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Breeding structure of a colonizing species: *Aedes albopictus* (Skuse) in peninsular Malaysia and Borneo

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The mosquito, *Aedes albopictus*, has recently become established in a number of cities throughout the United States. An initial survey of allozyme and genotypic frequencies in U.S. populations (Black *et al.*, 1988) revealed an extensive amount of local differentiation of populations and suggested that much genetic drift may have accompanied colonization. A study of gene flow was initiated in native habitats of *Ae. albopictus* in Malaysia to determine if the result observed in the U.S. was a consequence of colonization or simply followed the natural breeding structure of the species. Allelic and genotypic frequencies were monitored at ten enzymatic loci in 11 populations from peninsular Malaysia and Borneo. Multiple populations were sampled within the districts of Kuala Lumpur and Kuala Trengganu. Peninsular Malaysian and Borneo populations were strongly genetically differentiated. Allele frequencies were significantly different among and within districts in both regions. Variance in allele frequencies among all collections was partitioned into the variance among regions, districts within regions and collections within districts. Almost all of the variance within regions was attributable to local differentiation suggesting that genetic drift is an important component of the natural breeding structure of this species. This indicates that the large amounts of local differentiation found in U.S. populations was not a consequence of recent colonization.

INTRODUCTION

The native habitat of the medically significant mosquito, *Aedes albopictus* (Skuse), extends north from Madagascar throughout the Indomalayan and Oriental regions, China and Japan (Ho *et al.* 1973; Huang, 1972; Knight and Stone, 1977). It became established in Hawaii between 1830 and 1896 (Hardy, 1960; Joyce, 1961) and within the past twenty years has colonized the Solomon and Santa Cruz Islands in the South Pacific (Belkin, 1962; Elliott, 1980; Pashley and Pashley, 1983). During the summer of 1985 the species was discovered throughout Harris County, Texas (Sprenger and Wuithiranyagool, 1986). Since that time the species has become widely distributed in the United States, as far west as San Antonio, Texas, east to Jacksonville, Florida and north as far as Chicago, Illinois and Baltimore, Maryland. Comparison of allozyme frequencies (Black and Rai, unpublished) and diapause data (Hawley *et al.*, 1987) from U.S. populations with populations collected throughout the world range of *Ae. albopictus* suggest that the U.S. populations originated in Japan. Hawley *et al.*, (1987) present data indicating that the mosquito was introduced in commercial tyre shipments.

The population genetics of colonization is an integral component of many models of speciation (Dobzhansky, 1970; Lewontin, 1974; Mayr, 1963; Wright, 1978) and as such the recent introduction of *Ae. albopictus* may provide valuable insights into the types of genetic events which initially accompany colonization. Black *et al.* (1988) examined allozyme frequencies in adults and eggs from a number of U.S. cities during the summer and fall of 1986. In this initial survey we found large amounts of genetic differentiation among collections within the cities of New Orleans, Louisiana and Houston, Texas. The amount of variation within these cities was four times the amount found among cities. This suggested that drift may have initially played an important role in differentiating populations. However, no studies of local gene flow have been made of *Ae. albopictus* populations in its native habitat. It was therefore unknown whether the genetic drift observed in U.S. populations was a consequence of recent

colonization or characteristic of the natural breeding structure of the species.

Recently, the opportunity became available to collect *Ae. albopictus* in Malaysia. The species is thought to have originated in southeast Asia (Smith, 1956). While Malaysia is probably not the source of the U.S. population it nevertheless provided the opportunity to study the breeding structure of the species in native habitats. In this paper we describe gene flow among *Ae. albopictus* populations in native habitats in Malaysia. Collections were made from two districts in peninsular Malaysia and two districts on the island of Borneo. Numerous collections were made within the districts of Kuala Lumpur and Kuala Trengganu in peninsular Malaysia. By analyzing the amount of genetic differentiation within and among districts, we describe patterns of local gene flow. It was hoped that comparison of these results with those obtained in the U.S. would identify the types of genetic changes which accompany colonization.

