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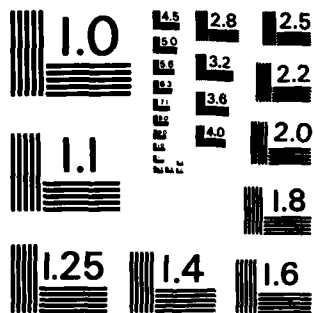
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Genetic Characterization of Insect Vectors of Disease

Annual Report

Jeffrey R. Powell

September 1, 1982

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

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We would initially like to point out that although, in name, this report represents progress up to the end of year 2, it can only present work done in the first 18 months of funding, due to the timing of the review panel's meeting in relation to the period of funding.

WORK ACCOMPLISHED

The proposed projects involved population genetic analysis of Aedes aegypti. The main technique employed is gel electrophoresis of soluble proteins followed by staining for specific enzyme activity. Using this method we have identified 22 enzyme loci in this mosquito, of which 11 are particularly variable (polymorphic) and useful for our studies. The objectives of the research are to study allelic variation at these loci in order to answer the following questions about A. aegypti: (1) How genetically variable are populations? (2) How much genetic differentiation exists between populations? (3) Can we define areas within the distribution of A. aegypti which have populations genetically similar and differentiated from other areas? (4) Do the world-wide patterns of genetic variation in this vector correspond in any way to patterns of diseases transmitted by A. aegypti? Rather than go into great detail here on the results obtained before this contract was in effect, the reader can consult earlier proposals and the following references: Tabachnick and Powell, 1978, 1979; Tabachnick et al., 1979, Powell et al., 1980. Here we shall be concerned only with new data collected under this contract and not yet published.

A total of 96 collections representing 63 localities from 21 countries (Table 1) have been analysed electrophoretically for 11 enzyme coding loci (8 enzymes). Many populations have been analysed for up to 22 loci, but these additional loci will not

be considered here as they are either monomorphic or otherwise uninformative. Where multiple collections have been received from the same locality, unweighted composite mean gene frequencies have been calculated, and are presented here. Table 2 lists collections that have been analysed since Tabachnick & Powell (1979) with their abbreviations and origins. Populations underlined are those that have been analysed since last year's progress report. We have restricted our analysis as far as possible to material collected directly from the field, but some colonised material of recent origin was included in the analysis. However, we have avoided making any generalisations or inferences from colony material alone.

Electrophoretic methods described elsewhere (Tabachnick & Powell, 1979) were used to score genetic variation at the following 11 enzyme loci in all collections: Gpd, Mdh, Me, Idh-1, Idh-2, Pgd, Hk-2, Hk-3, Hk-4, Pgm and Pgi, with the exception of seven earlier populations in which Idh-1 was not successfully resolved. D values were calculated by Nei (1972) and UPGMA tree construction was according to Sneath & Sokal (1973). Stepwise multivariate discriminant analysis was performed using a biomedical computer program package (BMDP7H: Dixon, 1977).

Tables 3-7 show allele frequencies at the eight most polymorphic loci (n = number of genes sampled). Gpd is largely monomorphic; only 12 of the 63 localities exhibit variation (5 East African formosus, 5 South-Eastern US, 1 West African, and 1 Mexican) and the frequency of Gpd¹⁰⁰ is above 0.91 in all cases except ABBE. Me is variable in only 6 localities (4 East African, 1 West African and 1 Texan) with Me¹⁰⁰ never below a frequency of 0.75. Data for Idh-1 may be found in Wallis & Tabachnick (1982). This locus is largely monomorphic outside of Africa, with the exception of the north coast of South America.

Variation for hexokinase (Table 3) is fairly concordant across all three loci (Hk-2, Hk-3, Hk-4). Genic variability is mostly restricted to the East African populations, and is highest of all in the aegypti subspecies. Some variation,

especially for Hk-4, is found in Asian and New World populations.

Tables 4-7 give gene frequencies at 5 loci for the 34 populations sampled since Tabachnick & Powell (1979); the descriptions given here refer to gene frequencies from all 63 localities. Malate dehydrogenase is of some geographic diagnostic use. Mdh⁸⁴ is essentially an *A. a. formosus* allele whereas Mdh¹²⁰ is only found at low frequencies in this subspecies. West African populations exhibit lowest heterozygosities and United States populations very much higher ones. Isocitrate dehydrogenase 2 is essentially under diallelic control with extreme disparate allele frequencies between regions making it of great diagnostic use. Idh-2¹¹⁶ is rare in all sylvan populations, but reaches high frequencies in domestic populations. *A. a. aegypti* from Texas, Central America and the Caribbean have highest Idh-2¹¹⁶ frequencies, but populations in the south-eastern United States resemble Asian populations having lower frequencies of this allele.

Phosphoglucuronate again supports the Hattingly (1957) subspecies division; all sylvan populations from West and East Africa are highly heterozygous whereas Pgm¹⁰⁰ predominates in domestic collections. Phosphoglucuronate dehydrogenase and phosphoglucose isomerase are generally weakly polymorphic. Exceptions are some East African *formosus* populations and the Caribbean where Pgd¹¹⁶ is common, and some New World populations where Pgi⁹³ and Pgi¹⁰⁵ sporadically reach higher frequencies.

Mean expected heterozygosity over 10 loci (excluding Idh-1) is highest in African populations, particularly East African ones, and lowest in Asia (Table 8).

From the gene frequency data for the 29 localities then available, Tabachnick & Powell (1979) described 4 geographic areas, and separated East African populations according to subspecies giving 5 groupings. With the addition of collections from 5 more localities, Powell *et al.* (1980) divided the New World group into 3: Caribbean, United States and South America. Using multivariate discriminant analysis it proved possible to delineate all 7 groups in two dimensions without overlap.

Since then, the number of localities from which data are available has approximately doubled, with most emphasis being placed on areas bordering the Gulf of Mexico. Nei's overall D values were calculated for every pairwise population comparison yielding a matrix of nearly 2000 data points. It became clear that the United States may be divided into south-east and south-west groupings resulting in a total of 8 geographic groups: East Africa aegypti (EAA), East Africa formosus (EAF), West Africa (WA), Asia (ASIA), south-eastern United States, (SEUS), south-western United States with Mexico (TEXMEX), south and central America with Trinidad (SCA) and the Caribbean (CAR). Table 9 presents a summarised version of the genetic distance matrix using these groupings, with intragroup distances on the diagonal. In doing this, three anomalous collections were omitted: WESTEX, TRIN, and GUAT. These have rather odd and extreme gene frequencies at one or more loci. In the case of GUAT, we have no neighbouring collections and it is therefore not known whether this is truly indicative of a separate genetic-geographic region, sampling error, or a gene pool isolate whose aberrant frequencies result from founder effect and drift. WESTEX appears to be an example of such an isolate; two good collections (large and directly from the field) show the same high frequency of Idh-1¹⁰⁰ and low heterozygosity. In the case of TRIN, sampling appears to be at fault as several other collections from the island agree with the overall picture. We feel that omitting these three samples is valid as it represents only a tiny fraction of the total data, and in such a large survey one must expect aberrant samples. Figure 1 is an UPGMA dendrogram generated from the data in Table 9.

Intensive sampling of A. aegypti from the southern United States has revealed a distinct discontinuity between populations from the east (Florida, Alabama, Mississippi, Louisiana and Beaumont TX) and those to the west (Texas and Mexico). This is most noticeable in terms of Idh-2 allele frequencies (Fig. 2). Considering the 8 south-eastern US populations, the frequency of Idh-2¹¹⁶ reaches 0.33 in HAMF,

but in all the other seven is below 0.22. Contrarily, Idh-2¹¹⁶ exceeds 0.28 in all 10 populations to the west of Beaumont (excluding the aberrant WESTEX), and in 8 of these exceeds 0.46. Furthermore, despite the proximity of these populations, the TEXIEX group is more genetically similar with the SCA and CAR groups than it is to SEUS (Table 9). Thus, for example, GALTEX and HOUTEX are more genetically similar to VEIEZ, PARA, SEALOTS and TRINCITY than they are to nearby BEAUTEX, DEQUIN and ABBE.

