The "African" Honey Bee

Edited by

Marla Spivak, David J.C. Fletcher, and Michael D. Breed

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INTRODUCTION

Marla Spivak,[1] David J.C. Fletcher,[2] and Michael D. Breed[3]

This book is the first review of the scientific literature on the Africanized honey bee. The African subspecies *Apis mellifera scutellata* (formerly *adansonii*) was introduced into South America in 1956 with the intent of cross-breeding it with other subspecies of bees already present in Brazil to obtain a honey bee better adapted to tropical conditions. Shortly after its introduction, some of the African stock became established in the feral population around São Paulo, Brazil, and spread rapidly through Brazil. It has since migrated through most of the neotropics, displacing and/or hybridizing with the previously imported subspecies of honey bees. Africanized bees have been stereotyped as having high rates of swarming and absconding, rapid colony growth, and fierce defensive behavior. As they have spread through the neotropics they have interacted with the human population, disrupting apiculture and urban activities when high levels of defensive behavior are expressed.

Our goal as editors was to bring together the large body of information that has become available concerning the Africanized bee and its spread and impact through the New World. Accordingly, we present chapters from a diversity of authors, include important research not previously reviewed in English, and cover a wide range of scientific methods including both basic and applied research. A couple important investigators were unwilling or unable to deliver promised chapters; nevertheless, we feel that this book is a success in that it brings some much needed objective balance and clarity to a subject that has often been clouded by emotions.

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While we as editors do not have a consensus even among ourselves as to the best solution to the "Africanized bee problem," we agree that there is sufficient variation in behavior within the honey bee population in the neotropics to provide the basis for future selection programs to yield manageable and productive honey bee populations that can coexist with humans. Others believe that the best way of ameliorating whatever undesirable traits these bees may display in the United States will be to modify them genetically by controlled hybridization with the honey bees already present in this country.

Our intention is to provide reviews of the major topics concerning Africanized honey bees. Recently, Needham *et al.* (1988) published a compendium of current research on Africanized honey bees and bee mites. Various other symposia and congresses are cited in Needham *et al.* (1988) or in this work. There is a large body of unpublished Master's theses and Doctoral dissertations from Brazilian universities on Africanized bees which provide a substantial base of knowledge concerning the biology of these bees. These have, unfortunately, been neglected in most English language reviews on Africanized bees; a list of these works is available from Dr. Lionel Gonçalves in Brazil.[4] Our hope is that readers will be able to identify common themes and principles concerning Africanized bees among the diverse views presented in this book.

WHAT SHOULD WE CALL THIS BEE?

The introduced bees from which our Africanized bees are derived were from a race distributed in southern and eastern Africa, *Apis mellifera scutellata* Lepeletier. Most of the other bees in the New World are derived from European races, *A. m. mellifera* L. (Germany), *A. m. ligustica* Spin. (Italy), *A. m. caucasica* Gorb. (Caucasus mountains, northern Europe and west central Russia), and *A. m. carnica* Pollman (southern Austrian alps, northern Yugoslavia, Danube Valley) (Winston, 1987).

Our opinion is that in general there has been too great of a tendency to pigeon-hole bees found in the New World as either European or African in origin. While the genetic origins of New World bees can be ascertained, the distinctions among the Old World subspecies, have been blurred by processes of hybridization, artificial selection, and natural selection in their current ecological context. Thus, knowledge concerning honey bees will probably be better advanced by considering New World bees as representing, to greater or lesser extents, new subspecies based on novel gene combinations and adaptations to local conditions.

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We have allowed the authors of the individual chapters to choose their own terminology when referring to the populations of bees discussed in this book. This book deals with a highly variable set of populations, some of which fit the stereotype of swarming, absconding and defense mentioned above and others which do not. As readers assimilate the information in this book, some statements may seem contradictory because they refer to different populations in different life zones that come under the general grouping of Africanized bees. We feel that, given the genuine differences of opinion among experts, this is the proper way to handle this difficult problem of systematics and nomenclature in this rapidly expanding and evolving population.

A BRIEF HISTORY OF THE INTRODUCTION OF AFRICAN BEES TO THE NEW WORLD

The most widely disseminated narrative of the introduction of African bees is presented by Kerr (1957). In this account, Professor W. E. Kerr brought 170 queens from the savannah of eastern and South Africa to Brazil. Forty-seven or 48 survived importation and were successfully introduced into colonies at Piracicaba (São Paulo state); one of these queens was from Tanzania (called Tanganyika at the time) and the others were from South Africa. These were moved in November of 1956 to Camapuã, Rio Claro, São Paulo state, Brazil, in a *Eucalyptus* forest of the Paulista Railroad. In early 1957, an ill-informed technician of the Railroad removed queen excluders from the entrances of 26 of these colonies, which soon swarmed (Nogueira-Neto, 1964; Kerr, pers. comm.; Kerr, 1966/67; Kerr, 1967; Gonçalves, 1974; Gonçalves and Stort, 1978). Coincidentally, Kerr (1957) characterized 26 of these colonies as "the most prolific, productive and industrious bees that we have seen up to now." It is not clear from the accounts whether this refers to the same 26 colonies which escaped.

There are several studies which support an argument that not all of the original colonies escaped, and which are not consistent with the commonly held view that the original spread was due to just 26 colonies. Kerr and Mendoça-Fava (1972) refer to experiments performed in 1957 using "hybrids" between African queens and *ligustica* drones, which indicates early rearing of African queens. Widely cited articles (Kerr, 1966/67, 1967; Portugal-Araújo, 1971) present tables in which honey production is compared among three races of bees, African, German, and Italian. Ten colonies of African bees were used in both 1958 and 1959 in these experiments, after the escape of the 26 colonies. In fact, African bees were propagated through queen rearing and artificial insemination during or after the 1958 and 1959 experiments and distributed widely among beekeepers in southern Brazil (Kerr, pers. comm.). By the time of the first International Congress of Apiculture held in Florianopolis in 1970, there were published

reports of African queens that were reared and inseminated with African drones (Kerr *et al.*, 1972).

Kerr (1966/67) presented a detailed discussion of the problems associated with the African bees and proposed a series of solutions, including enhancement of drone production in manageable stock, use of better protective equipment, placement of apiaries away from people and livestock, and use of Italian queens. The solutions presented in this article were of considerable interest, but unfortunately it was never translated and circulated in Spanish-speaking Latin America or in North America. Many of the solutions now being proposed stem from his original ideas, although his work is sometimes not cited.

In a survey of beekeepers Kerr (1966/67) found a slight preference for Africanized bees although some beekeepers reported quitting the business because of difficulties managing these bees. De Jong (1984) also reports a strong preference among the remaining beekeepers for African bees because of the bees' high productivity. Another reason beekeepers in some regions may prefer Africanized bees is that their highly defensive nature deters theft of equipment and honey. A preference for African bees is also probably dependent on the availability and affordability of both feral swarms and beekeeping technology that allows management of highly defensive bees. Many beekeepers in developing countries do not have access to movable frame hives, smokers, veils and other protective clothing.

Although problems with defensive behavior were quickly recognized in Brazil and Argentina, the first English language discussion in the scientific literature of a serious problem with defensiveness came from Nogueira-Neto in 1964. In 1969, Kerr published a map of the distribution of Africanized bees in Brazil (Kerr, 1969). Soon after, the National Research Council (USA) commissioned a report (Anonymous, 1972) on the developing problem. Professor C. D. Michener participated in the NRC study and published his assessment separately (Michener, 1975). Shortly thereafter, Taylor (1977) published maps which illustrated the predicted rate of spread of Africanized bees, their climatic limits in North America, and discussed the potential impact on beekeepers. Professor Taylor's predictions have been remarkably accurate, although the hypotheses about northern climatic limits have not yet been tested. The balance of the story concerning the spread, ecology, and demography of Africanized bees is dealt with in various chapters of this book.

HAS HYBRIDIZATION OCCURRED AMONG SUBSPECIES?

This critical and controversial issue is addressed in the first section of the book. Because of rapid advances in research, it cannot be conclusively reviewed in this book. Three major techniques have been applied to the question. Daly (Chapter 2) discusses the use of morphometrics and allozymes. Hall (Chapter 3) focuses on the use of DNA techniques. Some morphometric analyses have

supported the argument that intermediate phenotypes occur in Brazil and southern South America (Buco *et al.*, 1987; Rinderer, unpubl. data) while another study (Boreham and Roubik, 1987) has argued that the bee population in Panama has, over time, reverted to an African phenotype. Allozyme studies of the malate dehydrogenase locus have shown evidence of hybridization (Nunamaker and Wilson, 1981; Lobo *et al.*, 1989), however after initial hybridization in a given region, the alleles acquired from European bees may decline in frequency (O. R. Taylor, pers. comm.). Studies of mitochondrial DNA by Hall and Muralidharan (1989) and Smith *et al.* (1989) show similar restriction fragment patterns between the bees they sampled in the neotropics and African (*A. m. scutellata*) bees. These results are consistent with a study of nuclear DNA in Mexico in which the bees show a lack of persistent hybridization with European bees (Hall, 1990). However, these DNA patterns may be different in Argentina where the feral African population approaches its climatic limits in northern latitudes and European bees persist further south (Sheppard, unpubl. data). We hope these issues will be resolved in the near future.

