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A world-wide survey of genetic variation in the yellow fever mosquito, *Aedes aegypti**

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SUMMARY

Thirty-four populations of *Aedes aegypti* representing the world-wide distribution of the species, were analysed for genetic variation at 19–22 isozyme loci. The species has an average expected heterozygosity of $0{\cdot}129 \pm 0{\cdot}045$ based on 19 loci analysed in every population. Based on this genetic information, two major groups can be defined: the dark, often sylvan, African subspecies *formosus* and the light domestic subspecies *aegypti* in Africa and the New World. Asian populations do not fall easily into either group. These results are related to models which have been proposed for the evolution of this species. Although *A. aegypti* was introduced into the New World *c.* 350 years ago and has recently recolonized many areas following eradication programmes, no signs of founder effects are evident in this region. Asian populations, on the other hand, do show a significantly lower level of genetic variation compared to other populations. This may be related to the time of introduction of *A. aegypti* into Asia and historical absence of yellow fever on the Asian continent.

1. INTRODUCTION

The mosquito *Aedes aegypti* offers an intriguing opportunity for evolutionary genetic studies. *A. aegypti* is one of the most widely distributed insect species being found throughout the tropical and subtropical world (Fig. 1). It is a systematically complex species, exhibiting morphological and behavioural variation. Much of the species' recent evolution is closely tied to its association with humans. Mattingly (1957) was among the first to formally recognize the polytypic nature of *A. aegypti*, and proposed two subspecies based on morphology and geographic distribution. *A. ae. formosus* is a darkly coloured and usually sylvan form which is confined to Africa south of the Sahara Desert. The type form, *A. ae. aegypti*, is a pale to brownish-black, domestic mosquito, which is found on the coastal plains of Africa and is the form found outside Africa. The wide distribution of *A. aegypti* is a direct result of this 'domestic' subspecies' association with human habitats.

A. aegypti is an important insect vector of diseases such as yellow fever, dengue,

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and filariasis, and has been extensively studied for over 80 years. Reviews of its bionomics (Christophers, 1960), ecology (Petersen, 1977), systematics (Mattingly, 1957; McClelland, 1974), and physiology (Clements, 1963) are available. It has also been the subject of numerous genetic studies (Craig & Hickey, 1967; Rai & Hartberg, 1975).

A number of recent investigations have confirmed the polytypic nature of the species. Differentiation has been reported in colour variation (VandeHey, Leahy & Booth, 1978), oviposition site preference (e.g. Leahy, VandeHey & Booth, 1978), house entering behaviour (Trpis & Hausermann, 1978; Petersen, 1977), host

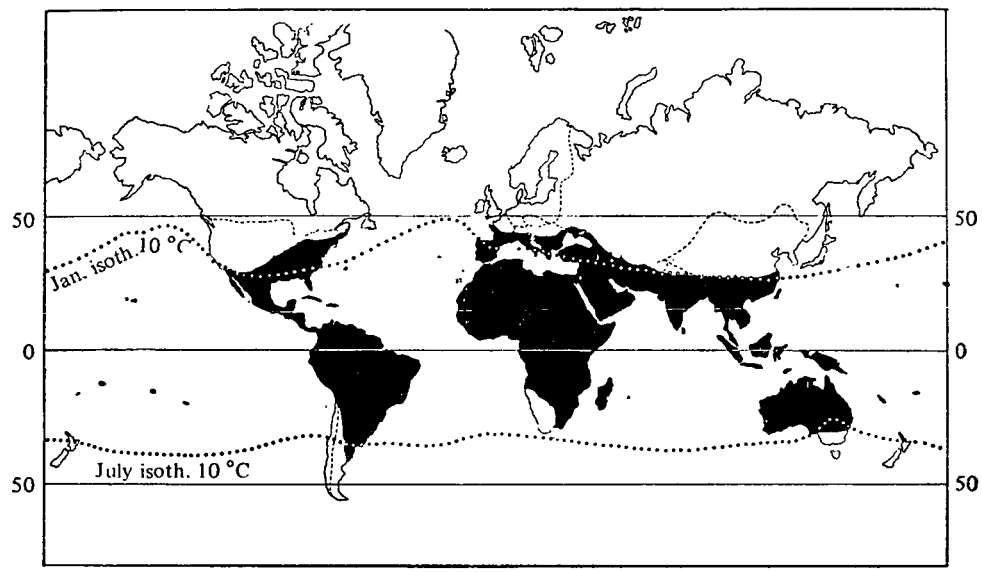

Fig. 1. Map of distribution of *Aedes aegypti* (from Christophers, 1960). While darkened areas show the complete reported range, continuously breeding populations exist only between the 10 °C isotherms approximately.

preference (Gouck, 1972), resistance to desiccation (Machado-Allison & Craig, 1972) and biochemical differences (Tabachnick, Munstermann & Powell, 1979; Scott & McClelland, 1975).

