BLOOD MEALS AND EGG PRODUCTION OF FOUR SPECIES OF ALASKAN *AEDES* IN CAPTIVITY (DIPTERA:CULICIDAE)

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Introduction. In the spring of 1968 a laboratory investigation of the feeding capabilities of wild-caught Alaskan mosquitoes was undertaken to determine their potential as biologic vectors of pathogens among animals. At least two blood meals would be required, with the interval between meals sufficient for multiplication of the pathogen in the mosquito. Accordingly the number of blood meals taken by individuals, interval between meals, and interval between first and last meals were determined. Information was also obtained concerning the number of blood meals required to produce each batch of eggs, and the interval between batches; number of eggs in each batch, total number of eggs and batches produced by each individual; and lifetime in captivity.

These data are presented for Aedes cinereus Meigen, A. communis (DeGeer), A. impiger (Walker) and A. punctor

(Kirby).

Conditions and Equipment. There was no natural light in the laboratory, which is below ground. In an attempt to simulate Alaskan summer conditions, overhead fluorescent lights were all turned on by day, and one quarter were left on at night. The cages were kept on shelves in low wooden cabinets, open only on the front. Thus the specimens received only indirect artificial light except during twice-daily examinations. Room temperature remained fairly constant at 18° C., rising occasionally to 22° for brief periods. Outside air was provided by a fan in the intake duct of the air-conditioning system.

Cages (Fig. 1, A) for single specimens were made from plastic culture tubes 10 cm long. The inner surfaces of the tubes

were scratched with a file to facilitate An oval oviposition hole approximately 2 cm long and 12 mm wide was made just below the middle of the tube, using the curved surface of a wood file 25 mm wide. On the opposite side of the tube, and 2 cm from its closed end, an air vent 5 mm in diameter was filed with a round, wood file 7 mm in diameter. A patch of nylon stocking mesh was glued over that air vent. Beside this, toward the end of the tube, a sugar-sop hole was made with an electric soldering iron. The open ends of the tubes were covered with reusable mesh caps made by wrapping a piece of masking tape, adhesive side out, loosely around the mouth of the tube and pressing to this, covering the mouth, a patch of nylon stocking mesh; then winding another piece of masking tape, adhesive side down, over the first piece.

Patches, 5 cm long and 4 wide, were cut from cotton gauze and from tan paper towels ("mechanic's hand-cloths") and made into pads composed of two single layers of gauze over one paper patch. A pad covered each oviposition hole and was held in place with two small rubber bands. The sugar-sop hole was plugged with absorbent cotton wetted with sugar water. A wet "mechanic's hand cloth," folded to make three layers, was placed in the bottom of a white porcelain pan (41 x 25 x 6 cm) on which were laid 30 cages, pads down.

At the end of the season, cages 9 cm long were made from 2.5 cm Clearsite ¹ display tubing which is easily cut with scissors. Holes were cut with cork borers and both ends of the tubing covered with mesh caps making a top vent unnecessary

(Fig. 1, B and C).

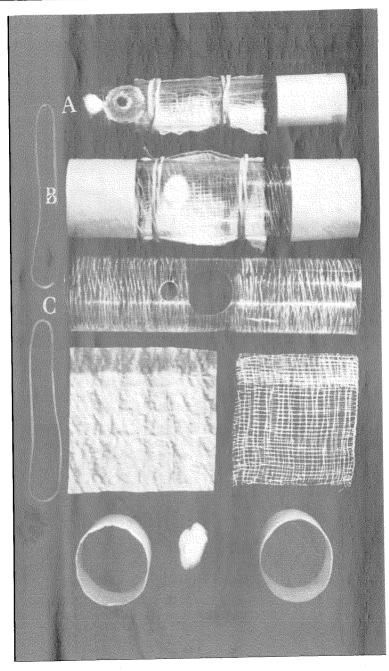


Fig. 1.—Cages and parts. A, culture-tube cage; B_t clearsite cage; C, same, unassembled.