THE SPREAD OF AFRICANIZED BEES AND THE AFRICANIZATION PROCESS

In the second section of the book, the Africanization process is considered. This consists of two distinct processes, migration of Africanized or African bees into new areas and gene flow among populations. To a considerable extent, workers in this area have been unable to distinguish between the migration of the bees into an area and subsequent evolutionary events. Some of these issues are covered in the chapters on identification, discussed above; in this section the chapters focus on ecological and behavioral mechanisms of the spread of Africanized bees. There are fundamentally differing views on how Africanization proceeds. For example, Rinderer and Hellmich, Chapter 5, argue that hybridization is a key factor, while one of us, Fletcher in Chapter 4, sees this population as African, rather than Africanized in nature. Fletcher (Chapter 4), proposes that a new introduction of African bees be undertaken, with pre- and post-introduction selection programs to insure that manageable and productive phenotypes are obtained. These differences may in fact be reconciled in part by Ratnieks (Chapter 6) approach which considers both that hybridization and selection processes may have taken place at and behind the migratory front. Another of the editors, Spivak, presents a case study of Africanization in Costa Rica (Chapter 7). This section of the book closes with a general review of honey bee genetics and breeding by Page and Kerr; this includes a review by Kerr of what measures have been taken in Brazil. Hellmich, Chapter 9, gives practical advice on preparing for Africanization.

POPULATION BIOLOGY AND ECOLOGY

Knowledge is somewhat better based concerning population biology and ecology than systematics and the Africanization process. Authors here provide the important basic data on colony dynamics (Winston, Chapter 10), population biology (Otis, Chapter 11), foraging (Rinderer and Collins, Chapter 12), ecology (Roubik, Chapter 13), and diseases (Shimanuki *et al.*, Chapter 14). These data have formed the fundamental characterization of Africanized bees and will provide the basis for further studies involving their ecology.

DEFENSIVE BEHAVIOR

The greatest cause of public concern over the Africanized bee has been its defensive behavior and particularly its stinging behavior. One of us, Breed, gives an overview of honey bee defensive behavior (Chapter 15). Collins and Rinderer (Chapter 16) and Stort and Gonçalves (Chapter 17) give detailed accounts of the genetic work on defensive behavior in European and Africanized bees. In our opinion there are two major gaps in knowledge of this area. First, we do not understand the range of defensive phenotypes in Africanized bees. It is difficult or even impossible to design rational selection programs without this knowledge. Second, there has been virtually no work on the ethology of colony defense of Africanized bees. We do not know if defense is organized in the same fashion as in European bees or if there are completely different modes of communication and division of labor in Africanized bees. The ethology of defense of European bees is reviewed in Breed's chapter. It is remarkable, given the span of time since the introduction of African bees and the public, agricultural, and scientific concern over defensiveness that more studies have not been done.

BEEKEEPING IN SOUTH AMERICA

To date the greatest experience with keeping Africanized bees has been in South America. We present three case studies (Brazil, Chapter 18; Peru, Chapter 19; and Venezuela, Chapter 20). In addition Spivak (Chapter 7) discusses issues related to beekeeping in Costa Rica. We view the Brazilian experience as being particularly important because they have had Africanized bees for 30 years and claim success in their selection programs. The Peruvian study also holds particular interest because the investigator brings a social scientist's viewpoint to his study. Our intent with these chapters was to bring together enough information so that research directions might become apparent. We also hope that the chapters will be useful to beekeepers in the United States.

CLIMATIC LIMITS OF AFRICANIZED BEES

The climatic limits of Africanized bees has been a controversial issue and there is no clear conclusion about survivorship of Africanized bees in temperate areas. Readers interested in this topic are referred to studies in Argentina (Kerr et al., 1982; Dietz *et al.*, 1985, 1988, 1990; Krell *et al.*, 1985), in Germany (Villa *et al.*, 1990) and predictions by Taylor (1977, 1985) and Taylor and Spivak (1984). The rapid spread of Africanized bees into North America may soon provide an empirical answer to this question. It is difficult to translate the experiences of South and Central American beekeepers into projections for North America because of differences in climate and beekeeping technology. Nevertheless, it is clear that Africanized bees have disrupted beekeeping and public activities wherever they have migrated and established permanent populations. The extent of the continued disruption appears to depend largely upon beekeepers and the implementation of selection programs.

CONCLUSIONS AND FUTURE DIRECTIONS

We have drawn together information on the identification, spread, ecology, defensive behavior, and practical implications of the Africanized honey bee in the New World. The most rapidly growing field of inquiry deals with identification and the extent of hybridization. How and why are European bees being hybridized and displaced while Africanized bees appear to have maintained a high degree of phenotypic and genetic similarity to bees from South Africa? More information should be available on these critical issues soon. We hope that this book stimulates further work on defensive behavior, particularly on phenotypic variation and the ethology of defense in Africanized bees. Many studies have assumed that morphometric or other identification techniques predict defensiveness; this linkage has not been firmly established and when studies of variability in defensiveness are conducted it will be important to pursue correlates between defensive traits and traits used in identification. Despite our use of the term "Africanized" we do not believe that this is a completely appropriate characterization of a population that has been subject to both natural and artificial selection since 1956 and which occurs in many different ecological zones. One of the challenges facing researchers on Africanized bees is to acknowledge that phenotypic differences among bees in different areas may explain apparent contradictions among studies. We hope that the focus of research will shift to unifying principles with an underlying comprehension of phenotypic variation.

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PART ONE

Systematics and Identification

2

SYSTEMATICS AND IDENTIFICATION OF AFRICANIZED HONEY BEES

Howell V. Daly[1]

The purpose of this chapter is to evaluate the current status of systematics of Africanized bees and of several methods of identification based on the phenotype. Both subjects are more complex than often appreciated, hence the need to discuss the history and background in some depth. The name "African bees" will be used for native bees of Africa and "Africanized bees" for their relatives in the Western Hemisphere.

Identification is the first step for all research and efforts to mitigate the problems created by Africanized bees. Furthermore, reports about Africanized bees are accurate only to the extent that the identifications are trustworthy. For these reasons, reliable methods are currently being developed for the purposes of scientific investigation, regulation (Stibick, 1984), and for future breeding and certification (Page and Erickson, 1985). For reports of recent progress in identification, see Needham *et al.* (1988).

SYSTEMATICS

Apis mellifera is well known for its remarkable communication and environmental control in the hive. Less familiar is its extraordinary biogeography. In contrast to other Apoidea where congeneric sympatry and limited distributions are common, *A. mellifera* occupies an immense and varied geographic area. Except for a narrow overlap with *Apis florea* in the east, *A. mellifera* coexists naturally with no other member of its genus. The distribution extends from southern Scandinavia south to the Cape of Good Hope and from Senegal east to about 60° E longitude (Ural Mountains; Mashhad, Iran; and coast of Oman) (Ruttner *et al.*, 1978). Colonies are found from sea level to about

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1000 m in the Alps in the temperate zone (F. Ruttner, pers. comm.) and, in the tropics, from sea level to 3100 m on Mt. Kilimanjaro in Africa. They are missing from extreme deserts, but survive as wild colonies in hot, arid Oman at 200-1500 m (Dutton *et al.*, 1981).

Populations throughout this vast distribution are believed to be largely interfertile. They are similar in morphology, have the same number of chromosomes, and exhibit low protein polymorphism. They probably share the same gene loci, but differ in allelic frequencies at some loci. Ruttner (1988a) has argued that the present distribution might be no older than the late Pliocene. Adaptation to local environments has created geographic races of greater or lesser distinction, depending mainly on physical barriers. Differences among races are found in morphometry, behavior, and physiology (Cornuet and Louveaux, 1981).

Beginning in 1956 in Brazil, African bees (one colony from Tanzania and 46 from Pretoria, South Africa) were said to be crossed with European bees (primarily *A. m. ligustica* and *A. m. mellifera*), to produce Africanized bees (Filho *et al.*, 1964; Kerr, 1967, 1969). This was a cross between distantly related races: bees of Europe and Southern Africa had evolved under different physical and biotic ecology and biogeographic history; they were separated by over 70^{o} latitude; and genetic exchange had been further restricted for at least the last 2,000 years by the Sahara desert (Ruttner, pers. comm.). The relative contributions of European and African ancestry in Africanized bees at the outset is unknown. Nor do we know the genetic consequences of subsequent hybridization with European bees and of natural selection in new habitats of the Western Hemisphere. During the period 1982 to 1985 in Panama, Boreham and Roubik (1987) found morphometric measurements of Africanized bees to become smaller or more African-like. It is clear that the entity we now call Africanized is not a singular population, but rather a series of variable populations.

