REVIEW

PROGRESS IN STUDIES ON THE OVERWINTERING OF THE MOSQUITO *CULEX TRITAENIORHYNCHUS*

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Abstract. A detailed review is presented of half a century's research in Japan, Korea and the temperate zone of China on the overwintering of *Culex tritaeniorhynchus*, the main vector of Japanese encephalitis. The evidence indicates that in the temperate zone, two types of overwintering strategy can be distinguished in *Cx. tritaeniorhynchus*, *ie* survival by adults *in situ* in a dormant state, or migration by individuals which are in reproductive diapause. The authors contend that the former strategy is not very effective, and individuals which overwinter in this way do not constitute the main source of the next season's mosquito populations. On the contrary, new mosquito populations seem to be established by groups of adults which appear suddenly, early in the year, and which are probably windborne migrants from the south. It is concluded that further research into the mode of overwintering of *Cx. tritaeniorhynchus* should focus on the *effective* source of the populations which appear in temperate areas in early spring, and that efforts should be made to increase our understanding of the migration patterns of this species.

INTRODUCTION

The flaviviral disease, Japanese encephalitis (epidemic encephalitis B), continues to be an important public health problem in eastern and southern Asia. The virus has been detected over a geographical area extending from the west coast of India eastwards to the Philippines, and from Indonesia in the south to southeast former USSR and Japan in the north (Rosen, 1986). The disease is very largely transmitted by mosquitos of the genus *Culex*, and by far the most important vector is *Culex tritaeniorhynchus*, a predominant mosquito in paddy-rice cultivations (Sirivanakarn, 1976; Zhang *et al*, 1982). To the south of about longitude 25°N, the species is active all the year round. However, in temperate areas, such as Japan, Korea and the Yangtze river basin and the area to the north in China, the mode of overwintering of *Cx. tritaeniorhynchus* is not clear, and there is controversy about the source of mosquitos of this species which appear in early spring. There are two opinions in this connection: the majority of authors appear to support the view that local overwintering by adult mosquitos is important (*eg* Bullock *et al*, 1959; Omori *et al*, 1965a; Wada *et al*, 1968, 1973; Buei *et al*, 1986), while others have suggested that there is significant seasonal invasion by migrants from elsewhere (Ree *et al*, 1976; Hayashi *et al*, 1978; Ming *et al*, 1989, 1993b). The question of how *Cx. tritaeniorhynchus* overwinters is central to our understanding of the epidemiology of Japanese encephalitis in temperate regions, and to the still unsolved problem of how the virus itself survives the winter. Here we review in detail the literature on the overwintering of *Cx. tritaeniorhynchus* in the temperate zone, dating from the late 1940s onwards (including evidence from the senior author's field observations and investigations over the last eleven years), and put forward the concept of the *'effective source'* of a mosquito population. In other words, the importance of an overwintering mechanism should be assessed by the contribution it makes to the new season's *Cx. tritaeniorhynchus* populations.

EFFECTIVE OVERWINTERING AND EFFECTIVE SOURCES OF MOSQUITOS

Some mosquito species are able to overwinter in temperate areas as hibernating adults-usually inseminated nulliparous females which have built-up a large fat reserve (Service, 1993). In some cases mosquitos may be in a 'quiescent' state where activity and development are arrested by the *direct* effect of adverse conditions (*eg* low winter temperatures), and activity will restart if conditions become temporarily favorable, *eg* individuals may emerge and feed. In other species, the overwintering generation appear to enter a true diapause which persists even if the winter environment becomes temporarily favorable. If *Cx. tritaeniorhynchus* females can survive cold winters locally in East Asia (*ie* they can survive through January and February) and then reproduce in spring, this would represent an *effective overwintering* strategy, and the source of mosquitos which establishes the new season's population could be described as an *effective source.*

REVIEW OF OVERWINTERING SURVEYS IN EAST ASIA

Japan

The overwintering of mosquitos has been investigated extensively in Japan since the late 1940s. In early studies (Anonymous, 1949-1956), no *Cx. tritaeniorhynchus* were found during eight years of surveys. Similarly, Makiya *et al* (1971) reported that among 900 mosquitos collected in nine cave dwellings in Aichi Prefecture, Japan, from November 1968 to May 1969, 67% were *Cx. pipiens pallens,* 13% were *Cx. hayashii,* and the other 20% included *Anopheles sinensis, Cx. orientalis* and *Cx. vorax,* but no *Cx. tritaeniorhynchus* were found.

