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ELECTROPHORETIC IDENTIFICATION OF AFRICANIZED HONEYBEES*

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Summary

A procedure for analysing the results of protein electrophoresis for taxonomic purposes is described. Its application to identifying Africanized honeybees is presented, using previously reported data for the loci coding for malate dehydrogenase (Mdh), alcohol dehydrogenase (Adh), and a general protein band (P-3). Mdh can be used alone to identify individual workers as Africanized or European, with a probability of more than 90% for the reported populations, but it does not meet the 99% probability criterion for use as a diagnostic locus. When the reported results for all three loci are combined, individual Africanized or European workers should be identifiable with a probability of more than 99%. This degree of accuracy may not be possible in other populations, not yet assayed electrophoretically.

Introduction

The ability of Africanized honeybees to spread throughout much of South America, and the likelihood of their spreading into the United States, has made them an object of much study, for both scientific and practical reasons. One of the problems in studying them is the difficulty of identifying unequivocally an unknown colony (and particularly an individual bee) as Africanized or European or some combination thereof. Rinderer and Sylvester (1981) have discussed the two primary methods currently available for identifying Africanization: morphometric analysis and electrophoretic analysis. A report of Africanized bees in Mexico and Guatemala (Morse, 1980) was based partly of electrophoretic data, homozygosis for one allelle at one locus being used as the diagnostic criterion. Electrophoresis, used in this way, is quite limited in its ability to identify Africanization. On the other hand, Ayala and Powell's method (1972) of analysing the results of electrophoresis leads to a much more efficient use of electrophoresis. In discussing genetic differentiation between populations, Ayala and Powell (1972) stated: 'The same amount of genetic differentiation may arise either by many loci that have a moderate amount of differentiation, or by complete or nearly complete differentiation at only a few loci, with identity of the other loci studied. For taxonomic purposes, however, these two situations have different implications. A locus at which complete differentiation exists between two populations can be used to diagnose the population to which an individual belongs. A locus at which only partial differentiation occurs cannot be so used. Many loci at which two populations are partially different can be used for diagnostic purposes, but statistical manipulations are more complex.' The application of these statistical manipulations for the differentiation of Africanized and European honeybees (*Apis mellifera*) is the subject of this paper.

Two enzyme systems and a general protein that have been analysed by electrophoresis have been reported to differ in allelic frequencies between Africanized and European (generally Italian) honeybees. These are malate dehydrogenase, Mdh (Sylvester, 1976; Contel et al., 1977), alcohol dehydrogenase, Adh (Martins et al., 1977) and a protein, P-3 (Mestriner & Contel, 1972). Studies of these systems have generally involved a fairly small number of colonies from a limited number of locations; hence, the reported allelic frequencies may not represent accurately those of either Africanized or European bees overall. Nevertheless, they are useful in examples to show the value of the method of Ayala and Powell (1972).

Procedure and Discussion

I will analyse in detail the calculations for one sampling at the Mdh-1 locus, and only discuss the results for the other reports. The reported allelic frequencies are given in Table 1. Assuming Hardy-Weinberg equilibrium, the genotypic frequencies may be calculated from these allelic frequencies according to the formula for 3 alleles: $1=p^2+2pq+q^2+2qr+r^2+2rp$,

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TABLE 1. Allelic frequencies at three loci in European (including Italian) and Africanized honeybees.

Loci code for proteins as follows: *Mdh-1*= malate dehydrogenase, *Adh-1*=alcohol dehydrogenase, *P-3*=general protein. Data in A, B & C from Sylvester, 1976; D from Contel et al., 1977; E from Martins et al., 1977; and F from Mestriner and Contel, 1972.

	Locus	*Population*	*Country*	*No. colonies*	*No. bees*	*Alleles and frequencies*		
A	Mdh-1	Italian	U.S.A.	24	52	0·50=0·703	0·63=0·099	1·00=0·198
		Africanized	Brazil	34	71	=0·007	=0·156	=0·837
B		European	Colombia	13	19	=0·395	=0·368	=0·237
C		European	Trinidad	10	15	=0·500	=0·133	=0·367
D		Italian	Brazil	34	136	C=0·710	B=0·154	A=0·136
		Africanized	Brazil	78	312	=0·030	=0·202	=0·768
E	Adh-1	Italian	Brazil	28	112	1=0·902	2=0·098	3=0·000
		Africanized	Brazil	78	312	=0·256	=0·697	=0·047
F	P-3	Italian	Brazil	7	28	F=0·005	S=0·995	
		Africanized	Brazil	68	272	=0·469	=0·531	