Partial reproductive isolation is known to exist between European and Africanized bees (Kerr and Bueno, 1970). As Africanized bees spread through South and Central America, their reproductive biology apparently has operated to perpetuate their African ancestry and give them sufficient advantage to replace European bees (Taylor, 1985; Rinderer, 1986). Africanized bees resemble their African parents more than their European parents in mitochondrial DNA, morphometry, hemolymph proteins, biochemistry of cuticular hydrocarbons, and behavioral characteristics. In view of this similarity, Taylor (1985) has speculated that Africanized bees are essentially African bees. The new DNA technology described in the next Chapter and elsewhere (Hall, 1988; Hall and Mulralidharan, 1989; Severson *et al.*, 1988; D. Smith, 1988; D. Smith *et al.*, 1989) will provide a method for assessing the genomes of these variable populations.

In the meantime, I provide here a graphic representation (FIGURE 1) of the morphometric relationships among African, Africanized, and European bees by

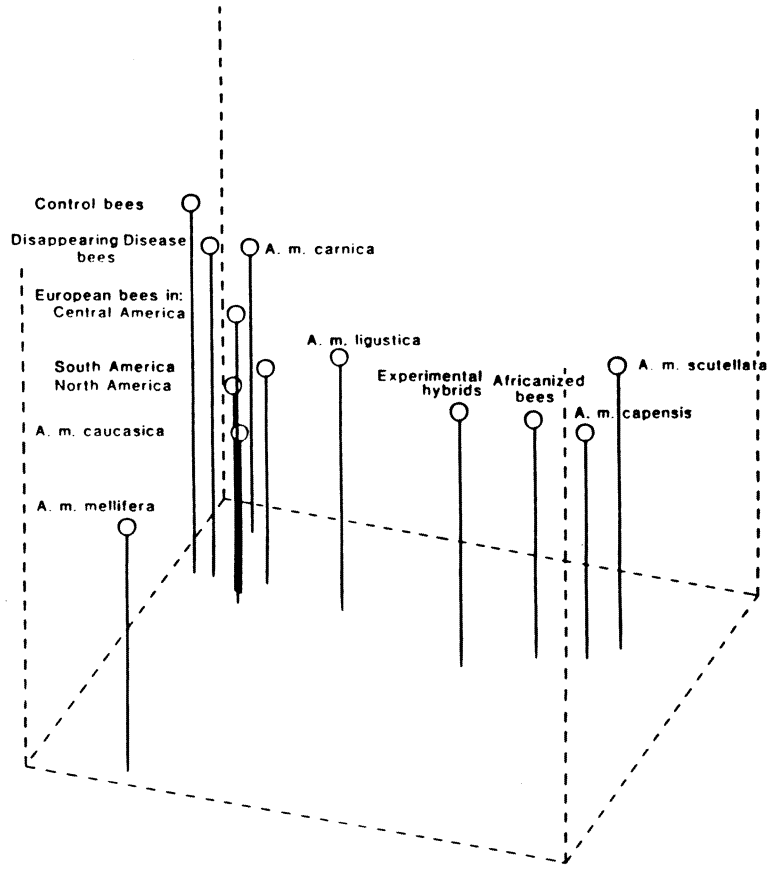

FIGURE 1. Morphometric relations among 13 groups of honey bees as shown in the first 3 dimensions of a discriminant analysis. Circles show positions of group centroids. The diameters of circles are arbitrary. See text and TABLE 1 for further explanation.

using a discriminant analysis of 25 characters (method discussed further on in this chapter). The characters were measured on ten bees from each of 820 colonies. Average measurements from each colony were used in the analysis. Colonies were divided into 13 groups on the basis of geography and known identity (TABLE 1). The "hybrids" were experimental colonies of miscellaneous origin in South America that were crosses of Africanized and European parents for one or more generations. Colonies designated as having "disappearing disease" and the "controls" were provided from the United States by W. C. Rothenbuhler (see Kulincevic *et al.*, 1984).

TABLE 1. Discriminant analysis of African, Africanized, and European honey bees.

No.	Name of groups and geographic origin	NC	CC	MC	(No.)
1.	*A. m. scutellata* (South Africa)	51	80.4	11.8	(3)
2.	*A. m. capensis* (South Africa)	12	91.7	8.3	(1)
3.	Africanized bees (South America)	191	68.6	16.2	(4)
4.	Hybrids of Africanized & European bees	10	90.0	10.0	(3)
5.	*A. m. carnica* (inbred lines, Germany)	8	87.5	12.5	(10)
6.	*A. m. liguistica* (Kangaroo Island, Austr.)	11	100.0		
7.	*A. m. mellifera* (Tasmania & S. Amer.)	36	100.0		
8.	*A. m. caucasia* (lab colonies, U.S.A.)	7	85.7	14.3	(9)
9.	European bees: North America	182	67.6	11.5	(10)
10.	European bees: Central America	135	69.6	8.9	(9,11)
11.	European bees: South America	118	66.1	15.3	(10)
12.	European bees: Disappearing Disease	36	73.9	13.0	(13)
13.	European bees: Control	23	86.1	11.1	(10)

Key: NC = number of colonies (10 bees measured in each colony). CC = per cent of colonies classified in the correct group out of total number of colonies in that group. MC = highest per cent of colonies misclassified in another group and the code number (No.) for the other group in parenthesis. See text for further explanation.

The graph shows positions of the centroids for the groups in the first three dimensions of the 12 dimensional space required for the 13 groups. The first three functions explain 62.8%, 15.76%, and 5.8% of the total variance, respectively, making a total of 84.4%. The graph is therefore an approximate representation of the morphometric relationships among the groups. Of interest here are the relations among African (*A. m. scutellata*), Africanized, and European bees (using the North American group as an example). For an exact measure of these relations, I computed Euclidean distances in the 12 dimensional space. The distance between African and Africanized bees is 2.56; between Africanized and European bees, 5.35; and between European and African bees, 6.65. From the approximate positions in the graph and the relative distances it can be seen that Africanized bees are closer morphometrically to African bees than to European bees in North America. Africanized bees, however, differ from African bees and are situated in the graph between African and European bees in a position that could indicate some hybridization has taken place or a "founder effect" has occurred (Mayr, 1963) or both.

After the analysis was completed, the original colonies were reclassified into the 13 groups (TABLE 1). All colonies of *A. m. mellifera* and *A. m. ligustica* were correctly classified into their respective groups, indicating that they formed discrete clusters. The clusters of the other groups overlapped to various degrees such that some colonies of a given group were misclassified as members of one or more other groups. The 191 Africanized colonies were reclassified as Africanized (131 colonies or 68.6%), hybrid (31 or 16.2%), *A. m. scutellata* (17 or 8.9%), *A. m. capensis* (10 or 5.2%), European bees: Central America (1 or 0.5%) and South America (1 or 0.5%). In this respect also, the Africanized bees show a closer morphometric relation to African bees and Africanized hybrids than to European bees. Buco *et al.* (1987) compared 24 of the same measurements among Africanized, African, and European bees. They report 18 measurements of Africanized bees are more similar to African bees than to European bees.

Disappearing disease among European bees in North America was hypothesized to be a genetic trait derived from Africanized bees (Roberge, 1978). The 13-group analysis indicates that such bees, like their controls, show no morphometric evidence of Africanization.

SCIENTIFIC NAMES FOR AFRICAN AND AFRICANIZED BEES

The concept of a widespread race of honey bees in subsaharan Africa that is closely related to the European races can be found in most reviews of bee classification. Before 1958, however, opinions differed as to whether subsaharan bees were distinct species (Smith, 1865; Ashmead, 1904; Friese, 1909; Skorikow, 1929a; Goetze, 1930, 1940; Maa, 1953) or an infraspecific form of *A. mellifera* (Buttel-Reepen, 1906; Enderlein, 1906; Ruttner and Mackensen, 1952; Kerr and Laidlaw, 1956). The modern era of bee classification started with

Kerr and Portugal-Araújo (1958) who applied the biological species concept to the problem. They cite genetic crosses among European and African races as justification that all belong to one species. All evidence to date supports their conclusion, but partial reproductive isolation may exist between some races (Kerr and Bueno, 1970).

The species name for the African bee, therefore, should be *Apis mellifera* Linnaeus (1758). Even this name has been subject to controversy. In 1761 Linnaeus changed the name to *Apis mellifica* because the original name means "honey carrier" rather than "honey maker" which he preferred. According to the Principle of Priority (Art. 23, International Code of Zoological Nomenclature, hereafter abbreviated as ICZN; see Ride *et al.*, 1985) the oldest available name is the valid name of a taxon. In spite of this long standing rule, the junior name still appears in some European literature.

