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Studies on the Egg Diapause and Overwintering of *Aedes albopictus* in Nagasaki

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Abstract: Diapausing eggs of *Aedes albopictus* are laid by females reared under the condition of low temperature and short photoperiod. The pupa and the adult are sensitive stages to temperature and photoperiod in the induction of egg diapause. In Nagasaki, diapausing eggs increase in mid September, and all eggs laid after October are in diapause. These diapausing eggs overwinter, and would not hatch until the diapause is broken in February of the next year. Hatching of diapausing eggs begins in mid March and continues until late May. Through some non-diapausing eggs can overwinter, it is sure that most overwintering eggs are in diapause in the field. It seems that the advantage of egg diapause is to prevent eggs from hatching before winter.

The larvae of *Aedes albopictus* breed mainly in small containers around the human habitation, and the adults rest in such a place as the thicket and attack men or animals coming nearby in the daytime. *Ae. albopictus* is known as a vector of dengue fever, and it is said that *Ae. albopictus* played a major role in the transmission of dengue fever prevailed in Japan during the World War II. Mori and Wada (1978) reported that in Nagasaki the adults of *Ae. albopictus* are found from May to October, and the larvae and pupae from March to December, but any larvae, pupae or adults are not found during winter. Even in winter the larvae hatch, when the eggs are collected from the bottom of breeding site and kept in a high temperature room (Abe et al., 1941 ; Nakata et al., 1953 ; Hong et al., 1972). Mori and Wada (1978) observed in spring the larvae immediately after hatching in containers in which all the larvae and pupae had died off

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from winter cold, and supposed that only eggs can pass the winter in Nagasaki and northward.

In Nagasaki, the number of newly hatched larvae begins to decrease in September, and from October any larvae do not hatch until next mid March when the newly hatched larvae are found again. This suggests that there is some mechanism to obstruct hatching of eggs, because the temperatute in September, when the number of the first instar larvae in the field begins to decrease, is much higher than 10–11 C, which is ecological temperature zero of the egg stage determined by Mastuzawa and Kitahara (1966). It is well known that eggs of aedine mosquitoes do not hatch in some environmental conditions, *e.g.* disappearance of water or increase of tension of dissolved oxygen. Some aedine mosquito species, *e.g. Ae. hexodontus* (Beckel, 1958), *Ae. canadensis*, *Ae. fitchii* and *Ae. stimulans* (Horsfall et al., 1958) enter diapause in the egg stage to pass winter. The diapausing eggs of these mosquitoes can hatch only after reactivation.

In the case of *Ae. albopictus*, Wang (1966) and Imai and Maeda (1976) showed that the factor inducing the egg diapause is low temperature under short photoperiod. The present studies made clear the sensitive stage of *Ae. albopictus* to temperature and photoperiod in the induction of egg diapause, the physiological state of overwintering eggs, and the process from the induction to the termination of diapause in eggs in the field of Nagasaki.

MATERIAL AND METHODS

The strain of *Ae. albopictus* used in this experiment was originated from Nagasaki and maintained in the laboratory over 90 generation at 25 C under 16 hr daylength.

Five hundred larvae were reared in a pan of 22 X 28 X 4cm, and were fed 0.4g of mixture of brewer's yeast and finely ground mouse pellets every day. Pupae were transferred to a laboratory dish containing clean water in a cage of 30 X 20 X 20cm of cheese cloth. Resulting adults were given 2% sugar solution. Three days after emergence of the last female, a chicken was given for blood meal. Five days after feeding on blood meal, filter paper was fixed on inside of laboratory dish containing a little water and given for their oviposition site. Soon after females laid eggs, the filter paper was taken away and the number of eggs was counted next day. Uñless otherwise stated the eggs on the filter paper were submerged in water in a glass vial, and newly hatched larvae were taken out and counted every day. The water used in all experiments was the tap water that was kept over 24 hr in a bucket. The number of observed eggs in each lot in all experiments was about 1500.

To make clear the factors inducing egg diapause in *Ae. albopictus* and their developmental stages most sensitive to these factors, newly laid eggs at 25 C under 16 hr daylength were transferred to 21 C under 10 hr daylength and *vice versa*, and then the hatching of eggs from females resulting in each lot was examined for 30 days.