Because a variety of criteria have been used to differentiate forms, some confusion arises in nomenclature. Mattingly's subspecies are most easily distinguished by differences in scale colouration on the first abdominal tergite. *A. aegypti* collected in sylvan habitats in Africa correspond morphologically to *A. ae. formosus*. However this morphological type can occasionally be found in domestic or urban habitats in Africa (Petersen, 1977; Trpis & Hausermann, 1975). The lighter scale pattern of *A. ae. aegypti* predominates in some populations in human habitats in Africa and all populations outside Africa. However within such populations there is considerable variation in scale colour ranging to patterns characteristic of *A. ae. formosus* (McClelland, 1974). In the samples we have studied there was little

ambiguity as to the subspecies based on morphology. Subspecies *aegypti* was always associated with human habitats. Sylvan collections were exclusively subspecies *formosus*. However some collections in human habitats in Africa (e.g. Dar Es Salaam) corresponded morphologically to *formosus*. Thus in describing the collections we indicate both the subspecies based on morphology and the type of habitat where collections were made (Table 1). (The term 'feral' has also been used to describe the form taken in sylvan habitats; it is almost certainly a misnomer.)

Our previous reports described the 'micro' and 'meso' geographic patterns of genetic variation in this species (Tabachnick & Powell, 1978; Tabachnick, Munstermann & Powell, 1979). Domestic (village) populations in coastal Kenya were shown to be genetically stable, panmictic units with relatively restricted migration between populations separated by distances as small as 1 km. The region of Kenya sampled in our studies is one of the few areas in the world where sylvan and domestic populations of *A. aegypti* occur sympatricly. Significant differences in allele frequencies at several isozyme loci indicate that restricted gene flow occurs between the forms.

The present paper extends our previous studies to a 'macro' geographic survey of *A. aegypti*. We present the results of our electrophoretic analyses of populations sampled from throughout the distribution of the species. In the discussion we have addressed the following points: (*a*) the polytypic nature of the species; (*b*) the genetic differences among geographic regions and how these differences may reflect the history of colonization of *A. aegypti* outside Africa; (*c*) the epidemiological consequences of observed genetic differences among mosquito populations; (*d*) the relationship of genetic differentiation and some hypotheses for the evolution of *A. aegypti*.

2. MATERIALS AND METHODS

Thirty-four different collections were analysed for genetic variation at 19–22 isozyme loci. Brief descriptions, and our symbols for the collections are shown in Table 1. The majority of collections were analysed using 'isofemale' lines (lines begun by single females collected from natural populations) as described by Tabachnick, Munstermann & Powell (1979). This generally involved assaying a single F_1 offspring per isozyme locus per female captured in nature. Several collections were analysed by directly electrophoresing field collected material. Four collections, designated MAYA, MEX-PN, VENEZ and SEMAR, represent field material from the respective region, which had been in laboratory culture for several generations. Several workers (Munstermann, pers. commun.; Tabachnick, unpubl.) have demonstrated that colonization in the laboratory may effect gene frequencies in *A. aegypti*. As a result, in the present study we have been careful to avoid making inferences which depend solely upon results from laboratory colonies. We have relied primarily on the samples analysed directly from nature, or at most the F_1's of field collected females.

Table 1. *Collection names, countries of origin, location and description of populations of* Aedes aegypti. f *is* formosus *subspecies morphology*; a *is* aegypti *subspecies morphology*

