ZOOPHILISM, HIBERNATION AND APPEARANCE OF MOSQUITOES OF JAPAN

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1. Zoophilism of Japanese mosquitoes

a. Observation of zoophilism by animal trap methods.

The zoophilism of mosquitoes, that is to say, which mosquito species bite predominantly on what animals, have been studied in details in anopheline mosquitoes by several foreign authors; as for the Japanese mosquitoes, little was known before the observations were done by us in Okayama, 1947, in connection with the activities of the Virus Commission, U.S. Army, directed by Dr. A.B. Sabin.

Our observations on this problem were made chiefly by the method of collection of mosquitoes by animal bait traps; in this, we kept various animals (goats, chickens, rabbits, etc.) in a cage, covered it with a single bed mosquito net, and made a small opening on one side

Table 1. Ratio of mosquito species in various traps.

	Number of tests	Total number	Mean number	Percentage of mosquito species						
				tri.	vish.	pip.	bit.	Ano.	Arm.	Other species
Human	13	1,665	125.1	72.6	0.6	23.1	0.4	2.8	0.1	0.4
Chicken	17	1,424	84.2	52.8	0.2	42.6	4.1	0.3	0.1	0.9
Canary	1	18	18.0	66.7	0	33.3	0	0	0	0
Rabbit- Chicken	9	5,295	599.3	86.4	1.6	9.2	0.7	1.6	0.1	0.4
Rabbit	6	1,435	239.2	92.5	3.0	3.0	0.1	1.2	0	0.1
Goat	15	3,816	255.4	85.9	1.5	3.2	0.9	8.1	0.2	0.1
Dog	3	222	74.4	63.1	0.5	26.1	2.3	2.8	1.4	4.1

Note: tri. (*Culex tritaeniorhynchus* Giles); vish. (*Culex vishnui* Theobald); pip. (*Culex pipiens pallens* Coquillett); bit. (*Culex bitaeniorhynchus* Giles); Ano. (*Anopheles hyrcanus sinensis* Wied.); Arm. (*Armigeres obturbans* Walker)

Table 2. Mean number of each mosquito species which actually
bite in a trap in one night.

	Number of test	Mean number	tri.	vish.	pip.	bit.	Ano.	Arm.
Chicken	13	28.6	5.1	0.2	21.2	2.0	0.1	0.0
Canary	1	9.0	3.0	0.0	6.0	0.0	0.0	0.0
Chicken- Rabbit	9	513.4	448.1	6.2	46.7	3.8	8.4	0.2
Rabbit	6	130.4	121.7	4.2	2.3	0.2	2.0	0.0
Goat	15	123.9	101.8	4.7	0.9	0.2	16.2	0.1
Dog	3	68.0	45.3	0.3	18.7	0.7	2.0	1.0

of it, so as to let the mosquitoes enter in the trap to bite the animals during the night; the openings were closed before sunrise, and the mosquitoes entered in the trap were collected with sucking tubes in the next morning. This trap was very successful to collect night-biting mosquitoes, and with chicken-rabbit traps, for example, more than 1,000 mosquitoes were collected in one night. It was, however, not adequate for the collection of day-time biting species, as most of the mosquitoes fled away from the same opening. Therefore, our data on the zoophilism of mosquitoes by this method were naturally restricted only to the night-time biting species in Okayama. The observations were continued from July to September, 1946; about two traps were put every night in a garden of the Medical College of Okayama, adjacent to the rice-field, using various animals as baits. The results of these collections are shown in Tables 1 to 3.

In table 1, the ratio of total mosquitoes which entered in various traps are shown; the mean number of mosquitoes collected in one night was highest in rabbit-chicken traps. (Two rabbits and three chickens were used in a single trap.) The number in goat or rabbit traps was also high, and always much higher than human or chicken traps. This seemed to be caused by the difference of the number of *Culex tritaeniorhynchus* attracted to each animal, as this species were the most abundant ones in Okayama in that season.

