COMPUTER ASSISTED IDENTIFICATION OF
HYBRID STRAINS OF WESTERN HONEY BEES

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Abstract

Standard, generally-agreed-upon principles of taxomony guide the naming of groups as species. Chief among these principles is that species-specific characteristics can exist because members of different species do not freely produce fertile hybrids.

The history of the nomencalture of subspecies of Western honey bees exemplifies 3 additional taxonomic principles. First, subspecies-specific characteristics have not been found in honey bees. Second, subspecies are distinguishable as groups by the multivariate techniques of principal component analysis or discriminant analysis. Third, subspecies identifications are statistically based and hence probabilistic. These characteristics of subspecies identification are ideally suited to computer applications. Computer systems can be used to collect data and then used to apply complex statistical procedures to provide identifications.

Such techniques have been used to analyze the honey-bee populations of the Americas. The honey bees in the Americas constitute populations resulting from subspecies hybridization. Populations of Africanized bees from South America are morphometrically quite distinct from populations of *A. m. scutellata* in South Africa. In every respect, the Africanized bees clearly show the genetical influence of their European parents. Additionally, F_1 progeny of Africanized and European honey bees provide further evidence of the hybrid nature of Africanized bees. It is clear that Africanized bees in the Americas constitute a "hybrid swarm". That is, they are "a continuous series of morphometrically distinct hybrids resulting from hybridization".

Resumen

Las normas, de acuerdo común, de los principios de taxonomía son la guía en el nombramiento de grupos como especies. Primero dentro de estos principios es que características específicas a la especie pueden existir porque miembros de diferentes especies no producen híbridos fértiles libremente.

La historia de la nomenclatura de sub-especies de la abeja melífera Occidental hace ejemplo de 3 principios taxonómicos diferentes. Primero, características específicas a sub-especies no se han encontrado en la abeja melífera. Segundo, sub-especies son distinguidas como grupos por las técnicas multivariantes de análisis de componente principal o análisis discriminante. Tercero, identificaciones de sub-especies son basadas estadísticamente y así probabilísticas. Estas características de identificación de sub-especies se prestan idealmente para la aplicación a computadoras. Systemas de computación pueden ser usados para recolectar datos y entonces aplicarles procedimientos de estadística complejos para identificarlos.

Tales técnicas se han utilizado para analisar la población de la abeja melífera en las Américas. Las abejas melíferas en las Américas se constituyen de poblaciones resultantes de hibridización de sub-especies. Poblaciones de abejas Africanizadas de America del Sur son morfométricamente muy distintas a la población de *A. m. scutellata* en Sur Africa. En todo respecto las abejas Africanizadas muestran claramente la influencia genética de sus padres Europeos. Adicionalmente, cria F_1 de abejas Africanizadas y Europeas proporcionan más evidencia de la índole híbrida de las abejas Africanizadas. Una comparación de abejas Africanizadas de Argentina y de Venezuela demuestra que

estos dos grupos son diferentes morfométricamente y proporciona más evidencia de hibridización. Queda claro que abejas Africanizadas en las Américas constituyen un "enjambre híbrido." O sea, son "una serie contínua de híbridos morfométricamente distintos resultando de la hibridización."

This discussion is first intended to describe the special taxonomic problems attending the subspecies identification of Western honey bees. Secondly, this paper will describe a developed system of identification that is reliant upon computer-assisted measurements of body parts and computer comparisons of measured data to baseline data through the application of multivariate discriminant analysis technologies.

SYSTEMATICS

A brief review of classical taxonomy reveals some rather clear concepts. First, the "species is the basic unit of taxonomy and evolution." (Mayr 1970). In order to name a group as a species, certain generally accepted questions are asked: (1) do similar groups have allopatric or sympatric occurrances; (2) do similar groups freely hybridize or not; and (3) do groups have species-specific characters?