For the dates of appearance of diapausing eggs in autumn and of their hatching in the next spring in the field, larvae were reared to adults each ten days from August to November in an outdoor insectarium in the camps of Nagasaki University School of Medicine (32°44′N. lat.), and the hatching of laid eggs were examined. Observation was continued until the end of May in the next year.

To know if non-diapausing eggs have the ability of overwintering in the field in Nagasaki, non-diapausing eggs of four days old were kept in two conditions, wet and dry, in the outdoor insectarium through winter. In the case of eggs kept dry, they were submerged in water in early March. Newly hatched larvae were counted every day until the end of May.

RESULTS

(1) Maternal effect of temperature and photoperiod on the hatchability of eggs.

Fig. 1 shows hatching curves of eggs that were laid by females resulting at 25 C under 16 hr daylength and at 21 C under 10 hr daylength. The hatching rate was apparently higher in eggs from high temperature under long photoperiod. Many eggs from females reared at 25 C under 16 hr daylength hatched between 4 and 16 days after oviposition, but only small number of eggs from females at 21 C under 10 hr daylength

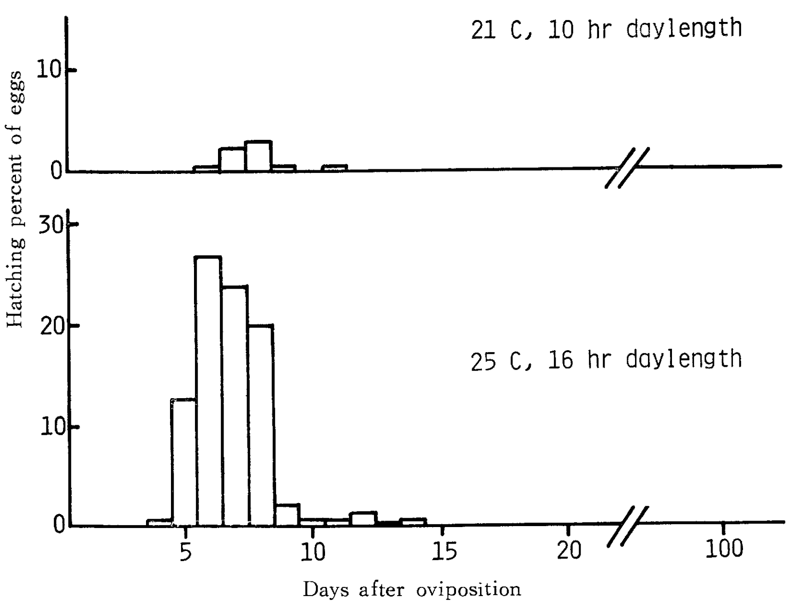


Fig. 1. Hatching curve of eggs laid by *Aedes albopictus* females resulting from 25 C under 16 hr daylength and from 21 C under 10 hr daylength.

hatched between 6 and 11 dayls after, and no eggs hatched thereafter until 100 days after oviposition. These unhatched eggs were considered as being in diapause. It can be said from the above that most non-diapausing eggs hatch by about 2 weeks after oviposition.

Larvae of each instar, pupae, adults and eggs that were reared at 25 C under 16 hr daylength were transferred to 21 C under 10 hr daylength, and conversely those at 21 C under 10 hr daylength were transferred to 25 C under 16 hr daylength. They were continuously reared, and the eggs were examined for hatching, of which results are shown in Table 1.

The hatching rate was low in eggs from females which were reared at 25 C under 16 hr daylength until 4th instar or younger larvae and transferred to 21 C under 10 hr daylength. The rate of hatching slightly increased in the eggs from females transferred at adults to 21 C under 10 hr daylength, and became high when only eggs were exposed to 21 C and 10 hr daylength.