where p=frequency of 1st allele (0·50), q=frequency of 2nd allele (0·63), r=frequency of 3rd allele (1·00). The alleles are identified by the relative rates of migration of the stained bands under the assay conditions used by Sylvester (1976); the fastest band found has arbitrarily been assigned the value of unity. Thus the band coded by the allele Mdh-$1^{0.50}$ moves one-half as far from the sample insertion point as the band coded by the allele Mdh-$1^{1.00}$ under the specific conditions. The expected frequencies of the genotypes that carry any one or two alleles were calculated from the allelic frequencies and are listed in Table 2. .

TABLE 2. Genotypic frequencies, at three loci in European (including Italian) and Africanized honeybees, calculated from the allelic frequencies in Table 1, assuming Hardy-Weinberg equilibrium.

			Genotypic frequencies					
	Locus	*Population*	0·50/0·50	0·50/0·63	0·63/0·63	0·63/1·00	1·00/1·00	1·00/0·50
A	Mdh-1	Ital.	0·494	0·139	0·010	0·039	0·039	0·278
		Afr.	0·00005	0·002	0·024	0·261	0·701	0·012
B		Eur.	0·156	0·291	0·135	0·174	0·056	0·187
C		Eur.	0·250	0·133	0·018	0·098	0·135	0·367
D		Ital.	0·504	0·219	0·024	0·042	0·018	0·193
		Afr.	0·001	0·012	0·041	0·310	0·590	0·046
			1/1	1/2	2/2	2/3	3/3	3/1
E	Adh-1	Ital.	0·814	0·177	0·010	0·000	0·000	0·000
		Afr.	0·066	0·357	0·486	0·066	0·002	0·024
			F/F	*F/S*	*S/S*			
F	P-3	Ital.	0·220	0·498	0·282			
		Afr.	0·00003	0·010	0·990			

The data for group A in Table 2 may be used as a specific example to estimate the overlap in genetic distribution of populations of Africanized bees from Brazil and Italian bees from California and the probability of assigning an individual to its correct population. The overlap in the genotypic distribution of these two populations is the sum of the smaller of the two frequencies for each genotype: 0·00005 + 0·002 + 0·010 + 0·039 + 0·039 + 0·012 = 0·102. To estimate the probability of assigning an individual to its correct population, assume that the two populations are equally common. Then assign individuals of each genotype to the population in which that genotype is the more frequent. This will lead to assignment errors with a frequency of half the amount of overlap of the genotypic distributions of the two populations. In this example, at the Mdh-1 locus the error frequency will be 0·102/2, i.e. 0·051. The probability of assigning an individual worker to the correct population is thus 94·9%. With the allelic frequencies reported by Contel et al. (1977) for Brazil, the same probability would be 92·8% (Table 2, D).

Ayala and Powell (1972), in discussing the use of allozymes to identify species of *Drosophila*, present 2 criteria for choosing loci as diagnostic. Their less stringent criterion of a diagnostic locus is '. . . when the probability of assigning an individual to the correct species is 99% or higher'. The Mdh-1 locus in the present example obviously does not meet this criterion for assigning an individual worker to one of the two populations. This is not surprising, since we should not expect two fully interfertile populations of one species to be as differentiated as two species. Nevertheless, because of the importance of identifying Africanized bees, some reasonably accurate electrophoretic identification method is desirable. The preferred choice would be to identify loci which are diagnostic by at least the 99% criterion. Since no such loci are known at present, a second choice would be to utilize loci at which Africanized bees are somewhat differentiated, though they do not meet the 99% criterion, and to combine these loci in such a way that Africanized bees can be identified.

Sylvester (1976) assayed honeybee enzymes with 30 staining systems (enzymes) in starch gel electrophoresis. Repeatable activity was found for 39 bands. If it is assumed that each band is coded by a different single gene locus (Ayala et al., 1975), these bands represent 39 loci. The only band which was found to vary was Mdh-1. Variation has been reported for Adh (Martins

et al., 1977) and a general protein, P-3 (Mestriner & Contel, 1972). Reported allelic frequencies for these three loci are not the same in Africanized and European bees (Table 1). Using these allelic frequencies to calculate the probability of assigning an individual worker to the correct population, the probabilities are 87·4% for Adh-1 and 85·4% for P-3. Mestriner and Contel (1972) also reported finding variation for an esterase. Unfortunately the reported allelic frequencies were the same for Africanized and European bees, so the locus is of no diagnostic value.