Certainly a group that has species-specific characters which does not hybridize with similar groups and has an allopatric distribution with other groups is deserving of species designation. The more clearly the information at hand provides these answers the more certain it is that a species designation is correct. The difficult problems of species-level taxonomy, for example, undescribed ecoclines and sibling species, are problems of incomplete information. As more information becomes available, clearer and more correct applications of the principles of taxonomy can be made.

Different taxonomic problems arise with the Western honey bee (*Apis mellifera*). In contrast to other Apoidea where congeneric sympatry and limited distributions are typical, the Western honey bee occupies an amazingly large and varied area. Naturally, it ranges from near the Arctic circle in Europe to the Cape of Good Hope to the south and from the North and South Atlantic coasts of Europe and Africa to the Ural Mountains in the north and the Arabian peninsula's coast of Oman in the south. Populations of Western honey bees freely interbreed with opportunity and produce fertile offspring. Indeed, honey bees may have only occupied this area since the last ice age of the late Pliocene (Ruttner 1987). In additon to this natural distribution, man in the last 300 years has spread honey bees throughout the New World areas of North and South America, Australia and New Zealand. Although clearly one species, *A. mellifera* shows substantial variation in morphology, behavior and physiology (Cornuet & Louveaux 1981). This subspecific variation has been the primary focus of honey-bee taxonomists within this century. Historically, rules governing the description and naming of varing populations of honey bees at the subspecies level have been non-existent. Guides to such nomenclatural issues have frequently included political and sociological elements as well as biological ones. As recent as 1953, Maa presented a list of 146 subspecific names of honey bees which he considered acceptable. This list comprised a revision of 600 named groups of *Apis mellifera* used previously. The difficulties of subspecies honey-bee taxonomy are obviously entangling. If it were not important for achieving various social and economic goals having to do with the public health and commercial characteristics of honey bees, subspecies taxonomy of honey bees would probably have collapsed.

Nonetheless, biological differences among honey bees that reflect adaptations to ecological differences do exist. A long tradition of biometry (Buttel-Reepen 1906, Alpatov 1929, 1948, Goetze 1930, 1940, 1964, Dupraw 1965a, 1956, Daly & Balling 1978, Ruttner 1986) has attempted to describe these differences. Biometrical applications of

modern statistical methods have lead to certain suggestions for taxonomic decisions at the subspecific level with *A. mellifera.* Ruttner (1986) suggests the "operational taxonomic units" called for by Sneath & Sokal (1973) can be the means of standard individual measures. The application of standard multivariate methods which are capable of describing the magnitudes of differences between populations, such as discriminant analysis or principal component analysis provides a basis for the acceptance of groups as subspecies. Applying these principles Ruttner (1986) described 23 subspecies from the Old World range of the species. In doing so, he observed that variation among groups was quantitative not qualitative and frequently occurred within narrow limits.

The application of modern computer technology to the collection and analysis of morphometric honey-bee data was begun by DuPraw (1965a, 1965b) and refined by Daly & Balling 1978 and Daly et al. 1982.

METHODS

Data collection was according to procedures adapted to the morphometric analysis of honey bees by Daly et al. (1982). Body-part images from wings, legs and sternites mounted on slides were projected with a standardized magnification from a projecting microscope to a digitizing tablet connected to a micro-computer (Fig 1, A and B). Various points on the projected images were identified by the operator with the aid of an electronic "mouse" or "cursor". The positions of the electronically marked points were transmitted to the micro-computer where they were converted to values representing actual lengths angles and counts of the 25 characters measured (Daly & Balling 1978).

In the numerical analysis of a sample, reductionist statistical procedures as used to calculate and then compare descriptive values of the sample to similar values for baseline groups. The analysis provides probabilities of a sample belonging to groups, based on these comparisons. At least two and probably more of the major suppliers of statistical software provide discriminant analysis packages that are appropriate for use in honey bee taxonomy.

