VARIATION IN THE VECTOR COMPETENCE OF GEOGRAPHIC STRAINS OF *AEDES ALBOPICTUS* FOR DENGUE 1 VIRUS[1]

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ABSTRACT. Eight geographic strains of *Aedes albopictus* from Asia and North America and one North American strain of *Aedes aegypti* were tested for their vector competence with dengue 1 virus. Three groups of *Ae. albopictus* were established based on their vector competence: a) the OAHU laboratory strain, b) the three Malaysian strains, and c) the TOKYO and three North American strains. The three North American strains were similar to the strain of *Ae. aegypti* from Houston, Texas in their ability to transmit dengue 1 virus. A comparison of barriers to infection and transmission suggests that *Ae. albopictus* HOUSTON represents an introduced strain distinct from the more similar MEMPHIS and NEW ORLEANS strains. Based on these studies the North American strains were seen as more similar to a northern Asian strain (TOKYO) than to the three Malaysian (southern Asia) strains, supporting the current hypothesis that the indigenous strains of *Ae. albopictus* recently introduced into the United States had a northern Asian origin.

INTRODUCTION

Historically dengue and dengue hemorrhagic fevers have probably been responsible for more human morbidity and mortality than any other arthropod-borne virus in tropical regions. *Aedes aegypti* (L.) has generally been considered to be the primary urban vector of the four dengue serotypes throughout the tropics. A second vector, *Aedes albopictus* (Skuse), has been responsible for dengue and dengue hemorrhagic fever epidemics in many Far Eastern and Pacific regions including Hawaii (Usinger 1944), Japan (Sabin 1952), Indonesia (Jumali et al. 1979), the Seychelles (Metselar et al. 1980), southern China (Qiu et al. 1981), Thailand (Gould et al. 1968), and Singapore (Chan et al. 1971).

Dengue and dengue hemorrhagic fevers pose serious public health problems in the Americas as well. Transmission of dengue viruses has remained at a relatively high level in recent years with epidemics of all four serotypes occurring in various parts of the Caribbean, Central, and South America. Recent indigenous transmission in Texas (Kappus et al. 1980), has also been reported. In Puerto Rico, dengue has persisted as an endemic disease and epidemics have occurred repeatedly since 1963 [Pan American Health Organization (P.A.H.O.) 1981]. The importance of dengue as a serious health problem

in the Western Hemisphere was underscored in 1981 with an epidemic in Cuba that caused hemorrhagic cases, with more than 116,000 hospitalized cases and 158 deaths reported (P.A.H.O. 1982). The total number of cases reported in the Americas in 1985 (68,998), with major epidemics in Mexico and Nicaragua (San Juan Laboratories 1985), more than doubled the 31,337 cases reported in 1984 and the 25,215 reported in 1983.

In August 1985, relatively large populations of *Ae. albopictus* were found throughout Harris County (Houston), Texas (Sprenger and Wuithiranyagool 1986). By the summer of 1986, this species was found to be widely distributed in 12 states from as far west as Laredo, Texas, east to Alabama, Florida, Georgia, Mississippi, and north throughout the Midwest including Indiana, Missouri, Ohio and Tennessee (Moore 1986, Rai 1986).

The introduction and establishment of *Ae. albopictus* in the United States has potentially serious implications for future dengue transmission. While transmission of dengue viruses in the Americas continues to be attributed to *Ae. aegypti*, the recent introduction of *Ae. albopictus* may alter the ecology of dengue by increasing the chances of these viruses becoming established in suburban and rural areas as well as their being transmitted by a vector other than *Ae. aegypti*. Rosen et al. (1985) demonstrated that Asian *Ae. albopictus* strains were more susceptible than *Ae. aegypti* to oral infection with each of the four dengue serotypes. They also noted that the susceptibility of strains of *Ae. albopictus* varied with different geographic strains of the virus serotypes. Marked variation among geographic strains of *Ae. albopictus* and *Ae. aegypti* for oral susceptibility to dengue virus infection was found in other studies (Gubler and Rosen 1976, Gubler et al. 1979, Rosen et al. 1985). The importance of intrinsic barriers to infection and transmission (Hardy et al. 1983) must be considered in vector competence studies

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particularly in the evaluation of the role a particular population may play in virus transmission in nature. To date however, no studies have evaluated these intrinsic barriers as they relate to dengue transmission.