Bullock *et al* (1959), however, reported that during winter surveys in 1957-1958, single specimens of *Cx. tritaeniorhynchus* were collected on 7, 13, 14 January, and 18, 21 February 1958, respectively, from brush- and wood-piles and a cave in the Kanto Plain area. The temperature in overwintering places ranged from 14° down to -1°C, but only a few days were below 0°C. The specimen caught on 21 February was placed in an insectarium, where it engorged on rabbit blood on 26 February, but it died on 6 March without ovipositing. A dissection revealed that its ovary was developed and fertilised, but no abdominal fat body had developed.

Wada *et al* (1968) reported that 8, 4 and 48 *Cx. tritaeniorhynchus* were collected respectively from an air-raid shelter in the south of the Izu Peninsula (local temperature 11.8°C) on 27 January 1967, from a stone pit in southeastern Japan (8.5°C) in 28 January, 1967 and from a stone pit, an air-raid shelter and an old tunnel in northwestern Japan in 29 January 1967 (10.4°C). All 46 females dissected were nulliparous, 45 with their ovarian development at stage I and one individual with an ovary at stage II. Wada *et al* (1973) captured a single *Cx. tritaeniorhynchus* by dry ice trapping in rice fields on 19 February 1966, and on 27 and 28 January 1969, respectively. In contrast, a total of 3,986 *An. sinensis* were captured by dry ice trapping, and by aspirator in pig sties and cow sheds, during the December to February period. As a result of the above-mentioned surveys of overwintering mosquitos in Japan, a total of only 68 *Cx. tritaeniorhynchus* have been collected in January and February. The most notable finding was the collection of 60 *Cx. tritaeniorhynchus* on three days in January 1968 in the Izu Peninsula. We note, however, that these females were not diapausing with their ovaries in the N stage, but appeared to be overwintering in a quiescent state, which does not seem to happen repeatedly every year in *Cx. tritaeniorhynchus* as it does, for example, in *An. sinensis.* Moreover, the occasional survival of a few quiescent mosquitos in some years seems unlikely to provide an effective mosquito source for the next generation.

Observations of mosquito activity in Yakushima and Tanegashima Islands (about 30°N) during the winter of 1991-1992 revealed that some mosquito species which overwintered in diapause in Kyushu (Japanese mainland) could continue to reproduce in these islands. No *Cx. tritaeniorhynchus* were found however (M. Mogi, personnel communication). Further south still, on the subtropical island of Amami (28°N), fairly large numbers of female *Cx. tritaeniorhynchus* have been found in winter (*eg* 1083 females caught between 3-18 February 1973) (Hayashi *et al,* 1975). The females caught in February 1973 were infected with the Japanese encephalitis virus, but in another year (1974) the virus was not detected in mosquitos until early July (Hayashi *et al,* 1975).

Korea

Whang (1961) found that among the 5,256 mosquitos captured between November 1959 and April 1960, mainly around Seoul, 45 were *An. sinensis,* 13 were *An. sineroides,* 5,178 *Cx. pipiens pallens* and *20 Cx. orientalis.* No *Cx. tritaeniorhynchus* were found. Similarly, Ree *et al* (1976) reported that no *Cx. tritaeniorhynchus* was found during intensive searches of a great variety of overwintering places during the November to February period, 1969 to 1974. Ree *et al* (1976) also mention earlier investigations, also unsuccessful, carried out each winter from 1960. It was concluded that any hibernating populations of *Cx. tritaeniorhynchus* must be extremely small, and that this species might immigrate into the Seoul area from outside.

China

Since 1978, extensive surveys of overwintering mosquitos have been carried out in China. The results from the areas to the north of the Yangtze valley were essentially similar to those from Japan: that is, in contrast to species like *An. sinensis*, only very small numbers of *Cx. tritaeniorhynchus* were captured early in the year, and in some years none were caught. Xue *et al* (1989), for example, reported that four *Cx. tritaeniorhynchus* were found in the straw wrapping around orange trees during a survey of overwintering mosquitos in the Wuxi and Yangzhou areas of Jiangsu Province in March 1987. Dissection of these females showed that their ovarian follicles had degenerated. Another *Cx. tritaeniorhynchus* was found in a straw stack in Fengxian, Shanghai, but no dissection report was available for this individual.