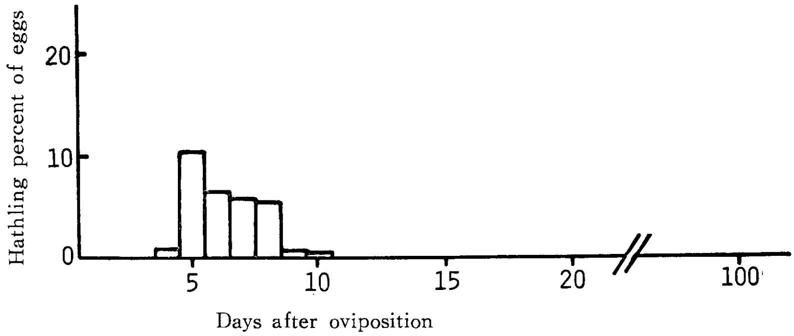
On the other hand, the hatching rate was high in eggs from females transferred at pupae or younger stages to 25 C under 16 hr daylength from 21 C under 10 hr daylength, but it was low when transferred at adults or eggs.

From these results, it seems that pupae and adults were more sensitive than younger stages in the maternal effect of temperature and photoperiod on the rate of egg hatching. It is also suggested that the females which pupate and emerge at low tem-

Table 1. Effect of high temperature with long photoperiod and low temperature with short photoperiod applied to given combinations of development on induction of embryonic diapause in the Nagasaki strain of *Aedes albopictus*.

Developmental stages exposed to specific temperature and photoperiod			% hatch of eggs laid by resulting females
25 C, L:D=16:8	21 C, L:D=10:14	25 C, L:D=16:8	
I	II III IV P A E		1.71
I II	III IV P A E		4.27
I II III	IV P A E		0.62
I II III IV	P A E		0.53
I II III IV P	A E		14.59
I II III IV P A	E		79.62
I II III IV P A E			90.96
	I	II III IV P A E	96.34
	I II	III IV P A E	80.39
	I II III	IV P A E	86.13
	I II III IV	P A E	85.10
	I II III IV P	A E	24.91
	I II III IV P A	E	39.39
	I II III IV P A E		5.56

* Roman numerals indicate larval instars, P=pupa, A=adult, E=egg



Fi.g 2. Hatching curve at 25 C under 8 hr daylength of eggs laid by *Aedes albopictus* females reared under the same condition from the first instar larvae.

perature under short photoperiod in autumn would oviposit diapausing eggs, even though they have experienced high temperature under long photoperiod until their pupation in the field.

In the above-mentioned experiments, hatching rates were compared in the eggs from combinations of high temperature with long photoperiod and of low temperature with short photoperiod. To know the effect of short photoperiod with high temperature on the embryonic diapause, the hatching curve of eggs from females resulting at 25 C under 8 hr daylength was observed. The result is shown in Fig. 2. Accumulative hatching rate was 29.6%. This result showed that even the combination of short photoperiod with high temperature is, to some extent, effective in the production of diapausing eggs. In other words, high temperature removes the e fect of short photoperiod from some eggs that might enter diapause at low temperature.

(2) The onset date of egg diapause under natural condition.

The larvae that hatched at 25 C under 16 hr daylength were transferred to an outdoor insectarium on the day of hatching and reared approximately every ten days from August to November, 1976, and the hatching of eggs from resulting females was observed. Rearing histories are given in Table 2. Since the developing speed of immature stages became slower with decreasing temperature in autumn, periods from the oviposition of an experimental lot to that of the next lot were varied. The first instar larvae that hatched after October 1 could not make any progeny, because air temperature remarkably decreased so that many larvae and pupae died and few females survived until feeding on blood meal.

Fig. 3 shows the accumulative rate of egg hatching by the end of the year by oviposition date and the time between sunrise and sunset at Nagasaki. It was shown that 78.9% of eggs hatched by the end of the year, when females oviposited in late August (experimental lot A). The rate of hatching by the end of the year was 12.7

Table 2. Rearing histories of *Aedes albopictus* in the experiments for the effect of the date of oviposition on the time of egg hatching, under outdoor conditions at Nagasaki, in 1976. (see Fig. 3 and Table 3 for results)