Many interesting problems in honey bee classification are found among the honey bees brought to the New World. These bees have come from a variety of sources and the populations of bees found in the Americas cannot be said to be members of identifiable sub-specific groups. Nonetheless, differences do occur among American populations of bees. Those populations derived in part from parents from the Highland population of East Africa (*A. m. scutellata*) are considered economically undesirable because of excess stinging (Collins et al. 1982) and inferior honey production (Rinderer et al. 1984, 1985). This parental stock was introduced to apiaries near Piracicaba Brazil from Africa in 1957 (Kerr 1967). Because of economic concerns, the identification of Africanized bees has become important.

As examples of the value of the discriminant analysis of morphometric measures, the results of two separate analyses are presented. The first compares a sample of a population of Africanized bees to a sample of the parental population in Africa. The second compares Africanized and European honey bees and F_1 progeny of crosses between these two.

RESULTS AND DISCUSSION

One use of multivariate discriminant function analysis is to compare two or more groups on the basis of a variety of quantitative measures. Such an analysis was done in a description of morphometric differences between South American Africanized and South African (*Apis mellifera scutellata*) honey bees (Buco et al. 1987). This study compared the two groups for each of the univariate measures with the use of t-tests.
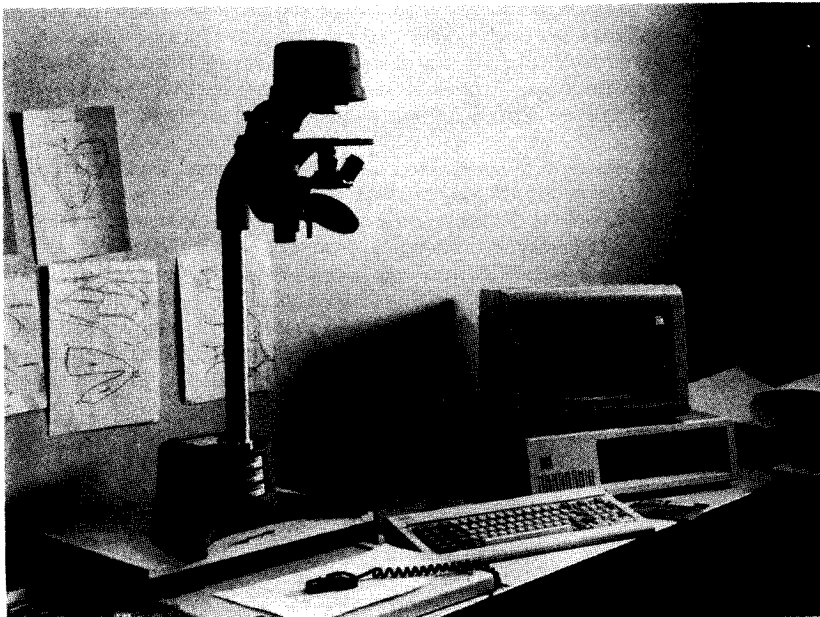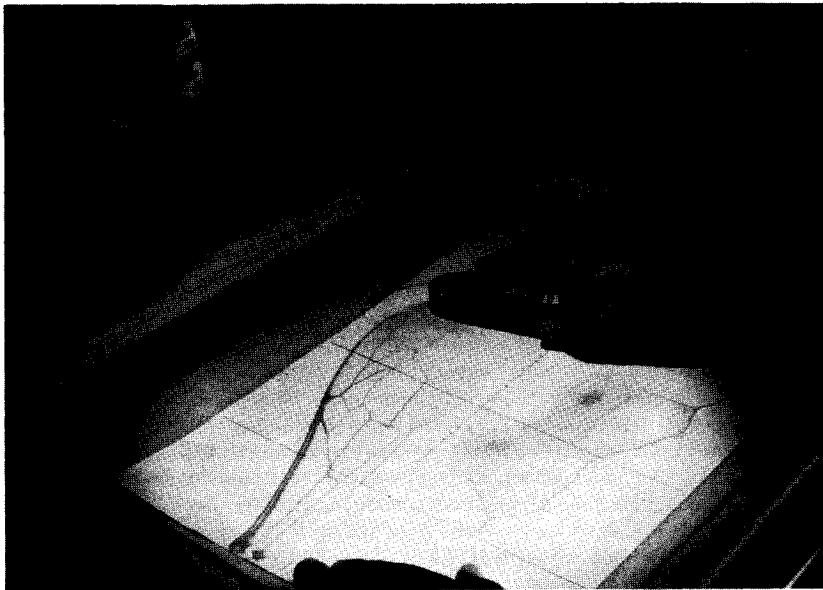
Fig. 1. Photographs of the data collection hardware. A. The projection of body part images onto the digitizing tablet. B. The data collecting assembly: projecting microscope, digitizing tablet and micro-computer.

