Identification of Africanized Bees[1]

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ABSTRACT

The expanding population of undesirable Africanized bees (Apis mellifera L.) in South America has made clear the need for an objective method of identifying them. Daly's technique of morphometric analysis uses measurements of 25 physical characters of bees. At present it is somewhat slow and expensive, but a new procedure should reduce both time and cost. Allozyme analysis is faster and cheaper and is more directly related to the genetics of the bees. However, at present, it is limited to the enzyme malate dehydrogenase and so is much less accurate than is theoretically possible. The addition of other enzymes will increase its accuracy to equal and possibly exceed that of morphometric analysis. The accuracy of both techniques is further limited in Central America at present because of lack of baseline information.

Because of the present limitations of allozyme analysis, we believe the report of Africanized bees in Mexico and Guatemala is very questionable.

Introduction

AFRICAN bees, *Apis mellifera,* L., were accidentally released in Brazil in 1956. Thereafter, a remarkable process of hybridization with European bees already in Brazil and natural selection for African characteristics (Kerr, 1967) caused enough problems to gain the attention of the U. S. Department of Agriculture (USDA) and the National Science Foundation (NSF). The NSF, with USDA funding, organized a committee to go to Brazil, assess the problem, and recommend what, if any, future action was needed.

These highly respected beekeepers and bee scientists conducted standardized tests of bees throughout the country. They documented the ability of Africanized bees to spread throughout a large part of a continent. They found those Africanized bees that had spread to the northern reaches of Brazil, which had comparatively little beekeeping, to be especially undesirable for commercial beekeeping by U. S. standards. In particular, the tendency of these bees to strongly defend their colonies was highly undesirable. The bees also swarmed excessively, absconded (left their hives completely), robbed excessively, killed queens when disturbed, and formed large populations of wild colonies that competed with beekeepers' colonies (Committee on the African Honey Bee, 1972).

The committee recommended that techniques be developed for identifying Africanized bees. In this way further movement of the bees in South America or further importation of the bees to other areas could be scientifically documented through the analysis of preserved specimens of bees. This paper reports the techniques that have been developed, their usefulness, and the interpretation of their results.

Morphometric analysis

Acting on the committee's recommendation, the USDA awarded a research grant to Dr. Howell Daly of the University of California at Berkeley. Daly, a highly respected bee taxonomist, developed a procedure that used body measurements to identify samples of bees as Africanized or European. His procedure is called morphometric analysis (Daly and Balling, 1978) and is based on the measurement of 25 physical characters of bees. The procedure includes a complicated mathematical treatment of the measurements. However, the reasoning underlying the mathematical procedure is not at all complicated.

An example of the procedure

Bees in a population vary for each of the measurements which are useful in distinguishing one population of bees from another. For example, if we went to many colonies in an area, collected fore-wings from 10 bees in each colony, and calculated a colony average for fore-wing length, our measurements could be summarized on a graph like the one in Fig. 1a. If we did the same in another area with a different population, it would look like Fig. 1b. When we superimposed these two graphs, we would produce Fig. 1c.

Fig. 1c clearly shows that the two populations have different ranges of wing lengths, but that the ranges overlap. For the purpose of this example, we have drawn this overlap to be 50% of the range of variation for each population. Thus, this character, by itself, is not a good measurement to distinguish members of the two populations. We can test our fore-wing measurements with additional samples of bees to see if we can tell if they come from Population A or Population B. If a sample of bees from a colony in Population A was given to us, we could compare the wing measurements with those in Fig. 1c. Half of the time these scores would match the area to the far left, and we could say the bees came from Population A. Half of the time the sample would match the area of overlap, and we could not say which population they came from.

Consequently, one character (e.g., fore-wing length) *by itself* is not enough to distinguish members of two populations. We can use two characters, such as fore-wing length and tongue length, on bees from many colonies in Population A and summarize the combined measurements on a graph like that in Fig. 2a. The circle is the line enclosing all the points from plots of both tongue and fore-wing measurements for each colony from Population A. The graph of scores from Population B would look like Fig. 2b. The scores for both populations on the same graph

