Macrogeographic genetic variation in a human commensal: 
*Aedes aegypti*, the yellow fever mosquito

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SUMMARY

Genetic variation at 11 enzyme loci in *Aedes aegypti* populations collected from 63 localities around the world is presented. A UPGMA tree based on genetic distance values clusters populations of sylvan *A. aegypti formosus* from West Africa and East Africa together, along with Asian and south-eastern U.S. populations. Domestic *A. aegypti aegypti* from East Africa and all other New World populations form the other major cluster. Multivariate discriminant analysis allows recognition of seven major global 'genetic–geographic' groups, which are consistent with the genetic distance data. Populations from the south-eastern U.S. are clearly genetically distinct from other U.S. and Mexican populations. This distinct gene pool may be indicative of reinfestation of areas where *A. aegypti* appears to have been absent in the recent past. Other evolutionary and epidemiological implications of the genetic population structure of *Aedes aegypti* are discussed.

1. INTRODUCTION

Human commensals have an important contribution to make to all areas of biology by their ready availability and general compliance to laboratory manipulation. In particular, they have great potential in increasing our understanding of several evolutionary processes as a result of their historical association with man. As we have expanded the region of our domain to include new environments, closely associated species have had to adapt to new pressures. Areas where man exists free of a certain species may suddenly become available to that species, and one may see rapid evolution of a recently introduced form. As man's patterns of migration and trade change, so does gene flow between populations of commensals. Secondary contact between two specialized forms may be monitored in the wild or imitated in the laboratory as an aid to understanding the speciation process. Commensals suffer direct and indirect interference from man, and have to respond appropriately to survive. In the case of an eradication attempt by chemical means, for example, the necessary genetic change in the population will rely on rebuilding from a few selected individuals, and the resulting population may well

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differ from the original one at a number of loci due to founder effects. In this situation, neutral or even detrimental genetic change may arise as a result of hitch-hiking by linked genes.

The house-mouse, *Mus musculus*, and the fruit-fly, *Drosophila melanogaster*, are good examples of human commensals that have been extensively studied genetically. Using large numbers of enzyme loci, local genetic differentiation (Selander & Yang, 1969) and subspecific differentiation (Selander, Hunt & Yang, 1969; Hunt & Selander, 1973) have been studied in *M. musculus*; Berry (1977) reviews work done on some island populations. Singh, Hickey & David (1982) present data for *D. melanogaster*. However, knowledge of world-wide isozyme variation in these species is not as extensive as it is for the mosquito species *Aedes aegypti*.

From a public-health viewpoint, mosquito-borne disease is of primary concern. *Aedes aegypti* is unusual in that its domestic form exists in strict association with humans, breeding in water containers inside and close to dwelling places, making it a commensal in a full sense of the word. Recent dengue epidemics in the Caribbean, and the ever-present threat of jungle and urban yellow fever in South America and Africa highlight the importance of *A. aegypti* as a target for control programmes, as it is the primary vector of these diseases. *A. aegypti* is one of the most widespread insect species, being found in close association with man throughout the tropical and subtropical world. The species is particularly interesting as one can study the extant ancestral form in Africa, where domestic and sylvan subspecies exist sympatrically (Petersen, 1977). Pioneer work by Craig and co-workers (e.g. Craig & Hickey, 1966) revealed considerable genetic heterogeneity in wild populations of *A. aegypti*. The species has latterly been shown to exhibit considerable morphological (McClelland, 1974; Petersen, 1977; VandeHey, Leahy & Booth, 1978), physiological (Machado-Allison & Craig, 1972), and behavioural (Trpis & Hausermann, 1975, 1978; Petersen, 1977; Leahy, VandeHey & Booth, 1978) differentiation. In such a polytypic species, there is also likely to be heterogeneity in those genetically controlled features of the vector that affect its overall ability to transmit disease. As efficiency of disease transmission can depend upon the geographic origin of the vector population (Aitken, Downs & Shope, 1977; Beaty & Aitken, 1979; Gubler, Novak & Mitchell, 1982; Tabachnick *et al.*, 1982), genetic delineation of geographic regions may be helpful in assessing the potential threat of invading populations. Routes of gene flow may also be elucidated by this means. The assumption made is that a gene pool that is an entity in terms of isozyme variability will possess other features common to the group as a whole. It has therefore been our contention that knowledge of the population genetics and evolutionary history of *A. aegypti* is of importance in understanding its role as a carrier of epidemic disease.

