

Computer-Assisted Measurement and Identification of Honey Bees (Hymenoptera: Apidae)¹

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ABSTRACT

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A procedure is described for rapid measurement and identification of honey bees, *Apis mellifera* L. Parts of bees are slide mounted, and the images are projected optically onto a digitizer tablet that communicates with a computer. Point coordinates for measurements are entered by hand with the digitizer. The computer calculates the various measurements and then determines by discriminant analysis whether the bees are likely to be European or Africanized in origin. The procedure significantly reduces the time and errors in making measurements. Blind tests of field identifications versus analytical identifications agreed in 95.6% of 135 collections. Five other collections with intermediate discriminant scores could have been caused by mixtures of Africanized and European bees, recent hybridization, or unusual environmental stresses that caused a reduction in the size of European honey bees.

This paper describes a procedure for the rapid measurement and immediate identification of honey bees and the storage of information about the bees for subsequent analysis. Despite the explosive growth of computer technology, we believe our procedure is the first to incorporate the semiautomatic accumulation of measurements and the use of mathematical decision functions in the routine identification of insects. Somewhat parallel procedures with different objectives are those of Vassiliev and Rostova (1977) for measurement of leaves for the study of morphogenesis, Hellenthal and Price (1980) for taxonomic analysis of lice, and Budai et al. (1980) for the retrieval of information on radiolarians. With the promise of image-analyzing techniques, the approach should be widely applicable to morphometric studies and to the identification of taxa that are best distinguished by morphometric characters.

The natural geographic races of *Apis mellifera* L. in Europe and Africa differ in behavior, color, and the morphometrics of adult bees (Ruttner 1968). If several characters are analyzed together by multivariate statistics, subtle distinctions can be shown among genetic lines (Louis et al. 1968) or intraracial ecotypes (Cornuet et al. 1975). In the Western Hemisphere, bees of European and Africanized ancestry are difficult to distinguish, especially in the case of quarantine interceptions where it is necessary to identify a few dead worker bees. When discriminant analysis was applied to this problem, Daly and Balling (1978) were able to distinguish between the races by using 25 morphometric characters (for explanation of discriminant analysis see Sneath and Sokal [1973]). The necessary coefficients were computed for both individual bees and collections of 10 bees from a colony. Identifications of the latter have a lower risk of misclassification. The procedure described here is a further development of their method.

Although the use of morphometrics in insect taxonomy is well established, the practice has been discouraged by the tedious procedure of measuring structures

under the microscope and the several transcriptions of the data by hand that usually are required. Computer hardware and software are now available that: (1) substantially reduce the handling of specimens and time required for measurements; (2) virtually eliminate accidental errors; (3) automatically analyze the data; and (4) store the data in a form that is easily retrieved.

Materials and Methods

Equipment and Programs

The computer used is a Digital Equipment PDP 11/34 with cartridge disk drive (type RK05JAA) and floppy disk drive (type RX01AA). Video display and keyboard are provided by a Tektronix scope terminal (type 4013) and printed copy by a Decwriter (type LA36). The digitizer, which communicates directly with the PDP 11/34 over a serial line, is a Summagraphics Corp. ID (model ID-2-CTR-1111; resolution 0.1 mm) with tablet active area, 11 by 11 in., and four-button cursor. Zeiss Luminar lenses, 16 mm and 25 mm, are mounted in a Bausch and Lomb Tri-simplex microprojector and project images of the specimens onto the digitizer surface.

The FORTRAN program Bequik (Fig. 1), written by Norman and Daly, receives and processes the point coordinates from the digitizer, computes and prints the identification information, and creates a permanent file on the disk for each collection. The collection files are periodically transferred to the larger IBM 4341 computer via a telephone link or floppy disk. The files are entered in a special file by the FORTRAN program Taxir. Taxir is an information storage and retrieval system based on Boolean algebra (Brill 1978). The measurements can then be retrieved for further analysis by any one or a combination of the items of information about the collections.

Methodology

The general procedure of Daly and Balling (1978) begins with the preparation of a numbered microscope slide mount of a forewing, hindwing, hind leg, and the third abdominal sternum of each bee. Ten bees from a colony is the usual collection, but a single bee can be

