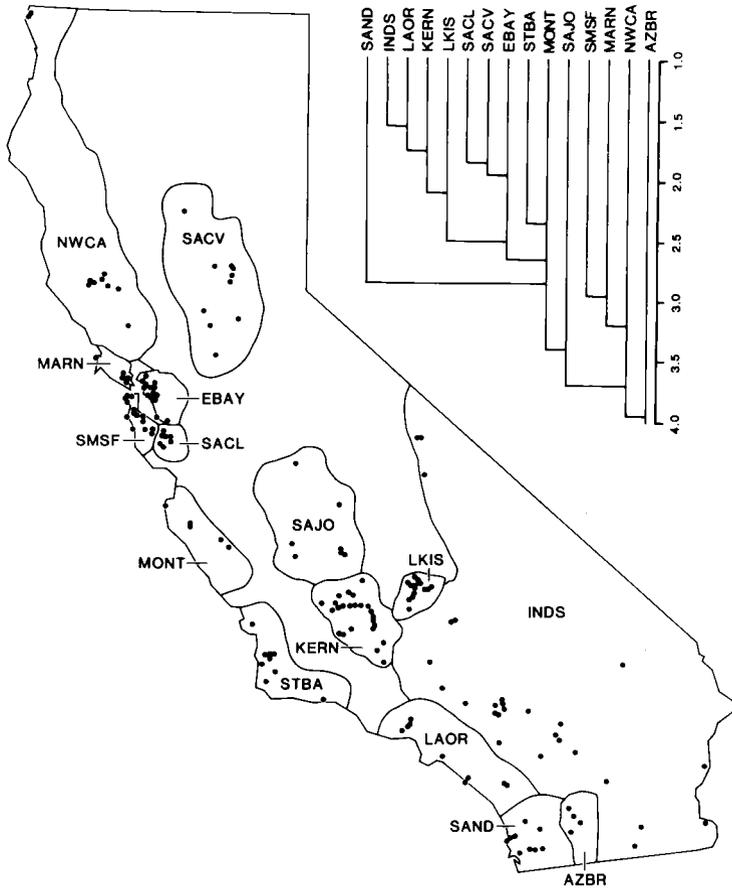
For the DFA based on all 25 variables, correct classifications of samples ranged from 100% (AZBR, MONT) to 26.7% (SACL) with an overall correct classification of 54.6% (compared to 6.7% if classified by chance alone). The phenogram prepared by UPGMA from the Euclidean distances among the centroids had a cophenetic correlation with the distance matrix of 0.8. A recognizable geographic pattern was depicted by the phenogram (fig 4):

4 clusters of OTUs were from geographically close, if not contiguous areas: ((INDS + LAOR) + KERN) + LKIS, (SACL + SACV) + EBAY and STBA + MONT at phenon 2.6, and (SMSF + MARN) + NWCA at phenon 3.7. The morphometric relationships of SAJO, SAND, and AZBR to geographic proximity were less clear in the phenogram, but inspection of the distance matrix indicated that each had its minimum distance in morphometric space with a contiguous geographic neighbor: SAJO with KERN, SAND with LAOR, and AZBR with SAND.

### **DISCUSSION**

The overall relationships of California feral bees to other populations were depicted best in the phenogram based on a discriminant function analysis (DFA) of 16 OTUs (fig 1). The relationships of the major groups of taxa were those that would be expected according to Ruttner's classification (1988): the three stems at phenon 6 correspond to his three major geographic groups of taxa in tropical Africa (Ruttner's group II; AFR in table I and fig 1), west Mediterranean (Ruttner's III-1; WME), and central Mediterranean (Ruttner's III-2; CME). The samples of feral honey bees from California exhibited greater morphometric similarity among themselves than with hive bees in the state and populations elsewhere (tables I, II). In contrast, the California hive bees were closer in morphometric space to samples of other hive bees in North America than to samples of feral bees in the state. This was shown in the phenogram where, among other North American OTUs, the California feral OTU's formed a discrete cluster and California hive bees first joined with those of North America outside the state.

Of all the subspecies in the DFA of 16 OTUs, the European OTUs in the Ameri-



**Fig 4.** Map of California showing 15 geographic/climate areas used in this study, 169 collection sites, and a phenogram of morphometric relations among samples when grouped by geographic/climate areas. See *Materials and Methods* for key to abbreviations and further description of geographic/climate areas.

cas were closest to *A m ligustica*, a relation to be expected by its favored status among beekeepers. However, no sample of California hive or feral bees was close enough to known samples of that subspecies to be classified as a member of that subspecies on the basis of morphometrics. Individual feral samples that closely re-

sembled other subspecies were also rare. Only one sample was classed as *A m mellifera* and one sample as *A m carnica* (table II), both with probabilities of membership of 0.8. Two other samples were classified as Africanized with probabilities of membership of 0.6. These samples were among several in southern California

that had unusually small worker bees. Allozyme and cuticular hydrocarbon analysis supported their identification as probably European in origin despite their small size.