Nineteen of the 24 characteristics differed between the groups (P = 0.05 or less). However, a full appreciation of the overall difference between the groups depended upon the multivariate discriminant further analysis.

The differences found in comparisons of individual characteristics were reflected in the multivariate discriminant function analysis. The two populations were completely separated by the discriminant function (Fig 2). The posterior probability of group membership for all cases indicated a correct classification of P = 0.9984 or greater using the derived discriminant function.

The feral bees of South America constitute a population which is morphometrically quite different from the parental population of *A. m. scutellata* in Southern Africa. Comparisons of individual characteristics, as well as the composite analysis represented by the discriminant function, indicated that Africanized bees are generally larger than their African parental stock.

The most likely explanation for these differences is that Africanized bees in the Americas are a subspecific hybrid swarm (King 1968) which has resulted from matings with European bees and then subsequent crossing and back-crossing. The generally intermediate morphometric character of Africanized bees suggests that a large number of loci are involved in the determination of the honey bee's morphometric make up. Predictable Mendelian events associated with crossing and then backcrossing between African and European bees would produce the Africanzed bees that were measured. Such bees clearly show the influence of both their European and their African parentage.

A different use for multivariate discriminant function analysis is to describe populations in terms of functions and use the functions to classify unknown samples. This was originally done for Africanized and European bees for the Americas by Daly & Balling (1978). However, the experimental production of F_1 progeny of European and Af-
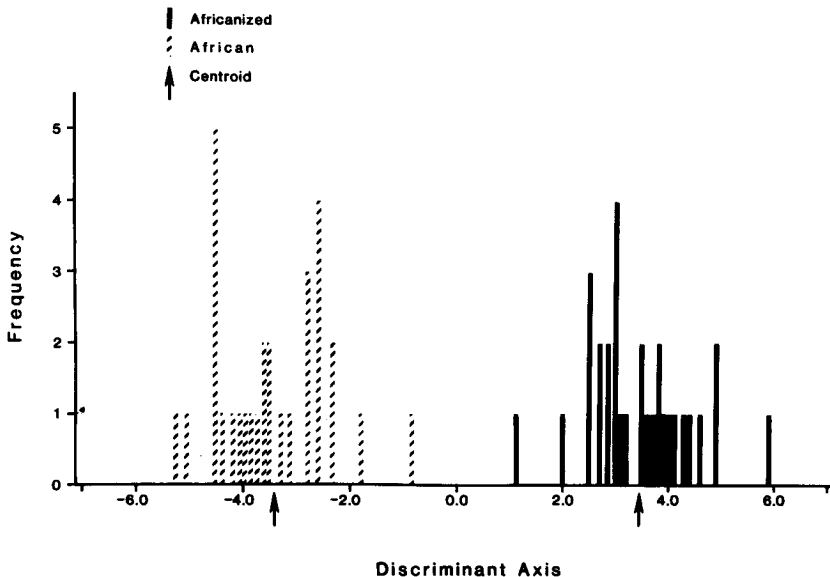

Fig. 2. Histogram of the results of the multivariate discriminant function analysis of African and Africanized bees.

ricanized parents made possible the development of discriminant functions for the three groups (Rinderer et al., unpublished).