METHODS

Aedes albopictus eggs were collected using ovitraps during November, 1987. Ovitraps consisted of 400 ml red plastic cups half-filled with tap water containing a 5×12 cm waterlogged balsawood oviposition paddle. In Kuala Lumpur and Kuala Trengganu district, West Malaysia (fig. 1), ovitraps were placed at ground level at sites positive for *Ae. albopictus*. Numerous *Ae. albopictus* males and females were attracted to workers within a few minutes, indicating the presence of a large *Ae. albopictus* population. In Sabah and Sarawak, traps were placed at locations where it was thought likely that *Ae. albopictus* would be present, but adult activity was observed only at Serian, Sarawak. No *Ae. aegypti* was observed at any site. Four or five ovitraps were placed from 5–100 m apart at every site. Ovitraps were left in place 3–4 days before paddles were collected. Paddles were transported to the University of Notre Dame

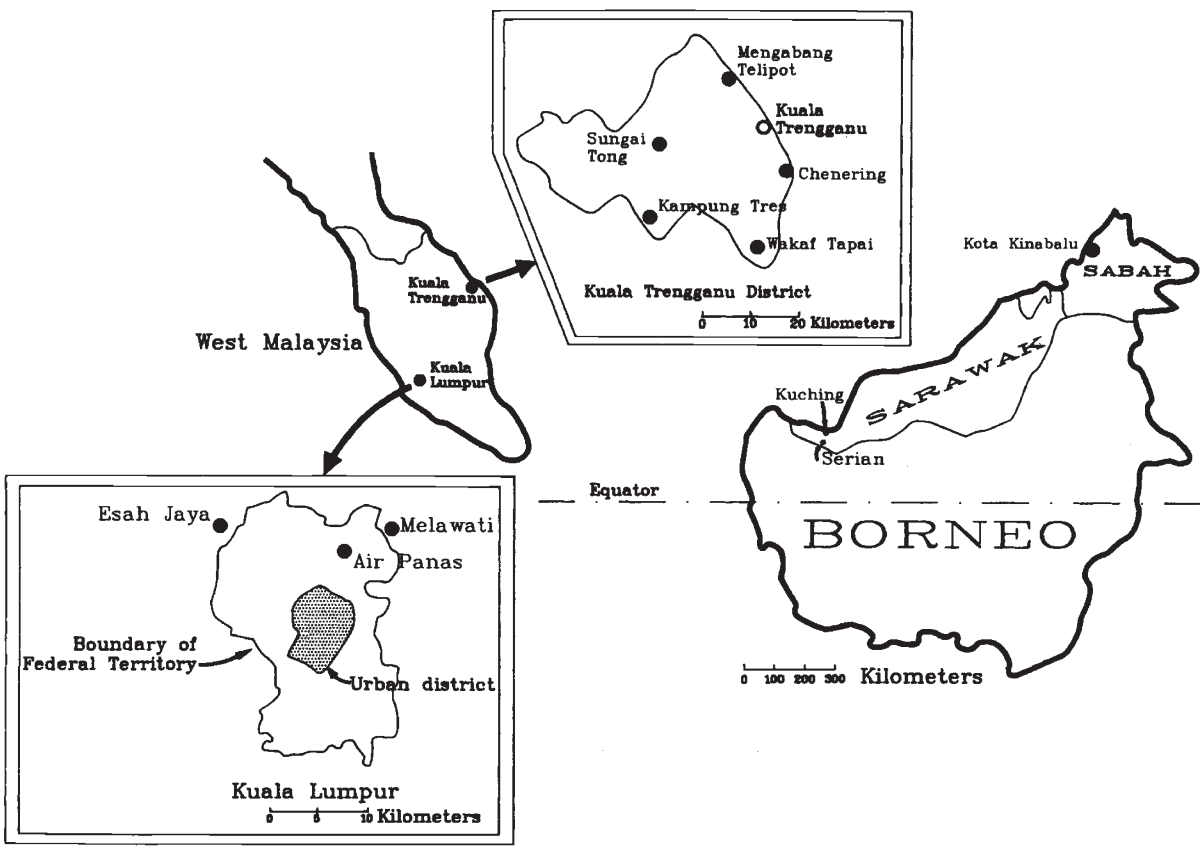

Figure 1 Sites in Kuala Lumpur and Kuala Trengganu in Peninsular Malaysia and Sarawak and Sabah in Borneo at which *Ae. albopictus* was collected for the present study.

for hatching and rearing in mid-December, 1987. Adult mosquitoes from all ovitraps at a collection site were pooled and transferred to a −70°C freezer to await electrophoresis.