The name *Apis mellifera*, however, does not distinguish African bees from all other forms of the species. We obviously need a name that is simple, stable, and communicates just which honey bees we intend. Zoological nomenclature is a system of scientific names for animals known to occur in nature. The system has been remarkably successful in providing unique names for species of animals. In the past, systematists attempted to extend the hierarchic classification to populations within species by naming subspecies, varieties, races, phases, forms, etc. In the twentieth century attention focused on biological variation among populations within a species. This led to the "New Systematics" of Huxley (1942) and Mayr (1942). Of several infraspecific categories, the subspecies traditionally has been favored as the category deserving formal recognition in nomenclature. All infrasubspecific categories are excluded from our formal nomenclature (ICZN Art. 1(b)(5)).

Earlier in this century, naming of subspecies became a major preoccupation of systematists. The practice drew criticism, of which the most influential was the critique of Wilson and Brown (1953). They argued that while criteria for species had proved to be objective and practicable, the delimitation of subspecies was not only subjective and arbitrary but also inefficient for reference purposes.

The intent of naming subspecies was to recognize genetically distinct, geographic segregates of a species that were capable of interbreeding along lines of contact. Yet the number of subspecies appeared to vary directly with the number of characters used to distinguish them. Wilson and Brown pointed out that popular quantitative methods still required an arbitrary decision on the degree of difference. There was essentially no lower limit to the definition of subspecies. More importantly, careful analyses of geographic variation of characters in various species of animals often revealed a lack of concordance among characters. Two populations having the same subspecies name have an implied similarity in characteristics. Likewise, two populations of the same species having different subspecies names have an implied discontinuity in character variation. Yet these implications may not have a basis in fact. Wilson

and Brown recommended the formal trinomial be replaced by the species name plus a simple vernacular name based on geographic origin. Following the paper of Wilson and Brown, interest in analysis of geographic variation continued to increase, but among professional systematists enthusiasm for naming subspecies markedly declined.

The difficulty in delimiting races of honey bees was already apparent in the last century. After comparing variation in size and color of specimens from diverse localities, Gerstaecker stated (in translation, 1863:342): "The variability of the coloration... gives transitions from one form to another; and thus it becomes impossible to define clearly limited varieties. Latreille and Lepeletier made eight species out of the Honey-Bee; with equal justice we might now, from the existing materials, make 20-30." He proposed six "main varieties" of *A. mellifera* of which one was in Egypt, one widespread in Africa and one in Madagascar.

Prior to 1958, 14 species or subspecies names had been proposed for honey bees in Africa, Madagascar and neighboring islands. In addition to a widespread race, many authors acknowledged variation among bees in the subsaharan region by including names for local varieties or distinct species. The small, scattered collections available for study undoubtedly had the effect of accentuating real differences among populations. In one instance, incorrect identification led to errors in biogeography. In 1906, both Buttel-Reepen and Enderlein stated that forms of *A. indica* (now under the name *A. cerana*) existed in West Africa. This Asian species is now known not to occur naturally in Africa.

Kerr and Portugal-Araújo (1958) recognized a single, widespread subsaharan subspecies and correctly named it *A. m. adansonii* Latreille (1804) according to the oldest available name for honey bees in this region of the African continent. For this reason, when African bees were introduced to Brazil, the name *A. m. adansonii* was applied to the bees in Brazil. They also recognized *A. m. capensis* Eschscholtz (1822) at the Cape of Good Hope and *A. m. unicolor* Latreille (1804) for bees on Madagascar and neighboring islands. Smith (1961) followed their classification and added *A. m. monticola* (Mt. Kilimanjaro and Mt. Meru) and *A. m. litorea* (coast of Tanzania). These were described as new "varieties," but are to be treated now as subspecies (ICZN Art. 45(g)(ii)(1)). Goetze (1964) recognized two subspecies: *A. m. capensis* for the Cape region and *A. m. adansonii* for the rest of Africa, Madagascar, and neighboring islands.

At this point, we have 19 names for species or subspecies in the Ethiopian Zoogeographic Region, of which 16 might apply in subsaharan Africa, Madagascar and neighboring islands. All are available names under the terms of ICZN, but much nomenclatorial housekeeping is needed in the future because most of the descriptions fail to include the designation and deposition of types, and types are missing.

The main issue still remains: How many geographic segregates deserve formal subspecies names? If we recognize one subspecies for subsaharan Africa

(excluding Madagascar and neighboring islands), then the name should be *A. m. adansonii*. Ruttner (1975a), however, presented evidence for six subspecies in subsaharan Africa: *A. m. adansonii* in West Africa from Senegal at least to the Republic of Congo, *A. m. scutellata* in Savanna of East and South Africa, *A. m. litorea* in East coast of Africa from Somalia to Mozambique, *A. m. monticola* in mountains of Ethiopia, Kenya, and Tanzania, *A. m. capensis* in Cape of Good Hope, and *A. m. unicolor* in Madagascar and neighboring islands.

Ruttner's subspecific classification is based on multivariate statistical analysis of 40 morphometric characters, plus distribution and behavior. In the statistical analysis, each subspecies forms a cluster that is partly or entirely separated from other such clusters. Overlapping clusters can be separated by detailed analysis of the subspecies involved (Ruttner, 1986, 1988a,b). Quantitative differences among subsaharan subspecies are similar in magnitude to those among European subspecies that have long been accepted by taxonomists and apiculturists. Future studies on intervening populations in Africa may show that these clusters intergrade to some extent. This is to be expected because no major physical barriers exist between the populations studied. Ruttner and Kauhausen (1985) explain the geographic diversification as adaptation to local environments.

Following their arguments, I agree that it is reasonable to recognize more than one subspecies in subsaharan Africa. The names can be justified both on grounds of convenience in communication as well as in recognition of distinctive populations for which the exact boundaries are still uncertain. If a separate subspecies in the savanna of East and South Africa is recognized, the name should be *A. m. scutellata* Lepeletier (1836). The name *scutellata* is based on specimens collected in "Caffrerie," a region in South Africa extending from the Great Kei River on the south to Natal Province on the north and between the Drakensberg Mountains and the coast. Populations near Pretoria are probably similar. According to Ruttner, *A. m. scutellata* extends north to East Africa.

In summary, we now have two choices for the scientific name of the African bees that were introduced to Brazil from Tanzania and Pretoria: If the classification of Kerr and Portugal-Araújo (1958) is followed, then the name should be *A. m. adansonii*. If we follow Ruttner (1975a) as I recommend and recognize six subspecies, then the name of the introduced bees is *A. m. scutellata*.

The issue of the correct name is further complicated by questions regarding the possible hybrid origin of Africanized bees. Mitochondrial DNA of Africanized bees is African in origin (Hall and Muralidharan, 1989; D. Smith *et al.*, 1989). This indicates that the spread has been by maternal migration and not by paternal gene flow into European populations. Additional research is needed to determine whether their nuclear DNA is similarly African in origin or is partly European through hybridization. If Africanized bees that exist now are not hybrids and still have essentially an African genome, then their scientific name

is *A. m. scutellata.* On the other hand, if Africanized bees are a genetic hybrid swarm (Buco *et al.*, 1987), then they do not have a convenient scientific name in zoological nomenclature.

Hybrids are sufficiently common among plants to receive special treatment in the International Code of Botanical Nomenclature (Voss *et al.*, 1983; Appendix I; see also Wagner, 1969). Several options are available to provide unique scientific names for hybrid plants at different taxonomic levels. The collective prefix "notho-" or hybrid form, as in nothosubspecies, can be used for any thriving population of hybrids between natural subspecies, whether F1, segregate or backcross.

In zoology, hybrids as such are explicitly excluded from the provisions of ICZN and do not receive separate scientific names (Art. 1 (b) (3)). Thus, if the Africanized bee is a genetic hybrid swarm between races originating in the Old World it cannot have a formal name that conveys that fact. Informally, animal hybrids are often designated by a formula indicating the parental taxa. In our case, the formula for the Africanized population would presumably be: *A. m. carnica* x *A. m. caucasica* x *A. m. ligustica* x *A. m. mellifera* x *A. m. scutellata.* This is obviously too cumbersome a name for practical use and fails to indicate the close similarity of Africanized and African bees. Unless and until the International Commission on Zoological Nomenclature comes to grips with the nomenclatural problems of hybrids, we will have to use common names such as "European" and "Africanized" even though these bees are permanent, identifiable additions to the fauna of the Western Hemisphere.

IDENTIFICATION

Identifications are made with various degrees of assurance. When specimens of a species have unique and clearly defined structural or other characters, then the identifications are irrefutable within the context of the current classification. For example, *A. mellifera* is distinguished structurally from its nearest relative, *A. cerana.* The latter species has two veinlets extending distad from the large basal cell of the hind wing rather than one as in *A. mellifera.* This and other key characters have proven to be consistent and species specific. Specimens of *A. mellifera*, therefore, can be conclusively identified (Daly, 1988).

The geographic races and other distinctive populations of *A. mellifera*, however, usually can not be conclusively identified. They exhibit characters that may overlap to some degree or may grade imperceptibly into adjacent populations. Based on comparison of samples known to be typical of two or more populations, one can estimate how often an identification based on certain characters is likely to be correct. If quantitative characters are used, statistical analysis can provide a statement about the probability that a new sample is correctly identified. In this case, the identification is probable rather than

conclusive. Identifications of subspecies, geographic races, genetic hybrid swarms, ecotypes, or biotypes are usually of this nature.