Of 25 morphometric variables, 21 had one or more significant correlations with one or more physical variables (table III). The physical variables themselves were intercorrelated. The long axis of the state is oriented southeast to northwest such that a correlation exists between latitude and longitude. Although the environmental isoclines are complex and do not coincide, the cooler, moister regions with reduced temperature range tend occur toward the north and toward the west while the warmer, drier regions with greater temperature range tend occur toward the south and toward the east. Within this context, bees tended to have shorter wings (FWLN, HWLN), a shorter vein (CUBB), shorter third sterna (STLN), smaller wax mirrors (WXLN, WXWD) that were farther apart (WXDS), and certain angles between veins of the forewing larger (AN30, AN32, AN35) or smaller (AN29, AN31, AN38) as one progressed from the north and west toward the south and east. Wings tended to be broader (FWWD, HWWD) with higher elevation; a relation also found for FWWD and certain other measurements of *Apis cerana* in the Himalayas (Singh *et al*, 1990).

The trend toward smaller size of some structures at lower latitudes corresponds to the cline toward smaller size among native honey bees in the old world (Alpatov, 1929; Ruttner, 1988). For example, most length measurements are smaller in African and Africanized bees than in European bees (Daly and Balling, 1978; Buco *et al*, 1987). The distance between wax mirrors increases at lower latitudes, a feature also exhibited in California feral bees. As a result of these trends in geographic variation, some colonies of feral bees in the

warmer and drier areas of California had unusually small workers that converged toward the morphometrics of bees at lower latitudes in the old world.

General body size as measured by component scores for samples on the first principal component (PC1) exhibited the same trend toward smaller size to the south and east, but not all morphometric variables that were highly correlated with PC1 did so. Most notably, the lengths of segments of the hind legs (FELN, TBLN, TRLN) were mostly uncorrelated with all physical variables except longitude. This is in contrast to Russian bees described by Alpatov (1929) where legs were absolutely shorter, but longer relative to the body at lower latitudes. Sample component scores for PC2 and to a lesser extent for PC4, both related to the venation pattern of the forewing, were also correlated with the physical variables. Here again not all variables highly correlated with the components had individual correlations with the physical factors.

Most of the morphometric variables that had significant correlations with one or more physical variables also had autocorrelation correlograms significantly different from those expected by a null hypothesis (fig 2). Exceptions were HAMU and TRWD which had no correlations with physical variables yet had significant correlograms. The correlograms for FWLN, HWLN\*, AN29, AN30, AN31, AN32\*, AN35, CUBB\*, and the 4 sternum variables\* were clines with positive autocorrelation coefficients in near distances and negative coefficients in far distances. The maximum, uninterrupted distances for significant positive coefficients were 258 km for AN35, 135 km for the preceding variables marked with an asterisk (\*), and 69 km for those remaining. The variables AN29, AN34, and AN36 had more complex correlograms with significant positive coeffi-

cients in both the near and far distances and with negative coefficients in the middle distances. TRWD, HAMU, HWWD, AN33, TRWD were roughly similar to these in configuration, but lacked significant coefficients at far distances. The lengths of the leg segments did not show a clinal pattern. Autocorrelations of sample scores on components 1-4 indicated only distant negative values for PC1, a strong cline for PC2 (related to pattern of forewing venation), no autocorrelation for PC3, and a complex correlogram for PC4 with near and distant positive values (fig 3).

The final analysis examined the correlation between morphometric distances among samples and the geographic distances (great circle distances) among collection sites. When samples were taken individually, the morphometric distances based on the 11 variables that exhibited simple clines gave the highest correlation with the geographic distances ( $r = 0.20$ ,  $F^2 = 4\%$ ). Cluster analysis of individual samples based on their morphometrics, however, did not yield many clusters of samples from adjacent collection sites. When the samples were grouped into 15 geographic/climate areas, the highest correlation with the geographic distances among areas ( $r = 0.344$ ,  $F^2 = 11.8\%$ ) was obtained when the morphometric distances were based on all 25 variables. The phenogram prepared from the morphometric distances of the grouped samples, gave clusters in which samples in a geographic/climate area more often resembled samples from adjacent or nearby areas rather than samples in distant areas (fig 4).