Ming *et al* (1993a) reported that six engorged *Cx. tritaeniorhynchus* were captured in pig sties in Fengxian by light trap in the evening of 11 February 1987. Their ovaries were all at the same developmental stage (Christophers' stage I). Ninety-three *An. sinensis* were captured in the same evening, and these showed differing degrees of ovarian development (of the 50 dissected, one was at stage I, 14 were at stage IIa, 34 at stage IIb, and three were parous). A meteorological analysis of this period showed that to the south of the Yangtze River, the 850 hpa (*ca* 1500 m) level was under the control of a southwest airflow with wind speeds at this altitude up to 12 m/s. The highest temperature between 8-10 February was 22.5°C, during which 12 *An. sinensis* were captured (two at stage IIa, four at stage IIb, three at stage IIIa, three at stage IIIb). On 11 February itself, the highest temperature was 27.6°C (unusually warm weather for this time of the year, and rarely seen during the last hundred years on meteorological records from Shanghai). The six *Cx. tritaeniorhynchus* appeared between 20:00 hours on 11 February and 08:00 hours on the 12th, when a cold front passed through. Another *Cx. tritaeniorhynchus* was trapped on the evening of 22 February 1988, when the highest temperature that day was 14.2°C.

Among the above-mentioned 12 *Cx. tritaeniorhynchus* reported in January and February, four showed follicle degeneration, six were associated with the cold front (and might well have had an exotic source) and two were not dissected and their ovarian development was not clear. Therefore, only the latter two mosquitos could conceivably have been overwintering in a state of diapause. We also note that from 12 February to 31 March 1987 there were still seven days with the lowest temperature below 0°C, and the minimum temperature was -2.7°C (28 February), so it was likely that the small numbers of *Cx. tritaeniorhynchus* present in the area may well have suffered further mortality. Again, it seems unlikely that locally overwintering *Cx. tritaeniorhynchus* would represent an effective mosquito source.

ANALYSIS OF THE APPEARANCE OF MOSQUITOS IN MARCH AND APRIL IN JAPAN AND CHINA

Observations in Japan

In the Nagaski area, Omori *et al* (1965a) found a *Cx. pipiens pallens* on 26 February 1965, hibernating in a hole in an embankment caused by the digging out of a tree stump. They thought that this might indicate that cracks in embankments and crevices in stone walls around terraced fields might be the main overwintering sites of *Cx. tritaeniorhynchus*. Also a specimen of *Cx. tritaeniorhynchus* was seen alighting on the stone wall of a dike on 18 March 1965, when the temperature was 15.4°C (Omori *et al*, 1965a). The dike was 3m wide and covered by thicket of bamboo grass, and it was suggested that hibernating mosquitos might come from these dikes. In order to confirm this, dikes and stone walls between fields were covered with emergence traps made of mosquito netting. However, only eight unfed mosquitos and one fed *Cx. tritaeniorhynchus* were captured by this method, while at the same time, a large number (total 203,022) of this species were captured by using the dry ice method, light trapping or repeated searching in cattle sheds, pig sties and chicken coops. Buei *et al* (1986) reported that similar results were obtained in Osaka Prefecture, Japan. During March to May of 1968-1970, no *Cx. tritaeniorhynchus* were captured in emergence traps placed over banks and walls between rice fields. However, a total of 5,075 *Cx. tritaeniorhynchus* were captured in dry ice traps during April to early June of the years 1967-1974.

We hold that if cracks in dikes or walls around paddy fields really were the main overwintering places of *Cx. tritaeniorhynchus,* large numbers of individuals would certainly be captured in the emergence traps, contrary to the above results. Adults are common from March and April, however, as large numbers can be caught by various methods, such as dry ice trapping, light trapping and animal baiting. Wada *et al* (1973) suggest that the reason for the difficulty in finding *Cx. tritaeniorhynchus* early in the year is that most of the overwintering mosquitos are diapausing at that time, and they do not emerge to take a blood meal even on warm days. They considered a seven year period (1965-1971), during which the cumulative daily mean temperature from 1 January to the day of first appearance of *Cx. tritaeniorhynchus* ranged from 621 to 710°C (Wada *et al,* 1973). However, we have examined meteorological data for the dates of first appearance in early spring (as reported by the Japanese authors) for six years between 1966-1971 (no 1969 data), and found that these all coincided with the date of the passage of a weather front. This is not merely a coincidence, because it has been repeatedly confirmed by over eleven years of observations in early spring in the Shanghai area (see below). Thus the day of "awakening from diapause" as defined by the Japanese scientists is equivalent to the "day of sudden appearance or abrupt increase in numbers" described by Chinese authors.

