OBSERVATIONS ON FECUNDITY IN *ORNITHODORUS MOUBATA*, MURRAY (*IXODOIDEA* : *ARGASIDAE*). RELATIONSHIPS BETWEEN MATING AND OVIPOSITION

BY

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1. INTRODUCTION.

The mating process of *Ornithodorus moubata* has been described by WAGNER (1958) and FELDMAN-MUHSAM (1967), the latter author having also recorded it by cinematography. FELDMAN-MUHSAM also gives a detailed description of the formation and the anatomy of the spermatophore. The outer envelope, the exospermatophore, remains on the surface of the female, during mating. The endospermatophore, which consists of two capsules, penetrates into the uterus of the female tick ; it contains the spermiophores. FELDMAN-MUHSAM as well as WAGNER stated that one female could mate serveral times. As a matter of fact we found up to eighty endospermatophore capsules in the uterus of a single female when dissected. This means that forty successive matings had taken place. These observations are from laboratory investigations, yet we still do not know how many matings occur in nature.

For these reasons, the question arises whether the number of matings occuring after a blood meal has any bearing on the number of egg-sets and on the number and the viability of the eggs. Normally egg-laying must be preceeded by the intake of a blood meal. It is admitted that mating occurs in *O. moubata* after feeding, when the host has been abandonned, and therefore on the ground where the ticks seek refuge. In connection with the problem of finding a male, it is uncertain, whether the females can lay several sets of eggs after a single mating, in other words : whether a single stock of spermiophores is sufficient to assure several sets of eggs after more than one blood meal or whether mating must be repeated after every blood meal in order to assure several sets of eggs. The laboratory observations reported here may furnish an answer to these questions.

2. MATERIAL AND METHODS.

The *O. moubata* strain comes from Tanzania (Ulanga district) and was bred several years in the Swiss Tropical Institute, according to the method of GEIGY & HERBIG (1955). Female

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ticks where isolated after their last nymphal blood meal, one tick per tube, so as to be sure of their virginity. The experiments were carried out with the following three samples :

Sample a (45 ♀♀) : One single mating was induced after the first blood-meal ; further blood meals were taken every three months, but the ticks had no further opportunity to mate.

Sample b (47 ♀♀) : Several matings (generally 3 or 4) were induced after the first blood meal ; further blood meals were taken every three months, but the ticks had no further opportunity to mate.

Sample c (55 ♀♀) : Mating was induced after each blood meal, about every three months. As not sufficient virgin ticks were available, the control experiment (*c*) was performed later, but from ticks of the same strain and bred under identical conditions (temperature 26-27°, relative humidity 80-90 %).

The experiment lasted several years until the death of the ticks. A single *O. moubata* female may lay eggs up to eight times. The number of egg sets was determined, as well as that of the eggs and of viable nymphs of the first stage (nymphs I) as a test of their viability.

3. Results.

3.1. *Total number of eggs laid during the whole life span of the female.*

Table I.
Total number of eggs laid during the whole life span of the female ticks.

Sample	*a*	*b*	*c*
Number of ticks (n)	45	47	55
Fecundity			
mean egg number laid by one female	243,8 ± 10,2 *	284,5 ± 15,1 *	498,5 ± 19,9
Viability			
mean percentage of nymphs I obtained from the eggs above	79,69 ± 2,88	75,21 ± 3,65	90,87 ± 1,78

* Value of t (Student t-test) for comparing the fecundity of sample *a* and sample *b* : t = 2,212 (P = 0,025).

We conclude from Table I that the number of eggs laid by females of *sample a* (one single mating after the first blood meal) and of *sample b* (3-4 matings after first blood meal) are not significantly different statistically. We used the Student t-test, after having proved by means of the χ^2 -test, that the distribution of the total egg numbers may be considered as a normal distribution (Kreyszig, 1968). As to the higher fecundity of the females of *sample c* (mating after every blood meal), we find that these differences remain within the normal range of biological variability in ticks.