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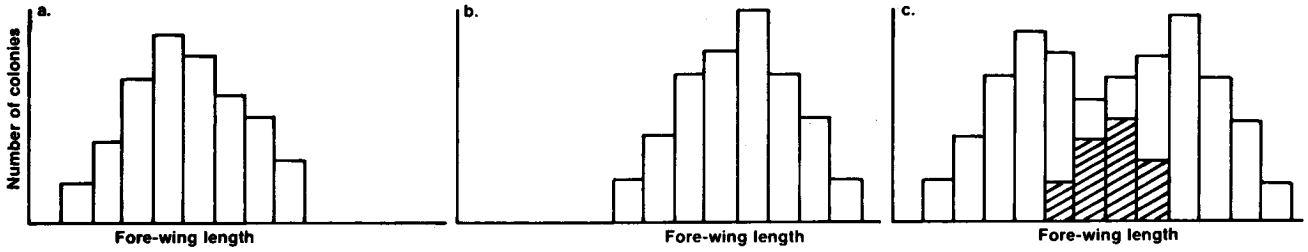

Fig. 1. Hypothetical example of colony averages for fore-wing lengths: a.) Population A, b.) Population B, c.) Populations A and B. Slashes mark area of overlap.

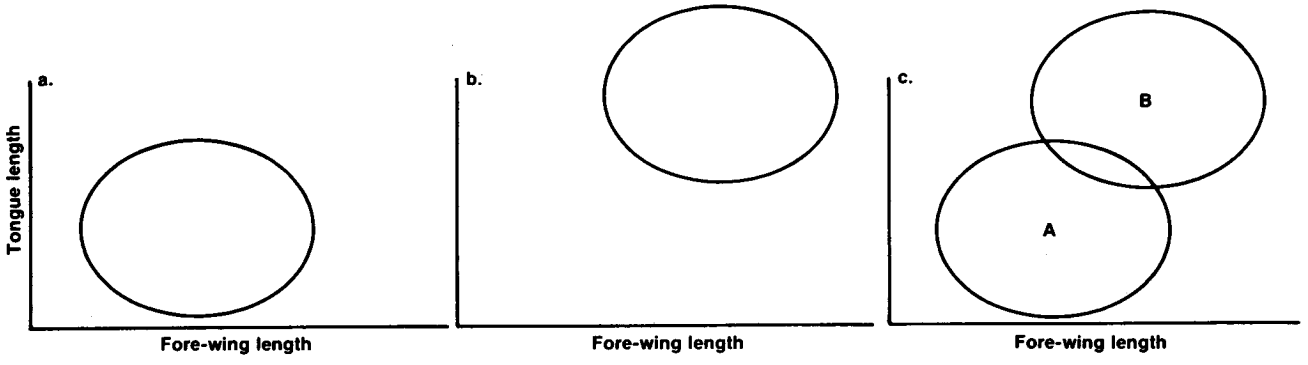

Fig. 2. Hypothetical example of colony averages for fore-wing and tongue lengths: a.) Population A, b.) Population B, c.) Populations A and B.

would look like Fig. 2c.

There is still an area of overlap between the two populations. But this time it is only 10% of the combined scores. We can compare sample values with those in the graph and say from which population a sample comes 90% of the time. Thus, the use of two characters greatly improves our ability to discriminate the origin of an unknown sample. Instead of using only two characters, Daly uses a computer and 25 characters. The discriminating power of the 25 characters he uses is enhanced by the power of modern statistics, which can consider relationships between them. Thus, the approach is better than a graphic demonstration with 25 characters.

Strengths of morphometric analysis

Morphometric analysis is a powerful tool. A collection of bees from a single colony can be processed and a valid determination between African or European specimens can be made 99.5 per cent of the time.

The power to make a determination on bees from a single colony is very important. For example, the Animal and Plant Health Inspection Service (APHIS) killed a swarm that arrived in Texas on a ship from Venezuela. Daly determined that this swarm was Africanized. This event has been quite helpful. First, we know that such a thing can occur. Second, APHIS port inspectors are now more alert about bee swarms. They are armed with the idea that they have kept an Africanized bee swarm out of the United States.

Weaknesses of morphometric analysis

Morphometric analysis is not an easy process. It requires specialized equipment that optically projects bee-body parts, a great deal of time and tedious work to make the necessary measurements, and a programmed computer to make the necessary calculations and comparisons. Consequently, morphometric analysis cannot be done everywhere, and at present it is an expensive and slow process. However, Daly is developing a new procedure that should allow the measurements to be made in 5 min. per bee.