Whilst Nei's D value is perhaps the most objective measure of genetic distance between populations, it is not necessarily quantitative distance per se that is most useful here. If we want to be able to define geographic-genetic groups of Aedes aegypti, then consistent qualitative differences between groups become important. Thus from a purely discriminatory viewpoint, one is interested in defining a group in the most productive manner, and a procedure is needed that takes account of the useful consistent discriminatory differences in such a way that they are not swamped by larger less meaningful randomly distributed variation. Of course, it is best that discriminatory features are large as this allows assignment of a population of unknown origin to a group with a higher degree of certainty, but consistency has to be balanced against magnitude. We have already demonstrated the use of stepwise multivariate discriminant analysis to this end (Powell et al.; 1980, 1982). Using the most discriminatory characters, the technique provides several uncorrelated composite variables from which canonical coefficients are calculated. These are then applied to every locality's gene frequency set and plotted two-dimensionally using the first two canonical variables.

When the gene frequency data from the 63 localities are analysed in this way, the situation demonstrated by Nei's D values and the dendrogram are essentially confirmed. This time only WESTEX is omitted; the very low frequency of Idh-2¹¹⁶ is an anomaly and disrupts the groupings. Using the gene frequency data from these 62 localities, figure 3 shows the canonical plot. EAA is well separated from all the

other groups which form a melee of largely indistinguishable regions to the left with lower X values. The reason for the three East African *A. a. aegypti* samples forming such a distinct unit is their diagnostic *Hk* gene frequencies (Table 3). *Hk-4* is particularly variable, and the low frequency of *Hk-4*¹⁰⁰ is of great discriminatory value.

In order to discriminate between the other geographic regions, the analysis is rerun omitting the three EAA members. Figure 4 is the resulting plot, showing fairly good separation of all groups. *Idh-1* was analysed in 52 of these 59 populations, and as it is of some discriminatory use, the analysis is run again for these populations including this locus, giving the plot in Figure 5.

Table 10 presents the coefficients for canonical variables in the latter two analyses for 59 and 52 localities respectively. Alleles are listed in order of decreasing contributory importance.

CONCLUSIONS

The patterns of world-wide variation described in previous publications have been supported and enlarged upon here. Sylvan and domestic forms of *A. aegypti* from East Africa clearly represent distinct sympatric gene pools, with a mean genetic distance of 0.0624 ± 0.0058 between populations based upon 10 isozyme loci. There is little genetic differentiation between populations within the West African subspecies (Table 9). There is three to four times as much differentiation between EAA and EAF, as well as EAA and WA, than there is between the two sylvan forms, EAF and WA. That is to say, most of the genetic differentiation in Africa is between subspecies rather than between east and west regions. It is of interest to note that we have been unable to locate domestic *A. a. aegypti* in West Africa. This presents a dilemma as most entomologists and epidemiologists believe that *A. a. aegypti* was introduced into the New World from West Africa via the slave trade commerce of the 16th - 18th centuries. Dispersal by human transport would seem more likely for the domestic

subspecies than the sylvan form. It is possible that *A. a. aegypti* was once present in West Africa but has for some unknown reasons been replaced. We will discuss this and other alternatives in a forthcoming paper on the evolution of *A. aegypti*.

Populations from Asia and the south-eastern USA are most closely related to the sylvan forms (Fig. 1) with average genetic distances of around 0.02 - 0.04. This similarity is to a large extent due to lower Idh-2¹¹⁶ frequencies. The high heterozygosity of EAA populations and low heterozygosity in Asia is consistent with the likely history of the species which probably spread from East Africa to Asia. Apart from the SEUS group, New World populations have the greatest affinity with the domestic East African group, forming the second cluster.

The canonical analysis confirms the existence of these groups as distinct genetic entities, and presents a means of "classifying" new populations. Any single population can be removed and replotted using the resulting new coefficients to examine its effect on overall distortion of the group. For instance, if the Asian looking WESTEX is included, the increased within group variance disturbs the groupings. We know that WESTEX is a real genetic anomaly, as two different collections from this locality have consistent gene frequencies, and are unlike any neighbouring localities. We believe this is likely to be a founder effect.

The most interesting new feature is the situation in the south-eastern USA. This could possibly be associated with the disappearance of and reinfestation by *A. aegypti* in areas around New Orleans over the last couple of decades (Trapido & Carmichael, 1974). An original founder effect coupled with the processes of drift and selection among the growing gene pool may be responsible for the genetic differences now observed. Aided by absence of competing populations, this SEUS type may have spread rapidly through Mississippi, Alabama and Florida to the east coast, and west through Louisiana. The zone of contact that now exists in eastern Texas may or may not be stable; if one population is spreading at the expense of the other, it

is of use to know something of the epidemiological parameters of each. TEXMEX populations are genetically more similar to Caribbean populations than they are to ones from SEUS, and dengue fever has recently been epidemic in the Caribbean (PAHO, 1979). It would be of use to obtain some more inland US populations to confirm and amplify our knowledge of the sudden discontinuity in eastern Texas.

Figure 4 demonstrates the distinctiveness of SEUS and TEXMEX, and how the former is rather similar to the EAF group. This sylvan element of SEUS is rather an enigma at present, but it is interesting to note that there are reports of New Orleans *A. aegypti* using tree holes as breeding sites, though this may merely be due to population overflow. The point on the plot that is closest to the EAF group is a composite of three collections from Mobile AL which have unusually high Pgd¹¹⁶ frequencies. When Idh-1 is included, the picture changes little, but it becomes easier to differentiate between EAF and SEUS, as the former has Idh-1 variants not commonly found in any US populations. The EAF population with the low canonical variable 2 is NYALI, collected near Mombasa. This displacement is a result of low heterozygosity at Pgd and Idh-1, which may indicate migration between the port of Mombasa and Asia by sea.

So at present we recognise seven distinct genetic-geographic groupings of *A. aegypti*. The New World is divided into Caribbean (Puerto Rico and Jamaica), south-eastern US (from Beaumont TX east to Vero Beach Florida) and the remaining regions bordering the Gulf of Mexico, the TEXMEX group (from Galveston TX west, Mexico, Guatemala, Venezuela, Guyana, Suriname, Trinidad). Some interesting epidemiological possibilities are suggested by these results. It has been known for some time that epidemic yellow fever has never existed on the Asian continent (Dudley, 1934), although dengue has been found there (Smith, 1956; Strode, 1951). Secondly for some unexplainable reason the southern United States has not suffered dengue fever for 40 years or yellow fever since 1905, although the Caribbean continues to experience

dengue, and in the case of Trinidad, yellow fever. Sporadic dengue cases have been reported in Mexico and south-west Texas in 1980. It is remarkable that populations of mosquitoes in Asia and the south-eastern US show such strong genetic affinity isozymically. It is of further interest to note that African subspecies formosus and New Orleans domestic form give low infectivity values for yellow fever and Dengue viruses respectively (Gubler et al., 1982; Tabachnick et al., 1982). This is consistent with the hypothesis that isozyme-based genetic-geographic groups share other genetic features in common, vectorial efficiency perhaps being one.

We feel that some of the heterogeneity within geographic regions reflects the recent history of Aedes aegypti populations with regard to their commensal association with humans. Compared to the other well studied commensals like the house mouse, A. aegypti has been more intensively subjected to human efforts for control and eradication. Insecticide control programs impose intense selection for increased resistance to the compound(s), and will concomitantly alter the genetic constitution of the local population due to hitch-hiking and stochastic loss of variability. Eradication over large areas may ensue, leaving isolated "island" populations. When control is relaxed, reinfestation by remaining isolates and invading migrants creates a new gene pool. The SEUS group appears to have arisen in exactly such a manner.

An intuitive notion that has prevailed in population genetics for many years is the swamping effect of gene flow upon potentially genetically divergent populations (e.g., Kimura & Maruyama, 1971). From this viewpoint, it may seem surprising that we are able to discern clear genetic-geographic regions, as levels of gene flow must surely be high for this prolific commensal. Our results would therefore seem to support circumstantially those who de-emphasize the role of gene flow as a mechanism for disrupting geographic differentiation (e.g., Endler, 1973). Alternatively, the notion that association with humans necessarily leads to high rates of gene flow

among populations may be wrong.