3.2. *Egg numbers at each oviposition.*

3.2.1. *Number of ovipositions.* The number of ovipositions exceeds never eight (Table II). Several ticks stop laying eggs rather earlier, as may be seen from Figure I which gives the percen-

tage of all females laying eggs ; it also shows that after the second oviposition no longer all females lay eggs.

TABLE II.
Fecundity.

number of egg-set	mean number of eggs laid			values of t comparing	"repeated oviposition"			second "repeated oviposition"
	a	*b*	*c*	*a* and *b*	*a*	*b*	*c*	*c*
1	83,82 ± 3,01 (n = 45)	97,36 ± 5,17 (n = 47)	138,71 ± 7,41 (n = 55)	2,2366 P = 0,025	12,50 ± 1,42 (n = 14)	14,77 ± 1,66 (n = 18)	15,06 ± 1,67 (n = 18)	16 (n = 1
2	75,90 ± 2,91 (n = 43)	71,93 ± 7,32 (n = 46)	126,51 ± 5,56) (n = 55	0,4912 P = 0,4	16 (n = 1)	27,8 ± 12,9 (n = 5)	14,78 ± 1,41 (n = 18)	
3	65,17 ± 3,67 (n = 40)	61,26 ± 5,09 (n = 42)	114,50 ± 5,37 (n = 54)	0,4918 P = 0,4	4 (n = 1)	11 (n = 1)	13,85 ± 3,27 (n = 7)	
4	38,80 ± 4,22 (n = 15)	48,80 ± 3,91 (n = 30)	78,77 ± 5,39 (n = 47)	1,587 P = 0,1	41 (n = 1)	30 (n = 1)	11 ± 1 (n = 2)	
5	44,28 ± 11,93 (n = 7)	45,85 ± 6,17 (n = 7)	61,00 ± 6,58 (n= 24)	0,116 P > 0,4				
6	41,66 ± 12,09 (n = 6)	41,62 ± 7,32 (n = 8)	55,53 ± 8,44 (n = 15)	0,003 P > 0,4				
7	5,5 ± 4,5 (n = 2)	13 (n = 1)	33,33 ± 8,56 (n = 3)	0,9623 P = 0,2				
8	14,3 ± 12,84 (n = 3)	42,44 ± 14,82 (n = 9)	0 (n = 0)	1,0293 P = 0,2				

3.2.2. " *Repeated oviposition* ". We also find that young females, following the first oviposition, may again lay a smaller set of eggs without taking in another blood meal. We propose to call this " *repeated oviposition* ". Whereas the normal pre-oviposition time (i.e. the time from the blood-meal — and mating — to oviposition) is 10 to 15 days (see 3.4. ; Table IV), " *repeated oviposition* " occurs after 50 days, and in one case, a second " *repeated oviposition* " occured after 114 days (see Table IV).

After the second and the following blood meals " *repeated oviposition* " is less important and it is no longer observed after the fifth blood meal (Table II).