Thus only 3 of the reported 42 loci show variation of potential diagnostic value. However, as Hung and Vinson (1977) discussed in their report on fire ants (*Solenopsis* spp.) and honeybees, positive results are dependent on proper combinations of buffer systems and supporting media. Further research on the reportedly monomorphic systems and other untested systems should yield more loci with diagnostic value.

By Ayala's method (Ayala, 1973; Ayala & Dobzhansky, 1974), loci at which the allelic frequencies are independent can be combined to reduce greatly the probability of an incorrect diagnosis. With the figures for Mdh-1, Adh-1, and P-3 from Brazil (Table 2, D, E, F), the probabilities of incorrect diagnosis of the population when these loci are treated individually are 0·072, 0·126 and 0·146, respectively. When they are combined, the probability of incorrect diagnosis of the population to which an individual worker belongs is $0{\cdot}072 \times 0{\cdot}126 \times 0{\cdot}146$, i.e. 0·00132 or 0·13%, which is a much more satisfactory identification than that provided by any one of the three loci individually. Therefore these three loci, none of which by the 99% criterion is diagnostic of Africanized bees, can be combined to yield a joint evaluation which is 99·87% correct.

Since this joint evaluation is a comparison of the gene frequencies in two sampled populations, a change in the gene frequencies in either population (or the use of other populations with different gene frequencies) will affect the accuracy of the evaluation. In the identification of Africanized bees, both types of change must be considered, because Africanized bees are expanding their range and so are moving into new areas with different populations of bees, and also they may be changing as they move. As Africanized bees spread into new areas, it will therefore be necessary to sample both the resident population before Africanization and the Africanized population in the new areas.

While no comparable surveys of allozymes have been reported for Central America, there is evidence that populations of European bees in northern South America have different gene frequencies from those of California, USA. Samples of European bees from Colombia and Trinidad have been analysed for Mdh-1 (Sylvester, 1976) and found to be less well differentiated from Brazilian Africanized bees than were Italian bees from California. The latter yielded a probability of correct diagnosis of 94·9%, and those from Colombia and Trinidad 86·6% and 86·8% respectively, relative to Africanized bees from Brazil. If the same reduced probability of correct diagnosis should be found to be true of Adh-1 and P-3 genotypic frequencies, the probability of correct identification of Africanized bees in these areas might be reduced to an unacceptably low level.

It has not been determined whether or not the Africanized bee population is receiving a gene flow as it progresses through South and Central America. The indications so far are of little change in the morphological, and particularly in the behavioural, traits characteristic of the original African stock (Taylor, 1981). However, there almost certainly has been some gene flow, since Nunamaker (1981) reported that all 940 bees studied from 18 colonies of *Apis mellifera adansonii* from South Africa were homozygous for the 'C' allele at the Mdh locus. It is likely that African bees introduced into Brazil had this same gene frequency. In contrast, Africanized bees from Brazil have been found to have allele frequencies of only 0·837 and 0·768 (Table 1) for this allele, demonstrating gene flow for at least one locus. Frequencies of the Mdh allele for populations more distant from the original release point in Brazil have not been reported. Thus there is no indication yet as to whether further gene flow is occurring at this or other loci.

Africanized and European bees are fully interfertile, so the expansion of the range of the Africanized population through areas already colonized by European bees is expected to result in gene flow, the Africanized bees becoming less and less distinct. However, this does not seem to be occurring with respect to morphological and behavioural traits. European bees in tropical South and Central America are generally found only where they are maintained by beekeepers. There is little or no feral population, probably because the European bees are not well adapted

to the tropics; the Africanized population, being better adapted, is expanding as a feral population. Therefore few European bees are present in much of the area where Africanized bees are expanding their range. Thus the opportunity for gene flow from European bees is limited.

It is thus impossible to predict at present whether or not gene flow into the Africanized population is occurring, or will occur. The probability of correct diagnosis by electrophoresis will need to be verified as the Africanized bee expands into each new area.

If other loci can be found at which Africanized and European bees can be differentiated, the probability of correct diagnosis can be further increased. This will be especially true if loci can be found which are themselves diagnostic at the 99% level.

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