The accuracy of probable identifications depends entirely on how representative the initial samples are with respect to the total populations to be identified. Both Africanized and European bees in the Western Hemisphere appear to be genetically heterogeneous. European bees are a mixture of races, including minor introductions from Africa even before the advent of Africanized bees (Morse *et al.*, 1973). Any procedure for making probable identifications should be based on a broad sampling of this heterogeneity.

The sample unit is usually a collection of bees from a colony and identification is based on pooled extracts or averages of characters of the collection. Some procedures can identify individual bees. A complication for all procedures is the possible mixture of Africanized and European workers in a single colony. This could occur by drift, or when an Africanized colony is in the process of taking over a European colony, or the queen may produce a mixture of daughters because she was inseminated by both kinds of drones.

Probability statements of identification must be interpreted within the context of the procedure. For example, with current methods in morphometrics, the statement that a colony collection is Africanized at 0.7 or 70% probability also indicates the sample is European at 0.3 or 30% probability. The sample could be of normal Africanized bees or normal European bees, but it is more likely to be the former based on previous analysis of known Africanized and European bees. The statement does not mean that the colony is composed of 70% Africanized bees and 30% European bees or that workers have 70% Africanized genes and 30% European genes. To make such statements, the procedures must be able to distinguish individuals or be based on genetic analyses, respectively. Furthermore, the statement that a new sample is Africanized at 1.0 or 100% probability is not a conclusive identification; it is still a probable identification based on the initial analysis of known Africanized and European bees.

All probable identifications carry the risk of actual misidentification. Any method (morphometric, biochemical, behavioral, genetic) that yields a probable rather than conclusive identification carries this risk. When large numbers of samples are being identified, even a small risk becomes an important consideration in terms of the numbers of samples that may be misidentified.

The problem of identifying Africanized bees can be considered at two extremes: "new introductions" or detection of the first Africanized bees to arrive in areas previously occupied by European bees; and "hybrids" or detection of genetic crosses and backcrosses between Africanized and European bees in areas where they have interbred. In the first situation, Africanized and European bees are relatively distinct and phenotypic methods are effective. However, special care must be exercised when one or a few Africanized samples are suspected in the midst of a large population of European bees. Because identifications are

based on probability statements, the suspected Africanized bees may, in theory, be indistinguishable statistically from the "tail" of a very large distribution of European samples. In this case, the best action would be to combine evidence from several methods of identification (Spivak *et al.*, 1988). In the second situation a spectrum of genotypes or hybrid swarm may exist in an area together with one or both parental types. Current phenotypic methods were not intended to discriminate among a series of genotypes and, even with further development, will never be as precise as genetic methods.

When Africanized and European bees are carefully compared, statistical differences in morphometry, behavior and physiology are not difficult to find. In behavior, for example, differences have been shown in defense of the nest (Collins *et al.*, 1982), weight of swarms and nest cavity selection (Rinderer, Tucker *et al.*, 1982), nectar foraging (Rinderer *et al.*, 1984), and hoarding (Rinderer, Bolten *et al.*, 1982).

Some of the differences between Africanized and European bees provide a practical basis for identification. Three approaches that are now used or might be used with further development will be discussed here: morphometrics, protein electrophoretic variants, and biochemistry of cuticular hydrocarbons. Average size of worker brood comb cells provides a useful character if natural comb can be obtained (Fletcher, 1978; Rinderer, Sylvester, Brown *et al.*, 1986; Spivak *et al.*, 1988). The collection of comb, however, from feral colonies is time-consuming and sometimes impossible because the host tree or building cannot be damaged. The comb may have been produced by the progeny of a previous queen and not the current queen. Furthermore, comb samples are often sticky and difficult to keep for later measurement without crushing. Unless fumigated or frozen, wax moths often infest comb samples. Other approaches that are still being explored range from the chemistry of venom and alarm pheromones (Mello, 1970; Shipman, 1975; Shipman and Vick, 1977; Blum *et al.*, 1978) to wing-beat frequency (Anonymous, 1986).

MORPHOMETRICS

Morphometrics is the measurement and analysis of form. In biology the forms measured are morphological structures of organisms and analysis is usually by statistics. Morphometrics is widely applied to problems in insect life history, physiology, ecology, and systematics (Daly, 1985). Because it is the phenotype that is measured, an insect's morphometrics includes both genetic and environmentally induced variation. To be useful in identification, the genetically determined racial differences between taxa must be large enough to provide distinguishing characters in spite of environmentally induced and local genetic variation within each taxon.

Historical background

Morphometrics of bees have been extensively analyzed, especially during the first third of this century when apiculturists sought bees with longer tongues that could reach nectar in flowers with deep corolla tubes. Without controlled matings, attention turned to natural variation of bees with the hope of finding useful stock for breeding. Although the objectives were not realized, these early studies gave us much information on geographic variation and inheritance of morphometrics and environmental influences on morphometrics (Merrill, 1922).

In 1929, Alpatov reviewed the pioneering studies of Russian scientists on the roles of genetics and environment in geographic variation in bees. By transplanting colonies to new localities in Russia and observing European races in the United States, he concluded the races and geographic variants within races had specific characters, including morphometrics, that were genetically determined. Unless artificially selected or crossed with other races, the characters were stable in new habitats.

Statistical analysis of individual morphometric characters (univariate analysis), therefore, formed the basis for early studies on bee races by Alpatov (1948), Goetze (1940, 1964) and Skorikow (1929a,b, 1936). The genetic basis for seven morphometric characters in bees was first established by Roberts (1961) who estimated heritabilities at 0.28 (number of hamuli) to 0.85 (wing width and tongue length). Morphometric characters are regularly used for breeding and certification in Europe (Ruttner, 1988a).

Careful measurements and experiments by Alpatov's contemporary, A. S. Michailov, also revealed environmentally induced variation within and among colonies. As summarized by Alpatov (1929), "the following conditions have a pronounced effect on the body size of worker bees: (1) the season of the development, (2) the temperature of the surroundings during the pupal stage, (3) the size of the cell, (4) feeding by nurse bees of different age, and (5) individuality of the colony." Alpatov noted that absolute body size and changes in some proportions could be related to reduced larval feeding.

During the same early period in the United States, Kellogg and Bell (1904), Casteel and Phillips (1903), and Phillips (1929) produced major papers on bee biometry and showed that drones were more variable than workers. This feature of drones was later explained by Brueckner (1976) to be the consequence of reduced developmental homeostasis that arises from their hemizygous genome. Grout (1937) demonstrated that workers reared from enlarged brood cells were significantly larger than workers from normal brood cells. Recently, Eischen *et al.* (1982, 1983) reared worker larvae with different numbers of nurse bees, finding positive correlations between the number of nurse bees and dry weight and life span of the progeny.

The use of morphometrics in bee classification was accelerated by DuPraw (1965a,b) who introduced the use of multivariate analysis. DuPraw's purpose

was to create a multidimensional framework based on discriminant analysis to show relationships of bee races. In this multivariate technique measurements of two or more characters are weighted and combined linearly to give maximal separation of two or more groups. For explanations of the method see Pimentel (1979) or a guide to mainframe computer packages such as for SPSS by Norusis (1985).

The multivariate approach, including principal component analysis, has since been applied to discriminate between genetic lines (Louis *et al.*, 1968), ecotypes or strains within a race (Louis and Lefebvre, 1968; Tomassone and Fresnaye, 1971; Cornuet *et al.*, 1978, 1982; Leporati *et al.*, 1983, 1984) and geographic races or subspecies (Louis and Lefebvre, 1971; Cornuet *et al.*, 1975; Gadbin *et al.*, 1979; Santis *et al.*, 1983). Ruttner *et al.* (1978) describe and illustrate 41 characters that are the basis for continuing analysis of all the geographic races of *A. mellifera* in the Old World. The results have appeared in a series of papers by Ruttner (1968, 1969, 1973, 1975a, b, 1981, 1986, 1988a,b) and Ruttner and Kauhausen (1985).

Univariate analysis of Africanized bees

The first efforts to distinguish Africanized from European bees with morphometrics were by univariate analysis. Kerr *et al.* (1967) and Kerr (1969) reported that Africanized bees are smaller than Italian bees except for number of hamuli, width of the basitarsus, and diameter of ocelli in which Africanized bees were said to be larger. Rinaldi *et al.* (1971) computed indices for various measurements of wings, mouthparts, and hind legs of Africanized, Italian, and Caucasian bees. Sarmiento *et al.* (1974) measured widths of abdominal (metasomal) sterna 3, 5, and 6 for Africanized and Italian bees. Authors of these papers do not provide sufficient statistical information to test differences between means.