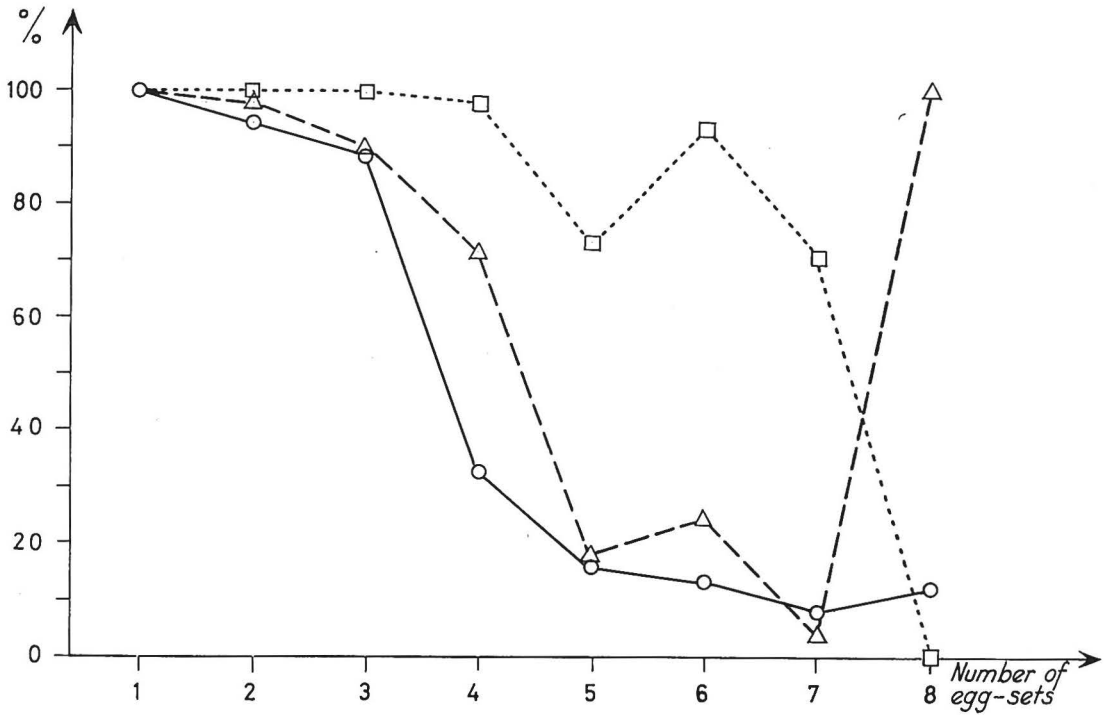

FIG. 1. *Percentage of females laying eggs.*

Ordinate : 100 % = number of living fed females ;
Abcissa : number of egg sets ; each egg-set is separated from the next by approximatively three months ;
o——o sample *a* one single mating after first blood meal ;
△- - - -△ sample *b* 3 to 4 matings after first blood meal ;
□. . . . .□ sample *c* matings after each blood meal.

3.2.3. *Differences between the samples a, b, c.* The difference observed at each oviposition correlate to those found for the total egg number.

There is no significant difference between the *sample a* (one mating at first blood meal) and *b* (3-4 matings at first blood meal), as proved by the use of the Student t-test. The values of sample *c* are higher than in both other samples, as already mentioned. Yet we should not expect any difference between samples *b* and *c* (in both cases several matings) after the first blood meal. For this reason we chose, in Figure 2, a common reference system by expressing as 100 % the value of the eggs layed at the first oviposition by each sample. With this interpretation, the difference disappears between sample *c* and the other two ; it appears therefore to be due only to the great biological variability of the ticks.

We may conclude that the number of the eggs laid is the same for the three samples, i.e. there are no statistically significant differences between the values if referred to a common reference system.

3.3. *Viability (number of nymphs I obtained).*

Figure 3 describes the mean viability, i.e. the mean number of nymphs I obtained from 100 eggs at each oviposition. The curves for sample *a* (single mating after first blood meal) and for sample *b* (3-4 matings after first blood meal)are identical, whereas they show a caracteristic difference with the curve of sample *c* (matings at each blood-meal). As values of the egg