Previous electrophoretic work on *A. aegypti* includes an investigation of the two sympatric East African subspecies (Tabachnick, Munstermann & Powell, 1979), examination of microgeographic and temporal variation (Tabachnick & Powell, 1978; Tabachnick, 1982) and world-wide surveys (Tabachnick & Powell, 1979; Powell, Tabachnick & Arnold, 1980; Powell, Tabachnick & Wallis, 1982). The formal genetics of *A. aegypti* is advanced enough to provide an extensive genetic...
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map of morphological, physiological and biochemical markers (Munstermann & Craig, 1979; Wallis & Tabachnick, 1982). In our analyses we use loci that are spread throughout the genome.

The aims of the present study are to characterise further the world-wide genetic structure of *A. aegypti* and illustrate the possible epidemiological implications. Previous studies (Tabachnick & Powell, 1979) have supported the description of the two subspecies, *A. aegypti aegypti* (domestic form) and *A. a. formosus* (sylvan form), made by Mattingly (1957), and the idea that *A. aegypti* has only recently been introduced into Asia (Smith, 1956). Here we give additional data supporting these observations, as well as focusing more closely on *A. aegypti* populations in the southern United States.

2. MATERIALS AND METHODS

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

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

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

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Tabachnick & Powell (1979) with their abbreviations and origins. Tables of gene frequencies presented here are for these new populations alone, with the exception of the three hexokinase loci (Table 3), the data for which were not presented in Tabachnick & Powell (1979). We have restricted our analysis as far as possible to Table 2.

| Sample name | No. of collections | Country | Location | Date collected | Subsp. | Habitat       |
|-------------|-------------------|---------|----------|----------------|--------|---------------|
| BWE         | 1                 | Uganda  | Bwerenga | 8/80           | f      | Outdoors, urban |
| GAMBIA      | 1                 | Gambia  | Diabugu  | 1/79           | 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’goyé   | 9/81           | f      | Outdoors, village |
| AKO         | 1                 | Ghana   | Akosombo  | 5/81           | f      | Outdoors, village |
| OUAGA       | 1                 | Indonesia | 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      | Outdoors, urban |
| GULF        | 1                 | U.S.    | Gulfport MS | 9/81 | a      | Outdoors, urban |
| ABBE        | 1                 | U.S.    | Abbeville LA | 9/81    | a      | Outdoors, urban |
| DEQUIN      | 1                 | U.S.    | De Quincy LA | 9/81    | a      | Outdoors, urban |
| MOBILE      | 3                 | U.S.    | Mobile AL | 9/81           | a      | Outdoors, urban |
| BEAUTEX     | 2                 | U.S.    | Beaumont TX | 9/81  | a      | Outdoors, urban |
| GALTEX      | 1                 | U.S.    | Galveston TX | 11/80  | a      | Outdoors, urban |
| AUSTEX      | 1                 | U.S.    | Austin TX | 7/79          | a      | Outdoors, urban |
| SANTEX      | 1                 | U.S.    | San Antonio TX | 12/79 | a      | Outdoors, urban |
| CCTEX       | 4                 | U.S.    | Corpus Christi TX | 5/80 | a      | Outdoors, urban |
| 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      | —              |
| MONMEX      | 1                 | Mexico  | Montemorelos | 5/81  | a      | —              |
| VICMEX      | 2                 | Mexico  | Victoria  | 8/80          | a      | —              |
| AREC        | 1                 | Puerto Rico | Arecibo    | 10/79         | a      | Outdoors, urban |
| BAR         | 1                 | Puerto Rico | Barcelona   | 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 |
| TRINCITY    | 1                 | Trinidad | Trinicity  | 6/81          | a      | Outdoors, urban |
| FELICITY    | 1                 | Trinidad | Felicity   | 6/81          | a      | Outdoors, urban |
| PARA        | 2                 | Suriname | Paramaribo | 5/81          | a      | Outdoors, urban |
| GTOWN       | 1                 | Guyana  | Georgetown | 10/81        | a      | —              |
| GUAT        | 1                 | Guatemala | Escuintla  | 9/80          | a      | —              |