Abbrev.	Country	Location	Date collected	Sub-species	Habitat
DAR	Tanzania	Dar Es Salaam	i. 1978	*f*	City suburb
KOM	Kenya	Kombeni Forest	xii. 1976	*f*	Sylvan
SHH	Kenya	Shimba Hills	xii. 1976	*f*	Sylvan
KDZ I	Kenya	Kwa Dzivo Village	ix. 1975	*f*	Outdoors, village
KDZ II	Kenya	Kwa Dzivo Village	iii. 1976	*f*	Outdoors, village
NYA	Kenya	Mombasa	xii.1976	*f*	City suburb
KBW I	Kenya	Kwa Bendegwa	ix. 1975	*a*	Indoors, village
KBW II	Kenya	Kwa Bendegwa	ix. 1975	*a*	Indoors, village
MAJ I	Kenya	Majengo	xii. 1975	*a*	Indoors, village
MAJ II	Kenya	Majengo	iv. 1976	*a*	Indoors, village
MGN I	Kenya	Mgandini	xi. 1976	*a*	Indoors, village
MGN II	Kenya	Mgandini	iv. 1976	*a*	Indoors, village
ENUGU	Nigeria	Enugu City	iv. 1978	*f*	Outdoors, urban
OGUI	Nigeria	Enugu Center	iv. 1976	*f*	Outdoors, urban
UKANA	Nigeria	Ukana	iv. 1976	*f*	Indoors, village
MAMUR	Nigeria	Mamu River Forest	iv. 1976	*f*	Outdoors, village
EGEDE	Nigeria	Egede Village	iv. 1976	*f*	Outdoors, village
ABORV	Nigeria	Abor Village	iv. 1976	*f*	Outdoors, village
BOBO	Upper Volta	Bobo-Dioulasso	x. 1976	*f*	Outdoors, village
KARI	Upper Volta	Kari Village	x. 1976	*f*	Outdoors, village
BWOM	Upper Volta	Bwombi Village	xi. 1976	*f*	Indoors, village
MOBAY	Jamaica	Montego Bay	iii. 1976	*a*	Outdoors, urban
MAYA	Puerto Rico	Mayaguez	i., iii. 1976	*a*	Outdoors, urban
SANJ	Puerto Rico	San Juan	iii. 1976	*a*	Outdoors, urban
HAMF	U.S.	Indian County, Fla.	iv. 1977	*a*	Outdoors, suburb
NORL I	U.S.	New Orleans	vii. 1976	*a*	Outdoors, urban
NORL II	U.S.	New Orleans	ix. 1976	*a*	Outdoors, urban
MEX-PN	Mexico	Piedras Negras	vi. 1977	*a*	Outdoors, urban
VENEZ	Venezuela	Maracay	vi. 1976	*a*	Outdoors, urban
CARAC	Venezuela	Caracas	vii. 1977	*a*	Outdoors, urban
GROGOL	Indonesia	Jakarta	v. 1976	*a*	Outdoors, urban
SEMAR	Indonesia	Semarang	v. 1976	*a*	Outdoors, urban
BANG	India	Bangalore	iii. 1977	*a*	Urban
TAI	Taiwan	Kaohsiung	ix. 1977	*a*	Indoors, urban

Table 2. *Isozymes studied in* Aedes aegypti; *life stage analysed and electrophoretic system*

Isozyme locus	Stage analysed	Gel system*	Chromosome linkage
1. Acetaldehyde oxidase (Ao)	Adults	A	—
2. Adenylate kinase (Adk)	Adults	III	—
3. Alcohol dehydrogenase (Adh)	Larvae	A	—
4. Alkaline phosphatase (Aph)	Larvae	I	1
5. Esterase-6 (Est-6)	Larvae	III	2
6. Fumarase (Fum)	Adults	A	—
7. Hexokinase-2 (HK-2)	Adults	III	3
8. Hexokinase-3 (HK-3)	Adults	III	3
9. Hexokinase-4 (HK-4)	Adults	III	3
10. Glucose-6-phosphate dehydrogenase (G6pd)	Adults	II	—
11. α-Glycerophosphate dehydrogenase (αGpdh)	Adults	III	2
12. Isocitrate dehydrogenase-1 (Idh-1)	Adults	III	—
13. Isocitrate dehydrogenase-2 (Idh-2)	Adults	III	2
14. Lactic dehydrogenase (Ldh)	Larvae	I	—
15. Leucine amino peptidase (Lap)	Larvae	I	—
16. Malic dehydrogenase (Mdh)	Adults	II	2
17. Malic enzyme (Me)	Adults	III	1
18. Phosphoglucoisomerase (Pgi)	Adults	II	3
19. Phosphoglucomutase (Pgm)	Adults	III	2
20. 6-Phosphogluconate dehydrogenase (6Pgd)	Adults	II	3
21. Tetrazolium oxidase-3 (To-3)	Adults	II	—
22. Xanthine dehydrogenase (Xdh)	Larvae	A	—

* A, 7% acrylamide; I, starch system A; II, starch system B; III, starch system C of Ayala *et al.* (1972).

A list of the isozyme loci we have surveyed is shown in Table 2. Twelve of the 22 loci have been mapped on one of the three *A. aegypti* chromosomes (Munstermann, 1979; Tabachnick, 1978; Tabachnick & Lichtenfels, 1978). Since the ten remaining loci are monomorphic (or nearly so), mapping is impossible or at least much more difficult. Since these loci are not variable they do not appreciably affect our conclusions; therefore the mendelian nature of their variants is not crucial. The majority of the isozymes have been analysed using starch gel electrophoretic techniques described by Ayala *et al.* (1972, 1974). Buffer systems, labelled I, II, and III in Table 2, conform to buffer systems A, B and C respectively of Ayala *et al.* (1972). Several of the isozyme systems were analysed using 7% acrylamide gels, with 0·5 M Tris-HCl gel buffer, pH 8·9, and a 0·04 M Tris-glycine reservoir buffer pH 8·3. This considerably improved the resolution of these isozymes over that obtainable using starch gel electrophoresis. The stain solutions are generally those of Ayala *et al.* (1972, 1974) with only some minor modifications (Tabachnick & Powell, 1978). Details of electrophoretic procedures will be supplied upon request.