Observations in China

Ming *et al* (1989) described the results of seven successive years of spring observations in Shanghai, China, which revealed that days of first appearance, 'sudden appearance' and 'sudden increase in numbers' were all associated with the passing of a front. These characteristics are in keeping with the pattern of northward migration in spring of other migratory insects in East Asia (*eg* Zhang, 1980; Kisimoto and Sogawa, 1995; Zhou *et al,* 1995). Furthermore, the authors have found that the *Cx. tritaeniorhynchus* females which suddenly appeared or increased in numbers in early spring were all at the same stage of ovarian development (*ie* stage I or stage II), in common with the immature ovaries found in most migrating insects (Johnson, 1969). In contrast, the *An. sinensis* which appeared during the same period showed quite different pattern of ovarian development (*ie* stages II-V), which is much less indicative of migration.

In general, we believe that the characteristics of (a) the small numbers of dormant overwintering mosquitos, (b) the uncertain overwintering sites, and (c) the relationship between the sudden increases in mosquito numbers in early spring and the movement of weather fronts, all indicate that the main sources of *Cx. tritaeniorhynchus* are not local overwintering populations.

EXPERIMENTS ON THE OVERWINTERING OF *CULEX TRITAENIORHYNCHUS*

In experiments carried out between December 1964 and February 1965, Omori *et al* (1965b) found that *Cx. tritaeniorhynchus* could successfully overwinter in cellars, animal houses and in corridors. In the experimental sites, the temperature range was 4.2-11.0°C and the relative humidity was 69.3-84.6%. The mosquitos were fed with 5% glucose, and their survival rate was found to be 20%. In experiments where the temperature was lowered down to 0°C, Zhang *et al* (1993) reported that all the *Cx. tritaeniorhynchus* died within 80 days, but diapaused *Cx. pipiens pallens,* on the other hand, had a survival rate of 69.5%. Xue *et al* (1989) described an experiment conducted in a simulated overwintering site in Fengxian, Shanghai, between September 1987 and April 1988. The results showed that all the mosquitos from pig sties and haystacks died, and those from ground pits had a survival rate, up until 10 March, of only 3.8%. All of the survivors were unfertilised females and could not reproduce their offspring. In areas around rice fields, only a few adult mosquitos were captured by the end on November, while in haystacks and pigsties only one engorged mosquito was captured in March (stage III follicles). The authors consider that neither the mosquitos captured before December nor the individual caught in March can be considered as representing an effective overwintering population, since the former were not shown to have survived the severest winter months (January and February), while by March 1987 the postulated first wave of mosquito immigration had occurred.

The above experiments demonstrated that *Cx. titaeniorhynchus* can overwinter successfully under experimental conditions, albeit with a relatively low survival rate (20%). However, the survival rate in simulated field overwintering experiments was much lower (3.8%), and it would appear that this species does not survive in places where temperatures fall below 0°C.

OBSERVATIONS OF ADULT MOSQUITOS IN LATE AUTUMN

Kawai (1969) and Wang *et al* (1989) pointed out that under conditions of short daylength, female *Cx. tritaeniorhynchus* appear to enter a reproductive diapause with the ovaries at the N stage. The ratio of first follicle to germarium area was less than or equal to 1.0, and the length of the first follicle was less than or equal to 40μ (Wang *et al,* 1989). Ming *et al* (1993a) reported that when mosquito larva and pupae collected in nature were bred under conditions of naturally declining room temperature and reduced illumination, females at stage N would appear in the last ten days of September. However, such diapausing mosquitos were not collected in the field. Ito *et al* (1986) were able to collect *Cx. tritaeniorhynchus* in Osaka in early September by using the dry ice method: numbers peaked in the middle of September, but then fell to zero in the first ten days of October. This indicated that the new generation of adults emerging at the end of September do not search for a bloodmeal at this time, and the fate of the stage N mosquitos is still unclear. As we have seen, no effective *Cx. tritaeniorhynchus* overwintering sites have been found, even after several decades of investigations, and no diapausing females with their ovaries in the N stage have been observed in field studies up to the present.

THE MIGRATION OF *CULEX TRITAENIORHYNCHUS* IN EASTERN CHINA: EVIDENCE FROM AERIAL SAMPLING WITH A BALLOON NET

Specimens of *Cx. tritaeniorhynchus* have recently been caught in a net suspended from an aerodynamically-shaped balloom (kytoon) at Jiangpu (Jiangsu Province) in September 1990 and at Dongxiang (Jiangxi Province) in October 1991, at heights ranging from 80-380 m above ground (Ming *et al,* 1993b). Most of the females which were dissected were found to have ovaries at stage N, and as mentioned above, females in this stage have not been found during ground investigations in either Japan or China. Most of the specimens caught at Dongxiang were flying in the subsiding air behind a cold front. These findings are significant because they provide direct evidence of the long-range southward migration of *Cx. tritaeniorhynchus* in autumn.