Ovitraps in Kuching and Kota Kinabalu were placed in wooded areas adjacent to large hospitals in these cities. In the large village of Serian (population approx. 10,000), traps were placed in undergrowth near human dwellings. Though collections from Kuala Lumpur (population one million) were made within or near its boundary, traps were placed in areas with a large amount of thick vegetation and low human population density. Traps in Air Panas were located in a wooded tract adjacent to an abandoned mining pool. Several dozen wooden houses were located 0·5 km from this site. Traps in Melawati were placed in a roadside strip of thick secondary forest located several hundred meters from a modern shopping complex. Both wooden (kampung style) houses and suburban tract housing were located within 1 km of this site. Ovitraps in Esah Jaya were placed in and near a small illegal dump site adjacent to a small muslim graveyard. This site was located about 100 m from a large expanse of suburban tract housing. Collection sites in Kuala Trengannu district in northeast peninsular Malaysia were rural. Chenering is a coastal roadside site with thick vegetation and a large number of discarded coconut husks (resulting in a large number of biting *Ae. albopictus* and *Armigeres* mosquitoes). Scattered wooden houses were located within several hundred meters of this site. Wakaf Taipei is an extensive, operational rubber plantation. Traps were placed in a narrow wooded strip on the plantation's border. Both the Kampung Tres and Sungai Tong sites were abandoned small scale rubber holdings with very few, scattered human dwellings within several kilometers. Ovitraps in Mengabang Telipot (population 4000) were placed in an approximately 1 hectare wooded area on the outskirts of the village.

Allozymes were resolved at 10 loci using vertical polyacrylamide gel electrophoresis on Hoefer SE600 gel boxes. Methods were derived from those of Munstermann (1979). A litre of 40 × Tris-Citrate buffer was made by dissolving 94·06 g (0·78 M) of Tris into 700 ml of distilled water, titrating to *p*H 7·1 with 1 M citric acid and bringing it to volume with distilled water. This buffer was diluted 1:39 with distilled water to make the electrode buffer and 1:19 for the gel buffer. A litre of 10× Tris-Borate-EDTA buffer was made by dissolving 98·31 g (0·81 M) Tris, 12·50 g (0·20 M) Boric acid, and 5·58 g (15 mM) EDTA (disodium) in enough distilled water to bring the volume to 1 litre. This buffer was diluted 1:9 with distilled water to make the electrode and gel buffers. Polyacrylamide gels (4·75 per cent Monomer, 0·25 per cent Bisacrylamide) were poured with the appropriate buffer, 0·5 per cent Photoflow, 0·15 per cent TEMED and 0·05 per cent Ammonium persulfate. Mosquitoes were ground in 25 μl of grinding buffer (10 per cent Sucrose, 0·01 per cent bromophenol blue, 1 per cent Triton X-100 dissolved in Tris-citrate electrode buffer diluted 1:5). 2·5 μl of sample were loaded per gel. Both systems were run at a constant 350 volts for 3 hours. ACON (aconitase), HAD (hydroxyl acid dehydrogenase), MDH (malic acid dehydrogenase), IDH (isocitrate dehydrogenase) and α-GPDH (α-glycerophosphate dehydrogenase) were resolved with the Tris-citrate system. PGI (phosphoglucoisomerase), 6-PGDH (6-phosphogluconic acid dehydrogenase), PGM (phosphoglucomutase), EST (esterase) and GOT (glutamate oxaloacetate transaminase) were resolved using the Tris-borate-EDTA system. Alleles were scored with reference to the most common allele (rf = 1·00) from the Memphis, Tennessee (U.S.A.) population.