The multivariate discriminant analysis correctly identified all samples as Africanized, European or F_1 hybrids (Fig. 3) Most classifications were at $P = 1.00$ and all but one were at $P \leq 0.090$. The remaining sample, an F_1 colony, was correctly classified at $P = 0.55$. As expected, the discriminant analysis procedure selected the F_1 category as the second most likely classification for all Africanized and European colonies. Interestingly, the second most likely category for the F_1 samples was European for all but 3 F_1 samples. Thus, the procedure was conservative in its classification of F_1 colonies in ways appropriate to regulatory needs. Only clearly Africanized or F_1 colonies would be so classified.

The identification of unknown samples by comparison to this baseline can be expected to correctly classify samples of Africanized and European worker bees and samples of worker bees that are completely or mostly F_1 hybrids. According to established practice, such identifications would be made with posterior probabilities of group membership of $P \geq 0.90$. Samples which are a mixture of bees would likely either be classified with a low probability of group membership ($P<0.90$) or the range for the majority of measurements would span 2 or 3 of the 3 group ranges.
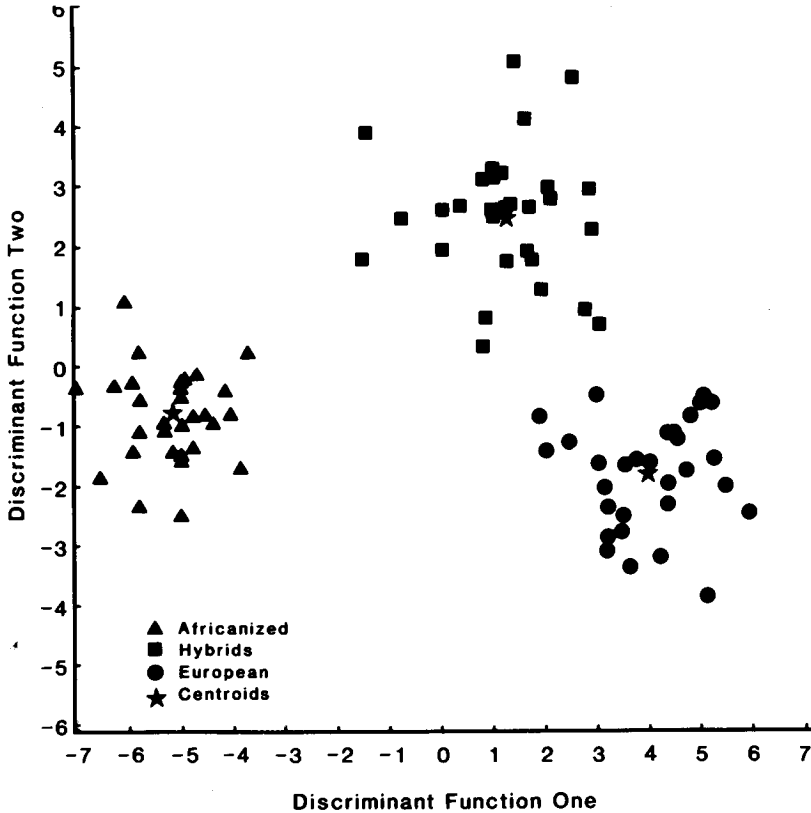

Fig. 3. Scatterplot of the results of the multivariate discriminant function analysis of Africanized, European and their F_1 hybrid bees.

Such samples would warrant close inspection. The classification of individual bees may provide evidence of mixed samples. Data supporting the classification of individual bees have been analyzed and discriminant functions of identifying individual bees have been developed.

Scientific and commercial issues regarding identifying Africanized bees and their hybrids have been well served by the computer-assisted discriminant function analysis of morphometric data. The process has permitted rather difficult taxonomic problems to be resolved clearly with numerical procedures. The success of the techniques with honey bee groups even at the subspecific level, suggests that other identification problems are within the capabilities of this tool to solve.

ACKNOWLEDGEMENT

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