

ON THE EFFECT OF MATING AND NUTRITION ON THE REPRODUCTION OF *CULEX PIPIENS FATIGANS* WIEDEMANN

by

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In the paper presented results are reported of some studies on mating and feeding of *C. fatigans* in the laboratory with particular reference to the influence of fertilization and nutrition on the reproduction of this mosquito.

1 - MATERIAL AND METHODS

The experiments were carried out with three laboratory colonies of *C. fatigans*. These were established in the Tropeninstitut in Hamburg in 1961 and 1962 with female mosquitoes collected in Rangoon (Burma/ South West Asia), Ifakara (Tanganyika/ East Africa), and Konakry (Guinea/ West Africa), respectively. The mosquitoes were reared under constant conditions of temperature (air 27°C, water 25° C), relative humidity (75-80%), and light (12 hours illumination). Standardized breeding in respect to density of larvae and amount of food yielded a uniform supply of vital adults. Birds and 10% sugar solution were offered to the adults. If no stated otherwise, 25 males and 25 females of the same age, kept in cages that measured 30 cm³, were used for the experiments.

2 - MATING

In order to obtain more accurate information on the time lapse between emergence from the pupal stage and mating, equal numbers of males and females were kept together in a cage for a certain period of time. The mating activity was measured by the insemination rates. These were determined at the end of each experiment by microscopic examination of the spermatocae of the females for the presence of sperms. The results obtained in two replicates of 25 males and 25 females each are given in table 1. They show that a certain number of *C. fatigans* mate already in the first 24 hours after emergence. However, the majority mates during the following 48 hours. Beyond the 5th day the insemination rate shows little increase.

In our experiments we have observed frequently a continuous decrease in the percentage of larvae that hatched from successive egg rafts laid by isolated females. In many cases the last egg rafts showed no hatch of larvae at all. Obviously, the amount of sperm transmitted to the female in a number of matings was not sufficient to ensure the production of viable eggs during the whole life span of the female.

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TAB. n° 1 MATING in laboratory colonies of *C. p. fatigans* in cages of 30 cm³.

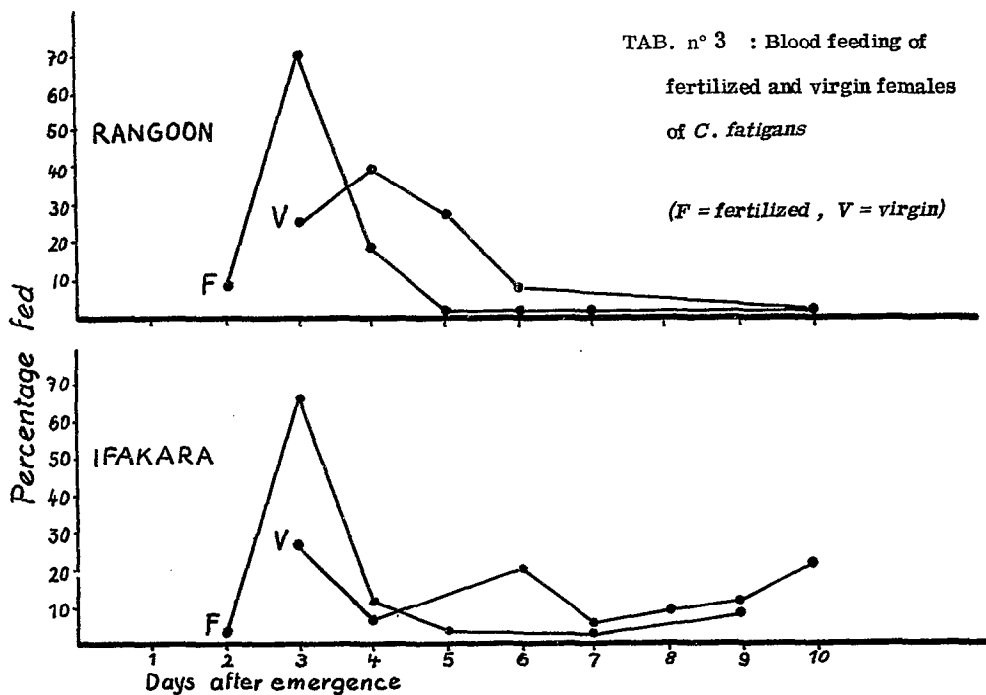
Duration of experiment in days	Percentage of females inseminated		
	RANGOON	IFAKARA	KONAKRY
1	15	6	0
2	78	57	14
3	83	92	50
5	87	98	90
10	92	98	90

It is known that *C. fatigans* can easily be colonized in the laboratory because of its stenogamy. This species swarms and mates readily in captivity in cages of 30 cm³ size. We have checked the degree of stenogamy present in our colonies of *C. fatigans* by keeping one male and one female soon after emergence in small cages of various size for a period of five days. Then the spermatecae of the females were examined. There were 20 to 30 replicates per experiment. The results obtained (see table 2) show that the females of all three colonies investigated were inseminated successfully in spaces much smaller than the cages commonly used for maintenance of laboratory colonies. The fact that *C. fatigans* is able to mate successfully in these small containers indicates, that it may be able to mate not only on the wing but also while resting on the wall, as observed in *Culex pipiens var. molestus* (TATE and VINCENT, 1936). The results show also that single pair matings, which are desirable for genetical studies, can easily be obtained with *C. fatigans*.