Genestats (Black and Krafsur, 1985*b*) and BIOSYS (Swofford and Selander, 1982) were used to analyze the data. Weir and Cockerham's (1984) χ^2 test for random mating was employed. Wright's (1978) analysis of allele frequencies in subdivided populations was used to partition variance among all samples into nested effects among regions, districts in regions and collections within districts. For this and the contingency χ^2 analysis, collections from Kampung Tres, Sungai Tong and Kuching were dropped because of small sample sizes.

RESULTS

Allozyme frequencies in populations of *Ae. albopictus* from peninsular Malaysia are listed in table 1. The frequencies of allozymes in Borneo populations are listed in table 2. Average expected heterozygosities were similar in all collections. Nei's unbiased genetic distances (1978) were calculated among all collections, among districts and between regions (table 3). Peninsular Malaysian populations were strongly genetically differentiated from the Borneo populations. Most of this differentiation was due to the inverse frequencies of α-Gpdh alleles in the two populations. Removal of this locus from the genetic distance analysis indicated that populations from the two regions were otherwise similar.

Table 1 Allele frequencies and sample sizes (N) at three collections sites in Kuala Lumpur and five sites in Kuala Trengganu in peninsular Malaysia

		Kuala Lumpur			Kuala Trengganu				
Locus	rf	Air Panas	Melawati	Esah Jaya	Telipot	Kampung Tres	Chenering	Wakaf Tapai	Sungai Tong
Pgm	*N*	24	57	21	43	5	20	0	0
	0·80	0·000	0·000	0·048	0·000	0·000	0·000	—	—
	0·86	0·188	0·018	0·143	0·012	0·000	0·000	—	—
	0·93	0·396	0·211	0·429	0·070	0·100	0·075	—	—
	1·00	0·417	0·553	0·357	0·581	0·600	0·625	—	—
	1·07	0·000	0·219	0·024	0·337	0·300	0·300	—	—
Pgi	*N*	25	57	23	44	5	22	15	8
	0·93	0·000	0·000	0·087	0·000	0·000	0·000	0·000	0·188
	1·00	0·840	0·956	0·739	0·864	1·000	0·886	1·000	0·813
	1·06	0·160	0·044	0·174	0·136	0·000	0·114	0·000	0·000
Acon	*N*	25	57	22	44	5	22	15	8
	0·87	0·000	0·026	0·000	0·011	0·000	0·023	0·000	0·000
	0·93	0·000	0·035	0·000	0·102	0·000	0·000	0·200	0·000
	1·00	0·820	0·895	1·000	0·807	0·500	0·932	0·667	1·000
	1·09	0·180	0·044	0·000	0·080	0·500	0·045	0·133	0·000
Est	*N*	24	35	16	35	5	21	14	8
	0·86	0·146	0·200	0·094	0·014	0·100	0·048	0·000	0·000
	0·91	0·146	0·257	0·156	0·114	0·300	0·119	0·464	0·000
	0·95	0·250	0·114	0·219	0·314	0·300	0·595	0·286	0·000
	0·97	0·292	0·371	0·063	0·400	0·300	0·048	0·179	0·000
	1·00	0·021	0·057	0·344	0·029	0·000	0·048	0·036	0·500
	1·03	0·083	0·000	0·125	0·129	0·000	0·119	0·036	0·500
	1·06	0·063	0·000	0·000	0·000	0·000	0·024	0·000	0·000
Had	*N*	25	57	19	43	5	22	14	8
	0·68	0·040	0·000	0·132	0·047	0·000	0·091	0·143	0·000
	0·79	0·400	0·211	0·500	0·000	0·400	0·409	0·393	0·563
	1·00	0·380	0·649	0·263	0·826	0·600	0·409	0·464	0·313
	1·20	0·180	0·140	0·105	0·128	0·000	0·091	0·000	0·125
Mdh	*N*	25	57	22	44	5	22	15	8
	0·63	0·000	0·000	0·000	0·011	0·000	0·000	0·000	0·000
	1·00	1·000	1·000	1·000	0·955	1·000	1·000	1·000	1·000
	1·37	0·000	0·000	0·000	0·034	0·000	0·000	0·000	0·000
Idh	*N*	25	57	22	44	5	22	15	8
	0·89	0·000	0·035	0·182	0·011	0·000	0·000	0·000	0·000
	1·00	1·000	0·965	0·614	0·909	1·000	0·977	1·000	0·750
	1·11	0·000	0·000	0·205	0·080	0·000	0·023	0·000	0·250
α-Gpdh	*N*	25	57	22	44	5	22	15	8
	1·00	0·980	1·000	1·000	0·989	1·000	1·000	1·000	1·000
	1·33	0·020	0·000	0·000	0·011	0·000	0·000	0·000	0·000
Got	*N*	25	57	22	44	5	22	14	8
	0·75	0·000	0·026	0·000	0·000	0·000	0·000	0·000	0·000
	1·00	1·000	0·974	1·000	1·000	1·000	1·000	1·000	1·000
	1·32	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000
6-Pgdh	*N*	24	57	19	42	5	22	14	8
	0·46	0·000	0·000	0·000	0·036	0·000	0·000	0·000	0·000
	0·71	0·104	0·061	0·000	0·048	0·000	0·000	0·036	0·313
	1·00	0·854	0·825	1·000	0·917	1·000	1·000	0·964	0·688
	1·21	0·000	0·114	0·000	0·000	0·000	0·000	0·000	0·000
	1·42	0·042	0·000	0·000	0·000	0·000	0·000	0·000	0·000
	2·04	0·000	0·000	0·000	0·000	0·000	0·000	0·000	0·000
	Het.	0·302	0·258	0·314	0·259	0·249	0·220	0·247	0·286
	(*SE*)*	(0·098)	(0·087)	(0·109)	(0·073)	(0·104)	(0·087)	(0·097)	(0·082)