PROCEDURE. Most of the A. impiger were collected in the Fairbanks area in late May and early June, though a few were taken in late July at Summit Lake, mile 195 on the Richardson Highway. Many of the A. cinereus were captured in the vicinity of Fairbanks in mid-June and a few in mid- and late-July. Half of the A. communis were caught near Fairbanks during June and most of the remainder at Summit Lake in the latter part of July. Most of the A. punctor were collected during August along the Richardson and Alaska Highways, in central Alaska.

wild-caught mosquitoes were brought to the laboratory in plastic culture tubes with pin holes in the lid. Each specimen was transferred to its cage. The cotton plugs were moistened with sugarwater, and wetted thereafter with distilled water twice a day. The pads on the cages were squirted with water and the cages placed on the wet towels in the porcelain pans. Once a day the mosquitoes were offered a meal if active or probing, and especially if they had recently deposited eggs. Each mosquito was fed by placing the mesh end of its cage against my arm. When biting they were allowed to take as much blood as they wanted. Biting without obviously taking blood was not considered a meal.

Eggs were deposited under the gauze in depressions formed in the moist paper pads. The 1 cm grid of nylon threads in the "mechanic's hand cloths" seemed to enhance the shirring effect in the moist pads. The specimens were transferred to clean cages a day or two after oviposition; also when the cages became dirty. The pads containing the eggs were removed and placed in Culpak 1 plastic culture plates until the eggs could be counted. Dates were recorded for blood meals taken and eggs deposited. The period for production of eggs was considered to be the time between the first blood meal involved and oviposition.

RESULTS. Table 1 gives the informa-

tion concerning total blood meals, time lapse, and longevity for the four species involved. Twenty-six A. cinereus took a total of 118 blood meals, averaging 4.5 meals each. Individuals took from 1 to 10 meals. The interval between first and last blood meal of each ranged from 6 to 119 days with an average interval of 62 days. Five specimens survived more than 110 days. Maximum survival time was 131 days, from June 19 to October 28.

Forty-nine A. communis took only 80 blood meals, averaging 1.7 meals per specimen. Individuals took from 1 to 5 meals. The interval between first and last meal ranged from 5 to 55 days with an average interval of 24 days. Six individuals survived 50 or more days. Maximum survival time was 71 days, from June 17 until August 27.

Thirty-two A. impiger took a total of 84 blood meals, averaging 2.6 meals per individual. They took from 1 to 5 meals each. The interval between first and last blood meal of each ranged from 7 to 78 days with an average interval of 31 days. Five individuals survived more than 60 days, with a maximum survival time of 92 days, from May 27 to August 27.

Twenty-one biting A. punctor took a total of 60 blood meals averaging approximately 3 meals each. Individuals took from 1 to 6 meals. The interval between first and last blood meal of each ranged from 10 to 55 days, with an average interval of 30 days. Two specimens survived more than 60 days, the maximum being 68 days, from August 22 to October 29. The one specimen that did not take a blood meal survived 71 days, outliving all the others.

Table 2 shows the minimum, average and maximum number of days between consecutive blood meals for the four species.

Table 3 gives the minimum, average and maximum number of eggs in successive batches and the number of individuals ovipositing. Twenty-three A. cinereus laid eggs, producing 81 batches totalling approximately 2,526 eggs; an average of 110 eggs per female. Nine batches were

¹ Endorsement by U. S. Department of Health, Education, and Welfare not implied.

TABLE 1.—Blood meals, time lapse, and longevity.

Total Meals Per Specimen	Specimens	Average Days Between First & Last Meal	Average Day Alive
Aedes cinereus	26	62	
I	T		97
2	7	26	52
3	3	42	61
4	3	60	77
	5	74	89
5 6	ī	43	53
	1	116	131
7 8	2	105	120
g	2	96	100
10		113	122
Aedes communis	49	24	••
I	30		26
2	10	22	36
3	7	24	34
4	I	47	52
5	I	18	50
Aedes impiger	32	31	
I	6	••	23
2	1 I	28	36
3	6	27	49
4	7	40	53
5	2	32	47
Aedes punctor	22	30	••
0	Ĭ.	• •	71
I	6	• •	14
2	4	14	24
3	3	27	40
3 4 5 6	5	36	49
5	2 1	39 55	57 56

the most deposited by an individual. The largest number of eggs laid by one specimen was 242, in 7 batches from 8 blood meals. Next largest was 218, in 6 batches from 9 blood meals. The largest single batch contained 114 eggs produced from one blood meal in 24 days. The shortest period for egg production was 6 days.

Thirty-six A. communis oviposited, producing only 42 batches totalling approximately 1,603 eggs; an average of 45 eggs per female. Three batches were the most deposited by an individual. The largest number of eggs laid by one specimen was 140, in two batches from 2 blood meals. The largest single batch contained 110 eggs produced from 1 blood meal in 22 days. The shortest period for production of eggs was 8 days.