In the context of a larger study, Woyke (1977) examined four colonies from South Africa, three colonies of Africanized bees, and nine colonies of Italian bees. He found an overlap in more than 30 characters, but counts of bristles were separated. Bristles on the upper surface of the wing were counted within a standard 0.8 mm x 0.5 mm area in the discoidal cell (2nd M cell). Bees with more than 80 bristles were classified as Africanized and those less than 80 were Italian bees. However, counts from one to three colonies in East and West Africa, and in northern and eastern Europe gave partial overlaps.

Multivariate analysis of Africanized and European bees

When Africanized and European bees are compared on the basis of single characters, the variation in characters usually overlaps between the groups. An intermediate specimen or sample from a colony, therefore, can not be identified

at a high level of probability by a single character. Multivariate discriminant analysis has features that are useful in identifying Africanized bees. When the same groups are compared in a discriminant analysis of many characters, the combination of characters often gives a clear separation of groups. In the simplest case where two groups are distinguished, new specimens can be identified by multiplying each of the measurements by a corresponding coefficient, summing the products, correcting with a constant, and comparing the resulting discriminant score to the expected values of the scores for the known groups. A probability of membership in each of the groups can be computed. A specimen or collection is usually assigned to the group with which it has a probability of membership greater than 50%.

The first multivariate analyses used 25 characters to demonstrate the feasibility of identifying Africanized bees by morphometrics (Daly, 1975, 1978). The characters were selected from those previously employed by Alpatov, Goetze, DuPraw, and Ruttner. Included were four linear measurements and ten angles between veins of the fore wing, number of hamuli and two linear measurements of the hind wing, four linear measurements of the hind leg, and four of the third sternum. Structures were dissected, cleaned, the sternum stained, and all mounted on a microscope slide. Images of the parts were projected on a table with an overhead projection microscope. Measurements were taken with ruler and protractor. Analyses gave good separation even though overlaps existed for each character. At the outset, it was anticipated that starved European bees might be misidentified if they were quite small in body size (Daly, 1975).

Daly and Balling (1978) describe the further analysis of samples of usually ten bees from each of 101 collections of Africanized bees and 297 collections of European bees. The collections came from diverse geographic areas and were from swarms, feral colonies, well managed and poorly managed colonies, and some were from flowers and other food sources. Analyses were made based on: (1) means of measurements for ten bees in each collection, and (2) on measurements of each individual bee. With all 25 characters in the analysis, all collections and 95.6% of individual bees were correctly identified. The expected rate of misidentification was computed at 0.5% for collections and 4% for individuals.

The 25 character analysis, though successful in identification, was tedious and time consuming to perform by hand, even with a computer for the analyses. The procedure was substantially improved by the addition of a digitizer to form a semi-automatic system for measurement and identification. Daly *et al.* (1982) describe the equipment and computer program for the system.

Blind tests of field versus analytical identifications gave agreement in 95.6% of 135 collections. Five other collections had intermediate scores which might have been genetic in origin (mixtures of Africanized and European bees within a colony or recent hybridization) or the result of environmental influences such as

those reported by Michailov that cause a reduction the size of European bees. In the latter case, a score based on atypical measurements that fell within the range of Africanized bees could lead to an intermediate score or misidentification (Daly *et al.*, 1982).

To test some environmental effects on morphometrics, Rinderer, Sylvester, Collins, and Pesante (1986) reared workers of Africanized and European bees under different combinations of nurse-bee genotype and comb cell size. Nurse-bee genotype had small and nonsignificant effects, but cell size had significant effects. The larger European comb resulted in larger bees and the smaller Africanized comb resulted in smaller bees. Despite these influences, the progeny could be correctly identified by the 25 character discriminant analysis. Similarly, Herbert *et al.* (1988) fed larvae various diets to induce nutritional stress with the result that the adult worker bees still were correctly identified by the morphometric procedure.

In summary, the 25 character analysis has been tested under several circumstances and found to give the most reliable identifications to date. The method requires about five hours for one person to process a sample of ten bees. A laboratory is required with stereomicroscope, light, slide making materials and a small chemical hood to remove solvent vapors, plus a projector with high quality optics, computer, digitizer, and computer program. Difficulties may be encountered in interfacing the digitizer and computer. The method requires skilled persons who must exercise care in making the slides and measuring bees. Applications of the method on abnormal bees and hybrids are continuing (Daly *et al.*, 1988; Rinderer *et al.*, 1989)

FABIS methods

Rapid techniques for identifying large numbers of collections have been developed by Rinderer, Sylvester, Brown *et al.* (1986). These are called FABIS for "Fast Africanized Bee Identification System." FABIS is a stepwise procedure leading to identification. To be "identified" in FABIS, the probability of membership must be 90% or greater; if less, then the collection is "unidentified" and is subjected to further analysis. Identifications are based on the means of measurements for 10 bees from a colony. In the latest version (Rinderer, Sylvester *et al.*, 1987), fore wing length, wet weight of freshly killed degastered bees, or dry weight of degastered bees is used as the first step. Bees that remain unidentified at the first step are then measured for one or two additional characters so that a combination can be used in a bivariate or trivariate analysis. Options are given to compute the scores and probabilities for various combinations of fore wing length, wet or dry weight, and length of hind femur.

FABIS has minimal requirements for equipment and can be performed in temporary quarters by unskilled persons. Linear measurements are made by mounting a wing and, if required, a hind leg on microslide coverslips with tape.

This in turn is mounted in a 35 mm slide mount and projected by a slide projector onto a wall at a standardized distance. The image is measured with a meter stick and converted by a magnification factor to the metric system. Weights are taken with a metric balance accurate to 0.01 gm (Sylvester and Rinderer, 1986).

Morphometrics by image analysis

Televised images can be converted to digital information by a suitably equipped computer and the images measured automatically. Of the various structures of bees that are often measured, the wings offer the best images with the current technology. In anticipation of the need to measure bees rapidly by image analysis, Daly and Hoelmer (unpubl. data) used a digitizer to measure 22 lengths of vein segments and 25 angles between veins on the forewings of 100 samples (ten bees each) of Africanized bees and an equal number of European bees. Discriminant analysis of the 47 measurements gave an unbiased estimate of correct classification of 99% for collections of ten worker bees/colony and 91% for single worker bees. This study was used by Batra (1988) as the basis for automatic image analysis of forewings, using an integrated system of optical, television, and computer instruments. The procedure is convenient and provides greater speed than hand-operated digitizers.

As with other phenotypic methods, the reliability of FABIS and the image analysis procedure depends on how similar the initial data sets are to the populations to be discriminated. To prepare for the identification of Africanized bees in a given area, a survey of European bees should be made in advance and compared with the identification standards.

PROTEIN ELECTROPHORETIC VARIANTS

The technique of electrophoresis makes possible the sorting of proteins taken from tissues of organisms. A homogenate of tissue is placed in a gel and an electrical current applied. Within the electrical field, different proteins migrate different distances from the point of origin depending largely on their net charge. The rate of migration may also be influenced by the sizes and shapes of the protein molecules, the properties of the sieve-like gel matrix, and other physicochemical conditions. The protein bands can be made visible in the gel by the addition of suitable stains.

The proteins of interest here are enzymes that can be identified by the use of histochemical stains that are specific for an enzyme. These stains generally couple a specific substrate or another aspect of an enzymatic reaction to a reaction that produces a visible dye. In this way, the localization of a specific enzyme can be determined despite the fact that hundreds or thousands of proteins may be present in a single organism.

Enzymes detected by this technique are called isoenzymes or isozymes. The application of a stain sometimes discloses multiple bands. These are alternate forms of the isozyme. Breeding experiments usually show the isozyme variants are inherited in a Mendelian pattern. Therefore, each variant is considered to be the direct product of an allele of the same gene coding for the enzyme. Such variants are called alloenzymes or allozymes. Although early workers often used terms such as slow, medium, and fast or alphabetic characters to designate allozymes, difficulties were encountered when new allozymes were found or when numerous allozymes were present. The use of "relative mobility" descriptions is much more likely to give unique names to particular allozymes and also to allow results from different studies to be more easily compared. Relative mobilities are calculated by simply measuring the distance a particular allozyme travels in the gel, relative to that of a reference allozyme. The reference allozyme is usually the most common allozyme in the population where the polymorphism was first described (Berlocher, 1980). In some cases, the reference allozyme is based on the slowest or fastest migrating allozyme. Thus, malate dehydrogenase in the honey bee is polymorphic and has five described allozymes: Mdh^{55}, Mdh^{65}, Mdh^{80}, Mdh^{87}, and Mdh^{100} (decimal points are often omitted). The gene responsible for the allozyme is designated similarly and italicized.

Electrophoresis permits analysis of the genetics of natural populations and even single insects in a manner never before possible and provides a valuable tool in systematics (Avise, 1974; Berlocher, 1984). The number of isozymes detected depends on the method employed. The most common are starch gel electrophoresis, polyacrylamide gel electrophoresis, and isoelectric focusing. Various modifications are possible within each method that may improve detection of isozymes. In a comparison of the common methods, Coyne *et al.* (1979) found each method to detect some variation not detectable by the other two.