We report here the variation in vector competence for dengue 1 virus by geographically diverse strains of *Ae. albopictus*, assess the vector potential for dengue virus transmission of some of the strains recently established in the United States, and compare the same with a strain of *Ae. aegypti* native to Houston, Texas.

MATERIALS AND METHODS

Mosquito Strains: Eight geographic strains of *Ae. albopictus* and one strain of *Ae. aegypti* were included in this study (Table 1). The mosquitoes were reared in enamel basins containing 1 liter of tap water with a density of 150 larvae per basin. The larvae were fed a standardized liver powder (National Biochemicals, Cleveland, OH) diet (Munstermann and Wasmuth 1985) and held in an insectary with a 16:8 hr light:dark photoperiod, 80% relative humidity, at 28°C to insure uniform size and physiological development. Adult mosquitoes were kept in one gallon plastic cages provisioned with honey-soaked cotton pads. The *Ae. albopictus* OAHU strain was chosen as our control since this strain has been used in earlier dengue studies by other workers (Gubler and Rosen 1976, Rosen and Gubler 1974, Rosen et al. 1985) and since this is the most inbred, hence most uniform, strain.

Virus Strain: An initial stock of Dengue 1 virus isolated from human serum in 1975 collected in Fiji was obtained from the reference bank at the Yale Arbovirus Research Unit (YARU; YARU 40130, passaged once in C6/36 *Ae. albopictus* cell line and maintained in mosquitoes). We used this single virus strain throughout the study and chose it for our initial trials since other investigators had demonstrated oral infection and transovarial transmission in mosquitoes with this strain (Rosen et al. 1983). A high-titered working stock of the virus was prepared by intrathoracic inoculation (Rosen and Gubler 1974) of *Ae. albopictus* OAHU with 0.34 μl (per mosquito) of the YARU stock; inoculated mosquitoes were incubated for 7 days at 28°C. Approximately 70 mosquitoes were chilled on ice, homogenized in a Ten Broeck grinder with 2 ml of *virus* diluent (PBS—pH of 7.2, 0.5% gelatin, 30% fetal bovine serum), centrifuged at 15,000 $\times$ g for 5 minutes at 4°C, and the supernatant stored in 0.1 ml aliquots at $-70°C$ until used for infectious bloodfeeding studies.

Infectious Bloodfeeding: To minimize age-related differences in transmission rates, all mosquitoes were infected approximately 1 week after emergence. An infectious bloodmeal was made using one part of fresh (homogenized alive) centrifuged mosquito supernatant obtained from *Ae. albopictus* OAHU inoculated 5 days earlier with 0.34 μl of a 10^{-5} dilution of the high-titered working stock virus to nine parts of defibrinated rabbit blood (Colorado Serum Co., Denver, CO).

Table 1. Origin and history of the geographic strains of *Aedes albopictus* and *Aedes aegypti* tested for dengue vector competence.

Species and strain	Geographic source (provider)	Year of colonization	Number of generations in the laboratory	Recent dengue history
Aedes albopictus				
OAHU	USA, Hawaii, Oahu Island (L. Rosen)	1971	100+	non-endemic
GERTAK	Malaysia, Penang Island, Gertak Sanggul (H. Yap)	1985	5	endemic
MINDEN	Malaysia, Penang Island, Minden Heights (H. Yap)	1985	5	endemic
PERTAK ROAD	Malaysia, Penang Island, Perak Road (H. Yap)	1985	5	epidemic
TOKYO	Tokyo, Japan (Y. Eshita)	1979	<20	non-endemic
HOUSTON	Harris County, Houston, Texas (D. Sprenger)	1986	4	non-endemic
MEMPHIS	Memphis, Tennessee (W. Black IV and J. Ferrari)	1986	3	non-endemic
NEW ORLEANS	New Orleans, Louisiana (N.O. Mosq. Control Dist.)	1986	3	non-endemic
Aedes aegypti				
HOUSTON	Harris County, Houston, Texas (D. Sprenger)	1986	2	non-endemic