Experimental lot	A	B	C	D	E	F
Date of hatch	Aug. 11	Aug. 21	Sep. 1	Sep. 11	Sep. 21	Oct. 1
Date of pupation	Aug. 15–18	Aug. 28–29	Sep. 7–10	Sep. 22–28	Oct. 2–8	Oct. 9–19
Date of emergence	Aug. 17–20	Aug. 30–31	Sep. 9–12	Sep. 26–Oct. 3	Oct. 7–13	Oct. 14–23
Date of feeding on blood meal	Aug. 24	Sep. 9	Sep. 16	Oct. 5	Oct. 23	Nov. 2
Date of oviposition	Aug. 30	Sep. 13	Sep. 21	Oct. 15	Nov. 3	Nov. 12

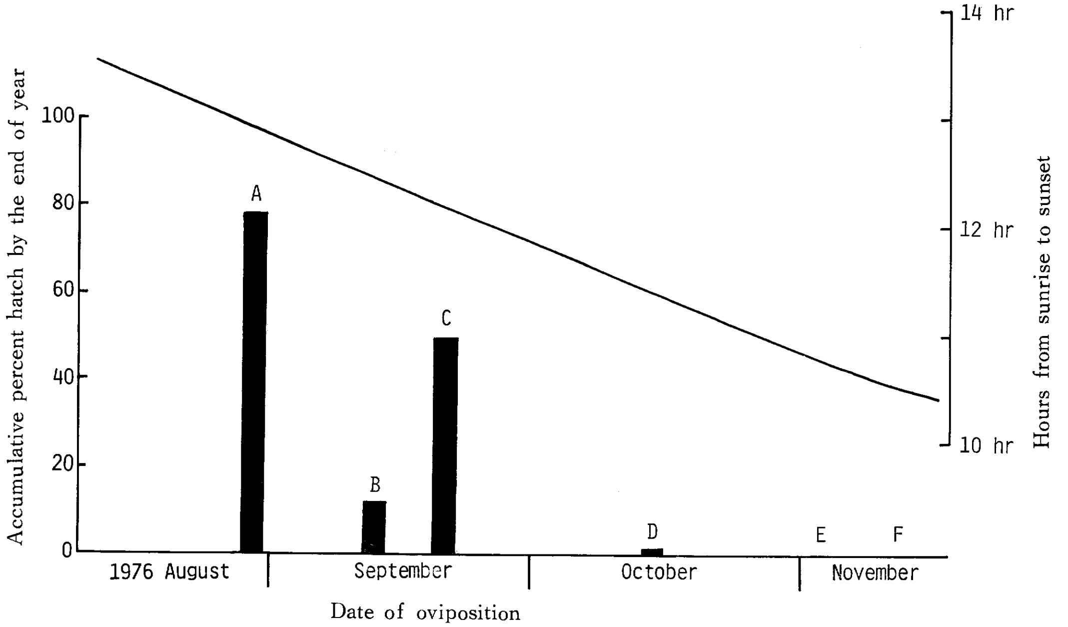


Fig. 3. Hatching by the end of the year under outdoor conditions of eggs laid from August to November by females of *Aedes albopictus* shown in Table 2, with seasonal change of hours from sunrise to sunset. Bars indicate accumulative rate of egg hatching by the end of the year, and alphabetical letters the experimental lots in Table 2.

% when eggs were laid in mid September (lot B), and 48.9% when oviposited in late September (lot C). The reason for revarsal between these two rates is not clear. When females emerged between mid September and early October and oviposited after mid October, most of eggs did not hatch by the end of the year. The embryos in these unhatched eggs developed completely, and the larvae could move in water when the egg shell was taken away. However, they were considered to be in diapause, because the temperature around the day of oviposition was much higher than the ecological temperature zero for the egg stage. It was suggested from the above that diapausing eggs began to be laid from mid September, and most of eggs laid after mid October were in diapause.

(3) The date of hatching of overwintering eggs.

The hatching of overwintering eggs in diapause that had been laid in autum, *i.e.* unhatched eggs in the lots A, B, C, D, E and F in the former experiment, was observed in spring. The rate of egg hatching in every five or six days is given in Table 3. The hatching of overwintering eggs began from mid March and continued till the end of May. Most of overwintering eggs hatched between late March and late April. A small number of eggs (1.4%) laid even on August 30 overwintered and hatched in the next spring. It was seen that the eggs laid in late autumn (lot E and F) hatched earlier in the next spring than those laid in early autumn (lot D). Eggs that enter diapause at relatively low temperatures may hatch easily, but details are not known.