WORK RECENTLY COMPLETED

In addition to this report, we are able to give the results of some very recent work on material collected this summer in the Caribbean. Table 11 lists populations analysed and those expected soon. Table 12 lists allele frequencies for the 7 loci polymorphic in this region.

As regards the data in terms of world-wide analysis, it fits in with the idea that the Caribbean, north coast of South America, Central America and Texas form a group of related gene pools distinct from the south-eastern U.S. type. However, on a microgeographic level, virtually all of the collections differ significantly from each other at one or more loci. We can say that there are no overall similarities that allow us to distinguish the Lesser Antilles as a distinct group in itself, and it appears that the differences observed from island to island result from the various control methods employed in each and their relative successes (see the enclosed Caribbean report).

A full computer analysis of the New World populations alone is now being undertaken, and should be greatly enhanced by the intended South American collecting trip.

SUMMARY OF CONCLUSIONS

1. Sylvan and domestic forms of Aedes aegypti from East Africa clearly represent separate gene pools.
2. All populations analysed from West Africa are genetically sylvan, and are much more similar to East African sylvan than they are to East African domestic.
3. There are two genetically distinct forms of Aedes aegypti in the southern US. The boundary lies somewhere between Beaumont and Houston.
4. Population from Asia and the south-eastern US are genetically most closely

related to sylvan forms. Where data is available, south-eastern US and sylvan forms also have similarly low infectivities for dengue and yellow fever viruses.

5. The other New World populations are genetically most similar to East African domestic populations.

6. Caribbean and South American populations are most similar to populations from Mexico and Texas.

7. Results so far are consistent with the hypothesis that isozyme-based genetic-geographic groups also possess features of vectorial efficiency in common.

U.S. ARMY INTERACTION

As stated in the original proposal we have maintained contact with personnel at Fort Detrick. We have advised them of the collections we have received, what data we have collected on them, and offered to send them whatever material they may find useful. We intend to continue this practice.

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Table 1. The 63 localities from which Aedes aegypti has been sampled.

| <u>Country</u> | <u>Population</u> | <u>Country</u> | <u>Population</u> |
|----------------|-------------------|----------------|-------------------|
| Kenya | Kwa Bendegwa | Taiwan | Kaohsiung |
| | Mgandini | Indonesia | Grogol |
| | Majengo | | Semarang |
| | Kombeni | | Tandjung Priok |
| | Shimba Hills | India | Bangalore |
| | Kwa Dzivo | Fiji | Walu Bay |
| Nyali | | | |
| Uganda | Bwerenga | | |
| Tanzania | Dar Es Salaam | Guyana | Georgetown |
| Nigeria | Ogui | Suriname | Paramaribo |
| | Ukana | Venezuela | Caracus |
| | Manu | | Maracay |
| | Egede | Guatemala | Escuintla |
| | Abor | Trinidad | Port of Spain |
| | Enugu | | Trinidad City |
| Upper Volta | Bobo | | Felicity |
| | Kari | | Sealots |
| | Bwombi | Puerto Rico | San Juan |
| | Ouagadougou | | Mayaguez |
| Senegal | Dakar | | Arecibo |
| | Kedougou | | Barcelonata |
| | N'goye | Jamaica | Montego Bay |
| Ghana | Akosombo | United States | Florida (2) |
| Gambia | McCarthy Island | | Alabama |
| | | | Mississippi |
| | | | Louisiana (3) |
| | | | Texas (9) |
| | | Mexico | Piedras Negras |
| | | | Victoria |
| | | | Montemorelos |

Table 2. Collections of *Aedes aegypti* analysed electrophoretically since Tabachnick & Powell (1979).

| Sample name | Number of collections | Country | Location | Date collected | Subsp. | Habitat |
|-------------|-----------------------|-------------|-------------------|----------------|--------|-------------------|
| BWE | 1 | Uganda | Bwerenga | 8/80 | f | Outdoors, urban |
| GAMBIA | 1 | Gambia | McCarthy Island | 79 | f | Outdoors, village |
| KED | 4 | Senegal | Kedougou | 9/80 | f | Outdoors, urban |
| DAKAR | 4 | Senegal | Dakar | 9/80 | f | Outdoors, urban |
| NGOYE | 1 | Senegal | N'goye | 9/81 | f | Outdoors, village |
| AZO | 1 | Ghana | Akosoambo | 5/81 | f | Outdoors, village |
| CEAGA | 1 | Upper Volta | Ouagadougou | 7/81 | f | Outdoors, village |
| TANP | 1 | Indonesia | Jakarta | 1/80 | a | Indoors, urban |
| FIJI | 1 | Fiji | Walu Bay | 9/80 | a | Outdoors, urban |
| VERO | 1 | U.S. | Vero Beach FL | 7/79 | a | " |
| GMLE | 1 | U.S. | Gulfport MS | 9/81 | a | " |
| ABBE | 1 | U.S. | Abbeville LA | 9/81 | a | " |
| DEQUIN | 1 | U.S. | De Quincy LA | 9/81 | a | " |
| MOBILE | 3 | U.S. | Mobile AL | 9/81 | a | " |
| BEAUTEX | 2 | U.S. | Beaumont TX | 9/81 | a | " |
| GALTEX | 1 | U.S. | Galveston TX | 11/80 | a | " |
| AUSTEX | 1 | U.S. | Austin TX | 7/79 | a | " |
| SANTEX | 1 | U.S. | San Antonio TX | 12/79 | a | " |
| CCTEX | 4 | U.S. | Corpus Christi TX | 5/80 | a | " |
| LARTEX | 3 | U.S. | Laredo TX | 1/80 | a | Outdoors, urban |
| EAGTEX | 1 | U.S. | Eagle Pass TX | 5/81 | a | " |
| WESTEX | 2 | U.S. | Weslaco TX | 9/80 | a | " |
| HOUTEX | 1 | U.S. | Houston TX | 9/81 | a | " |
| MONTMEX | 1 | Mexico | Montenorelos | 5/81 | a | " |
| VICMEX | 2 | Mexico | Victoria | 8/80 | a | " |
| AREC | 1 | Puerto Rico | Arecibo | 10/79 | a | Outdoors, urban |
| BAR | 1 | Puerto Rico | Barcelonata | 10/79 | a | Outdoors, urban |
| TRIN | 1 | Trinidad | Port of Spain | 6/80 | a | Outdoors, urban |
| SEALOTS | 1 | Trinidad | Port of Spain | 6/81 | a | Outdoors, urban |
| TRINICITY | 1 | Trinidad | Trincity | 6/81 | a | Outdoors, urban |
| FELICITY | 1 | Trinidad | Felicity | 6/81 | a | Outdoors, urban |
| PASA | 2 | Suriname | Paramaribo | 5/81 | a | Outdoors, urban |
| GTQIN | 1 | Guyana | Georgetown | 10/81 | a | " |
| GUAT | 1 | Guatemala | Escuintla | 9/80 | a | " |

Table 3. Allele frequencies at the hexokinase loci (Hk-2, Hk-3, Hk-4)