Infectious bloodmeals were provided for 30 min in a membrane feeding apparatus covered with a Badruche membrane as previously described (Rutledge et al. 1964). An aliquot of the infectious bloodmeal was frozen at $-70°C$ at the beginning of each bloodfeeding and subsequently titered in intrathoracically inoculated *Ae. albopictus* OAHU. The titer of the infectious bloodmeal was expressed as the median mosquito infectious dose $(MID_{50})/ml$ and calculated by the method of Reed and Muench (1938). Only fully engorged mosquitoes were saved and transferred to one gallon plastic cages and held in the insectary under the conditions described above for a 14-day incubation period.

In vitro *Salivation transmission*. Since there is no suitable vertebrate host with which to demonstrate transmission of dengue viruses, alternate methods must be employed. Capillary tubes partially filled with various fluids into which the mosquito's mouthparts are inserted have been used by others (Aitken 1977, Beaty and Aitkin 1979, Hurlbut 1966). In a preliminary study we compared the rate of transmission of dengue 1 virus by two strains of *Ae. albopictus*, OAHU and TOKYO, using fetal bovine serum and sucrose, or mineral oil as virus diluents in the capillary tubes (10% fetal bovine serum in 10% sucrose; mineral oil—E. R. Squibb & Sons, Inc., Princeton, NJ). We also titered each *capillary* diluent/virus mixture to determine if either might reduce the infectivity of dengue 1 virus in this assay system compared to a virus diluent/virus control. Mineral oil was subsequently chosen as the capillary diluent for all trials reported here.

In all experimental trials after the 14-day incubation, mosquitoes were chilled on ice and the first two pairs of legs and wings were removed. To demonstrate transmission, the proboscis was inserted into the end of a mineral oil-charged capillary tube filled with enough mineral oil to completely surround the mouthparts (Beaty and Aitken 1979, Hurlbut 1966). The capillary tube with the dangling mosquito was transferred to a holding rack and the mosquitoes were allowed to salivate over a 45 min period. The capillary tubes were examined for evidence of salivation before individual mosquitoes were removed from the tubes. Each mosquito was placed into a separate labeled vial and frozen at $-70°C$ and assayed later for the presence of dengue virus by indirect immunofluorescence (Kuberski and Rosen 1977). The capillary tube was snapped off at the mineral oil meniscus and triturated with a glass rod in a 1.5 ml conical polypropylene centrifuge tube (EPPENDORF®, Brinkmann Inc., Westbury, NY) containing 25 μl of diluent and then frozen at $-70°C$ for later assay.

Mosquito assay: Disseminated infection (infection beyond the midgut) was determined by indirect immunofluorescent head squash as previously described (Kuberski and Rosen 1977). Dengue 1 virus-specific mouse hyperimmune ascitic fluid obtained from Dr. D. J. Gubler and an FITC goat anti-mouse conjugate (Antibodies Incorporated, Davis, CA) were used for all head squash assays. For those individual mosquitoes whose head squash was negative, the remaining body was triturated in 50 μl of diluent and inoculated intrathoracically into 10 *Ae. albopictus* OAHU. Following a 10-day incubation at 28°C, the inoculated mosquitoes were killed by freezing and the heads of five individual mosquitoes were squashed and examined under a Zeiss epifluorescent microscope. In this manner it was possible to determine which mosquitoes were infected only at the midgut level and which had disseminated infections of those allowed to salivate (refeed) into the capillary tubes.

In vitro virus transmission was demonstrated by inoculating the supernatant of the triturated mineral oil-charged capillary tube into 10 *Ae. albopictus* OAHU, and after a 10-day incubation at 28°C head squashes were again assayed by immunofluorescence.

Statistical Analysis: A Chi-square contingency table analysis (with 1 d.f. and a correction for continuity; Snedecor and Cochran 1980) was used to determine if there were significant differences between strains in rates of infection and transmission, and in the proportion with barriers to infection and transmission.