Material collected directly from the field, but some colonized material of recent origin was included in the analysis. However, we have avoided making any generalizations 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.
3. RESULTS

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 U.S., 1 West African, and 1 Mexican) and the frequency of $Gpd^{100}$ is above 0.91 in all cases except ABBE. $Me$ is variable in only six localities (4 East African, 1 West African and 1 Texan) with $Me^{100}$ 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.

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

| Population | $n$ | $Hk$-2 | | | $Hk$-3 | | | $Hk$-4 | | |
|------------|-----|--------|---|---|---|---|---|---|---|
| KBW        | 170 | 0.762  | 0.230| 0.008| 0  | 0.766| 0.234| 0.667| 0.260| 0.073|
| MAJ        | 226 | 0.905  | 0.031| 0.064| 0  | 0.981| 0.019| 0.357| 0.064| 0.579|
| MGN        | 108 | 0.855  | 0.075| 0.070| 0  | 0.921| 0.079| 0.626| 0.124| 0.250|
| KOM        | 122 | 0.951  | 0.008| 0.004| 0  | 0.951| 0.008| 0.909| 0.008| 0.042|
| SHH        | 148 | 0.865  | 0  | 0.135| 0.135| 0.865| 0  | 0.865| 0  | 0.135|
| KDZ        | 256 | 0.931  | 0.005| 0.051| 0.013| 0.982| 0.005| 0.877| 0.019| 0.094|
| Nya        | 106 | 0.981  | 0  | 0.019| 0  | 1.0  | 0  | 0.962| 0  | 0.038|
| Dar        | 180 | 0.981  | 0  | 0.039| 0  | 0.961| 0.039| 0.961| 0.039| 0  |
| BWE        | 54  | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.976| 0  | 0.024|
| Gambia     | 118 | 0.966  | 0.017| 0.017| 0  | 1.0  | 0  | 0.898| 0  | 0.102|
| Ked        | 134 | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.988| 0  | 0.012|
| Dakar      | 274 | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.982| 0  | 0.018|
| Ouaga      | 60  | 0.983  | 0.017| 0  | 0  | 0.983| 0.017| 0.983| 0.017| 0  |
| Grogo      | 154 | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.942| 0  | 0.058|
| Semar      | 54  | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.944| 0  | 0.056|
| Tanp       | 120 | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.967| 0  | 0.033|
| Sante      | 108 | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.981| 0  | 0.019|
| Cctex      | 212 | 0.896  | 0.104| 0  | 0  | 0.896| 0.104| 0.888| 0.104| 0.008|
| Eagtex     | 116 | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.974| 0  | 0.026|
| Monmex     | 114 | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.965| 0  | 0.035|
| Carac      | 108 | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.926| 0  | 0.074|
| Mobay      | 86  | 0.733  | 0  | 0.267| 0  | 1.0  | 0  | 0.593| 0  | 0.407|
| May        | 150 | 1.0    | 0  | 0  | 0  | 1.0  | 0  | 0.987| 0  | 0.013|
| Sanj       | 52  | 0.923  | 0.019| 0.058| 0  | 0.962| 0.038| 0.808| 0.019| 0.173|

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

Population abbreviations appearing in this table but absent from Table 2 are defined in Tabachnick & Powell (1979).
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. $Mdh^{84}$ is essentially an $A. a. formosus$ allele whereas $Mdh^{120}$ 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. $Idh-2^{116}$ 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 $Idh-2^{214}$ frequencies, but populations in the south-eastern United States resemble Asian populations having lower frequencies of this allele.