Studies on proteins of honey bees span almost two decades. Considering the normal roles of enzymes in development and physiology and the potential variation of the genes responsible within and among populations, it is not surprising to learn that isozymes: (1) vary qualitatively and quantitatively over the life of the bee (Gilliam and Jackson, 1972a; Contel *et al.*, 1977; Bitondi and Mestriner, 1983; (2) are present in eggs (Nunamaker and Wilson, 1981a), larvae (Tripathi and Dixon, 1968, 1969; Nunamaker and Wilson, 1982), pupae (Mestriner, 1969; Mestriner and Contel, 1972) and adults (Gilliam and Jackson, 1972b); (3) vary among castes and sexes at the same level of development (Tripathi and Dixon, 1968, 1969; Kubicz and Galuszka, 1971); (4) vary among populations of the same geographic race (Cornuet, 1979; Badino *et al.* 1983a, 1985; Sheppard and Berlocher, 1984, 1985; Sheppard and McPheron, 1986); (5) vary among geographic races of *A. mellifera* (Mestriner and Contel, 1972; Martins *et al.*, 1977; Gartside, 1980; Sylvester, 1982; Badino *et al.*,1983b,

1984; Nunamaker *et al.*, 1984a; Sheppard and Huettel, 1988; (6) vary among species of *Apis* (Tanabe *et al.*, 1970; Nunamaker *et al.*, 1984b; Sheppard, 1985; and (7) vary among genera of the Apoidea (Contel and Mestriner, 1974; Snyder, 1975, 1977).

In comparison with other insects, honey bees and Hymenoptera generally have been reported to have a low level of isozyme polymorphism. For example, Sylvester (1976) used starch gel and 30 stains in his study of adult bees. Thirty-nine bands or loci were found of which only one, malate dehydrogenase (Mdh-1), was polymorphic. Nunamaker and Wilson (1980) used isoelectric focusing and 30 stains to reveal 28 isozymes of which Mdh and non-specific esterase were polymorphic. This unusual feature has stimulated numerous theoretical explanations of which a recent review is by Graur (1985). In contrast, a recent study of sawflies reveals levels of enzyme polymorphism consistent with diploid insects (Sheppard and Heydon, 1986).

The polymorphic isozymes of *A. mellifera* known to date are listed below. Because different techniques are used and different numbers of allozymes are reported, it is difficult to judge if authors are reporting the same allozymes. Furthermore, negative findings such as those of Brueckner (1974) may also be a result of the technique used (Hung and Vinson, 1977). The inheritance of allozymes based on breeding experiments has been determined for alcohol dehydrogenase (Martins *et al.*, 1977), esterase (Mestriner and Contel, 1972; Bitondi and Mestriner, 1983), malate dehydrogenase (Contel *et al.*, 1977), and P-3 protein (Mestriner and Contel, 1972).

•*Aconitase.* Acon-2: 100, 120 in adult worker bees in Czechoslovakia (Sheppard and McPheron, 1986).

•*Alcohol dehydrogenase.* Adh-1: 1, 2, 3 in drone and worker pupae, but absent in young larvae and adults of Italian and Africanized bees (Martins *et al.* 1977); F, S in worker larvae in Australia (Gartside, 1980).

•*Esterase.* Includes a series of loci, each of which has a suite of allozymes, e. g., the esterase loci 1, 3, 5, 6 in larvae and pupae of worker and drone Africanized bees (Bitondi and Mestriner, 1983). The most commonly reported locus is probably Est-3 (Sheppard, pers. comm.). Est: F, S in pupae of workers and drones of Africanized and Italian bees (Mestriner, 1969; Mestriner and Contel, 1972); F, S in worker larvae in Australia (Gartside, 1980); S, M, F in adult worker Italian bees (Badino *et al.*, 1984) and in Sicily (Badino *et al.*, 1985); 100, 130 in adult worker Italian bees (Sheppard and Berlocher, 1985); 70, 100, 130 in adult worker bees in Czechoslovakia (Sheppard and McPheron, 1986).

•*Hexokinase.* HK-1, the fastest allele, in higher frequency among European bees and lower in Africanized bees; other alleles not individually distinguishable found in higher frequency among Africanized bees and lower among European bees (Del Lama and Figueiredo, 1986; Del Lama *et al.*, 1988; Spivak *et al.*, 1988).

•*Malate dehydrogenase.* Mdh-1: A, B, C in larvae, pupae, and adult worker and drone Africanized bees and pupae of Italian worker bees (Contel *et al.*, 1977); a, b, c in adult worker bees in Guadaloupe (Cornuet, 1979); 0.50, 0.63, 1.00 in adult worker European, Italian, and Africanized bees (Sylvester, 1976, 1982); F, M, S in worker larvae in Australia (Gartside, 1980); 0.50, 0.63, 1.00 in adult worker bees in Guatemala and Mexico (Nunamaker *et al.*, 1984a), in Africa and Brazil (Nunamaker and Wilson, 1981b); 65, 80, 100 in adult worker bees in Norway (Sheppard and Berlocher, 1984); S, M, F in adult worker Italian bees (Badino *et al.*, 1983a); S, M, F, F1 in adult worker Italian bees (Badino *et al.*, 1983b, 1984) and in Sicily (Badino *et al.*, 1985); 65, 87, 100 in adult worker Italian bees (Sheppard and Berlocher, 1985); 55, 65, 80, 100 in adult worker bees in Czechoslovakia (Sheppard and McPheron, 1986).

•*Malic enzyme.* Me: 79, 100 in adult worker bees in Norway (Sheppard and Berlocher, 1984); 100, 106 in adult worker Italian bees (Sheppard and Berlocher, 1985); 79, 100 in adult worker bees in Czechoslovakia (Sheppard and McPheron, 1986).

•*Phosphoglucomutase.* Pgm: 75, 100 in adult worker bees in Czechoslovakia (Sheppard and McPheron, 1986; see also Del Lama *et al.*, 1985).

•*Protein.* P-3: F, S in pupae of workers and drones of Africanized and Italian bees (Mestriner, 1969; Mestriner and Contel, 1972).

Five isozymes have been compared between Africanized and European bees: alcohol dehydrogenase, esterase, hexokinase, malate dehydrogenase, and protein P-3. No isozyme has been found that gives complete separation of Africanized and European bees. In other words, we do not find the one allozyme exclusively in one type and another allozyme exclusively in the other type. However, except for the common esterase which is the same in both types, the other four isozymes exhibit partial separation because the frequencies of the alleles differ in each type. Of these, alcohol dehydrogenase was studied in larvae and protein P-3 in pupae, leaving hexokinase and malate dehydrogenase as the only isozymes currently available for use with adult worker bees.

Attention has concentrated on adult worker bees for the purposes of identifying Africanized bees because they are readily collected and have useful isozyme patterns that do not change with the bee's age (Gilliam and Jackson, 1972b). Bees intended for electrophoresis must be fresh or quickly frozen and stored frozen at -60°C. Homogenates of whole bees are prepared or, to avoid contamination from gut contents, the abdomens are discarded or only hemolymph is withdrawn.

Ayala and Powell (1972) propose a method by which partial differences in allozymes can be used as diagnostic characters to distinguish species of *Drosophila.* In brief, the first step is to compute the allelic frequencies from baseline data on the species to be distinguished. Then the expected frequencies of the genotypes in each species are computed by assuming the Hardy-Weinberg equilibrium. For each genotype, the frequency in one of the species will usually

be smaller than the other. The overlap of the two species is the sum of the smaller frequencies for each genotype. To identify new specimens by their genotypes, the two species are assumed to be equally common. An individual of a given genotype is assigned to the species with that genotype in the higher frequency. The probability of misidentification is half the computed overlap in the distribution of genotypic frequencies between the two species. Ayala and Powell considered a locus diagnostic if it had a probability of correct assignment at one of two levels: 99% or, more stringently, 99.9%. High probabilities of correct identification are possible only when the differences in genotype frequencies between the species are large.

The method of Ayala and Powell has been considered by Sylvester (1982), Nunamaker *et al.* (1984a), and Page and Erickson (1985) for the purpose of identifying Africanized bees. Sylvester computed the expected genotype frequencies for three alleles of malate dehydrogenase based on samples of (1) his own data for 34 colonies of Africanized bees from Brazil and 24 colonies of European bees from California, and (2) the data of Contel *et al.* (1977) for 78 colonies of Africanized and 34 colonies of Italian bees from Brazil. His evaluations of the probability of correct identification were 92.8 or 94.9%, respectively, depending on the baseline data. Sylvester (1982) and Rinderer and Sylvester (1981) considered the risk of misidentification with Mdh alone was too great for practical use. They propose that if new allozyme systems are discovered in adult bees, these could be combined in the identification procedure to give a joint probability of correct classification and thus reduce the uncertainty.