The strength of morphometric analysis depends on the strengths of its elements, specifically, measurements made on known populations. Daly has established these measurements with bee collections from beekeepers and bee scientists throughout the Americas. The people who have chosen Africanized bees for him may have been overly conservative in their determination of Africanization. Certainly, they wanted to be correct in their determination of Africanization; motivated by this desire, they may have collected only small bees that stung excessively.

Our laboratory has sent Daly a collection of bees from 300 colonies in Venezuela. Some of these bees are known F_1 hybrids; others are known Africanized bees reared on European cell-size comb. After Daly has finished measuring these bees, we can know the precision of his technique. If he has misidentified bees with his current data base, he can improve his data base with the measurements of these and other known materials and avoid similar misidentification in the future.

At present, Daly's data base consists of measurements of African bees from Africa, Africanized bees from South America, and European bees from South America, and European bees from Europe and North America. It is possible that a sample of bees from yet another population not adequately represented in this data base may be misidentified as Africanized. Such a misidentification can occur with samples of bees from Central America, because Central American bees at present are inadequately represented in the data base. This is especially true if only a few characters are used. However, use of the 25 characters reduces the chances of this kind of error.

Occasionally, a single bee in a sample of 10 from a known European col-

ony is misidentified as African with morphometric analysis. Daly suspects that poor colony nutrition or rearing of bees in undersized cells may cause this misidentification. He expects an overall misclassification rate of 4 per cent with analysis of single bees. This is no problem if we understand that the chances of a sample of about 10 bees per colony being misidentified is less than this 4 per cent. The possibility of error with such a sample of 10 bees is 0.5 per cent, a small chance. However, if a colony from outside the Africanized area is identified as African, it should be remembered that the chance of error is real. Certainly the colony should be killed as a precaution, but not until other colonies from the area are identified as Africanized should the area be considered to be Africanized.

Prospects for the future of morphometric analysis

Right now, morphometric analysis is a very good tool for the identification of Africanized bees. As the data base for this analysis grows, the tool can become still better. F_1 hybrids reared on European comb are now, or in the near future should prove to be, identifiable. However, extremely subtle Africanization probably cannot be determined by this method.

It is possible, although not documented, that the process of Africanization of a population first occurs by the slow intrusion of genes typical of Africanized bees (but not exclusive to them) into a non-Africanized population. At first morphometric analysis would not detect such "gene flow" if a few genes did not affect enough of the 25 characters to allow identification.

The genetic control of the body dimensions used in morphometric analysis is unknown, but most likely it is quite complex. The controls of the various dimensions are probably related, so that changes in one gene probably affect several measurements. Therefore, it is desirable to have an identification technique that detects the arrival of the first few genes indicating Africanization. The characters used should be under simple and direct genetic control to allow certain identification of the alternative forms of the characters.

Allozyme analysis

Such a technique does exist and the characters used are enzymes. Enzymes are proteins that act as catalysts for the chemical reactions necessary for life. The structural forms of enzymes are closely determined by their controlling genes, so changes in the genes produce corresponding changes in the enzymes. Allozymes are different structural forms of the same enzyme, produced by different forms (alleles) of the same gene (locus) (Prakash *et al.*, 1969). Some of these allozymes can be separated and specifically identified with a technique named electrophoresis or with a related technique called isoelectric focusing.

The USDA supported research to see if Africanized bees could be identified by allozyme analysis. This work was done by H. Allen Sylvester as his Ph.D. dissertation during his studies at the University of California at Davis (UCD).

An example of the procedure

Sylvester found that, in fact, bees did vary for the enzyme malate dehydrogenase (Mdh). He found three allozymes, the genes for which were named $Mdh^{1.00}$, $Mdh^{0.63}$, and $Mdh^{0.50}$. The frequencies of these allozymes in samples from 24 hives of UCD European bees were $Mdh^{1.00}$ — 20%, $Mdh^{0.63}$ — 10%, and $Mdh^{0.50}$ — 70%. In samples from 34 hives of Brazilian Africanized bees, the frequencies were $Mdh^{1.00}$ — 84%, $Mdh^{0.63}$ — 16%, and $Mdh^{0.50}$ — 1%. If certain genetic assumptions are met, such as Hardy-Weinberg equilibrium, these frequencies can be used to calculate the probability that a queen or worker bee is carrying any combination of two Mdh allozymes.