RESULTS

In the preliminary study, when fed essentially identical doses of virus in each of the two capillary diluents (8.8 $\log_{10}$ MID_{50}/ml in mineral oil vs. 8.7 $\log_{10}$ MID_{50}/ml in fetal bovine serum/sucrose), both the OAHU and TOKYO strain females showed slightly higher but not significant $(P > 0.75)$ rates of transmission (Table 2) using the mineral oil diluent as the recipient of

Table 2. Transmission of dengue 1 (Fiji strain) virus by two strains of *Aedes albopictus* using mineral oil or sucrose/fetal bovine serum as diluents.

| Mosquito strain | Rate of transmission in each diluent | |
	Mineral oil	sucrose/fetal bovine serum
OAHU	43% (13/30)[1]	39% (7/18)
TOKYO	14% (2/14)	12% (3/25)

[1] Percent transmission = (number transmitting/ number initially infected and refed on each diluent).

infectious saliva. There was no significant difference in virus titer ($P > 0.90$) between either of the two capillary tube diluents and the virus diluent control. With a dissecting microscope, saliva could also be seen in the capillary tubes containing mineral oil. The mineral oil's viscosity greatly facilitated the retention of each mosquito's mouthparts in the capillary tube during the 45 min salivation (transmission) period. Thus we chose to use mineral oil in all experimental trials.

The results of all refeeding trials are summarized in Table 3. All infectious bloodmeals titered between 8.1 and 8.5 $\log_{10}$ MID_{50}/ml. Given that virus titers of infectious bloodmeals fed mosquitoes via artificial feeders are generally 10–1,000 times less efficient in infecting these insects compared to viremic vertebrate hosts (DeFoliart et al. 1987), and adjusting for that reduced efficiency, all our infectious bloodmeals (Table 3) fell within the range of titer (3.8–8.0 $\log_{10}$ MID_{50}/ml) seen in natural human viremia (Gubler et al. 1981). Since there were no significant differences observed among replicates for any strains, data were combined and presented as a mean rate (Table 3); the GERTAK, MEMPHIS and NEW ORLEANS strains of Ae.

albopictus and the HOUSTON strain of Ae. aegypti were tested in one trial each.

Midgut Infections: All strains were relatively susceptible to oral infection with Dengue 1 FIJI virus. The HOUSTON strain was significantly lower in susceptibility at the midgut level than the OAHU, MINDEN, MEMPHIS ($P < 0.01$), PERAK and NEW ORLEANS ($P < 0.05$) strains of Ae. albopictus. The GERTAK and TOKYO strains of Ae. albopictus were not significantly different from any of the other strains or the HOUSTON strain of Ae. aegypti. The Ae. aegypti HOUSTON strain was also significantly lower in midgut infection than the PERAK ($P < 0.05$), OAHU, MINDEN, MEMPHIS and NEW ORLEANS ($P < 0.01$) strains of Ae. albopictus (Table 3).

Disseminated Infections: The eight strains of Ae. albopictus fell into three groups with respect to disseminated infection: a) OAHU, b) the Malaysian strains, and c) the North American and TOKYO strains. The disseminated infection rate of the OAHU strain was significantly higher than that of the GERTAK, PERAK ($P < 0.05$), TOKYO, HOUSTON, MEMPHIS and NEW ORLEANS ($P < 0.01$) strains of Ae. albopictus. The HOUSTON strain of Ae. albopictus was

Table 3. Rates of infection, oral transmission, and proportion of *Aedes albopictus* and *Aedes aegypti* with barriers to infection and transmission of dengue 1 (FIJI) virus.