The ratio of various mosquito species which entered in the traps varied with the bait used; most interesting was the opposite behavior of *Culex pipiens pallens* and *Culex tritaeniorhynchus* to the animals: the number of the latter was very high in goat or rabbit traps but relatively low in chicken traps, while that of the former species was very low in goat and rabbit, and relatively high in chicken. Human bait traps showed an intermediate value between birds and domestic animals and no such definite tendency was observed. The ratio of *Culex vishnui* was very similar to that of *Culex tritaeniorhynchus*, while that of *Culex bitaeniorhynchus* to *Culex pipiens pallens*. *Anopheles hyrcanus* showed an especially high rate in goat traps, much less in rabbits, and almost none to chickens.

In table 2, the mean number of mosquitoes which actually suck blood in each trap in one night is shown; in chicken traps, for example, the total number of *Culex tritaeniorhynchus* is in fact higher than *Culex pipiens pallens*, as shown in Table 1, perhaps owing to the extreme abundance of the former species in nature, but as most of the former species did not succeed in sucking the blood of chicken, the actual number of those which were engorged

Table 3. Blood sucking rate of mosquito species in various traps.
(Each number shows the percentage of mosquitoes which actually suck blood out of the total number of that species collected in each trap.)

	tri.	vish.	pip.	bit.	Ano.	Arm.
Chicken	11.4	17.6	52.3	56.9	5.6	/
Canary	25.0	/	100.0	/	/	/
Chicken-Rabbit	86.0	64.3	86.5	97.0	89.4	28.5
Rabbit	54.9	58.0	32.6	50.0	82.9	/
Goat	46.7	12.1	11.1	8.3	78.9	33.3
Dog	97.1	100.0	86.2	40.0	100.0	100.0

with blood was higher in the latter species. On the other hand, the number of *Culex pipiens pallens* which actually succeeded to suck blood in goat or rabbit traps was extremely low compared with that of *Culex tritaeniorhynchus*.

By the observation of the total number and the number of mosquitoes which actually engorged with blood, we can calculate the engorged rate of each species in various traps. This is shown in Table 3. The difference between *Culex pipiens pallens* and *Culex tritaeniorhynchus* is also shown in this Table; the former was high in chicken and very low in goat traps, while the latter was just the opposite. *Culex vishnui* was similar to the latter, and *Culex bitaeniorhynchus* to the former. The engorged rate of *Anopheles hyrcanus sinensis* was always much higher than *Culex tritaeniorhynchus* even in goat or rabbit traps and this species seemed to be much more voracious in their blood sucking action than the latter.

By these facts, we can conclude that, among the night-time biting species in Okayama, *Culex pipiens pallens* or *Culex bitaeniorhynchus* have a special affinity to chicken, but not to goat or rabbit; these mosquito species are not only attracted to the former predominantly, but also more easily succeed to bite chicken than goat or rabbit. Therefore, the actual number of mosquitoes which bite chicken is higher in *Culex pipiens pallens* than in *Culex tritaeniorhynchus* even though the absolute number in nature of the latter mosquito species is much greater than the former. It is just the contrary in the case of *Culex tritaeniorhynchus* or *Culex vishnui*. They are attracted predominantly to rabbits or goats (and also to horses or cattles), and more easily succeed to bite those animals, but less attracted or have more difficulty in biting chicken. *Anopheles hyrcanus sinensis* shows special affinity to goats (and also to large animals) but not to birds; the engorged rate of this species is highest among common mosquito species in Okayama.