By following the process described for morphometric analysis, we can produce a graph based on Sylvester's data for the Mdh allozymes in these two populations (Fig. 3). All three allozymes are found in both populations, and again, there is an overlap between populations. One bee or a group of bees, however large, from a single colony cannot with certainty be identified as belonging to one population or the other. This uncertainty is caused by the use of only one allozyme system (locus) and does not show that the method is defective. With identification of other independent allozyme systems the uncertainty can be further reduced in the way it was with morphometric analysis.

Strengths of allozyme analysis

Allozyme analysis is an easy process. It does require specialized equipment, but the procedures can be done nearly anywhere. Allozyme analysis is fast, and many bees per day can be analyzed with low labor costs. Finally, there is no confusion about what allozymes are present, so their genetic relationships can be clearly established. If enough allozyme systems showing differences are available, allozyme analysis can identify even single bees with a high probability of correctness.

Weaknesses of allozyme analysis

Despite the strengths of allozyme analysis and despite the apparent accuracy with which Africanized bees can be identified, **as presently used and with current baseline data, it cannot determine if bee populations are in the process of becoming Africanized.**

In identification of bees through allozyme analysis the frequency of Mdh allozymes in a sample of bees is compared with the observed frequency of the allozymes in previously surveyed populations of Africanized and European bees. Thus, this method distinguishes bees of known Africanized populations from those of known European populations. Sylvester (1976) studied the bee population of Colombia before it became Africanized and the bee population of Trinidad, which has yet to become Africanized. Both of these populations had a greater occurrence of $Mdh^{1.00}$ and a lower frequency of $Mdh^{0.50}$ than European bees. That is, they fell between California European bees and Brazilian Africanized bees, as measured with allozyme analysis. Yet, they were not Africanized populations; they were simply different populations of European bees. Therefore, Africanization of an area can only be unambiguously detected if the area has undergone allozyme analysis before Africanization has occurred.

Cornuet and Torregrossa (1977) studied the Mdh allozymes of the bees on the islands of Guadeloupe. They found that $Mdh^{0.63}$ was by far the most common allozyme. On Marie Galante Island the only allozyme found was $Mdh^{0.63}$. At the other locations surveyed, $Mdh^{1.00}$ and $Mdh^{0.50}$ were only found near apiaries in which known Italian and Caucasian queens had been introduced. Thus, the naturalized population of bees on the islands of Guadeloupe had in some way come to have only one allozyme rather than the three found in the populations so far reported. This further illustrates that baseline survey data are needed to document the introduction of other types of bees.

It is important to note that all Mdh allozymes found in Africanized bees exist in other populations of bees studied and that none of these enzymes can