TAB. n° 2 STENOGAMY in laboratory colonies of *C. p. fatigans*.

Type and size of cage in cm	Percentage of females inseminated after 5 days		
	RANGOON	IFAKARA	KONAKRY
30 x 30 x 30	95	72	95
15 x 10 x 10	72	68	48
12 x 4 (WHO-test tube)	10	35	5
7.5 x 2.5 (specimen tube)	0	3	0

In order to elucidate if and how mating can affect blood feeding and egg production in *C. fatigans*, experiments with virgin females and fertilized specimens have been carried out. For ten successive nights a bird was offered to both lots of mosquitoes apart from constant availability of sugar. The blood fed specimens were removed every morning. The results of two replicates of 25 virgin and 25 fertilized females each are shown in table 3. In both colonies the virgin females began the blood feeding later than the fertilized ones and feed more erratic. A number of the virgin females takes first an incomplete blood meal, which does not lead to the production of an egg raft. The comparison of the biting activity of virgin and fertilized females indicates that mating has a stimulating effect on the blood feeding activity.



Mating has also a marked influence on the production of eggs. This effect on reproduction becomes evident on comparison of the number of egg rafts laid by virgin and by fertilized females led to the development and deposition of egg rafts, while most of the virgin females took two or more blood meals before ovipositing. Only 27% of the blood meals taken by virgin females were followed by deposition of egg rafts. In the Ifakara colony virgin females and fertilized ones showed similar percentages of 81% and 82% of blood meals that were followed by development and oviposition of eggs. However, as blood feeding of the virgin females was delayed the total number of blood meals ingested during life time, was smaller than that taken by the mated specimens. Therefore, also in the Ifakara strain the egg production of the virgins was much smaller than that of the fertilized females. From the results of these experiments one can conclude that mating favours the egg production.

3 - NUTRITION

To study the influence of nutrition on reproduction of *C. fatigans*, in particular of blood feeding and sugar feeding, a number of experiments has been carried out. The objective of the feeding experiments with various host animals was twofold, first to see which hosts are preferred by the mosquitoes and, secondly, to study the quality of the various blood meals by the size of the egg rafts produced.

In order to investigate the host preference of our *C. fatigans* colonies under the prevailing laboratory conditions a certain host animal was offered to the mosquitoes at least five times a week until the last female mosquito of the experiment had died. The results obtained (see table 4) show a general preference of bird blood over that of mammals. This preference for birds is shown in all three mosquito colonies investigated. This is not only indicated by the much higher number of blood meals taken from this group of hosts, but also by the readiness with which the birds are attacked. In comparison with the feeding on chickens and canaries the feeding on mammals was markedly delayed and erratic. However, one of the birds, the paraquet, was completely rejected; not a single female fed on this bird.

TAB. n° 4 Host preference in laboratory colonies of *C. p. fatigans*. Number of blood meals taken by 50 females.

colony \ host	man	rabbit	guinea pig	white rat	chicken	paraquet	canary
RANGOON	70	27	31	8	151	0	98
IFAKARA		0	0	0	131	0	100
KONAKRY			2			0	76

The quality of the blood meal is - apart from other factors - of considerable influence on the fecundity of the mosquito females. In our feeding experiments with various hosts, this is clearly shown by the size of the egg rafts produced, i. e. the number of eggs deposited after each blood meal (see table 5). For *C. fatigans* the blood of birds has obviously better qualities than that of mammals. All 3 mosquito strains investigated produced markedly bigger egg rafts after blood meals on birds than on mammals including man.