| Population | n | <u>Hk-2</u> | | | | <u>Hk-3</u> | | <u>Hk-4</u> | | Null | Others | | |
|------------|-----|-------------|-------|-------|--------|-------------|-----|-------------|-------|-------|--------|-------|-------|
| | | 100 | 113 | Null | Others | 90 | 100 | 111 | 100 | | | 109 | |
| ZBW | 170 | 0.762 | 0.230 | 0.008 | 0 | 0 | 0 | 0.766 | 0.234 | 0.667 | 0.260 | 0.073 | 0 |
| NAJ | 226 | 0.905 | 0.031 | 0.064 | 0 | 0 | 0 | 0.981 | 0.019 | 0.357 | 0.064 | 0.579 | 0 |
| HGH | 108 | 0.855 | 0.075 | 0.070 | 0 | 0 | 0 | 0.921 | 0.079 | 0.626 | 0.124 | 0.250 | 0 |
| ZOM | 122 | 0.951 | 0.008 | 0 | 0.041 | 0.041 | 0 | 0.951 | 0.008 | 0.909 | 0.008 | 0.042 | 0.041 |
| SHH | 148 | 0.865 | 0 | 0 | 0.135 | 0.135 | 0 | 0.865 | 0 | 0.865 | 0 | 0 | 0.135 |
| KDZ | 256 | 0.931 | 0.005 | 0.051 | 0.013 | 0.013 | 0 | 0.982 | 0.005 | 0.877 | 0.019 | 0.094 | 0.010 |
| NYA | 106 | 0.981 | 0 | 0.019 | 0 | 0 | 0 | 1.0 | 0 | 0.962 | 0 | 0.038 | 0 |
| DAR | 180 | 0.961 | 0.039 | 0 | 0 | 0 | 0 | 0.961 | 0.039 | 0.961 | 0.039 | 0 | 0 |
| BJE | 84 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.976 | 0.024 | 0 | 0 |
| GAMBIA | 118 | 0.966 | 0.017 | 0.017 | 0 | 0 | 0 | 1.0 | 0 | 0.898 | 0 | 0.102 | 0 |
| KED | 134 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.988 | 0 | 0.012 | 0 |
| DAKAR | 274 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.982 | 0 | 0.018 | 0 |
| OUAGA | 60 | 0.983 | 0.017 | 0 | 0 | 0 | 0 | 0.983 | 0.017 | 0.983 | 0.017 | 0 | 0 |
| GROGOL | 154 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.942 | 0 | 0.058 | 0 |
| SEMAR | 54 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.944 | 0 | 0.056 | 0 |
| TANP | 120 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.967 | 0 | 0.033 | 0 |
| SANTEX | 108 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.981 | 0 | 0.019 | 0 |
| CCTEX | 212 | 0.896 | 0.104 | 0 | 0 | 0 | 0 | 0.896 | 0.104 | 0.888 | 0.104 | 0.008 | 0 |
| EAGTEX | 116 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.974 | 0 | 0.026 | 0 |
| MONIEX | 114 | 1.0 | 0 | 0 | 0 | 0 | 0 | 0.868 | 0.132 | 0.965 | 0 | 0.035 | 0 |
| CARAC | 108 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.926 | 0 | 0.074 | 0 |
| NOBAY | 86 | 0.723 | 0 | 0.267 | 0 | 0 | 0 | 1.0 | 0 | 0.593 | 0 | 0.407 | 0 |
| HAYA | 150 | 1.0 | 0 | 0 | 0 | 0 | 0 | 1.0 | 0 | 0.987 | 0 | 0.013 | 0 |
| SANJ | 52 | 0.923 | 0.019 | 0.058 | 0 | 0 | 0 | 0.962 | 0.038 | 0.808 | 0.019 | 0.173 | 0 |

No variants were detected at any Hk locus in the other 39 localities

Table 4. Allele frequencies at the Mdh locus

| Population | n | 84 | 100 | 120 | Others |
|------------|-----|-------|-------|-------|--------|
| BVE | 132 | 0.402 | 0.598 | 0 | 0 |
| GAIBIA | 122 | 0.090 | 0.910 | 0 | 0 |
| KED | 102 | 0.043 | 0.948 | 0.009 | 0 |
| DAKAR | 276 | 0.102 | 0.867 | 0.031 | 0 |
| NGOYE | 100 | 0.200 | 0.680 | 0.120 | 0 |
| AKO | 80 | 0 | 0.837 | 0.163 | 0 |
| OUAGA | 60 | 0.417 | 0.583 | 0 | 0 |
| TANP | 120 | 0.067 | 0.625 | 0.308 | 0 |
| FLJI | 202 | 0.059 | 0.292 | 0.599 | 0.050 |
| VERO | 120 | 0.159 | 0.683 | 0.158 | 0 |
| GULF | 42 | 0 | 0.167 | 0.809 | 0.024 |
| ABBE | 68 | 0 | 0.456 | 0.529 | 0.015 |
| DEQUIN | 46 | 0 | 0.609 | 0.391 | 0 |
| MOBILE | 164 | 0.118 | 0.646 | 0.236 | 0 |
| BEAUTEX | 130 | 0 | 0.484 | 0.516 | 0 |
| GALTEX | 240 | 0 | 0.313 | 0.687 | 0 |
| AUSTEX | 102 | 0 | 0.333 | 0.667 | 0 |
| SANTEX | 112 | 0 | 0.259 | 0.741 | 0 |
| CCTEX | 278 | 0 | 0.617 | 0.383 | 0 |
| LARTEX | 378 | 0.050 | 0.401 | 0.549 | 0 |
| EAGTEX | 116 | 0 | 0.422 | 0.578 | 0 |
| WESTEX | 158 | 0 | 0.677 | 0.323 | 0 |
| HOOTEX | 252 | 0 | 0.421 | 0.575 | 0.004 |
| MONNEX | 114 | 0 | 0.342 | 0.658 | 0 |
| VIGTEX | 168 | 0 | 0.384 | 0.616 | 0 |
| AREC | 194 | 0.026 | 0.361 | 0.613 | 0 |
| BAR | 80 | 0.013 | 0.474 | 0.513 | 0 |
| TRIN | 60 | 0 | 0.233 | 0.767 | 0 |
| SEALOTS | 40 | 0 | 0.350 | 0.650 | 0 |
| TRINICITY | 124 | 0 | 0.653 | 0.347 | 0 |
| FELICITY | 22 | 0 | 0.773 | 0.227 | 0 |
| PARA | 196 | 0.011 | 0.580 | 0.409 | 0 |
| GTOWN | 88 | 0 | 0.989 | 0.011 | 0 |
| GUAT | 362 | 0 | 0.055 | 0.945 | 0 |

Table 5. Allele frequencies at the *Idh-2* locus

| Population | n | 87 | 100 | 116 | 125 |
|------------|-----|-------|-------|-------|-------|
| EJE | 126 | 0 | 0.937 | 0.063 | 0 |
| GAMBIA | 122 | 0 | 0.918 | 0.082 | 0 |
| ZED | 116 | 0 | 0.966 | 0.034 | 0 |
| BARAR | 274 | 0 | 0.962 | 0.038 | 0 |
| NGOYE | 100 | 0 | 0.930 | 0.070 | 0 |
| AYO | 80 | 0 | 0.975 | 0.025 | 0 |
| CUJAGA | 60 | 0.033 | 0.967 | 0 | 0 |
| TAIP | 120 | 0 | 0.875 | 0.125 | 0 |
| FIJI | 202 | 0 | 0.881 | 0.119 | 0 |
| VERO | 120 | 0 | 0.792 | 0.208 | 0 |
| GULF | 42 | 0 | 0.833 | 0.167 | 0 |
| ABBE | 70 | 0 | 0.786 | 0.214 | 0 |
| DEQUIN | 46 | 0 | 0.891 | 0.109 | 0 |
| MOBILE | 170 | 0 | 0.870 | 0.130 | 0 |
| BEAUTEX | 136 | 0 | 0.833 | 0.167 | 0 |
| GALTEX | 240 | 0 | 0.463 | 0.537 | 0 |
| BYSTEX | 102 | 0 | 0.686 | 0.313 | 0 |
| SANTEX | 112 | 0 | 0.500 | 0.500 | 0 |
| CCTEX | 278 | 0 | 0.528 | 0.462 | 0.010 |
| LARTEX | 308 | 0 | 0.396 | 0.604 | 0 |
| EAGTEX | 116 | 0 | 0.509 | 0.491 | 0 |
| WESTEX | 106 | 0 | 0.953 | 0.047 | 0 |
| ECOUTEX | 254 | 0 | 0.343 | 0.657 | 0 |
| MONTEX | 114 | 0 | 0.491 | 0.509 | 0 |
| VICHTEX | 168 | 0 | 0.714 | 0.286 | 0 |
| AREC | 194 | 0 | 0.485 | 0.515 | 0 |
| BAR | 80 | 0 | 0.587 | 0.413 | 0 |
| TRDI | 60 | 0 | 0.067 | 0.933 | 0 |
| SEALOTS | 40 | 0 | 0.325 | 0.675 | 0 |
| TRINICITY | 118 | 0 | 0.551 | 0.449 | 0 |
| FELCITY | 22 | 0 | 0.364 | 0.636 | 0 |
| PARA | 198 | 0 | 0.247 | 0.753 | 0 |
| GTOWN | 88 | 0 | 0.693 | 0.307 | 0 |
| GUAT | 342 | 0 | 0.591 | 0.409 | 0 |