* The average expected genic heterozygosity and standard error among the 10 loci.

Table 2 Allele frequencies and sample sizes (N) at three collection sites in Borneo

Locus	rf	Kota Kinabalu	Kuching	Serian
Pgm	*N*	17	7	32
	0·80	0·000	0·000	0·000
	0·86	0·118	0·000	0·000
	0·93	0·353	0·000	0·266
	1·00	0·500	0·286	0·672
	1·07	0·029	0·714	0·063
Pgi	*N*	17	7	35
	0·93	0·235	0·071	0·200
	1·00	0·765	0·929	0·643
	1·06	0·000	0·000	0·157
Acon	*N*	16	7	34
	0·87	0·000	0·000	0·000
	0·93	0·000	0·143	0·000
	1·00	0·750	0·714	0·853
	1·09	0·250	0·143	0·147
Est	*N*	16	6	32
	0·86	0·000	0·000	0·000
	0·91	0·000	0·167	0·203
	0·95	0·000	0·000	0·297
	0·97	0·563	0·083	0·188
	1·00	0·219	0·250	0·063
	1·03	0·125	0·000	0·250
	1·06	0·094	0·500	0·000
Had	*N*	17	7	35
	0·68	0·000	0·000	0·029
	0·79	0·000	0·000	0·014
	1·00	1·000	1·000	0·929
	1·20	0·000	0·000	0·029
Mdh	*N*	17	7	34
	0·63	0·000	0·000	0·059
	1·00	1·000	1·000	0·941
	1·37	0·000	0·000	0·000
Idh	*N*	17	7	35
	0·89	0·206	0·286	0·014
	1·00	0·794	0·714	0·986
	1·11	0·000	0·000	0·000
α-Gpdh	*N*	17	7	35
	1·00	0·059	0·429	0·114
	1·33	0·941	0·571	0·886
Got	*N*	17	7	35
	0·75	0·000	0·000	0·000
	1·00	0·794	1·000	1·000
	1·32	0·206	0·000	0·000
6-Pgdh	*N*	17	7	35
	0·46	0·000	0·000	0·000
	0·71	0·000	0·214	0·029
	1·00	1·000	0·786	0·914
	1·21	0·000	0·000	0·043
	1·42	0·000	0·000	0·000
	2·04	0·000	0·000	0·014
	Het.	0·281	0·311	0·269
	(*SE*)*	(0·077)	(0·081)	(0·269)

* The average expected genic heterozygosity and standard error among the 10 loci.