Phosphoglucomutase again supports the Mattingly subspecies division; all
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 Sylvan populations from West and East Africa are highly heterozygous whereas Pgm\textsuperscript{100} predominates in domestic collections.

Phosphoglucone dehydrogenase and phosphoglucose isomerase are generally weakly polymorphic. Exceptions are some East African formosus populations and Table 5.

| Populations | n  | 87  | 100  | 116  | 125  |
|-------------|----|-----|------|------|------|
| BWE         | 126| 0.937| 0.063| 0    |      |
| GAMBIA      | 122| 0.918| 0.082| 0    |      |
| KED         | 116| 0.966| 0.034| 0    |      |
| DAKAR       | 274| 0.962| 0.038| 0    |      |
| NGOYE       | 100| 0.930| 0.070| 0    |      |
| AKO         | 80 | 0.975| 0.025| 0    |      |
| OUAGA       | 60 | 0.967| 0    | 0    |      |
| TANP        | 120| 0.875| 0.125| 0    |      |
| FIJI        | 202| 0.881| 0.119| 0    |      |
| VERO        | 120| 0.792| 0.208| 0    |      |
| GULF        | 42 | 0.833| 0.167| 0    |      |
| ABBE        | 70 | 0.786| 0.214| 0    |      |
| DEQUIN      | 46 | 0.891| 0.109| 0    |      |
| MOBILE      | 170| 0.870| 0.130| 0    |      |
| BEAUTEX     | 136| 0.833| 0.167| 0    |      |
| GALTEX      | 240| 0.463| 0.537| 0    |      |
| AUSTEX      | 102| 0.686| 0.314| 0    |      |
| SANTEX      | 112| 0.500| 0.500| 0    |      |
| CCTEX       | 278| 0.528| 0.462| 0.010|      |
| LARTEX      | 308| 0.396| 0.604| 0    |      |
| EAGTEX      | 116| 0.509| 0.491| 0    |      |
| WESTEX      | 106| 0.953| 0.047| 0    |      |
| HOUTEX      | 254| 0.343| 0.657| 0    |      |
| MONMEX      | 114| 0.491| 0.509| 0    |      |
| VICMEX      | 168| 0.714| 0.286| 0    |      |
| AREC        | 194| 0.485| 0.515| 0    |      |
| BAR         | 80 | 0.587| 0.413| 0    |      |
| TRIN        | 60 | 0.067| 0.933| 0    |      |
| SEALOTS     | 40 | 0.325| 0.675| 0    |      |
| TRINCITY    | 118| 0.551| 0.449| 0    |      |
| FELICITY    | 22 | 0.364| 0.636| 0    |      |
| PARA        | 198| 0.247| 0.753| 0    |      |
| GTOWN       | 88 | 0.693| 0.307| 0    |      |
| GUAT        | 342| 0.591| 0.409| 0    |      |

the Caribbean where Pgd\textsuperscript{116} is common, and some New World populations where Pgi\textsuperscript{93} and Pgi\textsuperscript{10S} sporadically reach higher frequencies.

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

(i) Genetic-geographic groupings

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, Tabachnick & Arnold (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

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 |
| AKO        | 80  | 0.225 | 0.238 | 0.325 | 0.212 |
| OUAGA      | 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    |
| HOUTEX     | 182 | 0    | 0.879 | 0.121 | 0    |
| VICMEX     | 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    |
| GTOWN      | 88  | 0    | 0.989 | 0.011 | 0    |
| GUAT       | 330 | 0.015 | 0.985 | 0    | 0    |

The following populations were fixed for $Pgm^{100}$: TANP, BEAUTEX, LARTEX, WESTEX, MONMEX, AREC, BAR, TRIN, and SEALOTS ($\bar{n} = 138.2$)