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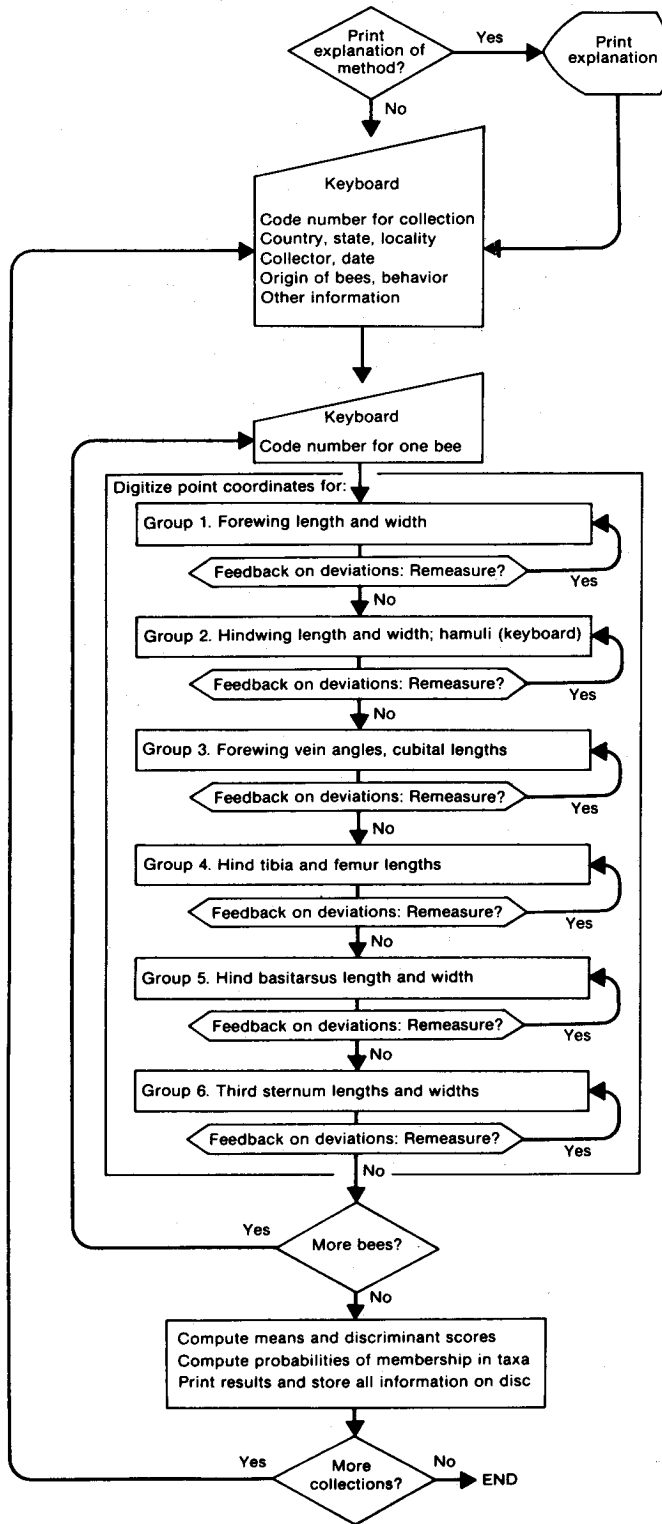


FIG. 1.—Flowchart of the program Bequik for measuring and identifying honey bees.

identified. Information about the collection (locality, date, behavior, etc.) is entered in the computer via the keyboard in a specific sequence with prompting on the video display. While viewing the magnified image of the specimen, the operator enters with the cursor the point coordinates for the measurements in a specific sequence. Prompts are given on the video display as well as bells to signal that the computer is ready to receive further coordinates. Numerical counts are entered via the keyboard. The 25 measurements are divided in six groups based on the lens used and the structure to be measured. Linear distances are computed and converted to true metric distances by a constant appropriate for the lens used. Angles are computed by trigonometry. Each measure is compared against the expected mean based on a set of previous measurements. Deviations greater than 1.96 SD units are shown on the video display, and a warning bell sounds. The operator has the option to remeasure a group if the deviations are either large or numerous. This protects against accidental errors in digitizing, while allowing odd bees to be recorded. From 1 to 30 bees can be measured in each collection. After the last bee is measured, discriminant scores are computed for each individual and for the means of the measurements for the collection as a whole. Different coefficients are used for individuals and collections. The probabilities of membership in the European or Africanized taxa are computed. The identities of individuals and collections are assigned to the taxa with greater than 50% probability of membership.

The collection data, discriminant scores, probabilities of taxa membership, and identities are printed, and a permanent computer file is created for this information, plus all the measurements and their means.

Results and Discussion

The honey bee has been the subject of numerous morphometric studies, beginning with Koshevnikov (1900-1905). Measurements usually were made with an ocular micrometer. Dupraw (1965) introduced the use of a microscope-slide projector and measured images with a ruler and protractor. Daly and Balling (1978) continued this practice. The use of a digitizer has several advantages over these procedures. In our experience, the average time to measure and record the length of a slide-mounted wing is greatly reduced: 42 sec with an ocular micrometer versus 19.2 sec with the digitizer. When many different measurements are made per slide, the average time falls to 7.2 sec per measurement. Precision is increased by using the digitizer and the projected image: at 16 \times magnification with a stereomicroscope and ocular micrometer, one can measure wing length to the nearest 0.05 mm, whereas the 23-fold image on the digitizer tablet can be measured to the nearest 0.004 mm. Other optical and computer equipment could be substituted in this procedure, but the digitizer must have high resolution. We also have found that differences among persons who make the measurements are reduced when they use the digitizer. Two experienced persons measured the same 27 wings by ocular micrometer and

again by digitizer. The mean difference between the ocular measures was $+0.0230 \pm 0.0069$ mm, range 0-0.11 mm, (significant difference from 0.0, probability < 0.01) vs. mean difference between the digitizer measures $+0.0070 \pm 0.0057$ mm, range 0-0.06 mm (not significant). Finally, the direct transmission of digital data to the computer eliminates the earlier transcription and checking of data by hand. In our experience with over 200,000 measurements by hand, most errors occurred during the manual transcriptions.