Table 6. Allele frequencies at the *Pgm* locus

| Population | n | 80 | 100 | 120 | Others |
|------------|-----|-------|-------|-------|--------|
| BWE | 126 | 0.254 | 0.381 | 0.333 | 0.032 |
| GAMBIA | 118 | 0.195 | 0.763 | 0.025 | 0.017 |
| KED | 132 | 0.039 | 0.549 | 0.339 | 0.073 |
| DAKAR | 276 | 0.061 | 0.578 | 0.335 | 0.026 |
| NGOYE | 100 | 0.100 | 0.660 | 0.180 | 0.060 |
| AYO | 80 | 0.225 | 0.238 | 0.325 | 0.212 |
| CUAGA | 60 | 0.083 | 0.517 | 0.400 | 0 |
| FIJI | 202 | 0 | 0.886 | 0 | 0.114 |
| VERO | 120 | 0.158 | 0.809 | 0.033 | 0 |
| GULF | 42 | 0.024 | 0.952 | 0.024 | 0 |
| ABBE | 70 | 0.014 | 0.814 | 0.143 | 0.029 |
| DEQUIN | 46 | 0.109 | 0.804 | 0.022 | 0.065 |
| MOBILE | 52 | 0.115 | 0.807 | 0.039 | 0.039 |
| GALTEX | 240 | 0 | 0.846 | 0.154 | 0 |
| AUSTEX | 102 | 0 | 0.990 | 0.010 | 0 |
| SANTEX | 112 | 0 | 0.946 | 0.054 | 0 |
| CCTEX | 278 | 0.020 | 0.926 | 0.054 | 0 |
| EAGTEX | 116 | 0 | 0.819 | 0.181 | 0 |
| EQUTEX | 182 | 0 | 0.879 | 0.121 | 0 |
| VIGTEX | 170 | 0.017 | 0.977 | 0.006 | 0 |
| TRINCITY | 124 | 0 | 0.871 | 0.129 | 0 |
| FELCITY | 22 | 0 | 0.864 | 0.136 | 0 |
| PARA | 196 | 0.011 | 0.989 | 0 | 0 |
| GTCVN | 88 | 0 | 0.985 | 0.011 | 0 |
| GUAT | 330 | 0.015 | 0.985 | 0 | 0 |

The following populations were fixed for *Pgm*¹⁰⁰:

TAMP, BEAUTEX, LARTEX, WESTEX, HOMIEX, AREC, BAR, TRIN, and SEALOTS ($\bar{n}=138.2$)

Table 7. Allele frequencies at the Pgd and Pgi loci.

| Population | <u>Pgd</u> | | | | | | <u>Pgi</u> | | | |
|------------|------------|-------|-------|-------|-------|--------|------------|-------|-------|-------|
| | n | 86 | 100 | 116 | 130 | Others | n | 93 | 100 | 105 |
| EJE | 124 | 0.024 | 0.895 | 0.081 | 0 | 0 | 126 | 0 | 1.000 | 0 |
| GAMBIA | 122 | 0 | 0.984 | 0 | 0.008 | 0.008 | 122 | 0 | 1.000 | 0 |
| KED | 122 | 0 | 0.979 | 0 | 0 | 0.021 | 144 | 0 | 1.000 | 0 |
| DAZAR | 248 | 0 | 0.926 | 0 | 0 | 0.074 | 286 | 0 | 1.000 | 0 |
| CUAGA | 60 | 0 | 0.967 | 0.033 | 0 | 0 | 60 | 0 | 1.000 | 0 |
| VERO | 120 | 0.017 | 0.933 | 0.050 | 0 | 0 | 120 | 0.100 | 0.892 | 0.008 |
| GULF | 42 | 0.071 | 0.929 | 0 | 0 | 0 | 42 | 0 | 0.881 | 0.119 |
| DEQUIN | 46 | 0 | 1.000 | 0 | 0 | 0 | 46 | 0.217 | 0.783 | 0 |
| MOBILE | 170 | 0.029 | 0.803 | 0.168 | 0 | 0 | 170 | 0 | 0.962 | 0.038 |
| BEAUTEX | 132 | 0.063 | 0.937 | 0 | 0 | 0 | 136 | 0.010 | 0.990 | 0 |
| GALTEX | 240 | 0 | 0.996 | 0.004 | 0 | 0 | 240 | 0.013 | 0.987 | 0 |
| AUSTEX | 96 | 0.031 | 0.969 | 0 | 0 | 0 | 102 | 0.078 | 0.922 | 0 |
| SANTEX | 112 | 0 | 1.000 | 0 | 0 | 0 | 112 | 0.009 | 0.991 | 0 |
| CCTEX | 274 | 0 | 1.000 | 0 | 0 | 0 | 276 | 0.077 | 0.883 | 0.040 |
| LARTEX | 394 | 0 | 1.000 | 0 | 0 | 0 | 394 | 0.187 | 0.812 | 0.001 |
| EAGTEX | 116 | 0 | 1.000 | 0 | 0 | 0 | 116 | 0.052 | 0.948 | 0 |
| WESTEX | 94 | 0 | 1.000 | 0 | 0 | 0 | 100 | 0.057 | 0.943 | 0 |
| ECOUTEX | 254 | 0 | 1.000 | 0 | 0 | 0 | 190 | 0.105 | 0.895 | 0 |
| MONIEX | 116 | 0 | 1.000 | 0 | 0 | 0 | 116 | 0.302 | 0.483 | 0.215 |
| AREC | 194 | 0 | 0.768 | 0.232 | 0 | 0 | 194 | 0.010 | 0.902 | 0.088 |
| BAR | 80 | 0 | 0.463 | 0.537 | 0 | 0 | 80 | 0.100 | 0.812 | 0.088 |
| TRIN | 60 | 0 | 1.000 | 0 | 0 | 0 | 60 | 0.250 | 0.750 | 0 |
| TRINICITY | 124 | 0 | 1.000 | 0 | 0 | 0 | 80 | 0.016 | 0.984 | 0 |
| PARA | 192 | 0 | 1.000 | 0 | 0 | 0 | 156 | 0.010 | 0.921 | 0.069 |
| GUAT | 184 | 0 | 1.000 | 0 | 0 | 0 | 188 | 0.293 | 0.707 | 0 |

The following populations are fixed for both Pgd¹⁰⁰ and Pgi¹⁰⁰:
 NGOYE, AKO, TANP, FIJI, ABDE, VICIEX, SEALOTS, FELICITY and GTOWN (n=90.3).

Table 8. Mean expected heterozygosity values with standard errors

for each genetic-geographic region based on 10 loci

(N=number of localities)

| Group | N | $\bar{H}_e \pm \text{S.E.}$ |
|---------|----|-----------------------------|
| EAA | 3 | 0.209 \pm 0.014 |
| EAF | 6 | 0.185 \pm 0.012 |
| WA | 15 | 0.107 \pm 0.004 |
| ASIA | 6 | 0.081 \pm 0.007 |
| SEUS | 8 | 0.137 \pm 0.008 |
| TEXMEX | 11 | 0.130 \pm 0.012 |
| SCA | 9 | 0.102 \pm 0.010 |
| CAR | 5 | 0.179 \pm 0.017 |
| Overall | 63 | 0.117 \pm 0.005 |

Table 9. Nei's overall D values within (on diagonal) and between

genetic-geographic groupings.

