RESEARCH NOTE

POLYGAMY: THE POSSIBLY SIGNIFICANT BEHAVIOR OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN RELATION TO THE EFFICIENT TRANSMISSION OF DENGUE VIRUS

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Abstract. The polygamous behavior of male *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) was investigated by co-habiting a newly-emerged male and females in a 30 cm^3 cage (1 male: 20 females) for up to 5 consecutive days. As determined by insemination rates, the results indicated that one *Ae. aegypti* and *Ae. albopictus* male could successfully mate with 1.10 (0-4), 4.10 (1-8), 5.40 (4-8), 5.10 (2-8), 5.15 (3-9) and 0.20 (0-3), 1.70 (0-3), 2.35 (1-4), 2.30 (0-4), 2.35 (1-4) *Ae. aegypti* and *Ae. albopictus* females, respectively on day 1,2,3,4 and 5 consecutively. The possibly significant role of their polygamy in relation to dengue virus transmission is discussed.

Dengue hemorrhagic fever (DHF) is of major international public health concern. The geographical distribution has expanded greatly and the number of cases has increased dramatically in the past 30 years. DHF is now endemic in more than 100 countries and threatens the health of about 40% of the world's population (2.5 billion people), especially in tropical and subtropical regions (WHO, 2000). The disease is caused by 4 antigenically related serotypes of dengue virus (DEN 1,2,3 and 4); *Aedes aegypti* (L.) is the important vector in urban areas, while *Ae. albopictus* (Skuse) is the vector in some suburban and rural areas (Gould *et al*, 1968; Rudnick, 1978; Self, 1979; Monath, 1994).

One of the crucial questions in the epidemiology of dengue is how the viruses are maintained in nature during the absence of viremic vertebrate hosts and/or when the climatic conditions are not favorable for mosquito population-density. Extensive studies have been made by several investigators, and their results demonstrated that the vertical transmission of virus from infected gravid females to the F_1-progeny occurred in both the experimental laboratory and mosquito field studies. This may be a possible factor involved in maintaining these viruses in nature (Khin and Than, 1983; Rosen *et al*, 1983; Mitchell and Miller, 1990; Bosio *et al*, 1992; Ahmad *et al*, 1997; Thenmozhi *et al*, 2000). The ability of *Ae. albopictus* and the possible mechanism of *Ae. aegypti* to transmit the dengue virus sexually from male to female have been demonstrated in the laboratory by Rosen (1987) and Tu *et al* (1998), respectively. The above results indicated that vertical as well as sexual transmission could contribute to the maintenance of dengue virus in nature. Further to previous studies, we report and emphasize on the highly polygamous mating habits of *Ae. aegypti* and *Ae. albopictus* males as a possible supportive factor for the sexual transmission of dengue viruses in the mosquito and/or invertebrate phase.

Laboratory-reared *Ae. aegypti* and *Ae. albopictus* (Chiang Mai, Thailand strain) were used in this study. Since the exact polygamous

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ability of these two mosquito vectors has never been recorded, an experimental design to determine the optimal ratio of one male to females and the suitable time for their co-habitation was investigated primarily with *Ae. aegypti*. A newly-emerged male was exposed to females in ratios 1:10, 1:20 and 1:30; the mosquitos co-inhabited a 30 cm^3 cage for up to 5 consecutive days. The result revealed that one *Ae. aegypti* male could mate successfully with 2-7, 4-8 and 7-14 females at ratios 1:10, 1:20 and 1:30, respectively (Table 1). Comparing the maximal mating of one *Ae. aegypti* male with 14 females at a ratio of 1:30 with the mating ability of 1 male with 20 females on day 3 (5 females), 4 (7 females) and 5 (8 females), no statistically significant difference was found (day 3/4, χ^2 = 0.48, p> 0.05; day 3/5, χ^2 = 1.03, p > 0.05). Therefore, in further experiments *Ae. aegypti* and *Ae. albopictus* were used at a ratio of 1 male: 20 females and a newly-emerged male and females co-habited in a 30 cm^3 cage for up to 5 consecutive days.

Table 1
Mating ability of one *Ae. aegypti* male with 10, 20 and 30 females during co-habitation in a 30 cm^3 cage.