Species and strain	N(R)*	Titer**	Percent infection		Percent transmission	
			MGI	DI	Modified	Population
Aedes albopictus						
OAHU	49 (2)	8.3	100	94	43	41
			(49/49)	(46/49)	(20/46)	(20/49)
Malaysia:						
GERTAK	28 (1)	8.1	89	71	45	32
			(25/28)	(20/28)	(9/20)	(9/28)
PERAK	31 (2)	8.3	97	74	43	32
			(30/31)	(23/31)	(10/23)	(10/31)
MINDEN	29 (2)	8.2	100	83	21	17
			(29/29)	(24/29)	(5/24)	(5/29)
Northern Asia:						
TOKYO	32 (3)	8.4	94	53	29	16
			(30/32)	(17/32)	(5/17)	(5/32)
North America:						
HOUSTON	21 (2)	8.2	71	38	38	14
			(15/21)	(8/21)	(3/8)	(3/21)
MEMPHIS	29 (1)	8.5	100	52	20	10
			(29/29)	(15/29)	(3/15)	(3/29)
NEW ORLEANS	27 (1)	8.3	100	59	6	4
			(27/27)	(16/27)	(1/16)	(1/27)
Aedes aegypti						
HOUSTON	20 (1)	8.3	70	30	17	5
			(14/20)	(6/20)	(1/6)	(1/20)

  * Number tested (number of replicates); data from replicates pooled since there were no significant differences observed when tested by Chi-square.

  ** Median mosquito infectious dose (MID_{50})/ml inoculated into Ae. albopictus OAHU strain mosquitoes and assayed by indirect immunofluorescence of head squashes after a 10-day incubation period.

  Abbreviations: MGI = midgut infection; DI = disseminated infection.

significantly lower in disseminated infection not only from the OAHU strain but also from the Malaysian GERTAK, PERAK and MINDEN strains ($P < 0.05$ for all comparisons). The MINDEN strain had a significantly higher disseminated infection rate than the TOKYO, MEMPHIS ($P < 0.05$) and HOUSTON ($P < 0.01$) strains but not from the OAHU ($0.25 > P > 0.1$) strain. However, the GERTAK and PERAK strains were not significantly different in disseminated infection than TOKYO and any of the North American strains with the exception of HOUSTON ($P < 0.05$). The TOKYO and MEMPHIS strains had a significantly lower disseminated infection rate than the OAHU ($P < 0.01$) and MINDEN ($P < 0.05$). However, the NEW ORLEANS strain was significantly lower in rate of disseminated infection only when compared to the OAHU strain ($P < 0.01$). The HOUSTON strain of *Ae. aegypti* was significantly lower in disseminated infection rate compared to the GERTAK ($P < 0.05$), OAHU, PERAK and MINDEN ($P < 0.01$) strains of *Ae. albopictus* (Table 3).

In this study, only the HOUSTON strains of *Ae. albopictus* and *Ae. aegypti* showed evidence of a significant midgut infection barrier to dengue 1 FIJI virus (Fig. 1). There were no significant differences among the Malaysian ($P > 0.05$) and among the North American strains ($P > 0.05$) of *Ae. albopictus* in the rate of disseminated infection, reflecting the relative presence of a midgut escape barrier in the respective populations (Fig. 1). Significant differences in prevalence of midgut escape barriers only occurred when members of the Malaysian strains or OAHU were compared with members of North American strains ($P < 0.05$). The *Ae. albopictus* TOKYO and the *Ae. aegypti* HOUSTON strains were comparable to members of the North American *Ae. albopictus* strains with respect to disseminated infection. The TOKYO and the three North American strains had a significant midgut escape barrier when compared to the OAHU strain ($P < 0.01$), while the MEMPHIS strain had a significant midgut escape barrier when compared to the MINDEN and GERTAK strains ($P < 0.05$). A salivary gland barrier (Hardy et al. 1983) was evident in those mosquitoes with a disseminated infection that did not orally transmit dengue 1 virus. In our studies the NEW ORLEANS strain had a significantly higher salivary gland barrier (Fig. 1) than did the OAHU, GERTAK or PERAK ($P < 0.05$) strains, perhaps accounting for the low rate of oral transmission seen with the former strain (Table 3). Thus, our data show that salivary gland barrier(s) are a major factor in limiting transmission and their pattern of oc-

currence is characteristic of the geographic strain used.