(n=number of pairwise population comparisons used)

| | WA | EAF | EAA | ASIA | SEUS | TEXMEX | SCA | CAR |
|--------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| WA | 0.0095 ±0.0007 n=105 | | | | | | | |
| EAF | 0.0181 ±0.0010 n=90 | 0.0185 ±0.0030 n=15 | | | | | | |
| EAA | 0.0695 ±0.0030 n=45 | 0.0624 ±0.0058 n=18 | 0.0197 ±0.0082 n=3 | | | | | |
| ASIA | 0.0239 ±0.0012 n=90 | 0.0193 ±0.0023 n=36 | 0.0398 ±0.0033 n=18 | 0.0113 ±0.0025 n=15 | | | | |
| SEUS | 0.0357 ±0.0019 n=120 | 0.0328 ±0.0033 n=48 | 0.0487 ±0.0035 n=24 | 0.0168 ±0.0020 n=48 | 0.0157 ±0.0022 n=28 | | | |
| TEXMEX | 0.0759 ±0.0017 n=150 | 0.0712 ±0.0033 n=60 | 0.0458 ±0.0032 n=30 | 0.0424 ±0.0022 n=60 | 0.0284 ±0.0014 n=80 | 0.0127 ±0.0012 n=45 | | |
| SCA | 0.0559 ±0.0028 n=105 | 0.0529 ±0.0048 n=42 | 0.0371 ±0.0032 n=21 | 0.0300 ±0.0032 n=42 | 0.0301 ±0.0024 n=56 | 0.0243 ±0.0020 n=70 | 0.0208 ±0.0031 n=21 | |
| CAR | 0.0910 ±0.0020 n=75 | 0.0762 ±0.0044 n=30 | 0.0500 ±0.0052 n=15 | 0.0536 ±0.0022 n=30 | 0.0453 ±0.0023 n=40 | 0.0332 ±0.0019 n=50 | 0.0357 ±0.0026 n=35 | 0.0370 ±0.0067 n=10 |
| | WA | EAF | EAA | ASIA | SEUS | TEXMEX | SCA | CAR |

Table 10. F-values and coefficients 1 and 2 (X and Y) for canonical variables in analysis of 59 localities (Fig. 4) and 52 localities (including Idh-1) (Fig. 5).

59 localities analysis

| Variable | Coefficient 1 | Coefficient 2 | F-value to enter or remove |
|-----------------------------|---------------|---------------|----------------------------|
| <u>Pgm</u> ¹⁰⁰ | - 8.860 | -6.680 | 38.2005 |
| <u>Idh-2</u> ¹⁰⁰ | 5.398 | -1.377 | 19.9160 |
| <u>Pgd</u> ¹⁰⁰ | -16.200 | -20.517 | 17.8895 |
| <u>Hk-2</u> ^{MULL} | -18.755 | 26.088 | 10.9124 |
| <u>Ma</u> ¹⁰⁰ | 14.026 | - 0.852 | 8.1005 |
| <u>Pgd</u> ¹¹⁶ | -25.740 | - 4.343 | 6.9890 |
| <u>Gnd</u> ¹⁰⁰ | - 1.841 | - 5.993 | 5.2055 |
| CONSTANT | 7.723 | 32.588 | |

52 localities analysis

| Variable | Coefficient 1 | Coefficient 2 | F-value to enter or remove |
|-----------------------------|---------------|---------------|----------------------------|
| <u>Pgd</u> ¹¹⁶ | -23.915 | 5.498 | 42.008 |
| <u>Idh-2</u> ¹¹⁶ | - 5.257 | - 0.099 | 38.104 |
| <u>Pgm</u> ¹⁰⁰ | - 9.061 | - 1.755 | 13.366 |
| <u>Idh-1</u> ¹⁶⁰ | 9.813 | - 0.595 | 8.308 |
| <u>Pgd</u> ¹⁰⁰ | -15.971 | -10.275 | 6.985 |
| <u>Ma</u> ¹⁰⁰ | 8.695 | -15.827 | 5.260 |
| <u>Idh-1</u> ⁸⁰ | 20.927 | 74.252 | 10.292 |
| CONSTANT | 15.941 | 26.398 | |

Table 11. New populations of *Aedes aegypti* samples and analysed electrophoretically.

| Country | Collection origins | Date collected | Abbreviation |
|-------------|-------------------------|----------------|--------------|
| Colombia | Malaga; Santander dept. | 4/82 | MALAGA |
| Antigua | St. Johns | 4/82 | ANTIG |
| Montserrat | Plymouth and elsewhere | 4/82 | MONT |
| Dominica | Fortune | 5/82 | DOIFOR |
| | Point Michel | 5/82 | DOMPH |
| | Soufriere | 5/82 | DOMSOU |
| St. Vincent | Kingstown and elsewhere | 4/82 | STVIN |
| Martinique | Fort de France | 6/82 | MART |
| Aruba | Oranjestad | 5/82 | ARUBA |
| Anguilla | Valley | 5/82 | ANGP |
| Jamaica | Kingston | 6/82 | JAMKING |

Also expected soon: Turks & Caicos, Barbados, Grenada, Bonaire, Curacao

Table 12. Gene frequencies at 7 polymorphic loci in the 10 populations of *Aedes aegypti* listed in Table 1. All populations were fixed (monomorphic) for the Idh-1, Hk-2, Hk-3 and Ma loci. (N= minimum number of genes sampled)

| Population | N | <u>Hk-1</u> | | | <u>Idh-1</u> | | | <u>Idh-2</u> | | | <u>Ma</u> | | | |
|------------|-----|-------------|-------|-------|--------------|-----|-------|--------------|-------|-------|-----------|-------|-------|-------|
| | | 100 | NULL | 200 | 100 | 200 | 100 | 116 | 85 | 100 | 116 | 70 | 80 | 100 |
| MALAGA | 144 | 0.993 | 0.007 | 1.000 | --- | --- | --- | 0.528 | 0.472 | --- | --- | --- | 0.604 | 0.396 |
| ANTIG | 216 | 1.000 | --- | 1.000 | --- | --- | --- | 0.648 | 0.352 | --- | --- | 0.037 | 0.495 | 0.468 |
| MONT | 212 | 1.000 | --- | 1.000 | --- | --- | --- | 0.363 | 0.637 | 0.067 | 0.160 | 0.646 | 0.127 | --- |
| DOMFOR | 140 | 0.908 | 0.092 | 1.000 | --- | --- | 0.077 | 0.416 | 0.507 | 0.086 | 0.107 | 0.664 | 0.143 | --- |
| DOMPH | 152 | 0.796 | 0.204 | 1.000 | --- | --- | --- | 0.579 | 0.421 | 0.138 | --- | 0.197 | 0.665 | --- |
| DONSOU | 216 | 0.954 | 0.046 | 1.000 | --- | --- | --- | 0.601 | 0.399 | --- | 0.079 | 0.324 | 0.597 | --- |
| STVIN | 18 | 1.000 | --- | 1.000 | --- | --- | --- | 0.778 | 0.222 | --- | --- | --- | 1.000 | --- |
| MART | 74 | 1.000 | --- | 0.975 | 0.025 | --- | --- | 0.387 | 0.613 | --- | --- | 0.463 | 0.537 | --- |
| ARUBA | 14 | 0.929 | 0.071 | 1.000 | --- | --- | --- | 0.714 | 0.286 | --- | 0.143 | 0.857 | --- | --- |
| ANGP | 108 | 1.000 | --- | 1.000 | --- | --- | --- | 0.759 | 0.241 | --- | 0.009 | 0.324 | 0.667 | --- |

| | <u>Per</u> | | | <u>Per</u> | | | <u>Per</u> | | |
|--------|------------|-------|-----|------------|-------|-------|------------|-------|-------|
| | 80 | 100 | 100 | 80 | 100 | 120 | 140 | 95 | 100 |
| MALAGA | --- | 1.000 | --- | --- | 1.000 | --- | --- | --- | 1.000 |
| ANTIG | --- | 1.000 | --- | --- | 1.000 | --- | --- | 0.222 | 0.778 |
| MONT | --- | 1.000 | --- | 0.005 | 0.995 | --- | --- | --- | 1.000 |
| DOMFOR | --- | 1.000 | --- | --- | 1.000 | --- | --- | --- | 1.000 |
| DOMPH | --- | 1.000 | --- | --- | 0.934 | --- | 0.066 | --- | 1.000 |
| DONSOU | --- | 1.000 | --- | 0.005 | 0.995 | --- | --- | --- | 1.000 |
| STVIN | --- | 1.000 | --- | --- | 0.833 | 0.167 | --- | --- | 1.000 |
| MART | --- | 1.000 | --- | --- | 0.875 | 0.025 | 0.100 | --- | 1.000 |
| ARUBA | --- | 1.000 | --- | --- | 1.000 | --- | --- | 0.429 | 0.571 |
| | 0.120 | 0.870 | --- | --- | 0.072 | 0.028 | --- | --- | 1.000 |

FIGURE HEADINGS.