3. RESULTS

Tables 3–6 show the gene frequencies for each population for four loci which show the most differentiation among populations; *Idh-2*, *Mdh*, *6-Pgd* and *Pgm*. The allelic frequencies at each of these loci exhibit differentiation between the major geographic regions sampled in our study. Our previous results (Tabachnick, Munstermann & Powell, 1979) showed genetic differences between sympatric sylvan *A. ae. formosus* and domestic populations of *A. ae. aegypti* in coastal Kenya. *Idh-2* was found to be significantly less heterozygous in sylvan populations. Table 3 shows that both West African *A. ae. formosus* and New World domestic populations show a similar effect. Asian populations, although morphologically and behaviourally domestic (and therefore conforming to Mattingly's description of *A. ae. aegypti*) exhibit *Idh-2* gene frequencies and heterozygosities which are more like *A. ae.*

Table 3. *Allelic frequencies and average expected heterozygosities* (H_e) *at highly differentiated loci in populations of* Aedes aegypti

Population	n	Idh-2^{100}	Idh-2^{116}	Others	H_e
DAR	180	0·805	0·167	0·028	0·323
KOM	122	0·951	0·049	0·0	0·093
SHH	150	1·0	0·0	0·0	0
KDZ*	262	0·893	0·103	0·003	0·190
NYA	104	0·904	0·096	0·0	0·174
KBW*	230	0·578	0·422	0·0	0·488
MAJ*	158	0·481	0·519	0·0	0·499
MGN*	218	0·518	0·482	0·0	0·499
ENUGU	118	1·0	0·0	0·0	0
OGUI	144	0·944	0·056	0·0	0·106
UKANA	144	0·965	0·035	0·0	0·068
MAMUR	100	0·890	0·110	0·0	0·196
EGEDE	68	0·955	0·044	0·0	0·084
ABORV	40	0·800	0·200	0·0	0·32
BOBO	166	0·966	0·034	0·0	0·066
KARI	114	0·921	0·079	0·0	0·145
BWOM	112	0·947	0·053	0·0	0·100
MOBAY	88	0·580	0·420	0·0	0·487
MAYA	152	0·230	0·770	0·0	0·354
SANJ	52	0·481	0·519	0·0	0·499
FLOR	112	0·670	0·330	0·0	0·442
NORL*	204	0·794	0·206	0·0	0·327
MEX-PN	112	0·402	0·598	0·0	0·481
VENEZ	86	0·698	0·302	0·0	0·422
CARAC	180	0·622	0·378	0·0	0·470
GROGOL	154	0·948	0·052	0·0	0·099
SEMAR	54	0·759	0·241	0·0	0·366
BANG	150	0·987	0·013	0·0	0·026
TAI	18	0·833	0·167	0·0	0·278

This table presents data for isocitrate dehydrogenase.
n, number of genes sampled from natural populations.
* Average of more than one collection from locality.

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Table 4. *Allele frequencies at phosphoglucomutase locus. Abbreviations same as Table 3*

Population	n	Pgm^{100}	Pgm^{120}	Others	H_e
DAR	176	0·750	0·091	0·159	0·404
KOM	90	0·678	0·220	0·100	0·478
SHH	138	0·877	0·080	0·043	0·223
KDZ*	262	0·817	0·114	0·069	0·315
NYA	104	0·770	0·115	0·115	0·381
KBW	230	0·930	0·013	0·057	0·132
MAJ*	156	0·981	0·019	0·0	0·037
MGN*	218	0·899	0·064	0·037	0·186
ENUGU	118	0·331	0·508	0·161	0·606
OGUI	148	0·568	0·338	0·095	0·556
UKANA	134	0·537	0·313	0·149	0·589
MAMUR	100	0·490	0·380	0·130	0·599
EGEDE	66	0·546	0·348	0·091	0·543
ABORV	40	0·550	0·300	0·150	0·585
BOBO	116	0·646	0·319	0·034	0·478
KARI	116	0·594	0·259	0·147	0·658
BWOM	112	0·669	0·169	0·170	0·511
MOBAY	88	0·955	0·0	0·045	0·086
MAYA	152	1·0	0·0	0 0	0
SANJ	52	1·0	0·0	0·0	0
FLOR	114	0·860	0·026	0·114	0·247
NORL*	—	0·789	0·132	0·078	0·352
MEX-PN	112	0·982	0·0	0·018	0·035
VENEZ	86	0·977	0·023	0·0	0·045
CARAC	180	0·989	0·0	0·011	0·022
GROGOL	150	0·920	0·0	0·080	0·147
SEMAR	29	1·0	0·0	0·0	0
BANG	100	0·950	0·0	0·05	0·095
TAI	18	1·0	0·0	0·0	0