Modified and Population Transmission Rates: The *modified* transmission rates (MT) (percentage of mosquitoes with a disseminated infection that transmitted) and the *population* transmission rates (PT) (percentage of all refed mosquitoes—whether initially infected by the virus/bloodmeal or not—that transmitted) were calculated for these experiments (Table 3). Only the NEW ORLEANS *Ae. albopictus* had a significantly lower MT rate than the OAHU, GERTAK and PERAK strains ($P < 0.05$). There were no other significant differences between strains of *Ae. albopictus* or with the *Ae. aegypti* HOUSTON strains. All strains of *Ae. albopictus*, except NEW ORLEANS, transmitted dengue 1 FIJI at moderately high rates once the virus had disseminated beyond the midgut. In summary, the MT rate of the NEW ORLEANS strain was significantly lower than the OAHU, GERTAK and PERAK strains, however, NEW ORLEANS was not significantly different in MT from the other North American strains or from TOKYO.

As with the MT rate, the PT rates for OAHU, GERTAK and PERAK strains were significantly higher than that of the NEW ORLEANS *Ae. albopictus* (Table 3). The OAHU strain's PT rate was also significantly higher than that of the MEMPHIS, TOKYO, and NEW ORLEANS strains. However, the Malaysian strains were not significantly different from each other with respect to PT rates. Significant differences occurred when OAHU or the Malaysian strains were compared with TOKYO and the North American MEMPHIS and NEW ORLEANS strains. Finally, the PT rate of the HOUSTON *Ae. aegypti* was significantly lower than the OAHU ($P < 0.01$) and PERAK ($P < 0.05$) strains of *Ae. albopictus*.

DISCUSSION

The eight geographic strains of *Ae. albopictus* showed marked variation in susceptibility to oral infection with dengue 1 (FIJI) virus. Earlier work had also demonstrated marked variation in susceptibility to oral infection of *Ae. albopictus* strains from Asia to dengue viruses and that work suggested that the barrier to infection was at the midgut level (Gubler and Rosen 1976). The importance of midgut and salivary gland barriers in numerous vector-virus relationships has been postulated (Hardy et al. 1983, Grimstad et al. 1985). While midgut barriers were of importance in our study, a salivary gland barrier was also present. We did not determine if this was a salivary gland infection or a salivary gland
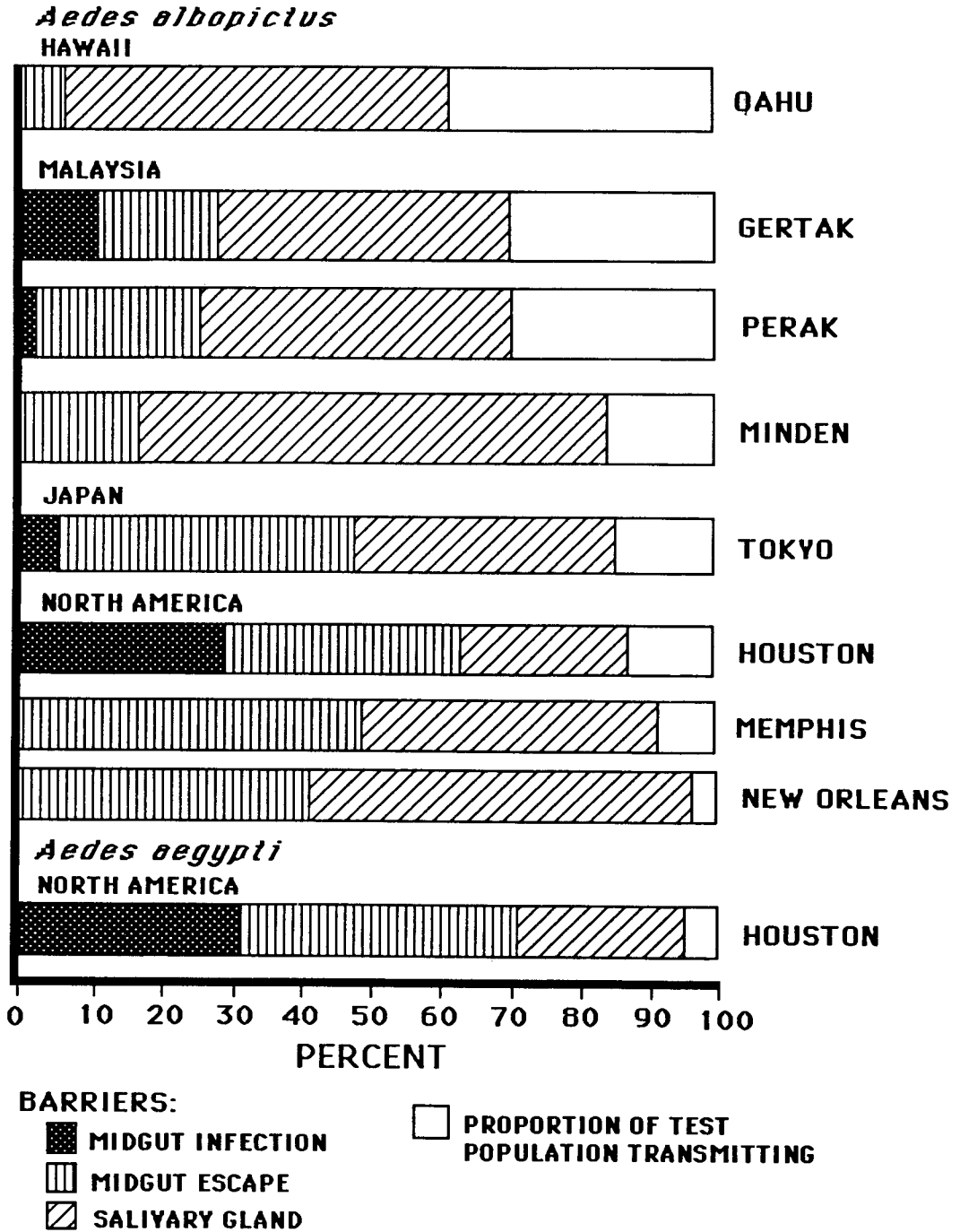
Fig. 1. Comparison of the proportion of strains of *Aedes albopictus* and *Aedes aegypti* with barriers to infection and transmission and proportion transmitting dengue 1 (FIJI) virus.