Pairwise contingency χ^2 tests indicated that allele frequencies were significantly different among all districts (analyses not shown). Table 4 lists the results of contingency χ^2 analysis to test for homogeneity of allele frequencies among collections within Kuala Lumpur and Kuala Trengganu. Populations within both districts were significantly differentiated.

The variance in allele frequencies among all collections was partitioned using a nested random effects model following Wright (1978). Collections were nested within districts and districts were nested within regions. Table 5 shows that, with all loci included, 52 per cent of the total variance among all collections was attributable to differences among regions. But with the α-Gpdh locus removed, the variance among regions dropped to 2 per cent of the total. In a third analysis, esterase was removed because in a locus by locus analysis (data not shown) it was determined that variation at this locus contributed most to the local variance. Removal of this locus had no effect on the amount of variance attributable to local differentiation. In all analyses, the amount of variance among collections within a district was consistently 4–5 times as large as the amount of variance among districts.

Observed genotypic frequencies were compared with those expected under random mating (Hardy-Weinberg expectations). We found 8 of 71 tests to show significant deviations from expectations. This was not significantly greater than expected with a type I error rate of 5 per cent ($0{\cdot}05 \times 71 = 3{\cdot}55$ significant observations, $\chi^2[1\ \mathrm{df}] = 3{\cdot}36$, $P \geqq 0{\cdot}05$). Significant results were homogeneously distributed among collections. Four of the significant results were observed at the *Est* locus, 3 at the *Had* locus and 1 at the *Pgm* locus. No consistent trend towards heterozygote excess or deficiency was noted among significant results. In general, mosquitoes appeared to mate at random within populations.

DISCUSSION

The large genetic distance observed between peninsular Malaysia and Borneo populations was chiefly attributable to differentiation at the *α-Gpdh* locus. A survey of allozyme frequencies among populations throughout the world range of *Ae. albopictus* (Black and Rai, unpublished) indicates that this variant is unique to Borneo. This suggests either that very little migration exists between populations in the two regions or that selection acts to maintain alleles in almost inverse frequen-

Table 3 Nei's unbiased genetic distances among collections, districts and regions in Malaysia

	All collections									
	1	2	3	4	5	6	7	8	9	10
Peninsular Malaysia										
Kuala Lumpur										
1. Air Panas										
2. Melawati	0·019									
3. Esah Jaya	0·030	0·059								
Kuala Trengganu										
4. Telipot	0·045	0·016	0·088							
5. Kampung Tres	0·024	0·019	0·081	0·025						
6. Chenering	0·028	0·034	0·044	0·034	0·025					
7. Wakaf Taipai	0·030	0·021	0·064	0·032	0·000	0·018				
8. Sungai Tong	0·072	0·074	0·044	0·093	0·098	0·060	0·078			
Borneo										
Sabah										
9. Kota Kinabalu	0·208	0·185	0·254	0·173	0·199	0·251	0·234	0·285		
Sarawak										
10. Kuching	0·177	0·118	0·203	0·101	0·126	0·159	0·132	0·176	0·099	
11. Serian	0·164	0·149	0·212	0·130	0·159	0·163	0·169	0·224	0·035	0·103

	Districts		
	1	2	3
1. Kuala Lumpur	0·036		
2. Kuala Trengganu	0·041	0·028	
3. Sabah	0·215	0·219	—
4. Sarawak	0·175	0·154	0·035

All loci	Regions	
	1	2
1. Peninsular Malaysia	0·037	
2. Borneo	0·191	0·035
α-Gpdh removed		
	1	2
1. Peninsular Malaysia	0·043	
2. Borneo	0·072	0·039

Table 4 Contingency χ^2 test for homogeneity of allele frequencies among collections in Kuala Lumpur and Kuala Trengganu in peninsular Malaysia

	Kuala Lumpur		Kuala Trengganu	
Locus	df	χ^2	df	χ^2
Pgm	8	48·69***	6	1·78
Pgi	4	24·04***	2	4·48
Acon	6	19·95**	6	11·93
Est	12	50·35***	12	41·08***
Had	6	34·99***	6	50·63***
Idh	4	54·29***	4	4·84
Gpdh	2	3·18	2	0·85
Got	2	2·51	—	—
Mdh	—	—	4	3·45
Pgdh	6	21·01***	4	—
Total	50	259·01***	46	123·88***

** $P \leqq 0{\cdot}01$.
*** $P \leqq 0{\cdot}001$.

cies. Given the large genetic distances among these two relatively close regions, it is likely that a great deal more differentiation exists among islands throughout Indonesia and east through the Solomon Islands. This highlights the need for more extensive examination of breeding structure throughout these regions.