Twenty-seven A. impiger laid eggs, producing 32 batches totalling approximately 926 eggs; an average of only 34 per female. Two batches were the most deposited by a single specimen. The greatest number of eggs laid by one female was 90, in one batch, produced from 4 blood meals in 38 days. Another deposited 90 eggs in 2 batches produced from 3 blood meals in 57 days. The next largest number was 70 eggs in one batch produced from 2 blood meals in 25 days. The shortest egg production period was 9 days.

Nineteen A. punctor laid eggs, producing 46 batches totalling approximately 1,591 eggs; an average of 84 per female. Five batches were the most deposited by an individual. The largest quantity of eggs laid by a single specimen was 279

TABLE 2.—Period between consecutive blood meals.

Meal Count	Days between Consecutive Blood Meals			
	Minimum	Average	Maximum	Specimens
Aedes cinereus				26
1-2	4	25	51	25
2-3	7	14	31	18
3-4	5	18	74	15
.4-5	5	13	35	12
5-6	3	9	. 15	7
6-7	2	12	28	6
7-8	6 -	10	15	5
8-9	8	13	19	3
9-10	15	15	15	ĭ
Aedes communis				49
I-2	4	17	35	19
2-3	2	11	22	9
3-4	8	9	10	2
4-5	2	2	2	I
Aedes impiger				32
1-2	4	20	62	26
2-3	2	13	46	15
3-4	2	10	19	9
4-5	3	4	5	2
Aedes punctor				21
1-2	10	13	20	15
2-3	2	10	22	11
3-4	6	11	13	8
4-5	10	13	15	4
56	15	15	` 15	ī

in 4 batches produced from 5 blood meals. Next largest was 238 eggs in 4 batches from 4 blood meals. The largest single batch contained 108 eggs produced from 2 blood meals in 14 days. The shortest egg production period was 7 days.

Usually there was a decrease in the number of eggs in each successive batch, as shown in the column headed "Average" in Table 3. Note that only A. cinereus and punctor produced more than three successive batches of eggs. Table 4 shows that the average interval between successive batches of A. cinereus eggs decreased to the fifth batch and then fluctuated; but this trend was not always true of individual specimens. The batch-interval for A. punctor individuals fluctuated, though the averages indicate uniform periods.

From Table 5 it is obvious that only from one meal did all four species produce eggs in each batch category. For the most

part column 1 indicates a gradual decrease in the quantity of eggs in each successive batch, and in production time. Of the 81 batches produced by A. cinereus, 83 percent were from 1 blood meal, 10 percent from 2 blood meals, 4 percent from 3, 1 percent from 7 blood meals and 2 percent apparently from no additional meal-possibly because of an interrupted and delayed oviposition, or failure to record a meal. Of the 42 batches of eggs laid by A. communis, 88 percent were produced from 1 blood meal, 7 percent from 2 meals, 2 percent from 5 meals, and 2 percent from no additional meal, apparently. Thirty-two batches of eggs were deposited by A. impiger, 50 percent of which were produced from 1 blood meal, 28 percent from 2 meals, 12 percent from 3, 6 percent from 4, and 3 percent from 5 blood meals. Forty-six batches were laid by A. punctor, 91 percent of which were produced from I blood meal and the rest from 2 meals.

TABLE 3.—Egg production.

	Eggs Per Batch				
Batch Category	Minimum	Average	Maximum	Females Ovipositing	
Aedes cinereus					
1st	2	50	114	23	
2nd	3	33	74	18	
3rd	12	30	60	14 8	
4th	4	25 18	51		
5th		18	26	7	
6th	9 6	13	21	5 3 2	
7th	7	18	30	3	
8th	7	I 2	17		
9th	12	12	12	I	
Aedes communis				26	
1 st	I	40	110	36	
2nd	8	28	47	5 1	
3rd	14	14	14	1	
Aedes impiger				A.W	
ıst	3	32	90	27	
2nd	I	15	25	5	
Aedes punctor			. (**	
ıst	2	39	96 - 18	19 12	
2nd	13	39 38 28	108	10	
3rd	. 6	28	55		
4th	13	26	51	4 1	
5th	17	17	17	1	

Table 4.—Days between egg batches.