The morphometrics of worker honey bees are known to be influenced by a variety of environmental factors during development (review by Daly, 1991). Although direct environmental induction of the variation just described cannot be excluded, the correlations of variables with physical fac-

tors, clinal patterns of autocorrelation, and similarity to natural geographic trends among honey bees in the Old World points to an underlying genetic differentiation among feral populations in California. Additional circumstantial evidence of a genetic basis as opposed to a temporary environmental influence was obtained by sampling a second time some colonies of small worker bees. The average fore wing lengths of worker bees from these colonies were consistent over time.

A comparable case has been described for the house sparrow that was introduced to North America from Europe in 1852-1860. Although genetic evidence is limited, the phenotypic differentiation and clinal variation of the birds in North America have been interpreted as adaptive mainly because of the similarity to clines in the Old World (Johnston and Klitz, 1977). Genetic evidence of rapid adaptive evolution in new habitats has been discovered in *Drosophila subobscura*. The flies, native to the Palearctic, were first found in South America in 1978 and in North America in 1982. Chromosomal-inversion polymorphisms were shown to be correlated with latitude in the Old World and in a parallel manner in both hemispheres of the New World. Furthermore, latitudinal clines in body size have been analyzed and are known to be related to chromosomal arrangements (Prevosti *et al*, 1988).

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**Résumé — Variation géographique progressive chez les abeilles sauvages de Californie.** La preuve est faite que les abeilles sauvages de Californie sont des populations métisses, qui se différencient partiellement des colonies exploitées par les apiculteurs d'une part, et des races européennes d'autre part par la morphométrie. La plupart des variables morphométriques sont faiblement mais significativement corrélées avec les facteurs du milieu et présentent des variations progressives (clines). En particulier des clines distincts ont été observés pour la vénation de l'aile antérieure. Certaines mesures en relation avec la taille du corps suivent la loi

de Bergman, mais la première composante (PC1), qui est représentative de la taille générale, est apparue faiblement corrélée à la latitude et ne présente qu'une autocorrélation spatiale négative entre échantillons éloignés. De même, la plupart des segments des pattes postérieures ne présentent pas de corrélation avec les variables physiques ni d'autocorrélation spatiale et, par conséquent, la loi d'Allen n'a pu être retrouvée. Cette variation géographique naissante s'est développée au cours d'une période de 138 années et en présence d'un nombre élevé de colonies exploitées de diverses origines. Certains aspects de la variation peuvent être comparés avec les tendances géographiques des abeilles indigènes de l'Ancien Monde et avoir une base génétique. La tendance vers une diminution de la taille de certaines variables morphométriques dans les basses latitudes aide à comprendre la présence de colonies ayant de petites ouvrières dans les régions plus chaudes et plus sèches de Californie. De telles colonies peuvent représenter un écotype adapté aux conditions désertiques.

***Apis mellifera* / colonie sauvage / morphométrie / variation géographique / loi de Bergmann**

**Zusammenfassung — Schrittweise geographische Variation bei wilden Bienenvölkern in Californien.** Es wird der Beweis vorgelegt, daß wildlebende Bienenvölker in Californien Bastardpopulationen bilden, die sich in ihrer Merkmalsausprägung teilweise von den Völkern der Imker und von europäischen Rassen unterscheiden. Die meisten morphometrischen Variablen zeigen geringe, aber signifikante Korrelationen mit Umweltfaktoren und schrittweise veränderte Muster der räumlichen Autokorrelation mit der Entfer-

nung. Deutliche allmähliche Veränderungen ("clines") wurden besonders beim Muster der Adern des Vorderflügels beobachtet. Bestimmte Maße der Körpergröße ließen die Bergmann'sche Regel erkennen, aber die Gesamtkörpergröße, gemessen nach der Komponenten-Gewichtung auf die Gesamtgrößenkomponente (PC1), war schwach mit der geographischen Breite korreliert und zeigt nur eine entfernte, negative räumliche Autokorrelation. Diese beginnende geographische Variation hat sich in einem Zeitraum von nur 138 Jahren und in Gegenwart einer großen Anzahl von Wirtschaftsvölkern verschiedener Herkunft entwickelt. Einige Aspekte der Variation können mit geographischen Trends bei einheimischen Bienen der Alten Welt verglichen werden und besitzen möglicherweise eine genetische Grundlage. Der allgemeine Trend zu geringerer Körpergröße in niedrigen geographischen Breiten hilft bei der Erklärung des Vorkommens von Völkern mit kleinen Arbeiterinnen in den wärmeren und trockeneren Teilen Californiens. Solche Völker können als Repräsentanten eines Ökotyps gelten, der an Wüstenbedingungen angepaßt ist.

***Apis mellifera* / wildes Bienenvolk / Morphometrie / geographische Variation / Bergmann'sche Regel**

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