Table 3. Hatching of eggs laid by *Aebes alpoictus* females from August to November, under outdoor conditions. Alphabetical letters indicate experimental lots in Table 2.

% hatch of eggs	A	B	C	D	E	F
Before winter	78.91	12.68	49.80	0.04	0.00	0.00
Mar. 16–20	0.00	0.13	0.05	0.00	0.13	0.14
Mar. 21–25	0.00	0.00	3.81	0.00	32.11	31.22
Mar. 26–31	0.00	0.00	0.05	0.00	0.36	0.18
Apr. 1– 5	0.00	18.13	4.72	0.09	6.71	0.00
Apr. 6–10	0.23	3.31	0.91	0.04	1.39	0.14
Apr. 11–15	0.00	13.44	11.84	45.52	19.86	0.45
Apr. 16–20	0.36	13.00	3.81	20.22	6.35	0.00
Apr. 21–25	0.14	1.56	0.14	9.98	4.92	0.00
Apr. 26–30	0.05	13.49	4.58	2.36	7.29	11.30
May 1– 5	0.23	0.31	2.00	2.36	0.49	1.41
May 6–10	0.05	0.00	0.14	0.40	0.00	0.32
May 11–15	0.32	0.04	0.14	2.54	0.00	0.32
May 16–20	0.05	0.04	0.36	0.18	0.00	0.77
May 21–25	0.00	0.04	0.05	0.00	0.00	0.18
May 26–31	0.00	2.28	0.05	0.00	0.00	0.00
Total in spring	1.43	65.77	32.65	83.60	79.61	46.43
Total	80.34	78.45	82.45	83.64	79.61	46.43

(4) Date of reactivation of overwintering eggs.

Diapausing eggs from females resulting in the outdoor insectarium in autumn were divided into two groups, and one was kept wet and the other dry. Eggs were sampled and tranferred to 25 C under 16 hr daylength at irregular intervals from November to April, and when kept dry, they were submerged in water. The number of hatched larvae were counted every day for 30 days after the transfer. Since most of eggs that were kept wet hatched already on April 24 in the outdoor condition, they were excluded from observation. Fig. 5 shows the accumulative hatching rate of eggs that were kept wet, and Fig. 6 shows those of eggs kept dry. Whether eggs were kept wet or dry, when transferred to 25 C under 16 hr daylength on March 4, they began to hatch on the next day, and the rate of egg hatching went up considerably in 3 days. However, when eggs were transferred before February, they scarecely hatched within a few days after the transfer. From these results, it seems that the overwintering eggs were various in the extent of diapause, but most of the overwintering eggs were reactivated in late February. The hatching rate was low in the eggs that were kept dry compared with the eggs kept wet. It is not clear if these unhatched eggs were alive or dead.

