

VARIATION IN OVIPOSITION, HATCH RATE AND SETAL MORPHOLOGY IN LABORATORY STRAINS OF *Aedes albopictus*

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ABSTRACT. The egg-laying habit and the larval hatch process were observed for 2 strains of *Aedes albopictus*, one from a natural forest and the other from an urban area. The latter females laid eggs easily on a piece of sponge containing tap water. Ninety-five percent of these eggs hatched in tap water within 10 days after being laid. Under the same conditions, the forest strain females laid 80% fewer eggs,

which hatched sporadically over several months. However, the forest strain gradually became similar to the urban one during colony maintenance in the laboratory. There was a concomitant increase in the average number of branches of larval seta 1-VII. The evolutionary significance of these phenomena is discussed.

INTRODUCTION

Widely distributed mosquito species are often variable in morphological and physiological characters. Some of these variations are treated as subspecies or geographical races with allopatric distributions, or clines where characters change gradually through a continuous population. Some characters, on the other hand, appear to vary in accordance with more local ecological conditions. An example is *Aedes aegypti* (Linnaeus) which is composed of the type-form and the feral one, *formosus* (Walker), with almost allopatric distributions (Mattingly 1957). McClelland (1974) reported that in this species "in any geographical region, there was a tendency for populations closely associated with man to be relatively paler than those less associated with man."

Aedes albopictus (Skuse) is distributed widely in the Oriental region with extensions into the Palaearctic, Australian and Ethiopian regions. Gubler and Rosen (1976) detected variation in susceptibility to dengue viruses among geographical strains of this species, but little has been studied on other aspects of its variations. This report presents evidence for the occurrence in *Ae. albopictus* of ecological strains which appear to reflect local differences in larval breeding habitats.

MATERIALS AND METHODS

Tsushima strain (*Tsushima* hereafter). Origin: Ca. 20 adult females attracted to man in the natural forest of evergreen broad-leaved trees on Sao-Zaki, Tsushima, Japan (34°39'N., 129°19'E.) on August 22, 1975.

Rearing conditions: 27°C, 15 hr light.

Laboratory host: Man.

Generations at the initiation of the experiment: 4.

Nagasaki strain (*Nagasaki* hereafter). Origin: Adults collected in Nagasaki City, Japan (32°46'N., 129°52'E.) in 1968.

Rearing conditions: 27°C, 14.5 hr light.

Laboratory host: Mouse.

Generations at the initiation of the experiment: 55.

EXPERIMENT-1. Forty females were exposed to a piece of sponge saturated with tap water 2 days after feeding on man. Eggs deposited were counted daily. Observations were made at 27°C and 14.5 hr light.

EXPERIMENT-2. Eggs obtained in Experiment 1 were submerged in tap water and the daily number of hatched larvae was recorded at 27°C and 14.5 hr light for 250 days. The water was usually renewed every day. For one-half of the eggs, larval food (mixture of dried yeast and ground mouse pellets) was added to stimulate

embryos not hatching within 30 days after oviposition.

RESULTS

EGG-LAYING HABIT. Table 1 shows differences in the egg-laying habits of the 2 strains. *Tsushima* females laid less than one-fifth of the number of eggs of *Nagasaki* females. Daily mortality among females was higher in *Tsushima* than in *Nagasaki*, but was not a main cause of the large difference in the numbers of eggs laid since the difference occurred during the first 10 days when many adults of *Tsushima* were alive. In *Nagasaki* most (97%) of the eggs were laid in the first 10 days. In *Tsushima* 40% of the total were laid in the second or later 10-day period. Dissection of dead females of *Tsushima* demonstrated mature eggs (even over 100) and spermathecae containing sperm. *Nagasaki* females actively fed on human hands 5 days after the start of oviposition, but *Tsushima* females did not. Therefore, the difference shown in Table 1 was attributed to the differential preference for the oviposition site. *Nagasaki* females oviposited readily on sponge-tap water substrates, but *Tsushima* with mature eggs often died before ovipositing on them.

HATCHING OF LARVAE. *Nagasaki* larvae hatched easier than *Tsushima* under the test conditions (Fig. 1). In *Nagasaki*, larval hatch rates were very high. Hatching began 3 days after oviposition and 95% of eggs hatched within 10 days. However, the remainder (3.6% of tested eggs) hatched sporadically over 200 days. Larval food added to tap water appeared to induce an earlier hatch from the persistent eggs when it was applied one month

after the eggs were laid, however the second application given 2 months later was ineffective. In *Tsushima*, the duration from egg-laying to larval hatch was more variable and the final hatch rate was lower than in *Nagasaki*. In tap water, about one-half of the larvae hatched after 100 days. Larval food clearly induced an earlier hatch from the persistent eggs.