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

| Population | n   | 86 | 100 | 116 | 130 | Others |
|------------|-----|----|-----|-----|-----|--------|
| BWE        | 124 | 0.024 | 0.895 | 0.081 | 0 | 0 |
| GAMBIA     | 122 | 0 | 0.984 | 0 | 0.008 | 0.008 |
| KED        | 122 | 0 | 0.979 | 0 | 0 | 0.021 |
| DAKAR      | 248 | 0 | 0.926 | 0 | 0 | 0.074 |
| OUAGA      | 60 | 0 | 0.967 | 0.033 | 0 | 0 |
| VERO       | 120 | 0.017 | 0.933 | 0.050 | 0 | 0 |
| GULF       | 42 | 0.071 | 0.929 | 0 | 0 | 0 |
| DEQUIN     | 46 | 0 | 1.000 | 0 | 0 | 0 |
| MOBILE     | 170 | 0.029 | 0.803 | 0.168 | 0 | 0 |
| BEAUTEX    | 132 | 0.063 | 0.937 | 0 | 0 | 0 |
| GALTEx     | 240 | 0 | 0.996 | 0.004 | 0 | 0 |
| AUSTEx     | 96 | 0.031 | 0.969 | 0 | 0 | 0 |
| SANTEx     | 112 | 0 | 1.000 | 0 | 0 | 0 |
| CCTEx      | 274 | 0 | 1.000 | 0 | 0 | 0 |
| LARTEX     | 394 | 0 | 1.000 | 0 | 0 | 0 |
| EAGTEX     | 116 | 0 | 1.000 | 0 | 0 | 0 |
| WESTEx     | 94 | 0 | 1.000 | 0 | 0 | 0 |
| HOUTEx     | 254 | 0 | 1.000 | 0 | 0 | 0 |
| MONMEX     | 116 | 0 | 1.000 | 0 | 0 | 0 |
| AREC       | 194 | 0 | 0.768 | 0.232 | 0 | 0 |
| BAR        | 80 | 0 | 0.483 | 0.537 | 0 | 0 |
| TRIN       | 60 | 0 | 1.000 | 0 | 0 | 0 |
| TRINCIty   | 124 | 0 | 1.000 | 0 | 0 | 0 |
| PARA       | 192 | 0 | 1.000 | 0 | 0 | 0 |
| GUAT       | 184 | 0 | 1.000 | 0 | 0 | 0 |

The following populations are fixed for both Pgd and Pgi: NGOYE, AKO, TANP, FIJI, ABBE, VICMEX, SEALOTS, FELICITY and G Townsend (n = 90-3).

Table 8. Mean expected heterozygosity values with standard errors for each genetic-geographic region based on 10 loci

| Group | N  | \(H_e \pm \text{s.e.} \) |
|-------|----|------------------------|
| EAA   | 3  | 0.209 ± 0.014          |
| EAF   | 6  | 0.185 ± 0.012          |
| WA    | 15 | 0.107 ± 0.004          |
| ASIA  | 6  | 0.081 ± 0.007          |
| SEUS  | 8  | 0.137 ± 0.008          |
| TEXMEX| 11 | 0.130 ± 0.012          |
| SCA   | 9  | 0.102 ± 0.010          |
| CAR   | 5  | 0.179 ± 0.017          |
| Overall | 63 | 0.117 ± 0.005          |

result from founder effect and drift. WESTEx appears to be an example of such an isolate; two large collections directly from the field show the same high frequency of \(Idh-2^{100} \) 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. Fig. 1 is an UPGMA dendrogram generated from the data in Table 9.

Intensive sampling of \textit{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 \textit{Idh-2} allele frequencies (Fig. 2). Considering the 8 south-eastern US populations, the frequency of \textit{Idh-2}^{116} reaches 0.33 in HAMF, but in all the other 7 is below 0.22. Contrarily, \textit{Idh-2}^{116} 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 TEXMEX 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 VENEZ, PARA, SEALOTS and TRINITY than they are to nearby BEAUTEX, DEQUIN and ABBE.