Nunamaker and Wilson (1981b) compared Mdh in bees from ten colonies of pure African bees from South Africa and 12 colonies of Africanized bees from Brazil. They found the African bees to be monomorphic for Mdh 100 and the Africanized bees polymorphic with the Mdh 100 very high at 93%. From this they conclude the homozygous Mdh 100 genotype is characteristic of African bees. In a subsequent paper, Nunamaker *et al.* (1984a) compared European bees from Australia (4 colonies), Denmark (3), Finland (11), France (2), New Zealand (2), Norway (3), Sweden (3), and Tasmania (3) versus African bees from South Africa (16 colonies). The computed probabilities for correct classification were 99.2-100% This is sufficient to qualify Mdh as a diagnostic locus in the sense of Ayala and Powell (1972). Their choice of samples, however, largely omitted native bees from central and southern Europe.

In Europe, several studies now report Mdh 100 in much higher genic frequency than previously known: northern Italy, 0.073- 0.446, southern Italy 0.521-0.962, Sicily 0.690-1.00 (Badino *et al.*, 1983a,b; 1984); Emilia region of northern Italy, 0.19- 0.46 (Sheppard and Berlocher, 1985); Norway 0.00-0.41 (Sheppard and Berlocher, 1984); Czechoslovakia 0.00-0.53 (Sheppard and McPheron, 1986). Furthermore, Badino *et al.* (1984) showed an inverse

relationship between Mdh F (=100) and S in Italy, with F increasing toward the south and warmer winter climates.

It is now clear that homozygous Mdh 100 genotypes can be expected to occur widely in Europe, especially in southern Italy, and are not restricted to southern Africa. The presence of such genotypes in the Western Hemisphere could be from bees imported from either Europe or Africa. They are not exclusively "African" genes. The Mdh 100 allele may confer higher fitness in warmer regions as suggested by Rinderer and Sylvester (1981) and Badino *et al.* (1984). Populations of bees in warmer regions that were established by introducing colonies from cooler regions might be expected to exhibit shifts over time in the relative frequencies of the Mdh allozymes.

The use of allozymes to identify Africanized bees remains a viable option in need of improvement. A distinct advantage is that allozymes are immediate products of structural genes and independent of environmental influences. A disadvantage is the need to kill bees directly by freezing and keep the samples frozen at ultralow temperature before the analysis. Under field conditions, this may be difficult. To be of practical use in the future, one or more additional allozyme systems must be found and added to the Mdh system to meet a stringent joint probability of correct classification. Most important is the development of an adequate baseline on the expected genotype frequencies in critical geographic areas. The method requires special electrophoretic equipment, wet laboratory with chemical hood (some stains are highly toxic) and skilled personnel. If starch gels are made the previous day, run time and incubation time are about four hours each. A number of individual bees can be analyzed at the same time.

BIOCHEMISTRY OF CUTICULAR HYDROCARBONS

Insects contain lipids that are derived partly from their diet and partly from synthesis (Lockey, 1980). Lipids can be extracted from an insect's body by immersion in a fat solvent such as hexane or methylene chloride. Lipids extracted by relatively short immersion are probably derived mainly from the epicuticle and the underlying exocuticle that is also rich in lipids (Hendricks and Hadley, 1983). Longer immersion extracts lipids from cuticular glands and from tissues inside the insect's body. Hemolymph, for example, is rich in hydrocarbon (Chino and Kitazawa, 1981).

In addition to the cuticular lipids, worker bees secreting comb wax may have wax scales on the abdominal sterna that contribute to the extract. The extract also could conceivably include contaminants from the bee's environment such as lipids in honey (Smith and McCaughey, 1966), lipids that have rubbed off from other bees or the combs (Tulloch, 1980), as well as plant lipids from plant cuticular waxes, propolis (Ghisalberti, 1979) and pollen (Stanley and Linskens, 1974).

The extractable lipids from insect cuticle are a complex mixture of compounds in which hydrocarbons often predominate (Hadley, 1986). Analysis of the extract usually involves partitioning the extract followed by gas chromatography and often gas chromatography-mass spectrometry. The hydrocarbons of interest are mostly the long, unbranched chains of saturated (alkanes), mono-unsaturated (alkenes), and di-unsaturated (alkadienes) carbon molecules in an odd-numbered series from C15 to C43. The degree of unsaturation is indicated by "carbon number:degree of unsaturation," e.g., the mono-unsaturated C35:1.

Blomquist *et al.* (1980) determined the cuticular wax of European bees to be 58% hydrocarbon in contrast to comb wax which is predominantly monoester at 31-35%, with hydrocarbon at 13-17%. They further demonstrated that the composition of wax synthesized varies with the age of the bee and with season. In winter, hydrocarbon is the major fraction extracted from the cuticle. In summer months when comb is constructed, hydrocarbon was the major component in younger and older bees, while monoester was the major component in bees 11-18 days following emergence as adults.

Tulloch (1980) pointed out that although comb wax of African and European bees is similar in composition, African wax has proportionately less unsaturated C31 and more C35 hydrocarbon than European wax.

Carlson and Bolten (1984) first reported qualitative and quantitative differences between extracted cuticular hydrocarbons of Africanized and European bees. The samples of bees were of unknown age and varied in method of preservation. Differences in proportions between the two kinds of bees were found in C35:1, C35:2, C37:1, C37:2, C39:1, C39:2, C41:1, C41:2, and C43:2. These totalled 22.4% of hydrocarbons from Africanized bees, but only 1.1-3.1% of hydrocarbons from European bees. Africanized bees had much more C35:1 than European bees. The latter had small, trace, or undetected amounts of the C35 to C43 series. Subsequently, Lavine and Carlson (1987) used multivariate statistics to improve the separation of the two types of bees by the hydrocarbons. Carlson (1988) reported that individual drones of African, Africanized, and European bees could be identified by hydrocarbon patterns.

McDaniel *et al.* (1984) identified and quantified the hydrocarbons extracted from the whole sting apparatus, sting shaft, and general body cuticle of European bees. The samples were of foragers (workers more than 21 days old) collected in plastic bags and frozen. They conclude the sting apparatus is a sufficient source of hydrocarbons for identification of single bees and is relatively free of contamination from extraneous sources. Extracts of the apparatus can be readily made and yield the series C15 to C38 components in contrast to the cuticle with only C23 to C36 components. They did not compare Africanized bees in their analysis.

Francis *et al.* (1985) compared extractable hydrocarbons from workers and drones of four *Apis* species, including samples from native bees in Africa and

Africanized bees from South America. My review is confined to their study of *A. mellifera*. Random aged and newly emerged bees were frozen. Dehydration during storage did not affect the quantity of hydrocarbon extracted. In both random aged and newly emerged bees, they confirmed the higher proportion of unsaturated C35:1 and C35:2 in native African and Africanized bees versus the European subspecies. They also found chain lengths longer than C35 only in random aged African bees. In contrast to previous studies, newly emerged European bees had unsaturated components with chain lengths longer than C35 in amounts sometimes greater than in Africanized bees.

Analyses published to date have used packed column gas chromatography. The most recent analyses by R. K. Smith (1988) utilize the increased resolution of capillary column gas chromatography-mass spectrometry. With this instrument, isomers can be distinguished among molecules of the same chain length and degree of unsaturation. The position of the double bond is counted from the nearest end of the chain and indicated by a numerical prefix, e. g., 14-C35:1.

R. K. Smith (pers. comm.) examined random aged bees that had been collected and stored in isopropanol. Among the mono-unsaturated components, he found the proportions of the following isomers to differ between Africanized and European bees: 9-, 8-, and 7-C29:1; 10-, 9-, and 8-C31:1; and 14-, 12-, and 10-C35:1. Africanized bees exhibited predominantly 9-C29:1 (7-C29:1 also present; 8-C29:1 not observed), only 9-C31:1 (8- and 10-C31:1 not observed), and predominantly 10-C35:1 (12-C35:1 also present; 14-C35:1 not observed). European bees had predominantly 8-C29:1 (9- and 7- C29:1 also present), equally frequent 10- and 8-C31:1 (9-C31:1 not observed), and predominantly 12-C35:1 (14- and 10-C35:1 also present).

In summary, analyses of extractable hydrocarbons have demonstrated a number of differences in composition that are of potential use in identification. The analysis requires a wet laboratory with a chemical hood, appropriate instruments, and skilled personnel. Identification of single bees is possible. About one hour is needed to prepare the sample and one hour for the analysis, but the analytical work can be automated (R. K. Smith, pers. comm.).

Important questions remain to be answered. Do useful hydrocarbons exist that are independent of the bee's age? Will comb secreted by bees of a different geographic type contaminate resident bees with hydrocarbons such that they will be misidentified? Can hybrids and backcrosses between Africanized and European bees be identified?

Procedures for collection, storage, and extraction should be standardized. Precautions must be taken to avoid contamination or loss of lipids. R. K. Smith (pers. comm.) recommends live capture, killing by freezing or cyanide, and storage in dry air either loose or mounted on insect pins. Contact of the bees with fat solvents, petroleum distillates, halocarbons, ethyl acetate, acetone, benzene, ether, etc. will probably render the specimens useless for analysis.

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