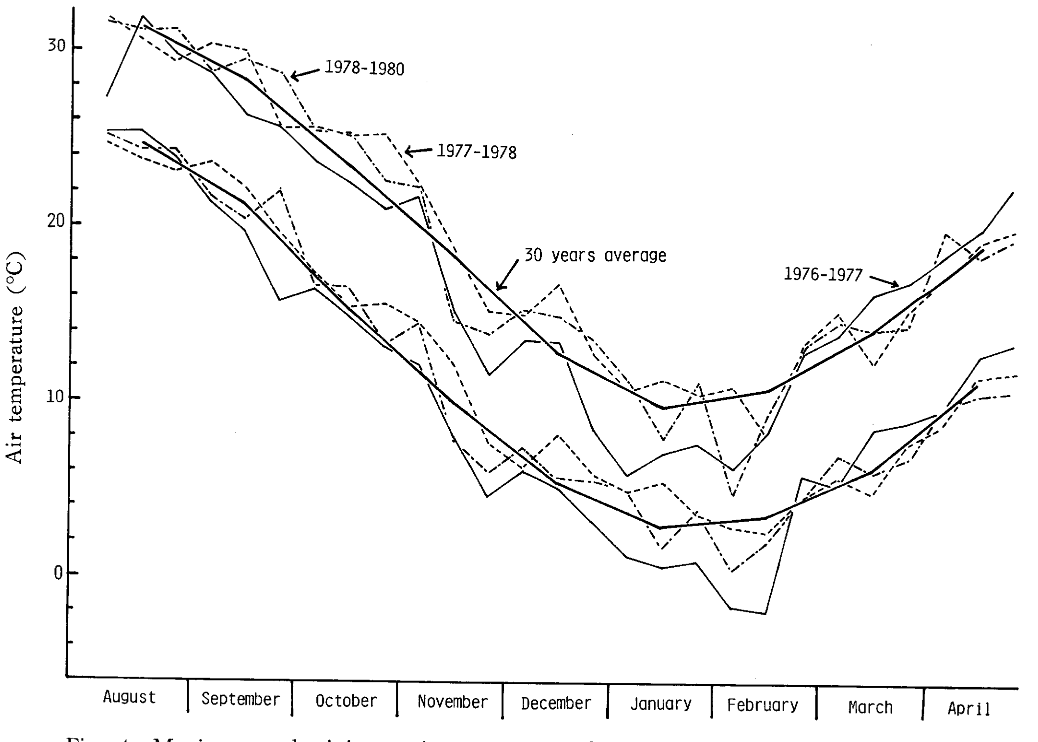


Fig. 4. Maximum and minimum air temperature of ten days averages at Nagasaki from August to Arpil in 1976–1980, with 30 years averages from 1941–1970 at Nagasaki. Obtained from the Nagasaki Marine Observatory.

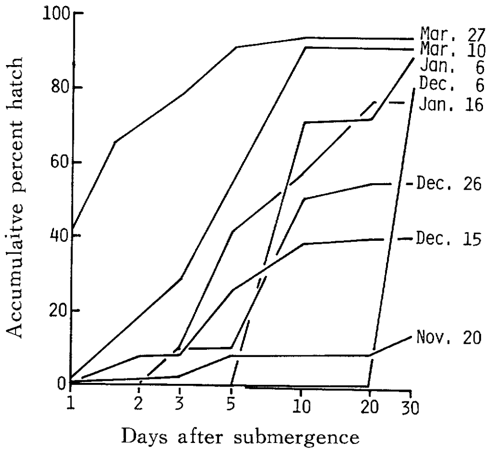


Fig. 5. Hatching of diapausing eggs of *Aedes albopictus* kept wet under outdoor condition and transferred to 25 C under 16 hr daylength on the date shown in the figure.

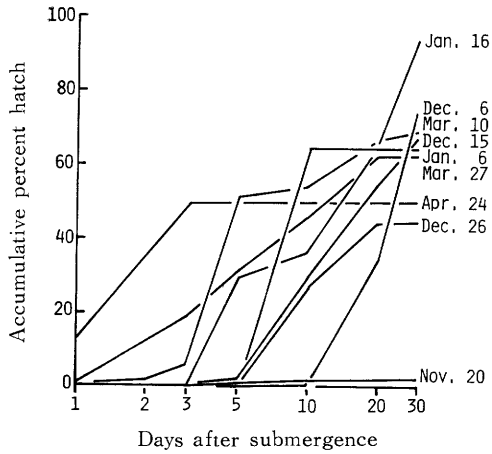


Fig. 6. The same as in Fig. 6 excepting that diapausing eggs were kept dry, and submerged in water after transfer to 25 C under 16 hr daylength.

(5) The overwintering possibility of non-diapausing eggs.

Non-dianpasuig eggs from females resulting from 25 C under 16 hr daylength were kept wet or dry and submerged in water in the next spring. The rates of egg hatching were given in Table 4. Some eggs laid in December, 1979 and kept at 15 C for one day before they were transferred to outdoor hatched in the next spring, whether they were kept wet or dry. In the case of eggs laid in October, 1977 and kept dry, the hatching rate in the next spring was more than 70%. But any eggs that were laid in December, 1976 or January, 1977 and kept wet during winter did not hatch. It is not certain whether this failure of hatch was due to the particularly cold winter of 1976/1977 (see Fig. 4) or to the complete dryness of the eggs during the period. But it is considered that non-diapausing eggs can stand, to some extent, coldness of winter.