formosus populations in West and East Africa. The allelic frequencies observed at the *Mdh* locus (Table 5) show differentiation which follow Mattingly's subspecies identification. Heterozygosities and the frequency of the most common allele, Mdh^{100}, are not significantly different among different regions. When examining the frequencies of rare alleles however, one finds that in the majority of domestic populations (East African domestic, New World and Asia) the Mdh^{120} allele is the second most common. In all *A. ae. formosus* collections, Mdh^{84} is the second most frequent allele. Table 6 shows that *A. ae. formosus* populations from both East and West Africa are significantly more heterozygous than the *A. ae. aegypti* collections from other regions at *6Pgd*. Also there is differentiation in the gene frequencies of *6Pgd* among populations. However the allelic differentiation of *6Pgd* appears to be independent of subspecies identification. East African sylvan populations are significantly more heterozygous at *6Pgd* than populations from any of the other regions. However *A. ae. formosus* populations from West Africa are less heterozygous at *6Pgd* than New World domestic populations. Domestic populations from

Table 5. *Allele frequencies at malate dehydrogenase locus. Abbreviations same as Table* 3

Population	n	Mdh^{84}	Mdh^{100}	Mdh^{120}	Others	H_e
DAR	200	0·055	0·910	0·035	0	0·168
KOM	94	0·298	0·681	0·021	0	0·447
SHH	108	0·185	0·787	0·028	0	0·346
KDZ*	232	0·185	0·763	0·052	0	0·381
NYA	100	0·280	0·580	0·140	0	0·566
KBW*	176	0·080	0·710	0·210	0	0·445
MAJ*	152	0·066	0·704	0·224	0	0·438
MGN*	192	0·068	0·688	0·245	0	0·464
EGUGU	104	0·192	0·673	0·135	0	0·492
OGUI	88	0·187	0·778	0·034	0	0·357
UKANA	146	0·110	0·808	0·982	0	0·328
MAMUR	26	0·154	0·846	0	0	0·261
EGEDE	16	0·062	0·938	0	0	0·116
ABORV	20	0·500	0·500	0	0	0·500
BOBO	104	0·115	0·875	0·01	0	0·221
KARI	102	0·147	0·853	0	0	0·251
BWOM	98	0·061	0·888	0·051	0	0·205
MOBAY	98	0·041	0·367	0·592	0	0·528
MAYA	84	0·024	0·797	0·178	0	0·332
SANJ	54	0·019	0·611	0·370	0	0·489
FLOR	102	0·088	0·716	0·186	0·01	0·445
NORL*	286	0·154	0·476	0·378	0	0·623
MEX-PN	90	0	0·567	0·433	0	0·491
VENEZ	86	0	0·602	0·398	0	0·479
CARAC	170	0·053	0·841	0·106	0	0·270
GROGOL	106	0·198	0·594	0·208	0	0·565
SEMAR	84	0·111	0·788	0·111	0	0·374
BANG	106	0·255	0·670	0·075	0	0·480
TAI	18	0	0·778	0·222	0	0·345

Asia are monomorphic at *6Pgd*. Table 4 shows the allelic frequencies found at *Pgm*. In the New World, Asian, and East African populations Pgm^{100} is found with higher frequencies than in West African and most *A. ae. formosus* populations in East Africa. Bullini & Coluzzi (1972) found very similar results in a survey of 19 populations of *A. aegypti* for variation at the *Pgm* locus. This attests to the stability of the *Pgm* polymorphism over a 6–7 year period.

The average expected heterozygosities (H_e) for each population are shown in Table 7. (*Ao, Idh-1* and *Ldh* were omitted in calculating H_e because these loci were not assayed in all populations.) The results show that the Asian populations are significantly ($P < 0{\cdot}05$) less heterozygous than East and West African sylvan populations and New World domestic populations.