Blind tests of the analytical identifications were conducted during 1974 to 1977. Our initial discriminant coefficients were obtained by comparing bees whose ancestry was reasonably certain, based on geographic location and the observations of experienced persons. We then received collections of bees from O. R. Taylor, University of Kansas, with code numbers and no other information. After our identifications, Taylor gave us the collection data and the identifications in the field made by himself, G. W. Otis, and K. van Deursen. The bees were usually taken from artificial hives, wild colonies, or swarms so that colony defense behavior was observed as well as the size and color of the bees. The collections were made in Brazil, French Guiana, Guyana, Surinam, and Venezuela. The bees were from areas occupied exclusively by European bees or by Africanized bees or where the latter were invading.

The independent field and analytical identifications agreed without qualification in 95.6% (129) of 135 collections and disagreed in 4.4% (6). Our analyses of the 135 collections gave probabilities of membership near 100%. The separate computed identities for the collections and for the 10 individual bees comprising each of the collections agreed in 110 collections; they differed by 1 bee in 14 collections, and 2 bees in 11.

The field and analytical identifications of seven other collections had certain reservations attached. Of these we disagreed on two collections, but the field identifications were unsure. Our analyses of the remaining five (three agreements, two disagreements) were unsure, because either the probability of membership for a collection was much less than 100% or 3 to 7 individual bees differed from their own collection in their probable identity. The collections, therefore, appear mixed or intermediate between European and Africanized bees.

The intermediate collections illustrate the difficulty in using phenotypic characters to identify genetically different, but closely related populations. Such collections can be expected to occur if: (1) some Africanized bees in adjacent colonies drift into European colonies or vice versa; (2) Africanized bees are in the process of taking over a European colony; (3) genetic hybridization between Africanized and European bees has recently occurred; or (4) unusual environmental stresses (food shortage, small cell size in the comb) have altered the normal growth of European bees so that they become smaller adults and thus resemble Africanized bees in some measurements. Additional information on the history and nature of the colony would be required to help determine their identities. If specimens had been frozen,

then allozyme analysis (Rinderer and Sylvester 1981) would be especially useful. As a practical matter, however, most collections are preserved dry or in alcohol.

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REFERENCES CITED

- Brill, R. C. 1978.** The Taxir primer, 3rd ed. Computing Center, University of Michigan, Ann Arbor. 179 pp.
- Budai, A., W. R. Riedel, and M. J. Westberg. 1980.** A general-purpose paleontologic information device. *J. Paleontol.* 54: 259-262.
- Cornuet, J. M., J. Fresnaye, and L. Tassencourt. 1975.** Discrimination et classification de populations d'abeilles a partir de caractères biométriques. *Apidologie* 6: 145-187.
- Daly, H. V., and S. S. Balling. 1978.** Identification of Africanized honeybees in the Western Hemisphere by discriminant analysis. *J. Kans. Entomol. Soc.* 51: 857-869.
- Dupraw, E. J. 1965.** The recognition and handling of honey-bee specimens in non-Linnean taxonomy. *J. Apic. Res.* 4: 71-84.
- Hellenthal, R. A., and R. D. Price. 1980.** A review of the *Geomydoceus subcalifornicus* complex (Mallophaga: Trichodectidae) from *Thomomys* Pocket Gophers (Rodentia: Geomyidae), with a discussion of quantitative techniques and automated taxonomic procedures. *Ann. Entomol. Soc. Am.* 73: 495-503.
- Koshevnikov, G. A. 1900-1905.** Materials to the natural history of the honey bee. Vol. 1 and 2. Soc. des Amateurs des Sciences Naturelles, Moscow.
- Louis, J., J. Lebevre, R. Moratille, and J. Fresnaye. 1968.** Essai de discrimination de lignées consanguines d'Abeilles domestiques (*A. m. mellifica* L.) obtenues par insemination artificielle. *C.R. Acad. Sci. Paris, Ser. D.* 267: 526-528.
- Rinderer, T. E., and H. A. Sylvester. 1981.** Identification of Africanized bees. *Am. Bee J.* 121: 512-516.
- Ruttner, F. 1968.** Les races d'abeilles. In R. Chauvin [ed.], *Traité de biologie de l'abeille*, vol. 1. pp. 27-44.
- Sneath, P. H. A., and R. R. Sokal. 1973.** Numerical taxonomy. W. H. Freeman and Co., San Francisco. 573 pp.
- Vassiliev, B. R., and N. S. Rostova. 1977.** On some correlations of leaf characters of *Bryophyllum* (Crassulaceae). Experience in automatic system usage for object measurements and data treatment in morphological investigations. *Botanical J. Leningrad*, 62: 319-329. (in Russian, with English summary)