As for the human beings, it seemed to be intermediate in this respect between domestic animals and chickens, since of mosquitoes which actually did biting this period, 61 % were *Culex tritaeniorhynchus*, 16% *Culex pipiens pallens* and 13 % *Anopheles hyrcanus sinensis*. As shown in Table 1, the number of mosquitoes "attracted" to human beings on the average is relatively high, about $\frac{1}{2}$ of the number in goats. It must be noted, however, that the number of mosquitoes which actually bite human beings is much lower than those biting domestic animals; as will be shown in the next chapter, horses or cattles are bitten by thousands of mosquitoes in one night; human beings knows how to prevent the the attack of mosquitoes.

By the fact that both mosquito species, which attack birds or domestic animals, have equal affinity to human beings, we can imagine that the main vectors of some virus from birds to human beings may be *Culex pipiens pallens* or *Culex bitaeniorhynchus*, while from domestic animals to human beings *Culex tritaeniorhynchus*, *Culex vishnui* or *Anopheles hyrcanus sinensis*, if those have the same affinity in the development of the virus in their body. These facts may be of value in the explanation of epidemiology of Japanese B encephalitis in Japan.

With the animal trap method above mentioned, we can observe the activities of mosquitoes quantitatively, under complete objective condition; this method is also available for the ob-

servation of seasonal or climatic influence on the activities of mosquitoes; the observations of this kind have already been started this year.

b. Observation of zoophilism of mosquitoes by the collection in animal sheds.

The wall of sheds of various domestic animals are one of the best places for the collection and observation of mosquitoes. Here we can find large number of mosquitoes engorged with blood, which attacked the animals during the night before. It must be noted that the mosquitoes resting on the wall may not be the all which did the biting; most of the day-time biting species and some of the night-time biting species do not stay in the shed after engorged, but fly away outdoors. The difference of the data observed by animal trap method and the collection in animal sheds will be explained by this fact.

Table 4. Ratio of mosquito species on the wall of animal sheds.
(August, 1946, Okayama)

	Number of tests	Total number	tri.	vish.	pip.	bit.	Ano.	Arm.	Other species
Horse	5	1,558	84.0	0	0.4	0.1	15.3	0.3	0
Goat	5	1,831	77.9	0.3	0.2	0	12.0	9.6	0
Cow	4	367	78.2	1.4	1.9	0	16.6	1.9	0

Table 4 shows the ratio of mosquitoes collected on the wall of various animal sheds during August, 1946, in Okayama. *Culex tritaeniorhynchus* was the most common species in horse, cow and goat sheds; *Anopheles hyrcanus* and *Armigeres obturbans* could be found also commonly, but other species, such as *Culex pipiens pallens* were very rare. In horse barns or cow sheds of mountain districts, *Culex vishnui* was the most common species and *Culex whitmorei* was also found rather commonly. By these facts, it may be well to assume the special affinity of these species to domestic animals.

On the other hand, the collection in horse barns in the same place of Okayama Prefecture in the end of September, 1947, yielded quite different data; *Anopheles hyrcanus sinensis* was the most abundant and *Culex tritaeniorhynchus* much less. This is caused by the difference of the seasonal activity of both species; with this and other observations, we found that the active season of the latter is very short, only from July to the beginning of September, while that of *Anopheles hyrcanus sinensis* and *Culex pipiens pallens* continues from May to October at least.

Similar facts were observed in the collection of mosquitoes in animal sheds of northern parts of Japan, in the summer of 1947. In Tohoku district, the northern part of Honshu, the ratio of *Culex tritaeniorhynchus* is much less than in Okayama even in summer, and that of *Culex pipiens* and *Anopheles hyrcanus sinensis* much higher: *Aedes vexans nipponii*, which were very rare in Okayama, became one of the most abundant mosquitoes in houses of domestic animals.

In Hokkaido, the extreme north island of Japan, *Culex tritaeniorhynchus* nearly disappeared and the most abundant mosquitoes were *Anopheles hyrcanus sinensis* even in August; next to this, *Aedes vexans nipponii*. By the collection in animal sheds of various parts of Japan in various seasons, we may suggest that *Culex tritaeniorhynchus* is active only in hot weather,

while *Anopheles hyrcanus sinensis* and *Culex pipiens pallens* are active in cooler temperature. *Aedes vexans nipponii* is a species especially abundant in northern parts of Japan, and also active in low temperature.