In high latitude regions there are basically two adaptive strategies for insects to pass the winter: one is to overwinter *in situ* in an inactive dormant state, such as occurs in *Cx. pipiens pallens.* The other strategy is long-distance migratory flight, usually by sexually immature individuals, to overwintering areas at lower latitudes. *Cx. tritaeniorhynchus* appears to belong to the latter case, with the N stage females embarking on high-altitude windborne migration, which in late autumn in eastern China would tend to result in southward displacement on the northeast monsoon winds. Movement in the opposite direction would tend to occur in spring and early summer, with infectious females being displaced to the north with the then-prevailing southwest monsoon winds.

OTHER EVIDENCE FOR THE WINDBORNE MIGRATION OF *CULEX TRITAENIORHYNCHUS*

Captures of insect species on ships far from land show that at least some individuals of the species concerned can maintain continuous flight for a long period, and are thus capable of long-distance migration. Asahina (1970) reported that single specimens of *Cx. tritaeniorhynchus* were caught on 21 September and 18 October 1967, and 10 September 1968, respectively, on board a weather ship in the northwest Pacific, 500 km south of Cape Shionomisaki. The wind directions in September to October 1967 and 1968 when the mosquitos were captured were from NNW, N or NE, and thus these movements were similar to southward autumn migration of *Cx. tritaeniorhynchus* mentioned above. On 30 June 1969, 91 *Cx. tritaeniorhynchus* were collected from fisheries survey boats 200 km to the east of Shanghai (Asahina, 1970). On this day the wind direction was WSW, and so this appears to be an example of the northward migration of this species. Other records of the capture of *Cx.*

tritaeniorhynchus on the East China Sea (on July 24/25 1973 and on 1 July 1977) are given by Hayashi *et al* (1978).

Another type of evidence for long-range wind-assisted movement is the capture of insects high in the air, or on mountain tops. Apart from the *Cx. tritaeniorhynchus* specimens caught by the aerial net suspended from a balloon (described above), we note that Li *et al* (1986) caught one individual of this species in a net attached to a remotely-controlled aircraft at a height of 500 m above Xuzhou (Jiangsu Province) on 8 September 1980. This coincided with the southward migration of *Cx. tritaeniorhynchus* in autumn. On 15 June 1985, four *Cx. tritaeniorhynchus* were caught in a net at high elevation on Huangshan Mountain (1,840 m): this appears to be a further example of the northward movement of this species (Wang *et al*, 1989).

Incidentally, *Cx. tritaeniorhynchus* has also been caught at altitude in the tropics. Reynolds *et al* (1996) caught this and other *Culex* species in a balloon-supported net at 150 m height in West Bengal, India.

CONCLUSION

Based on the present review on research on the overwintering of *Cx. tritaeniorhynchus* in the temperate zone, the authors conclude that this mosquito can overwinter in two ways, namely, by local hibenation or by seasonal migration. However, in spite of much effort over many years, only a few locally hibernating *Cx. tritaeniorhynchus* have been found in January and February in some specific years. Therefore, it seems most unlikely that the individuals surviving the winter *in situ* provide the main source of the new season's populations in northern areas. The observation that few dormant mosquitos survive the winter in the wild, coupled with the failure of *Cx. tritaeniorhynchus* to survive in simulated overwintering experiments, does not explain the phenomena of repeated sudden increases of adult *Cx. tritaeniorhynchus* densities in March and April in both Japan and China, which do not occur in other species of mosquito. Furthermore, it has been confirmed that in the Shanghai area, such sudden increases are associated with weather fronts and are characteristic of immigration by *Cx. tritaeniorhynchus* from the south. It is the progeny of these early spring immigrants which develops into the new mosquito population in any given year. The origins of these migrants and the characteristics of their migration remain to be elucidated. Such studies may also explain the long-standing mystery of how and where the Japanese encephalitis virus survives the winter.

Our research has demonstrated that the adult females emerged in late autumn every year in temperate zone are mainly in reproductive diapause with the development of their ovarian follicles arrested at the N stage. However, these females have not been found in field surveys, except by the aerial netting of individuals actually engaged in migration. This demonstrates that the majority of stage N diapause mosquitos probably undertake southward migration in late autumn. Further research is required to identify the destinations of these migrants.

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