We have used Nei's (1972) statistic to find the genetic distances (D) among all populations. Rather than present the entire data matrix of all 650 pairwise comparisons, we have averaged these D values for within region comparisons and for between region comparisons. The complete data matrix as well as more detailed gene frequency data are available upon request. Table 8 shows the average D

Table 6. *Allele frequencies at 6-phosphogluconate dehydrogenase locus. Abbreviations same as Table 3*

Population	*n*	$6Pgd^{100}$	$6Pgd^{116}$	Others	H_e
DAR	198	0·859	0·116	0·025	0·248
KOM	92	0·793	0·174	0·033	0·340
SHH	86	0·709	0·093	0·198	0·449
KDZ*	214	0·743	0·079	0·178	0·410
NYA	104	0·913	0·067	0·020	0·161
KBW*	226	0·991	0·009	0·0	0·018
MAJ*	154	1·0	0·0	0·0	0
MGN*	212	1·0	0·0	0·0	0
ENUGU	108	0·981	0·019	0·0	0·037
OGUI	68	0·985	0·015	0·0	0·030
UKANA	156	1·0	0·0	0·0	0
MAMUR	28	1·0	0·0	0·0	0
EGEDE	16	1·0	0·0	0·0	0
ABORV	54	1·0	0·0	0·0	0
BOBO	104	0·933	0·0	0·067	0·125
KARI	104	1·0	0·0	0·0	0
BWOM	52	0·885	0·038	0·077	0·209
MOBAY	130	1·0	0·0	0·0	0
MAYA	94	0·797	0·179	0·024	0·332
SANJ	52	0·596	0·404	0·0	0·482
FLOR	108	0·991	0·009	0·0	0·018
NORL*	208	0·930	0·043	0·029	0·136
MEX-PN	108	0·991	0·009	0·0	0·018
VENEZ	86	1·0	0·0	0·0	0
CARAC	108	0·863	0·137	0·0	0·236
GROGOL	52	1·0	0·0	0·0	0
SEMAR	29	1·0	0·0	0·0	0
BANG	106	1·0	0·0	0·0	0
TAI	18	1·0	0·0	0·0	0

values obtained as a result of comparing all populations. The D values in this table represent the average genetic distance of all populations within any geographic region from all populations in a second region. These results agree with our previous findings (Tabachnick, Munstermann & Powell, 1979). The average D values between Mattingly's subspecies are approximately $\frac{1}{5}$ the distance of values obtained for subspecies of *Drosophila* (Ayala, 1975). Using the average D values in Table 8 we performed an unweighted-pair group cluster analysis (Sokal & Sneath, 1973) to produce the dendrogram shown in Fig. 2. This Figure and Table 8 shows that, with the exception of Asia, domestic populations of *A. ae. aegypti* in the New World and in East Africa, form a genetically distinct group, differentiated from other geographic regions, especially *A. ae. formosus* populations. Of particular interest is the placement of Asian *A. ae. aegypti* with a group composed of East and West African *A. ae. formosus*. We will return to this finding because of its implications concerning the method of colonization of *A. aegypti* outside of the African continent.

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Table 7. *Average expected heterozygosities (H_e) based upon 19 genetic loci in populations of* Aedes aegypti

East African *formosus*		East African *aegypti*		West African *formosus*		New World		Asian	
Pop.	$H_e \pm$ S.E.	Pop.	$H_e \pm$ S.E.	Pop.	$H_e \pm$ S.E.	Pop.	$H_e \pm$ S.E.	Pop.	$H_e \pm$ S.E.
DAR	0·134 ± 0·046	KBW*	0·167 ± 0·051	ENUGU	0·134 ± 0·054	MOBAY	0·177 ± 0·052	GROGOL	0·084 ± 0·043
KOM	0·185 ± 0·046	MAJ*	0·109 ± 0·040	OGUI	0·139 ± 0·046	MAYA	0·116 ± 0·040	SEMAR	0·100 ± 0·040
SHH	0·164 ± 0·044	MGN*	0·153 ± 0·052	UKANA	0·139 ± 0·051	SANJ	0·157 ± 0·047	BANG	0·092 ± 0·046
KDZ*	0·156 ± 0·044			MAMUR	0·107 ± 0·049	FLOR	0·100 ± 0·036	TAI	0·067 ± 0·035
NYA	0·136 ± 0·040			EGEDE	0·103 ± 0·049	NORL*	0·122 ± 0·047		
				ABORV	0·169 ± 0·058	MEX-PN	0·108 ± 0·043		
				BOBO	0·119 ± 0·044	VENEZ	0·117 ± 0·042		
				KARI	0·117 ± 0·049	CARAC	0·132 ± 0·039		
				BWOM	0·137 ± 0·049				
H_e	0·155 ± 0·009		0·140 ± 0·016		0·129 ± 0·007		0·129 ± 0·009		0·086 ± 0·007

* Average of collections from the same location.