TAB. n° 5 Size of egg rafts produced by *C. fatigans* fed on various hosts

colony \ host	man	rabbit	guinea pig	rat	chicken	canary
RANGOON	129 33-197	166 141-204	136 61-219	123 46-190	193 43-300	214 31-326
IFAKARA		0	0		228 78-345	219 63-335
KONAKRY			121 111-131			227 132-299

Despite this general difference in the size of the egg rafts deposited after blood meals on birds and on mammals, it is not possible to conclude from the number of eggs produced on the type of the foregoing blood meal with accuracy, because the ranges of the various categories overlap widely. Moreover, we have observed many times, that the size successive egg rafts laid by a single female decreases considerably. The average size of the egg rafts produced by a number of females fed on canaries decreased from the first raft to the 8th oviposition as follows : 229, 207, 205, 183, 181, 178, 85 and 31 eggs. However, it is uncertain, that this high number of ovipositions occurs in nature frequently enough to be of some importance. For practical purposes it seems reasonable to consider field collected rafts with more than 200 eggs as a result of a blood meal on a bird.

In nature juices containing sugar are not only the principal food of male mosquitoes but are also ingested by females. In the laboratory fruits or sugar solutions are commonly used in the maintenance of colonies to keep the mosquitoes alive. In our experiments and in those recently reported by DE MEILLON and SEBASTIAN (1964) this nutrition with sugar has proved to be of great importance for the survival of the mosquitoes and has shown a noticeable effect on the feeding activity and oviposition of *C. fatigans*.

We have observed, that females which are provided with sugar solution soon after emergence, usually take their first blood meal one to two days later than those which are offered

water only, apart from the host animal. It is customary to withdraw the sugar supply from laboratory colonies one or two days before a blood meal is offered in order to achieve a better success. Obviously, nutrition with sugar decreases the biting activity to a certain extent.

The nutrition with sugar has a considerable influence on the longevity of the mosquitoes, both males and females. In our experiments (see table 6) the mosquitoes kept without access to sugar showed a high mortality a few days after emergence. Additional supply of blood did not increase the longevity of the females markedly. The females died usually after the first oviposition. If the mosquitoes are fed on sugar, the length of life of both sexes is significantly prolonged. Additional blood meals are obviously of little influence on the longevity of the females. These results indicate that nutrition with sugar is of paramount importance for the length of life in *C. fatigans* and possibly of other mosquitoes too.

TAB. n° 6 LONGEVITY of *C. p. fatigans* provided with different food.

Colony	RANGOON				IFAKARA	KONAKRY
	supply	water only	water + bird	sugar only	bird + sugar	bird + sugar
♂ range	3 - 6	3 - 11	4 - 41	3 - 38	2 - 43	3 - 46
average	4	6	20	18	15	23
♀ range	3 - 6	2 - 10	13 - 34	3 - 68	5 - 61	6 - 46
average	4	6	26	28	29	26

Nutrition with sugar has also a marked effect on oviposition. Females which have constant access to sugar show a delayed and erratic oviposition. While the mosquitoes without supply of sugar laid their eggs usually 2 to 4 days after the blood feeding, those fed additionally on sugar continued to oviposit up to two months or more after their last blood meal. This phenomenon has been observed also in laboratory colonies of American *C. fatigans* (*quinquefasciatus*) by FARID (1949) and is a common observation in our *C. p. pipiens*. As pointed out recently by de MEILLON and SEBASTIAN (1964) it is of great importance to know whether the kind and concentration of sugar ingested by the mosquitoes in nature have the same influence on biting activity longevity and oviposition of *C. fatigans*.

However, in this respect attention is drawn to the possibility that delayed oviposition, which we have observed not only in our *Culex* strains but also in various colonies of *Anopheles*, might be influenced also by a low attractiveness of the breeding water provided. We have observed, that for *C. fatigans* a fermenting blood solution is much more attractive as oviposition medium than other suspensions and plain water normally used in maintaining laboratory colonies.

SUMMARY

Three colonies of *C. p. fatigans* originating from West Africa, East Africa and Asia have been investigated in respect to mating, feeding and reproduction.

Mating occurred already during the first 24 hours after emergence, but the majority of mosquitoes mated during the following two nights. A considerable degree of stenogamy was observed which indicates, that *C. fatigans* is able to mate not only on the wing. Experiments with virgin and mated females have shown that mating has a stimulating effect on blood feeding and on the egg production of *C. fatigans*.

In feeding experiments with various hosts a pronounced preference for birds over mammals was observed. The birds were not only more readily attacked by the mosquitoes but also their blood has obviously better qualities for the development of eggs in *C. fatigans*. The effect of the type of blood meal on fecundity is clearly demonstrated by the size of the egg rafts produced subsequently. The numbers of eggs laid after blood meals on mammals including man were considerably smaller than those laid subsequent to a blood meal on birds.

It has been observed that nutrition with sugar reduces the biting activity of the mosquitoes to a certain extent. - Nutrition with sugar has proved to be of paramount importance for the longevity of the mosquitoes. Without supply of sugar, both sexes of *C. fatigans* show a high rate of mortality. - Sugar fed females show a considerable delay of oviposition up to more than two months after the last blood meal.

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