The amount of variation in allele frequencies among collection sites within districts was 4–5 times as large as the variation among districts. The same phenomenon was observed among local populations of *Ae. albopictus* in the United States (Black *et al.*, 1988). We attributed this variation in local U.S. populations to genetic drift associated with restricted gene flow among newly established populations.

In the current study there are three possible explanations for the observed local differentiation. It is possible that we collected offspring represent-

Table 5 Nested analysis of variance of allele frequencies among collection sites in peninsular Malaysia and Borneo. Collections are nested within districts and districts are nested within regions

	All loci		*α-Gpdh* removed		*Est* removed	
Source	Variance	% Total	Variance	% Total	Variance	% Total
Regions	0·316	52·2%	0·007	2·4%	0·335	63·4%
District (Regions)	0·055	9·1%	0·054	18·3%	0·032	6·1%
Collections (District (Regions))	0·234	38·7%	0·234	79·3%	0·161	30·5%
All collections	0·605		0·295		0·528	

ing few families such that the observed differentiation is a consequence of sampling. We consider this unlikely because multiple ovitraps were collected at each collection site, high densities of *Ae. albopictus* existed at each site, and *Ae. albopictus* does not oviposit whole egg batches in a single container (Rozeboom *et al.*, 1971). However, in principle, little migration is required to make local populations panmictic within a district and, as such, either selection or genetic drift must be acting to create this local differentiation.

Under a model of selection, populations in districts might be panmictic but local selection pressures act to differentiate subpopulations. We consider this model unlikely because there was less variance among districts than among collections within districts. For selection to create this result there would have to be a similar range of microhabitats in both districts and these habitats would have to be sampled equally. A more parsimonious explanation is that much genetic drift accompanies the establishment of local populations. Under this model, local subpopulations are founded by few parents and the effective migration rate is sufficiently restricted to maintain local differentiation. Studies on dispersal of laboratory reared *Ae. albopictus* in the field in both Hawaii (Bonnet and Worcester, 1946) and Japan (Mori, 1979) indicate that individuals may migrate a maximum of 200 metres in their lifetime. However, no studies have been done on wild *Ae. albopictus*, nor have any such investigations been performed in southeast Asia. It is therefore difficult to judge the likelihood that the observed local differentiation is maintained by limited migration.

These results indicate, contrary to original predictions (Black *et al.*, 1988), that the amount of differentiation observed among local populations of *Ae. albopictus* in the United States is not a consequence of recent colonization but rather an attribute of the natural breeding structure of the species. However, the similarity of breeding structures in Malaysia and the U.S. is unexpected given the marked differences in the distribution and quality of breeding resources in the two regions. Tyres and other discarded containers constitute the majority of oviposition sites for *Ae. albopictus* in the U.S. The distribution of these varies a great deal from city to city, but in general these constitute sparse oviposition resources. It is easy to envisage genetic drift developing under these circumstances. A few gravid females may locate and oviposit in a group of discarded tyres. The offspring, failing to locate other oviposition sites, oviposit in the same tires. In contrast, Malaysian *Ae. albopictus* breed in a large variety of commonly found containers. Any artificial container, coconut husk, bamboo stump, or treehole is quite likely to contain *Ae. albopictus* (Ho *et al.*, 1973). Thus, the distribution of oviposition sites in Malaysia is considerably more continuous than that in the U.S., making this scenario much less plausible. Our results emphasize the need for a more extensive study of breeding structure coupled with ecological and behavioral investigations to identify local barriers to gene flow in this and other native habitats of *Ae. albopictus*.

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