Table 8. *Average genetic distances (D values) within and between geographic regions of* A. aegypti ± *standard errors. Abbreviations as in Fig. 2*

	EA-O	WA-O	Asia	New World	EA-I
EA-O	0·01474 ± 0·00118 $n = 10$	0·01757 ± 0·00103 $n = 45$	0·01422 ± 0·00109 $n = 20$	0·03265 ± 0·00191 $n = 40$	0·03807 ± 0·00368 $n = 15$
WA-O		0·01100 ± 0·00089 $n = 36$	0·02119 ± 0·00109 $n = 36$	0·04263 ± 0·00185 $n = 72$	0·04895 ± 0·00296 $n = 27$
Asia			0·00926 ± 0·00185 $n = 6$	0·02547 ± 0·00206 $n = 32$	0·02983 ± 0·00418 $n = 12$
New World				0·01489 ± 0·00174 $n = 28$	0·02813 ± 0·00136 $n = 24$
EA-I					0·02056 ± 0·00686 $n = 3$

n, number of pairwise comparisons.

4. DISCUSSION

The pattern of genetic variation among populations of *A. aegypti* throughout the world is consistent with the pattern described previously in coastal Kenya. Tabachnick, Munstermann & Powell (1979) showed that sylvan and domestic *A. aegypti* represent two distinct gene pools existing sympatricly in coastal Kenya. The world wide pattern of genetic variation also shows distinct gene pools. Numerous studies have demonstrated the polytypic nature of *A. aegypti* in East Africa (see Trpis & Hausermann, 1978). The forms of *A. aegypti* (or Mattingly's subspecies) show strong preferences for different habitats (Petersen, 1977) and

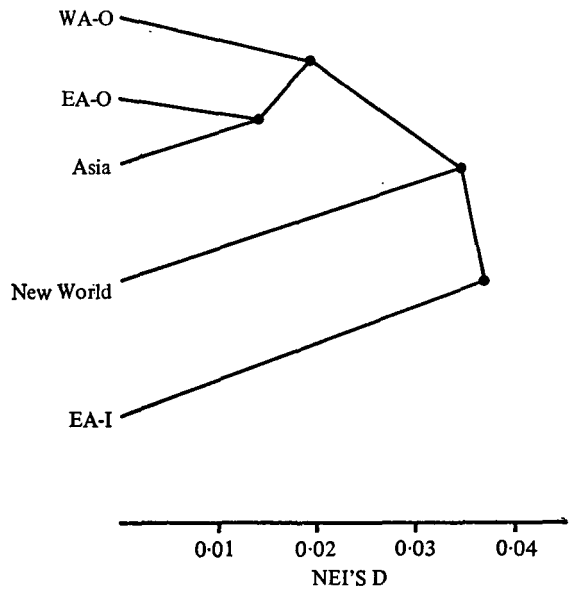

Fig. 2. Dendrogram based on Nei's (1972) genetic distance measure. Each branch point represents the mean genetic distance between the populations of the two joined regions. This figure is based on the 19 loci surveyed in all populations. EA-O, East Africa outdoor (*formosus*); EA-I, East Africa indoor (*aegypti*) form; WA-O, West Africa *formosus*.

exhibit associated behaviours adapted to those habitats (Trpis & Hausermann, 1978; Leahy, VandeHey & Booth, 1978). These behavioural and habitat differences are crucial to interpreting our genetic data. Our results show a strong correlation between electrophoretic variation among populations with similar behavioural, morphological and ecological differentiation, regardless of geographic proximity. For example, domestic *A. ae. aegypti* in East Africa show greater genetic relatedness to domestic populations in the New World than they do to sylvan populations less than a kilometre away. On the other hand, West African *A. ae. formosus* show greater genetic similarity to East African sylvan populations than to all other domestic populations throughout the world.

We believe that the important mechanism which maintains these distinct gene pools, even in areas of sympatry, is the habitat selection of each subspecies. At present there is little evidence in support of pre- or postmating isolating mechanisms. Moore (1979) failed to detect any evidence for such isolating mechanisms in laboratory tests between freshly collected strains of the two subspecies.

It is most probable that the ancestral form of *A. aegypti* was sylvan. Of the 34 African species of the subgenus *Stegomyia* only one (*A. ae. aegypti*) is found occurring outside the Ethiopian region, and it is also the only African mosquito preferring to breed in man's drinking water (Petersen, 1977; Trpis & Hausermann, 1978). The evolution of traits allowing the species to breed in man-made habitats was crucial in its spread throughout the tropical world. Some of the more important behavioural traits which are associated with domesticity are: (1) house entering behaviour (Trpis & Hausermann, 1978), (2) preference for man-made oviposition sites (Leahy, VandeHey & Booth, 1978; Petersen, 1977), and (3) preferences for human blood meals (Gouck, 1972; McKenna, 1973).