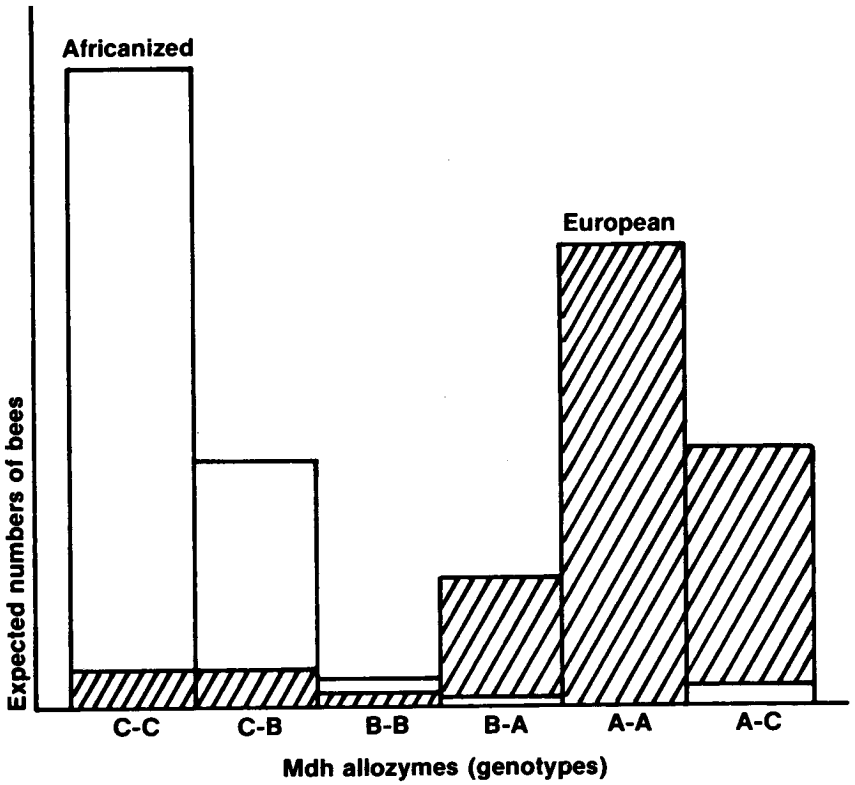

Fig. 3. Example of the probability that a female bee from two populations, Brazilian-Africanized and California-European, carries any combination of two Mdh allozymes. (A = $Mdh^{0.50}$, B = $Mdh^{0.63}$, C = $Mdh^{1.00}$)

be thought to be caused by an "African bee gene." If a truly African bee gene exists, it has not yet been found.

The results of Sylvester (1976) and Contel *et al.* (1977) suggest that the expression of $Mdh^{1.00}$ may be especially beneficial for bees in tropical areas. If this is true, natural selection can operate on European bees in tropical areas and raise the occurrence of $Mdh^{1.00}$. It also follows that an attempt to identify European bees from tropical areas with current procedures for allozyme analysis would most likely yield false conclusions that the bees were Africanized.

Finally, allozyme analysis requires the use of live or frozen bees in order to have active enzymes. This may make sample collection difficult in remote areas.

In summary, *at present* allozyme analysis can identify whether a sample of bees taken from different colonies belongs to a well-studied population of Africanized bees or a well-studied population of European bees. It cannot make identifications of Africanization in populations for which no baseline data have been collected. It cannot correctly identify individual bees or colonies often enough to be of any use.

Prospects for the future of allozyme analysis

The current problems with allozyme analysis stem from two areas. (1) Only Mdh allozymes are used in the analysis, and (2) accurate baseline information is not available on the occurrence of allozymes for most bee populations. If one or both of these problems are overcome, this method can be a very useful tool in the identification of Africanized bees. A single bee could be classified with very high probability if enough allozyme systems were included. This probability and the efficient use of allozymes in honey bee identification will be discussed by the second author in a separate paper.

Two other allozyme systems with differing frequencies in Africanized and European bees are already known to exist (P-3 protein — Mestriner and Contel, 1972; alcohol dehydrogenase — Martins *et al.*, 1976). Alcohol dehydrogenase is not currently used, because it only occurs in pupae and not in adults, and is therefore more difficult to sample. With further study additional allozyme systems should be found. Even without the use of additional systems, allozyme analysis could become useful. We know that occurrences of known allozymes in Africanized populations differ from occurrences of the same allozymes in European bees. A thorough survey through Central America would establish the necessary baseline data, and additional surveys at later dates could then monitor the process of Africanization in Central and North America. With this procedure, properly conducted surveys could detect changes in allozyme occurrence as low as 6.5%.

The recent identification of Africanized bees in Mexico and Guatemala

A recent report (Morse, 1980) states that Africanized bees are in Mexico and Guatemala. According to our analysis, many questions remain to be answered before the report can be considered a fact. The identifications were based on allozyme analysis. We have already discussed the weakness of this method of identification as it is now used. Morphometric analysis of the Mexican and Guatemalan bees has not conclusively shown that they are Africanized (Daly, personal communication). One bee in 10 from Mexico has been identified as Africanized with morphometric analysis. On single-bee analysis, this method misidentifies 4 of 100 bees, so one of 10 is not unexpected. Analysis of the bees from Guatemala gave confusing results, such that they were not identified clearly as African or European bees. They might have been neither, since baseline data do not exist for Guatemalan bees. According to the report, no Africanized behavioral characteristics were observed in either Mexico or Guatemala. We think that Africanized bees are not at all difficult to identify in the field. Thus, if the Central American bees do not appear to be Africanized, it is most likely that they are not Africanized.

We mention the report and our objections to it because: 1) we believe that the American public (and especially that portion of the American public that owns bee yards) should not think Africanized bees are closer to North America than they actually are; 2) we believe that beekeepers and government officials in Central American countries who do not have African bees should not think that they do have them; and 3) we think that beekeepers anywhere should not think that the Africanization of their bee populations may result in greater or lesser problems than those predictable.

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