CHANGES OF CHARACTERS IN THE TSUSHIMA STRAIN. The maintenance of *Tsushima* was difficult initially. To obtain eggs in sufficient numbers, repeated blood-feeding, cut bamboos as oviposition containers and larval food as a hatching stimulus were required. However, maintenance became easier with each successive generations. At the 10th generation, the maintenance with tap water and sponge was possible. After the 15th generation, eggs laid on the inside wall of glass vials containing water and sponge became common. The average branch number of larval seta I-VII increased during this acclimatization to laboratory rearing conditions (Table 2). Apparently, the 2 branched I-VII seta was predominant (70%) and the 3-branched one was minor (30%) in the first laboratory generation of *Tsushima*. In the 3rd generation, the 2-branched decreased (52%) while the 3-branched increased (48%). The ratio reversed in the 5th generation (33% to 67%) and the 3-branched I-VII seta remained predominant to the 22nd generation. The difference between the first generation and the succeeding ones was statistically significant. The I-VII seta with 3 branches was also predominant in *Nagasaki*, while the 2-branched one was predominant in the *Tsushima* strain of *Aedes riversi* Bohart and Ingram.

Table 1. Numbers (and %) of eggs laid by 40 engorged females of *Aedes albopictus* of each strain.

Strain	Days after blood-feeding				Total	Average per female
	3-10	11-20	21-30	31-40		
<i>Tsushima</i>	361 (59.9)	178 (29.5)	37 (6.1)	27 (4.5)	603 (100.0)	15.1
<i>Nagasaki</i>	3141 (97.4)	79 (2.4)	5 (0.2)		3224 (100.0)	80.6

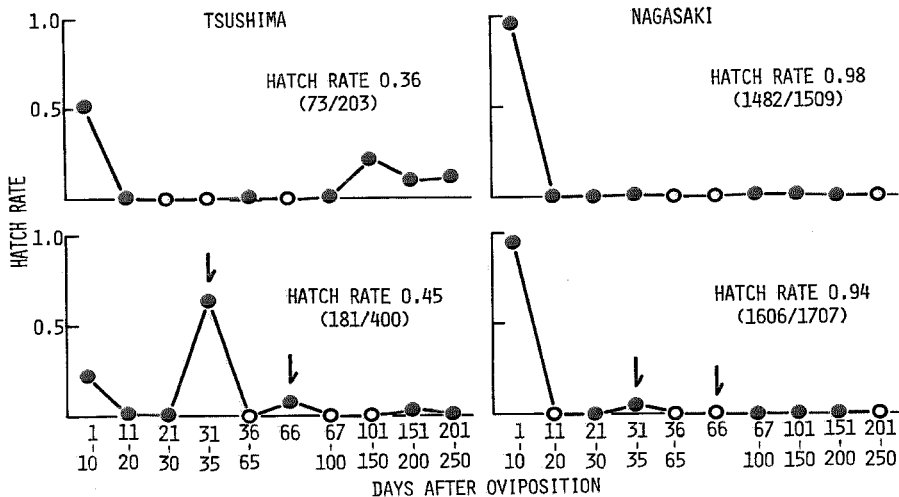


Fig. 1. Days from oviposition to hatch in *Aedes albopictus*. Hatch rate (%) on the ordinate: Hatch number in the indicated duration/ Total hatch no. Open circle: Hatch number was zero. Arrow: Mixture of dried yeast and ground mouse pellets was added.

DISCUSSION

Nagasaki and *Tsushima* in this experiment probably represent opposite extremes insofar as characters examined are concerned. Most populations of this species in Japan are expected to fall within these extremes. For example, it was observed that the females collected at a forest of cultivated bamboos in a rural area of Saga (a prefecture adjacent to Nagasaki) did not oviposit on the sponge-tap water substrate as readily as *Nagasaki*, but most hatched quickly in tap water (Mogi, unpublished observations). Ito (1959) and Imai and Maeda (1976) observed that embryonated summer eggs from Kyoto City hatched in tap water without any delay. The establishment of laboratory strains of this species from individuals collected in the man-made environment (urban or rural) is very easy compared with the difficulties encountered at the initial stage of maintenance of *Tsushima*. It appears that *albopictus* in the

man-made environment of Japan requires less specialized stimuli for egg-laying and larval hatch than the forest population of *Tsushima*. Similar inter-population differences in oviposition site selection and larval hatch were reported for *Ae. aegypti* (Leahy et al. 1978, Gillett 1955) and *Ae. triseriatus* (Say) (Means et al. 1977).

Artificial containers around human dwellings in Japan are fairly diverse in shape, size, color and materials. The quality of water supporting larval development also varies from clean to very turbid. Therefore, the wide preference in the selection of oviposition sites would be advantageous in the man-made environment, where the gain due to the expanded number of breeding places is expected to exceed the loss due to oviposition at places unsuitable for larval growth.