Figure 1. UPGMA dendrogram generated from the genetic distance data given in Table 10.

Figure 2. Idh-2¹¹⁶ allele frequencies (shaded areas) in the southern US.

Figure 3. Canonical plot of 62 Aedes aegypti populations based on 10 polymorphic loci. Each population is represented by the initial letter of its group (except "X" for EAA); overlap of populations from different groups is represented by ".

Figure 4. Canonical plot of 59 Aedes aegypti populations based on 10 polymorphic loci.

Figure 5. Canonical plot of 52 Aedes aegypti populations based on 11 polymorphic loci.

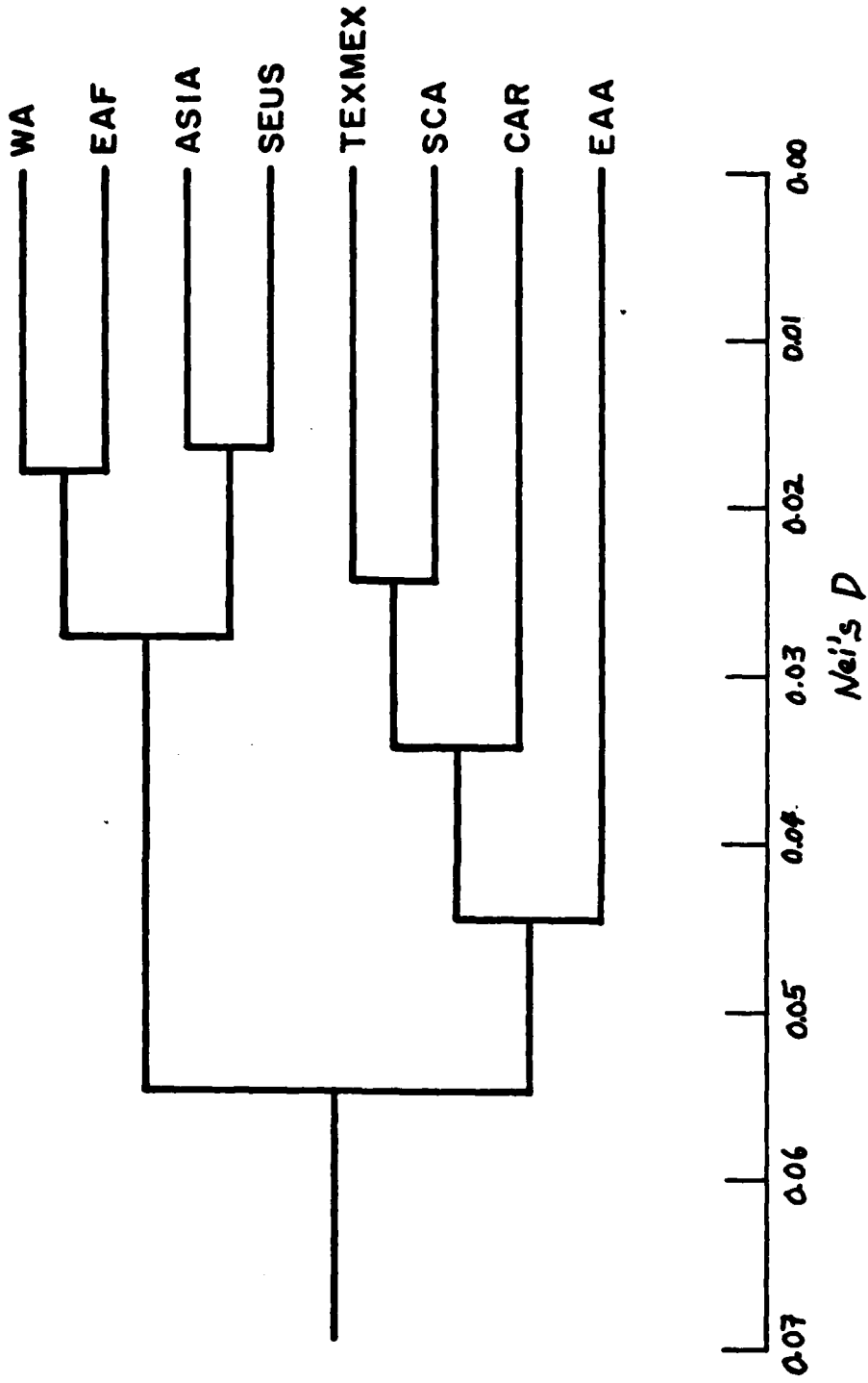


FIGURE 1

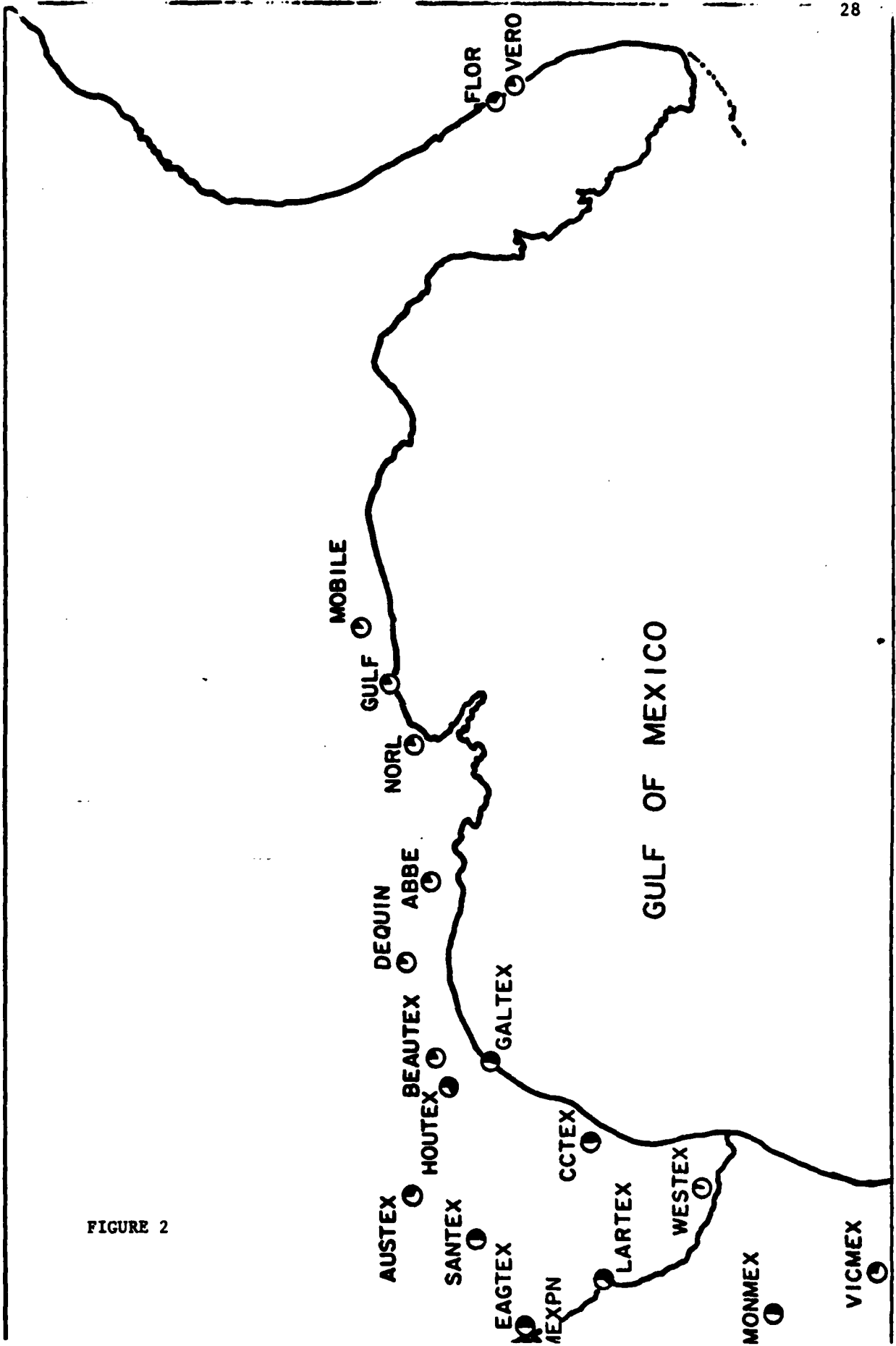


FIGURE 2

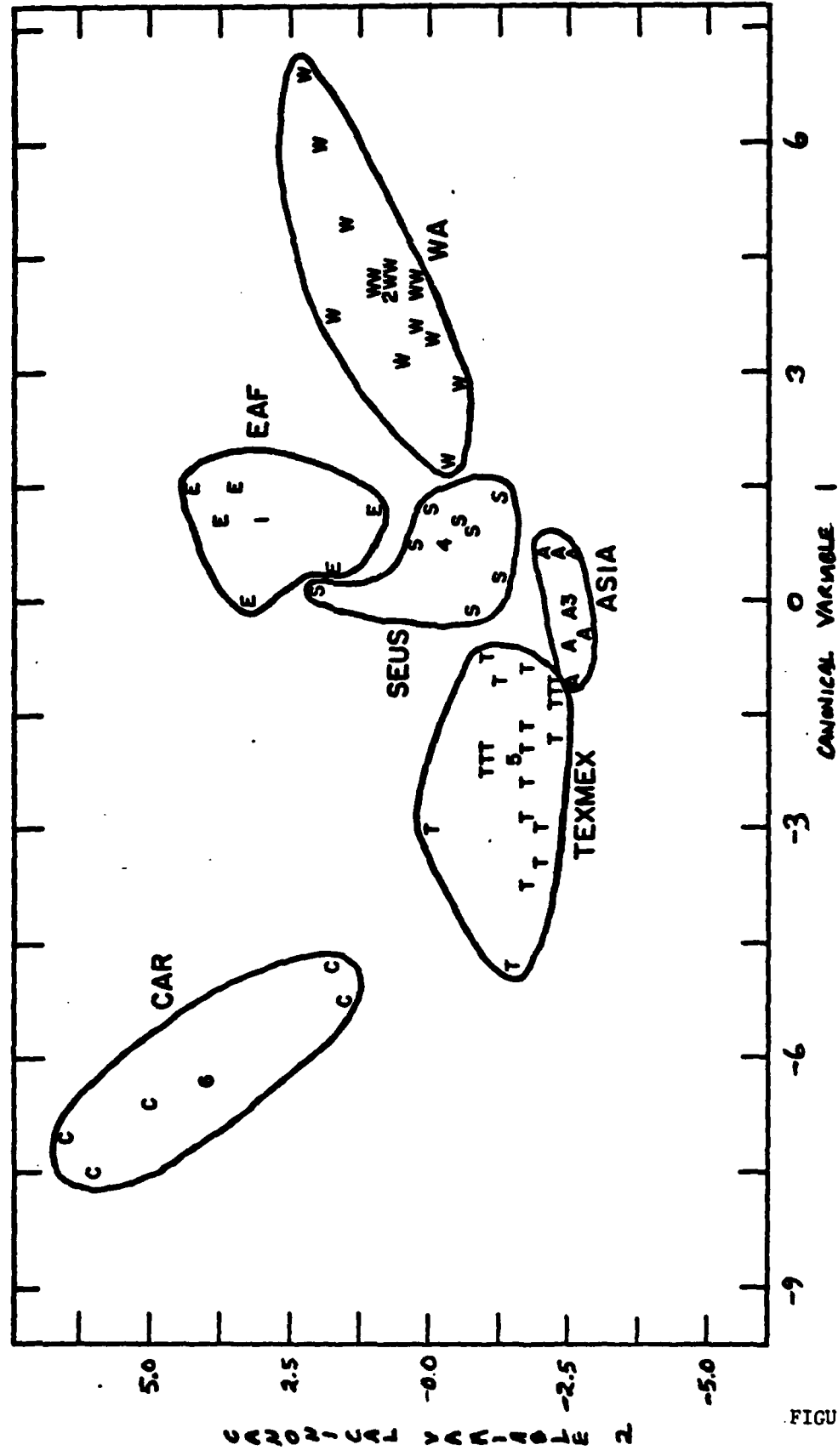


FIGURE 4

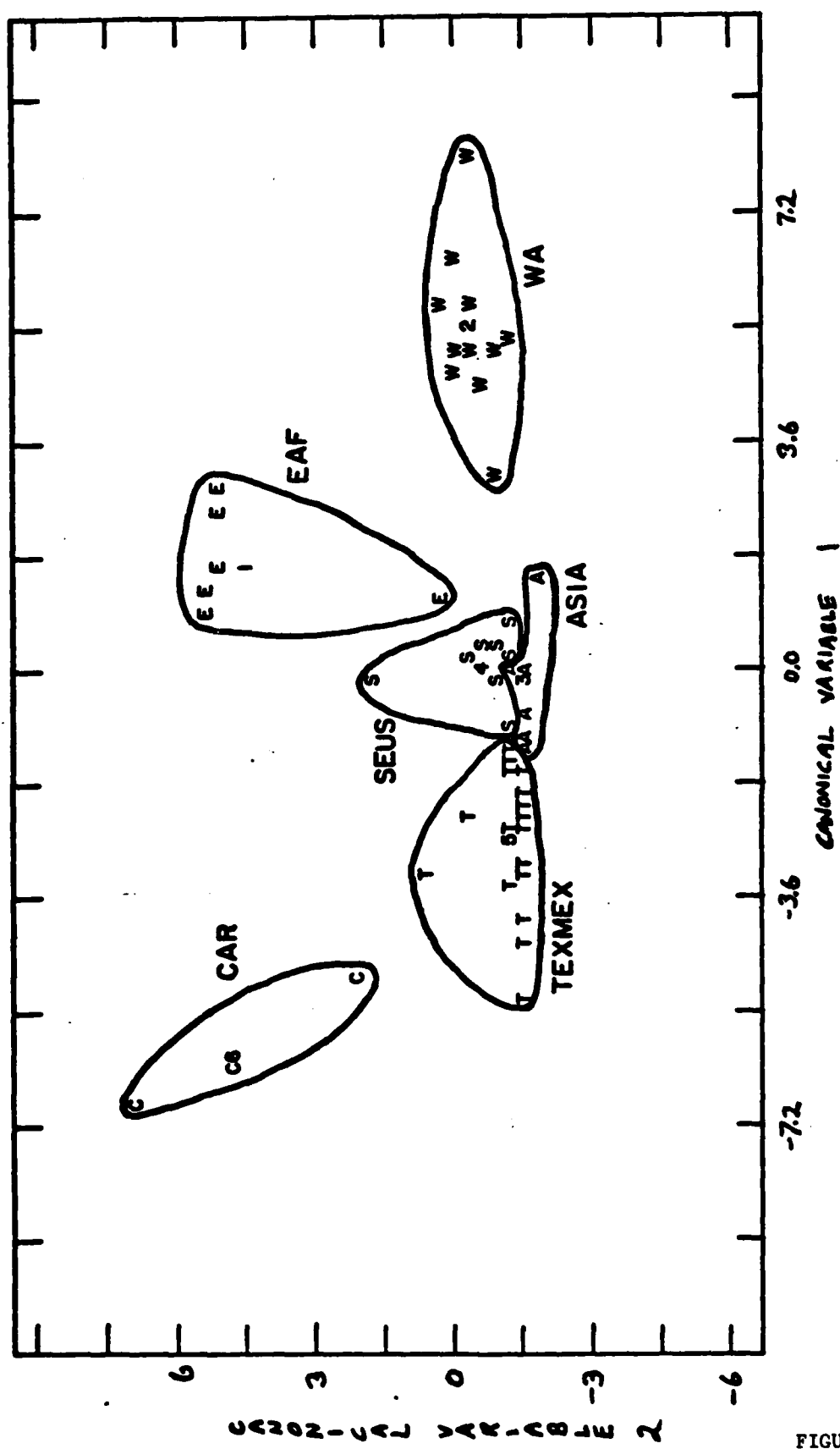


FIGURE 5

END

FILMED

1-85

DTIC