Batch Category	Minimum	Average	Maximum
Aedes cinereus		31	71
*-1	6	19	67
1-2	9 8 6	15	29
2-3	8		25
3-4		8	9
4-5 5-6 6-7 7-8 8-9	7 6 8	9	14
5–6	0	11	14
6-7		12	15
7-8	9 10	10	10
8–9	10		
Aedes communis			
*-I	10	24	41
1-2	16	18	22
2-3	22	22	22
Aedes impiger		22	6т
4-1	9	32 18	26
1-2	12		
Aedes punctor			
*-I	7	12	18
	7.	12	26
1-2	9	12	22
2-3	11	12	. 13
3-4	12	12	12
4-5			

^{*} First blood meal.

TABLE 5.—Quantity and rate of egg production in relation to number of blood meals involved.

Batch Category	Average Eggs Per Batch: Average Days Between First Meal Involved * and Oviposition		
	r Meal	2 Meals	3 or More Meals
Aedes cinereus			
1 St	52:26	51:41	27154
2nd	32:14)···+·	33:54 74:68
3rd	30:10	24:16	48:29
4th	23:09	36:22	40.29
5th	18:08		• •
6th	13:07		• •
7th	18:07	• • • • • • • • • • • • • • • • • • • •	• •
8th	13:09	• • • • • • • • • • • • • • • • • • • •	• •
9th	12:19	•••	•••
Aedes communis			
1st	39:23	63:30	2.1.79
2nd	28:18	V3·3V	24:18
3rd	14:15	• • •	· · · · · · · · · · · · · · · · · · ·
Aedes impiger			
1 st	29:30	23:30	
2nd	18:14	-3.30	51:38 1:23
Aedes punctor			1.23
Ist	39:12		
2nd	33:10	45175	
3rd	28:09	47:17	• •
4th	26:11	• •	• •
5th	17:09	• •	y .*

⁴ A meal taken just the day before oviposition was counted as contributing to the production of eggs in the next subsequent batch.

Usually a blood meal was taken soon after oviposition, but on 5 occasions (impiger twice, and cinereus thrice) specimens took a blood meal the day before depositing eggs, and no additional meal afterward. These meals were counted as contributing to the production of eggs in the next subsequent batch. Some specimens appeared to pass much of a blood meal with little or no digestion taking place.

Discussion. Conditions in the laboratory were far different from those in nature. There was no natural light, nor any great daily fluctuation in temperature; and there was only a small choice in relative humidity. Once feeding started, the mosquitoes could take as much blood as they wanted without interruption. They could not get much exercise and could become stuck in condensation or a drop of excrement. The large-diameter cages

used at the end of the season solved the condensation problem and allowed the mosquitoes horizontal flight.

The oviposition site probably lacked the specific properties attractive to each species in nature, but the basic conditions—moisture, substrate, and niches for deposition of the eggs—were available. Some specimens died full of eggs, which suggests that perhaps the conditions offered for oviposition left much to be desired for certain individuals, although other factors may have been involved. This did not happen with *Culiseta alaskaensis* (Ludlow) (Sommerman 1969).

It is possible that by chance the cages were better suited to the needs of certain species. Also, early capture, resulting in short exposure to natural field conditions, may make it possible for some to withstand confinement better. About half of the A. communis were captured later in

the season and they took fewer meals and laid only one batch of eggs, suggesting that they may have been older specimens when captured and had fed and deposited eggs previously; or possibly, having been free for a longer period, were more severely affected by confinement. Or this may have indicated only that the feeding habits of this species vary in different localities, as noted by Hocking (1954), Hopla (1964–1965), and Jenkins (1948).

As evident in Table 1, 96 percent of A. cinereus, 81 percent of A. impiger, 68 percent of A. punctor and 38 percent of A. communis took two or more blood meals. The average time lapse between first and last meals appears great enough to be significant in terms of pathogen development within the mosquito even though the average period between any two consecutive meals (Table 2) may be too short.

The high percentages of egg batches produced from only one blood meal suggest this to be the usual pattern, at least for punctor, communis and cinereus. indicated in Table 3, more than 50 percent of the ovipositing females of punctor and cinereus deposited 3 or more batches of eggs. If the average periods required to produce the first two batches (Table 4) are added, then at the age of 50 days a large percentage of cinereus would be seeking its third blood meal, and the same would be true of punctor at the age of 24 days. Similarly, at the age of 42 days a small percentage of communis would be seeking its third meal-all ages covering considerable periods of time. Likewise, using the information in the "1 Meal" column of Table 5, the age of cinereus at the time of its third meal would be 40 days, communis 41 days, and punctor 22 days; all reasonably close to the above periods, so this may be significant in terms of pathogen development in the mosquito.