Rapid hatch increases the potential for population growth since the capacity for increase (r_c) is inversely proportional to

Table 2. The branch number of the larval seta I-VII of *Aedes albopictus* and *Aedes rivarsi*.

Species and strain	Generation in laboratory	No. of specimens examined	Branch no. in pairs of right and left setae							Average branch number \pm S.E.	Difference from The preceding generation	Tsuchima-1			
			4-4	4-3	3-3	3-2	2-2	2-1	1-1				3-1		
<i>Ae. albopictus</i> Tsushima	1	70			11	20	39					2.30 \pm 0.04	—	—	
	3	28			8	11	9					2.48 \pm 0.07	*	*	
	5	79			42	22	15					2.67 \pm 0.04	*	**	
	7	66		1	26	20	19					2.57 \pm 0.04	n.s.	**	
	9	345		1	159	88	97					2.59 \pm 0.02	n.s.	**	
	11	59			30	17	12					2.65 \pm 0.04	n.s.	**	
	13	71		1	39	18	13					2.70 \pm 0.04	n.s.	**	
	15	92		2	52	25	13					2.73 \pm 0.03	n.s.	**	
	16	22			10	5	7					2.57 \pm 0.08	n.s.	**	
	17	8			5	1	2					2.69 \pm 0.12	n.s.	**	
	18	14			7	3	4					2.61 \pm 0.09	n.s.	**	
	19	30		1	2	17	5	2	1		2	2.80 \pm 0.08	n.s.	**	
	20	25				12	7	5	1			2.60 \pm 0.08	n.s.	**	
	22	41		1	1	19	9	12				2.61 \pm 0.06	n.s.	**	
	55	90		3	68	8	9	1	1			2.83 \pm 0.04	—	**	
	Nagasaki <i>Ae. rivarsi</i> Tsushima	1	58			3	19	32	2	2	2		2.16 \pm 0.05	—	*

* P < 0.05 by t-test.
 ** P < 0.01.
 n.s. not significant.

the mean generation time (Laughlin, 1965):

$$r_c = \frac{\log_e R_0}{T_c}$$

where R_0 is the net reproductive rate and T_c is the mean generation time. Therefore, less specialized requirements for larval hatch also can be a great advantage in the man-made environment where breeding places are diverse.

On the other hand, in natural forests of temperate Japan, breeding places of *albopictus* are limited primarily to tree holes which are sufficiently dark and rough to attract and stimulate gravid females of *albopictus*. The water in tree holes contains a variety of chemical and biological stimuli inducing oviposition and larval hatch. Therefore, even such a strain as *Tsushima* would easily express its reproductive potential despite its strict requirements for oviposition and larval hatch.

Aedes albopictus probably originated in Southeast Asia where many species closely related to *albopictus* occur (Huang 1972). It is fairly common in the man-made habitat in the lowland of southwest Japan but rare or absent in the natural forest of evergreen broad-leaved trees (Eshita and Kurihara 1978; Miyagi and Toma 1978, 1980). Further, the species is not found in some small uninhabited islands where natural evergreen broad-leaved tree forests remain (Mogi, unpublished). These points suggest that man's activity was indispensable for the species to establish itself in Japan. Therefore, it may be reasonable to assume that forest populations such as *Tsushima* resulted from readaptation of a man-associated strain to the forest habitat.

Huang (1972) considers the branch number of the abdominal seta I-VII to be useful in discriminating *albopictus* from some species of the *Scutellaris* subgroup. She wrote, "The larva of *albopictus* can be distinguished from *alcasidi*, *malayensis* and *riveri* by having abdominal hair I-VII usually 4-, sometimes 3-branched, and

always much shorter and stronger; in the 3 other species hair I-VII usually with 2 (2-3) long branches." Because *Ae. riveri* occurs in the original habitat of *Tsushima* (Mogi 1976), the setal branch number was carefully documented (Table 2). The data support the view of Tanaka et al. (1979) that seta I-VII is a poor discriminating character due to the wide overlapping at least between *albopictus* and *riveri* of Japan. The branch number of I-VII is different among local populations of *albopictus*, bifurcation being predominant in some populations. Furthermore, Sakakibara (1963) observed that *albopictus* larvae reared in tap water or filtered bamboo stump water have less developed setae than those reared in wood, bamboo or straw infusion. Colless (1956) observed a similar tendency for *albopictus* from Singapore. However, Sakakibara (1963) noticed that even his hairy specimens corresponded to the range of variation in the normal type of Colless. This suggests that Japanese *albopictus* might be different from the Singapore strains with respect to the response of the hairiness factor.

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