escape barrier (Grimstad et al. 1985) or if both were present. The salivary gland barrier appeared to be most important in limiting transmission by the Malaysian strains, while both the midgut escape and the salivary gland barriers were equally important in the TOKYO and three North American strains of *Ae. albopictus* (Fig. 1).

It was shown earlier that *Ae. albopictus* was more susceptible to oral infection with each of the four dengue serotypes than was *Ae. aegypti* (Rosen et al. 1985). The MEMPHIS and NEW ORLEANS strains of *Ae. albopictus* had significantly higher MT rates than the *Ae. aegypti* HOUSTON strain. We recognize that the ability of a mosquito to ultimately transmit is of greater epidemiological significance than midgut susceptibility. However, none of the North American *Ae. albopictus* strains tested significantly transmitted dengue 1 virus more efficiently at the virus dosages we used than the *Ae. aegypti* HOUSTON.

The population transmission rate is the most relevant for potential transmission to human hosts when assessing a strain's overall vector competence. Other studies emphasizing the transmission rate of the few females with disseminated infection ignore the epidemiologic relevance of the vector population's performance as a whole. If the midgut barriers preclude dissemination of virus in all but a low percentage of females ingesting a bloodmeal from a viremic human host, obviously the vector competence of the field population will be minimal. The lack of widespread indigenous transmission of dengue in Texas following the introduction of dengue (Kappus et al. 1980) is a reflection, perhaps, of the low vector competence seen with the Texas strain of *Ae. aegypti*. Our results suggest that the introduced strains of *Ae. albopictus* could conceivably foster a dengue outbreak in the southern United States, however, their overall vector competence indicates that indigenous transmission would probably not be much more significant than it might be with the native *Ae. aegypti*. Furthermore, our data strengthen the argument that one must speak of vector populations of *Ae. albopictus* rather than of "vector species" for dengue viruses and a complete evaluation of the effect of the barriers on the population's vector performance is essential.

The profile for vector competence among the three North American strains tested showed considerable differences between the HOUSTON strain and the MEMPHIS/NEW ORLEANS strains more or less paralleling allozyme differentiation among the HOUSTON and NEW ORLEANS strains (Black et al. 1987). Large and independent introductions of *Ae. albopictus* into these two cities have been postulated based on significant differences in allele frequency and the existence of unique alleles in each city (Houston vs. New Orleans) (Black et al. 1987).