By these observations it may well be concluded that *Anopheles hyrcanus sinensis*, *Culex tritaeniorhynchus*, *Armigeres obturbans* and *Aedes vexans nipponii* have special affinity to domestic animals, at least. *Culex pipiens pallens*, on the other hand, was extremely rare in animal sheds in Okayama, but relatively abundant in northern parts of Japan, and may take in some parts the place of *Culex tritaeniorhynchus* in northern districts or in cool season.

c. Mosquitoes which bite cold-blooded animals.

There are some mosquito species which bite only cold-blooded animals, such as frogs, snakes or lizards, and not the warm-blooded ones. They have, therefore, almost no relation to the epidemiology of human diseases, and can be excluded from the control-works of mosquitoes in the standpoint of human or veterinary hygiene. These are such species as *Culex hayhii*, *Culex rubithoracis*, *Culex pallidothorax* and *Uranotaenia bimaculata*. we suppose that *Culex rubensis*, the species we found in Hokkaido in 1947, belongs to this category.

2. Hibernation and appearance of Japanese mosquitoes.

The hibernation, appearance and seasonal occurrence of each mosquito species is a problem closely related to the epidemiology of various mosquito born diseases. For example, species which hibernate during winter as adults have the possibility of keeping virus until the next year, while in other mosquitoes, which pass through winter as larvae or eggs cannot be the reservoir of virus except when the transovarial infection occurs. The season of the appearance is also different in each species, and also closely related to the appearance and seasonal occurrence of each mosquito born diseases, which run parallel with it.

Our observation on this problem was made systematically in the year of 1947, in Tokyo, Okayama and Kumamoto.

The results will be summarized as follows.

The most interesting fact observed by us during this season is that each mosquito species in Japan hibernate during the winter in different stages of their life history. In short, most anopheline and culicine species are found in winter only as adults, while most aedine species only as eggs. There are also several species, the larvae of which are so resistant to the cold as can hibernate through the winter of Japan (*Aedes togoi*, *Armigeres obturbans* and *Tripteroides bambusa*). The nymphs, in general, are weak to the cold than larvae and even that of *Aedes togoi* die out during the winter in Tokyo districts.

a) Genus *Culex*

1. *Culex pipiens pallens* Coquillett, the most common house mosquito in urban districts of Japan, hibernates during the winter only as adults, while larvae and pupae die out in early December in natural conditions. The adults of this species often appear in our houses even in winter, when it is warm. They began to bite animals (especially chickens) from the beginning of April, under the temperature of 11° to 14°C., and the larvae were observed

for the first time in the middle of April (Tokyo). The first emergence of adults from these new larvae took place in the beginning of May (Tokyo). In Okayama, the activity of hibernated adults began in the later part of March and a great number of larvae hatched out from eggs laid by them appeared in cesspools and drains in the middle of April. The first emergence of new adults from these larvae took place on about April 25th.

2. *Culex tritaeniorhynchus* Giles, the most common house mosquito in rural districts of Japan, seems to enter in activity much later than *Culex pipiens pallens*. It is sure that this species hibernates during winter only as adults, but they are scarcely found in human dwellings: the most favorite resting place of this species during the winter is also horse and cattle barns. Larvae of this species disappear much earlier than those of *C. pipiens* and they reappear much later next year. We tried in vain to find the larvae of this species in Okayama from April 20 to May 5. We could not find them even in the beginning of June, in Tokyo. In Kumamoto, we found small number of first instar larvae of this species in a drain (May 8). We suppose that these are the new hatched larvae from the eggs which were laid by over-wintered females. In the same area, we could collect adults, nymphs and larvae of *C. pipiens pallens*, 4th instar larvae of *Anopheles* and also nymphs and larvae of *Aedes togoi*, *Aedes japonicus*, etc. By these facts we would imagine that *C. tritaeniorhynchus* appears much later than these species.