The time required for development of eggs tended to decrease as these Aedes aged, but the periods increased for Culiseta alaskaensis (Sommerman 1969).

Conclusions. In the localities men-

tioned, the females of A. cinereus, communis, impiger, and punctor are capable of taking multiple blood meals over an extended period if extrinsic conditions are favorable. With the exception of communis in certain localities, the majority of specimens are capable of depositing two or more batches of eggs, the production of each requiring at least one blood meal. These feeding capabilities, in terms of number of blood meals taken and intervals between meals, suggest the potential for biologic transmission of pathogens among animals.

SUMMARY. In the summer of 1968 wildcaught specimens of four species of Aedes from central Alaska, mostly from the Fairbanks area, were isolated, each in a plastictube cage in the laboratory, and were allowed to feed on a human at intervals. Twenty-six Aedes cinereus Meigen, 49 A. communis (DeGeer), 32 A. impiger (Walker), and 21 A. punctor (Kirby) furnished the following data. Almost all specimens took more than one blood meal. The maximum number of blood meals per specimen for each species in the order listed above was 10, 5, 5, and 6. The interval between meals varied considerably among individuals of each species. The days between the first and last blood meal of each individual, by species, averaged 62, 24, 31, and 30 respectively. The number of specimens ovipositing was 23, 36, 27, and 19; the most batches of eggs laid by an individual of each species were 9, 3, 2, and 5; and the maximum number of eggs per specimen was 242, 140, 90, and 279. Production of a single batch of eggs required from 1 to 7 blood meals, but for each of the species, the percentage of egg batches produced from one blood meal only was 83, 88, 50, and 91. There was usually a decrease in the number of eggs in each successive batch, and a blood meal was usually taken soon after each oviposition. The number of blood meals taken per individual, and the time lapse between first and last blood meals, (if not between successive meals), indicate that

in these respects all four species have the

potential for biologic transmission of pathogens among animals.

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SWARMING AND MATING BEHAVIOR IN CULEX PIPIENS QUINQUEFASCIATUS SAY 1

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The sterile male technique is being studied as a means of controlling Culex pipiens quinquefasciatus Say (= C. p. fatigans) one of the principal vectors of Wuchereria bancrofti. In order to employ the sterile male technique, as with any control program, a thorough knowledge of the biology of the insect vector must be known. In a control program dependent upon breeding characteristics it is necessary to investigate the mating behavior of the species. Unfortunately, there is disagreement among researchers as to the interrelationships of the two behaviors in mosquitoes. The most common idea (Bates, 1949) is that copulation is almost invariably associated with the swarming of males and that the two are interrelated. On the other hand, Nielson and Haeger (1960) stated that swarming and mating are independent activities, even though they normally occur simultaneously. The purpose of this research was to determine what factors influence swarming and how this was related to mating in C. p. quinque fasciatus. A study was also made of the copulatory behavior within the swarm.

METHODS AND MATERIALS. Outdoor

Cage Study—A cage, 12 m x 4.8 m and 3.6 m high, consisting of an aluminum frame covered by 20-mesh plastic screen was used for the outdoor studies. It was located in a wooded area near the Laboratory for Insects Affecting Man, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Gainesville, Florida. Within the cage a wooden shed, 3.6 m x 2.4 m and 2.1 m high was constructed with the south side covered with 20-mesh screen. The northern end and northern half of the east side were covered with plywood and the remaining left open. A small plastic-lined "pond" (0.9 m x 0.9 m) surrounded by grass and flowering plants was located in the southern half of the shed.

Periodically 1500 C. p. quinquefasciatus pupae obtained from the laboratory colony were placed in the pond for emergence. All observations of adult behavior and light conditions were made from the east side of the pond and facing west. Light readings were made with a Luna-Pro® electronic system exposure meter.

Adult female mosquitoes were removed each day at random from the cage with a mechanical aspirator. Samples were taken of 2-, 3-, and 4-day-old mosquitoes for spermathecal examination. Females obtained from the cages were

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