Whilst Nei’s \textit{D} value is perhaps the best 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

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

Fig. 2. *Idh-2*\(^{216}\) allele frequencies (shaded areas) in the southern U.S.

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
Fig. 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 *.

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)

| Variable  | Coefficient 1 | Coefficient 2 | F-value to enter or remove |
|-----------|---------------|---------------|---------------------------|
|           | 59 localities analysis | 52 localities analysis |                      |
| *Pgm*100  | -8.860        | -6.680        | 38.2005                  |
| *Idh*-2100| 5.398         | -1.377        | 19.9160                  |
| *Pgd*100  | -16.200       | -20.517       | 17.8895                  |
| *Hk-2NULL*| -18.755       | 26.088        | 10.9124                  |
| *Me*100   | 14.026        | -8.852        | 8.1005                   |
| *Pgd*116  | -25.740       | -4.343        | 6.9890                   |
| *Gpd*100  | -1.841        | -5.993        | 5.2055                   |
| Constant  | 7.723         | 32.588        |                           |

|           |               |               |                          |
| *Pgd*116  | -23.915       | 5.498         | 42.008                   |
| *Idh*-2118| -5.257        | -0.099        | 38.104                   |
| *Pgm*100  | -9.061        | -1.755        | 13.366                   |
| *Idh*-1180| 9.813         | -0.595        | 8.308                    |
| *Pgd*100  | -15.971       | -10.275       | 6.985                    |
| *Me*100   | 8.695         | -15.827       | 5.260                    |
| *Idh*-1180| 20.927        | 74.252        | 10.292                   |
| Constant  | 15.941        | 26.398        |                           |
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Fig. 4. Canonical plot of 59 *Aedes aegypti* populations based on 10 polymorphic loci.

Fig. 5. Canonical plot of 52 *Aedes aegypti* populations based on 11 polymorphic loci.
the use of stepwise multivariate discriminant analysis to this end (Powell et al. 1980; Powell et al. 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 a two-dimensional plot is made using the two canonical variables that account for the highest proportion of total dispersion.

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, Fig. 3 shows the canonical plot. EAA is well separated from all the other groups, which form a mêlée 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 high frequency of $Hk-4^{108}$ is of great discriminatory value.

In order to discriminate between the other geographic regions, the analysis is re-run omitting the three EAA members. Fig. 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 Fig. 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. In these analyses, SCA was not upheld as a distinct group; this group is now merged with TEXMEX, and henceforth referred to as such.

4. DISCUSSION

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 $0.0624 \pm 0.0058$ between populations based upon 10 isozyme loci. There is little genetic differentiation between populations in West Africa (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 sylvan groups EAF and WA. It is of interest to note that we have been unable to locate domestic type $A. a. aegypti$ 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 sixteenth-eighteenth 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 U.S.A. are most closely related to the sylvan forms (Fig. 1) with 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 unlike any neighbouring localities and believe that this is an example of founder effect.

The most interesting new feature is the situation in the south-eastern U.S.A. This could be associated with the disappearance of and reinfection 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 in 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 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 U.S. 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, Alabama 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 population. 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 U.S. (from Beaumont TX east to Vero Beach Florida) and the remaining regions bordering the Gulf of Mexico, the TEXP 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, it is interesting that 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 interesting that populations of mosquitoes in Asia and the south-eastern U.S. have a high genetic affinity with regard to isozymes. This is consistent with the hypothesis that isozyme-based genetic–geographic groups share other genetic features in common, vectorial efficiency perhaps being one.

Heterogeneity within some geographic regions may reflect the recent history of *Aedes aegypti* populations with regard to their association with humans. Compared to other well-studied commensals like the house mouse, *A. aegypti* has been more intensively subjected to human efforts for control and eradication. Insecticide control programmes 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 may have arisen in 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 are generally thought to be high for prolific commensals. 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.

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