These data demonstrated that the comparison of infection and transmission rates between the eight geographic strains of *Ae. albopictus* resulted in the formation of three groups: OAHU,

the Malaysian strains, and the TOKYO and North American strains. Although the delineations between these three groups were not always statistically distinct, the general pattern seemed to be a reasonable interpretation of the data. Other workers have hypothesized that the North American strains of *Ae. albopictus* originated in northern rather than southern tropical Asia based on diapause response, cold-hardiness of eggs, the importation of tires into North America (Hawley et al. 1987) and allozyme analyses (Black et al. 1987). In our study, *Ae. albopictus* TOKYO, a representative of northern Asia, was most similar to the North American strains both in the rates of infection and transmission of dengue 1 virus and in midgut escape barrier and salivary gland barrier patterns (Fig. 1) than were the OAHU or Malaysian strains representing tropical areas.

The PERAK strain of *Ae. albopictus* was from a dengue epidemic area while both GERTAK and MINDEN came from dengue endemic areas of Malaysia (Table 1). There were no significant differences between any of the Malaysian strains with respect to oral infection and transmission. The susceptibility of our Malaysian strains was higher than that obtained by other workers using *Ae. albopictus* from that general region of Asia and may be due in part to our use of unfrozen (freshly homogenized) virus stock for initial oral infection of adult mosquitoes. Assuming that the Asian strains were representative of the geographical location from which they were collected, they probably provide a profile of epidemic/endemic region *Ae. albopictus* strains from southeast Asia in contrast to the TOKYO strain from a non-endemic region. The TOKYO strain is a laboratory colony and may not be representative of *Ae. albopictus* from northern Asia. However, it was considerably less passaged than OAHU (Table 1). In addition, the effect of laboratory colonization in this species on the genetic variability of oral susceptibility to infection with dengue viruses over time is not known. Even with our freshly collected field strains, we cannot be sure as to what effect the initial colonization had on genetic variability. Nevertheless, assuming that our data are representative of natural field populations, the above comparisons can be made. Further investigation of this relationship would be enhanced if fresh uncolonized field strains were available and isofemale lines were used to limit founder effects and genetic drift (Tabachnick et al. 1982).

Future studies must address the effect of ambient field temperatures (including mean, fluctuating) on vector competence of *Ae. albopictus* relevant to North American cities. The study of Watts et al. (1987) of the effect of temperature

on the vector competence of *Ae. aegypti* suggests that "temperature-induced variation in the vector efficiency of *Ae. aegypti* may be a significant determinant in the annual cyclic pattern of dengue hemorrhagic fever epidemics in Bangkok." Similar studies need to be undertaken in order to further assess the potential importance of the North American *Ae. albopictus* strains in indigenous dengue transmission.

Furthermore, it was demonstrated earlier that *Ae. albopictus* exhibited a higher rate of transovarial transmission of dengue viruses than *Ae. aegypti* (Rosen et al. 1983). If the North American strains of *Ae. albopictus* transovarially transmit dengue viruses at a rate higher than the indigenous *Ae. aegypti*, the introduction and establishment of *Ae. albopictus* has potentially serious implications to U.S. public health. Given the apparent ability of indigenous *Ae. albopictus* populations in the U.S. to tolerate cold and short daylength by diapausing (Hawley et al. 1987), and the similar vector competence shown by these strains in comparison with *Ae. aegypti*, dengue virus entering the southern United States would not only be transmitted by *Ae. aegypti* but by additional vector populations of *Ae. albopictus*. This latter species is a vector that may be better adapted at maintaining dengue virus overwinter in the field through transovarial transmission, as has been demonstrated in the laboratory. Our future studies will evaluate the vector competence of additional United States *Ae. albopictus* populations as they become available for dengue serotypes 2, 3, and 4; emphasis will be placed on the use of new Caribbean-region isolates and eventually include transovarial transmission.

ACKNOWLEDGMENTS

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