Duration of co-habitation (Day)	No. females that succeeded in insemination at		
	1 : 10	1 : 20[a]	1 : 30
1	0	4	0
2	2	8	11
3	5	5	14
4	7	7	7
5	7	8	7

[a]Day 3/4, χ^2 = 0.48, p > 0.05; day 3/5, χ^2 = 1.03, p > 0.05

Details of the mating ability of one male *Ae. aegypti* and male *Ae. albopictus* with 20 females after co-habitation in a 30 cm^3 cage for up to 5 consecutive days are shown in Table 2. The average number of female *Ae. aegypti* that mated with the one male on day 1 was 1.10 (range 0-4). The mating ability increased markedly from day 2 to 5 with the average number of females that mated with the one male ranging from 4.10-5.40 (range 1-9). The statistical analysis of the average number of females that mated with the one male using the F-test demonstrated that days 3,4 and 5 produced a similar mating ability which was, however, more significant and gradual than during days 1 and 2 (F = 29.62, p < 0.01).

Table 2
Mating ability of one male *Ae. aegypti* and male *Ae. albopictus* with 20 females during co-habitation in a 30 cm^3 cage.

Duration of co-habitation (Day)[a]	No. females that succeeded in inseminating		Mean ± SD No. females mated by one male (range)		*t*, p
	Ae. aegypti	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	
1	22	4	1.10 ± 1.33 (0-4)	0.20 ± 0.70 (0-3)	2.68, =0.01
2	82	34	4.10 ± 1.41 (1-8)	1.70 ± 0.80 (0-3)	6.62, <0.001
3	108	47	5.40 ± 1.10 (4-8)	2.35 ± 0.88 (1-4)	9.73, <0.001
4	102	46	5.10 ± 1.71 (2-8)	2.30 ± 1.13 (0-4)	6.10, <0.001
5	103	47	5.15 ± 1.69 (3-9)	2.35 ± 1.04 (1-4)	6.30, <0.001

[a]Twenty experiments for each duration.

Similar results were also found for *Ae. albopictus* [day 1 (0.20; range 0-3) < day 2 (1.70; range 0-3) < day 3 (2.35; range 1-4) = day 4 (2.30; range 0-4) = day 5 (2.35; range 1-4), F = 20.15, p < 0.01], except that its mating ability was significantly less than that of *Ae. aegypti* given the same length of co-habitation (t = 2.68-9.73, p < 0.01).

Laboratory and field studies have demonstrated that dengue viruses may be transmitted vertically by *Ae. aegypti* and *Ae. albopictus* (Khin and Than, 1983; Rosen *et al*, 1983; Mitchell and Miller, 1990; Bosio *et al*, 1992; Ahmad *et al*, 1997; Thenmozhi *et al*, 2000). Rosen (1987) demonstrated that the virgin females of *Ae. albopictus*, which had taken prior blood meals and contained fully-developed eggs enclosed in the chorion, then mated with dengue 1 virus infected males and the F_1-progeny was infected with the virus by sexual transmission. Similar results were obtained for Japanese and St Louis encephalitis viruses in *Ae. albopictus* (Rosen, 1988). Additional details of *Ae. aegypti* were stated by Tu *et al* (1998): the results of their studies revealed that dengue 2 virus particles were detected in the matrix, epithelial cells and peripheral fat body of the testis; secretory droplets of columnar cells of the accessory glands; and the epithelial and muscle cells of the seminal vesicles. However, none was found in the germ cells (spermatogonia, spermatocytes, spermatid, spermatozoa), which suggested that the sexual transmission of the dengue virus from infected male to female mosquitoes is mainly through male accessory gland fluids. These fluids possibly enter the egg accompanied by semen via the micropyle at fertilization. Male mosquitos do not take blood meals naturally, so they have no pathway in which to obtain the dengue viruses from viremic vertebrate hosts. Nevertheless, they still have an opportunity to be infected with the virus by vertical transmission from infected parents (Khin and Than, 1983; Ahmad *et al*, 1997; Thenmozhi *et al*, 2000), and naturally infected males may be the main force initiating sexual transmission. Combining these observations with the strongly polygamous mating of *Ae. aegypti* and *Ae. albopictus* males, it appears that sexual transmission of the dengue virus in mosquito vectors could be one of the effective mechanisms for maintaining dengue viruses in nature, particularly during the absence of viremic vertebrate hosts and/or at times of low mosquito population-density. Another remarkable point is that one female *Ae. aegypti* and female *Ae. albopictus* could mate successfully with 4 and 3 males respectively during co-habitation 5-day-old 1 female: 10 males in a 30 cm^3 cage under observation for 1 hour. The repeated mating of *Ae. aegypti* and *Ae. albopictus* females with the same and/or different males is enough to guarantee the successful transfer of accessory fluids containing viruses from males to females, and this could be an additional mechanism in the sexual transmission of dengue viruses. Although our study was not concerned with the dengue virus-polygamous systems, the present results call for the further investigation of natural sexual transmission of the viruses.

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