Table 4. Effect of winter cold on the hatching till late May of non-diapausing eggs of *Aedes albopictus*

Date of oviposition	Dec. 24, 1976	Jan. 20, 1977	Oct. 25, 1977	Dec. 24, 1979	Dec. 24, 1979
Date of transfer to outdoors	Dec. 28, 1976	Jan. 24, 1977	Oct. 29, 1977	Dec. 28, 1979*	Dec. 28, 1979*
Period of keeping eggs dry	None	None	Oct. 29, 1977–Apr. 23, 1978	None	Dec. 28, 1979–Mar. 20, 1980
Hatching rate (%)	0.00	0.00	73.03	46.04	28. 27

*Eggs were kept at 15 C under 10 hr daylength for one day before they were transferred to outdoors.

DISCUSSION

The distribution of *Ae. albopictus* spreads from Southeast Asia to the northern part of Honshu Island of Japan. In Amami-Oshima Island located south of Kyushu Island, and southward, the immature stages and adults of *Ae. albopictus* are seen through the year (Takenokuma, 1966; Wada et al., 1976). But in Kyushu Island and northward, any of larvae, pupae and adults can not overwinter, because the cold in winter is too severe for their survival, and only eggs survive winter (Makiya, 1968; Mori and Wada, 1978). When the females of *Ae. albopictus* were reared in the condition at low temperature under short photoperiod, they laid eggs in diapause (Wang, 1966; Imai and Maeda, 1976). This was confirmed in the present experiment, too. It was also suggested that the females that have emerged in autumn lay diapausing eggs in the field.

The eggs in the condition of diapause do not hatch, even when the temperature goes up, or rather high temperature days continue in autumn or winter. The time for diapausing eggs to begin to appear in outdoor experimental conditions is coincident with the time of decrease in the number of newly hatching larvae in natural populations in the fie'd.

A few eggs laid on August 30 hatched in the next spring. As these eggs are considered to be in diapause, it seems that diapausing eggs begin to be laid in company with non-diapausing eggs as early as in August.

In this experiment, a few eggs that were laid on October 1 hatched during autumn, but they would not complete their development and die in winter. So, their hatching may be insignificant in the population dynamics in the field.

All eggs of the univoltine species of aedine mosquitoes are in the condition of obligatory diapause in winter. On the other hand, the multivoltine species lay eggs in facultative diapause under certain euvironmental conditions. If in the multivoltine species the sensitivity to the change of environment is different within the population, the extent of facultative diapause in eggs would be various. For example, *Ae. albopictus* females from 21 C under 10 hr daylength mostly lay diapausing eggs. If eggs at 21 C under 10 hr daylength were transferred to 25 C under 16 hr daylength just after oviposition, more larvae would hatch than when kept continuously at 21 C under 10 hr daylength. The difference of the hatching rate was probably caused by the hatching of eggs in the condition of weak diapause. When eggs were laid in the outdoor condition in autumn and transferred to 25 C under 16 hr daylength before winter, a small number of eggs hatched over a long period. Therefore, it is considered that *Ae. albopictus* lay eggs in various conditions from complete diapause to non-diapause in autumn.

It is well known that *Ae. aegypti* do not lay diapausing eggs even at short photoperiod or at low temperature. But, when the eggs were kept dry during winter, a few eggs overwintered in outdoor conditions in Nagasaki (Ofuji, 1963). In the case of *Ae. albopictus*, many non-diapausing eggs from females resulting at 25 C under 16 hr daylength can overwinter, if they kept dry. From this result, it seems that some non-diapausing eggs that are laid in early and mid September in the field have a chance to succed in overwintering, when they are in a dried condition. Of course, most of overwintering eggs are in diapause by the maternal effect of low temperature and short shotoperiod. Because even non-diapausing eggs have the ability, to some extent, to withstand the cold, the advantage for the eggs to enter diapause lie mainly in prohibiting them from hatching in autumn lest larvae should die during cold winter.