3. Other species of the Genus *Culex*.

It is supposed that most of the other species of the Genus, *Culex* hibernate only as adults: *Culex vishuni* and *Culex bitaeniorhynchus* were found in Okayama as adults early in the spring. Larvae of this and other species were not found in nature during this period. Little is known about the manner of hibernation of other *Culex* species.

b) Genus *Aedes*

1. *Aedes togoi* (Theobald). It is a well known fact that the larvae of this species hibernate during winter in every concrete pools or stone holes. They are so resistant to the cold as can survive even when enclosed in ice for some time. This species pass winter also as eggs, in the same manner as other aedine species do. This fact was also assured by the following experiment; we collected, in winter, muds or decayed leaves at the bottom of the breeding place, and put them in an incubator of 24° to 28°C., and found larvae of various *Aedes* species in a few days which hatched out from eggs contained in these materials.

It was April 15 that we first found new larvae of this species just hatched out from the hibernated eggs, about 10 days later than that of *Aedes japonicus*. Adults of this species first appeared in the beginning of May, in Tokyo, in the end of April, in Okayama.

2. *Aedes japonicus* (Theobald). This is also a very common *Aedes* species in Japan. It resembles very much to *A. togoi*, morphologically as well as ecologically. Larvae of this species also relatively tolerate the cold, and we could find them in their breeding places until the end of December. So far as we could observe, all the breeding places of this species became dry during the dry season of winter and they all disappeared until the end of December. We suppose that larvae of this species hibernate through winter at least in

warmer part of Japan. Experimentally, they are a little weaker to the cold than larvae of *Aedes togoi*.

It is sure that the eggs of this species are able to pass winter in natural conditions and hatch out in of next spring. The first larvae of this species appear about 10 days earlier than *Aedes togoi*. In Tokyo, the first emergence of adults from these new hatched larvae was April 19, about 10 days earlier than *Aedes togoi*. Among various mosquitoes in Tokyo, this is the species which appear earliest in nature.

3. *Aedes albopictus* Skuse. This species seems to be adapted in much warmer temperature than most of the other species of Japan. Larvae and adults of this species disappear much earlier than other *Aedes* mosquitoes in late autumn, when the lowest temperature of night time becomes 6°C., and hibernate during the winter as eggs, principally. However, we observed that the adults of this species can live through winter until next spring in special warm conditions. For example we also observed in our laboratory that the adults of this species die out in late autumn in natural outdoor conditions, as the atmospheric temperature became lower than 6°C.

The first appearance of larvae from the hibernating eggs in spring is always later than the other *Aedes* mosquitoes. In 1945, it was April 19, and in 1947, it was April 26 that we first found the 1st instar larvae of this species in Yanaka cemetery, Tokyo. In Okayama, they were found only as 4th instar larvae (not yet as pupae) during the period we stayed there (from April 22 to May 4). Other *Aedes* species, such as *togoi* or *japonicus*, were already in activity as newly hatched adults. In Kumamoto, we found some adults of this species already emerged from a stone hole, the water of which was in special warm condition under direct sunshine (From May 5 to May 12). However, most of the breeding places of this species contained only pupa and 4th instar larvae, and never the skin casts of pupae at the time. (The presence of the skin casts of pupae in water shows that the adults of that species had already emerged).

The first emergence of adults of *A. albopictus* in Tokyo took place on June 4, in natural conditions (Yanaka cemetery).

4. Other *Aedes* species. Very little is known about the manner of hibernation of other aedine species in Japan. *Aedes niveus* Ludlow, a relatively rare species, were found to hibernate as eggs: we could get several larvae of this species by putting the debris from the bottom of stone holes of cemetery into warm water. However, *Aedes dorsalis* and *Aedes vexans nipponii* were caught as adults very early in spring by our members in Okayama; it is probable that these species hibernate as adults in winter, in the same manner as most of the culicine species.

c) Genus *Armigeres*.