The genetic distance values (D) detected between subspecies of *A. aegypti* are small when compared to similar values obtained in species of *Drosophila* (Ayala, 1975). The relatively small distances are expected since the genetic differentiation among populations is the result of allelic frequency differences and not allelic substitutions, No single locus is 'diagnostic' for either subspecies or geographic area. Apparently, in spite of the large morphological and behavioural differences, protein evolution in *A. aegypti* has not been rapid. Nei (1972), Sarich (1977), Carson (1976) and others have argued that there is a more or less direct correlation between the genetic distance among groups and the time since they began diverging. The lack of large genetic differences as determined by electrophoresis between forms of *A. aegypti* may be the result of their having diverged relatively recently. This is consistent with the argument above that association with human habitats was the cause of the divergence.

One model which has been proposed for the evolution of *A. aegypti* agrees remarkably well with our results. Petersen (1977) proposed that domestic *A. ae. aegypti* originated in North Africa. As the Sahara Desert expanded and more of North Africa became arid there was intense selection for behavioural traits adapted to human habitats. Eventually the Sahara Desert became an isolating barrier between the newly evolving domestic form along the north coast of Africa and the ancestral sylvan form south of the Sahara. During more recent times human trade activity introduced domestic *A. ae. aegypti* to urban coastal regions of Africa and the rest of the world.

The introduction of *A. ae. aegypti* into the New World was likely the result of the extensive slave trade between the New World and Africa during the 15th to 19th centuries. Since *A. aegypti* colonised the New World in this manner, one might anticipate the possibility of finding founder effects in some of the New World populations. These were not detected. New World populations were not significantly less heterozygous than East African domestic populations and show very similar allelic frequency distributions. The lack of founder effects in the New

World is probably due to the extent of the trade which occurred during the past 350 years and to the capability of *A. ae. aegypti* to migrate aboard ship.

During the 1960s extensive efforts were made to eradicate *A. aegypti* from the New World (PAHO, 1971). Several areas (Mexico, Brazil, and most of the U.S.) were presumedly free of *A. aegypti*, at least for a while. Since about 1970, eradication efforts have ceased and the species has re-expanded to more or less its previous distribution. Our samples of *A. aegypti* from the recently recolonised areas show no decrease in genetic variation indicative of founder effects. The recolonisation must have occurred by multiple introductions and/or eradication was never really achieved in these areas, resulting in a reservoir of genetic variation in the surviving resident populations.

Table 9. *Average number of alleles ± standard errors found in different habitats throughout the range of* Aedes aegypti

Region	Subspecies	*n*	Average number of alleles ± S.E.
East Africa	*Formosus*	5	2·14 ± 0·01
West Africa	*Formosus*	9	1·71 ± 0·05
East Africa	*Aegypti*	3	1·92 ± 0·07
New World	*Aegypti*	8	1·73 ± 0·05
Asia	*Aegypti*	4	1·46 ± 0·09

n, number of populations.

The introduction of *A. aegypti* into Asia has been a subject of discussion for many years (Dudley, 1933, 1934). There is no historical evidence of yellow fever ever having occurred in Asia and Asians do not show titres of antibodies for yellow fever virus (Strode, 1951). As a result, many epidemiologists have speculated that Asian *A. aegypti* populations are not as efficient vectors as populations outside Asia. Aitken, Downs & Shope (1977) have shown that an Asian strain of *A. aegypti* does not transmit yellow fever as well as a New World domestic or an East African sylvan strain (see also Beaty & Aitkan, 1979). Our results support the idea that Asian *A. aegypti* are genetically differentiated from the other populations. The electrophoretic data show Asian populations to be, on the average, significantly less heterozygous than *A. aegypti* found elsewhere (Table 7) and have a fewer number of alleles per locus (Table 9). In addition, although morphologically and behaviourally domestic, Asian *A. aegypti* populations have *Idh-2* gene frequencies similar to sylvan *A. ae. formosus*. This genetic 'peculiarity' of Asia *A. aegypti* suggests that research into the genetics of the vectorial capacity may well provide an explanation for the absence of yellow fever in this part of the world.

The low heterozygosity of Asian *A. aegypti* may be the result of their recent introduction. Based on the history of dengue fever epidemics in Asia, C. E. G.

Smith (1956) proposed that *A. aegypti* was introduced to Asia during the latter part of the 19th century when dengue epidemics in urban centres first began to occur. Since *A. aegypti* is the principal urban vector of dengue, its introduction at this time presumedly precipitated the epidemics. Our results support this interpretation. Nei, Maruyama & Chakraborty (1975) have shown that for new populations begun by two to ten founders, heterozygosity recovers rather slowly after the initial decrease. A minimum of about 10^5 generations is needed to significantly restore heterozygosity to the level of the original populations. For *A. aegypti* this means a minimum of about 4000 years, Thus the level of genetic variation in Asian populations is consistent with the idea of relatively recent (certainly less than 4000 years ago) introduction of a few founders. Moreover, such considerations as these further support the conclusion of multiple introductions of *A. aegypti* into the New World rather than regeneration of genetic variation after introduction.

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