There is a variation in the extent of diapause in the egg, from females reared at low temperature under short photoperiod, while at high temperature under long photoperiod resulting females laid a few diapausing eggs, besides a number of non-diapausing ones. It can therefore be said that *Ae. albopictus* has a variety in the physiology of diapause. This is advantageous for the maintenance of species and the spread of distribution.

REFERENCES

1) Abe, I., Iwasawa, S. & Nakahashi, Y. (1941): [The experimental studies on the overwintering of *Aedes albopictus*, vector mosquito of the dengue fever, and on early eradication of this mosquito.] Navy Med. J., 32(5), 333−341. (in Japanese)

2) Beckel, W. E. (1958): Investigations of permeability, diapause, and hatching in the eggs of the mosquito *Aedes hexodontus* Dyar. Canad. J. Zool. 36, 541−554.

3) Hong, H. K., Shim, J. C., Shin, H. K. & Yun, Y. H. (1972): Hibernation studies of forest mosquitoes in Korea, 1971. Trop. Med. News 38, 4−8.

4) Horsfall, W. R., Lum, P. T. M. & Henderson, L. M. (1958): Eggs of floodwater mosquitoes (Diptera : Culicidae). V. Effect of oxygen on hatching of intact eggs. Ann. Ent. Soc. Amer. 51, 209−213.

5) Imai, C. & Maeda, O. (1976): Several factors effecting on hatching of *Aedes albopictus* eggs. Jap. J. Sanit. Zool. 27(4), 363−372. (in Japanese with English summary)

6) Makiya, K. (1968): Population dynamics of mosquito larvae overwintering in southern Japan. Jap. J. Sanit. Zool., 17(4), 232−235. (in Japanese with English summary)

7) Matsuzawa, H. & Kitahara, N. (1966): Some knowledge on the biology of *Aedes albopictus* Skuse. Jap. J. Sanit. Zool., 19(4), 223−229. (in Japanese with English summary)

8) Mori, A. & Wada, Y. (1978): The seasonal abundance of *Aedes albopictus* in Nagasaki. Trop. Med., 20(1), 29−37.

9) Nakata, G., Matuo, K. & Ito, S. (1953): Ecological studies on mosquitoes about Kyoto City. (I) On the successions of larvae breeding in minute inland water found in graveyards and bamboo groves. Jap. J. Sanit. Zool., 4(3, 4), 67−72. (in Japanese with English summary)

10) Ofuji, K. (1963): Possibility of establishment of yellow fever mosquitoes, *Aedes aegypti* (L.) in Japan. 2. Cold-and dry-resistance of eggs, ecological zero point of larvae, development of

larvae in early spring, and general summary. Endem. Dis. Bul. Nagasaki Univ., 5(4), 209−222.
11) Takenokuma, K. (1966): Ecological study on *Aedes albopictus* in Amami-Oshima. Jap. J. Sanit Zool., 17(2), 108−112. (in Japanese with English summary)
12) Wada, Y., Mogi, M., Oda, T., Mori, A., Suzuki, K. & Miyagi, I. (1976): Notes on mosquitoes of Amami-Oshima Island and the overwintering of Japanese encephalities virus. Trop. Med., 17(4), 187−199.
13) Wang, R. I. (1966): Observation on the influence of photoperiod on egg diapause in *Aedes albopictus* Skuse. Acta Ent. Sinica, 15(1), 75−77. (in Chinese with English summary)

長崎産ヒトスジシマカの卵休眠と越冬について

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ヒトスジシマカの休眠卵は低温短日下で育った雌成虫によって産まれるが，卵休眠を誘起する温度，日長条件に最も感受性が高いのは蛹と成虫期であった．長崎では9月中旬から休眠卵が多くなり，10月下旬には産まれる卵がすべて休眠卵であった．休眠卵は翌年2月頃覚醒するまでは孵化することはなく，屋外では3月中旬から5月下旬にかけて孵化する．しかし，非休眠卵でもある程度の耐寒性を備えており乾燥していたりすると越冬も可能である．それゆえ休眠卵は秋に卵が孵化し冬の寒さで幼虫が死滅することを防ぐのに役立っていると考えられる．

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