The only one species of this genus in Japan, *Armigeres obturbans* Walker, has also a special manner of hibernation. Larvae of this species pass through winter in the bottom of their breeding places. The most common breeding places of this species in Japan is cesspools and bamboo-holes, and we could collect even in winter some larvae of this species in the mud of the bottom. They can survive even on decayed leaves of humid condition.

Larvae of this species, however, are not so resistant to the cold as *Aedes togqi* larvae; some of the former die if the ice formed on the surface of their breeding water, and most of the larvae do not come back to life if enclosed in ice.

Eggs of *Armigeres obturbans* have very similar structure to those of *Aedes*. They are very strong to the cold as well as to the dryness. It is probable that this species pass through the winter as ovae, but this is not yet proved in nature, as in *Aedes* species.

The appearance of this species as adults in spring is also very late. The adults of this species we first observed in nature on June 16.

d) Other Genera of Tribe Culicini.

Very little is known about the manner of hibernation of other mosquito genera of Japan. *Mansonia* is said to hibernate as larvae in Europe (Martini). As for *Mansonia uniformis*, the only species of this genus found in Japan, we tried very hard to find the larvae, but we could get none at least in their breeding places in Yuga (Okayama prefecture). On the other hand, some of our students staying in Okayama districts, caught several adults of this species early in spring. It is therefore, very probable that this species pass through winter as adults.

Tripteroides is a genus similar to *Aedes* ecologically. In Japan, only one species, *Tripteroides bambusa* Yamada is recorded. Larvae of this species are relatively strong to the cold and it is sure that they are able to pass through winter until next spring, though we had no chance of finding them in winter in their natural breeding places. About species of the other two genera of Japan (*Theobaldia* and *Uranotaenia*), we have as yet no experience on the manner of their hibernation.

e) Tribe Anophelini.

Anopheles hyrcanus sinensis, the most common species of this tribe in Japan, hibernates during winter as adults. We often find them resting on the wall or on the ceiling of horse or cattle barns in midwinter; larvae of this species are also relatively strong to the cold, and we find them in their breeding places until December, but in January, they all disappear from our sight. In Kagoshima, the southernmost place of Japan, several authors state that they hibernate as larvae in the mud of their breeding places. Ovae of this species are weaker to the cold and to the dryness than those of *Aedes*, and seem to be unable to pass the winter of Japan. About the other species of *Anopheles*, we suppose that the mode of hibernation is the same as that of *A. hyrcanus sinensis*, but this is not yet confirmed in natural conditions.*

The adults of *A. hyrcanus sinensis* seem to appear in spring later than *Culex pipiens*, but earlier than *Culex tritaeniorhynchus*. New larvae of this species attain to maturity in the beginning of May in Okayama as well as in Kumamoto.

* Prof. Sawada and others found larvae of *Anopheles lindesayi japonicus* in winter of 1947, in Kyushu. I also found several larvae of this species on the surface of a stream in Hakone-mountains, near Tokyo, in January, 1949.

Table. 5. List of hibernation and appearance of main Japanese mosquitoes in Tokyo districts in 1947.

	Hibernation	Season of appearance							
		March		April		May		June	
Culex pipiens	adults		A°		L		A		
Culex tritaen.	adults				A°		L		A
Aedes togoi	larvae. ovae	L°			L		A		
Aedes japonicus	ovae (larvae)				L		A		
Aedes albopictus	ovae						L		A
Armigeres obturb.	larvae, ovae	L°							A
Anoph. hyrc. sin.	adults			A°			L		A

Note : A° appearance of hibernated adults.
 L° Presence of hibernated larvae.
 A appearance of new adults.
 L appearance of new larvae.