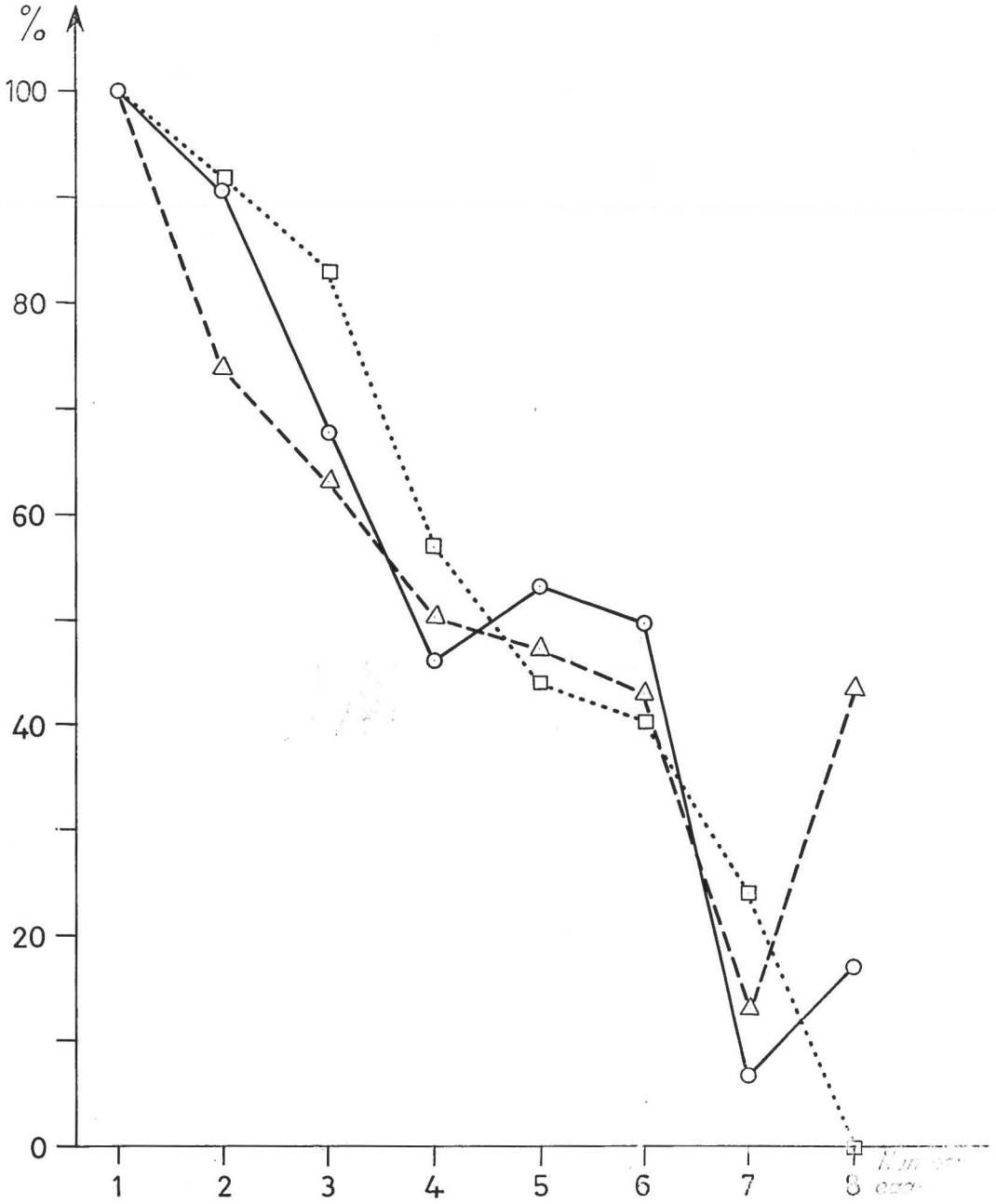

FIG. 2. *Number of eggs layed at each oviposition*, as referred to the number of eggs laid at the first oviposition ;

o———o sample *a* one single mating after first blood meal ;
△- - - -△ sample *b* 3 to 4 matings after first blood meal ;
□.....□ sample *c* matings after each blood meal.

TABLE III.
Viability.

number of egg-set	mean %-age obtained from eggs			"repeated oviposition"			second "repeated oviposition"
	a	*b*	*c*	*a*	*b*	*c*	*c*
1	87,31 ± 2,78 (n = 45)	88,82 ± 2,70 (n = 47)	92,58 ± 1,50 (n = 55)	87,71 ± 3,75 (n = 14)	78,72 ± 5,52 (n = 18)	70,61 ± 8,81 (n = 18)	94 (n = 1)
2	85,95 ± 3,32 (n = 43)	86,27 ± 3,00 (n = 46)	91,15 ± 2,43 (n = 55)	88 (n = 1)	72,40 ± 17,38 (n = 5)	75,44 ± 7,07 (n = 18)	
3	78,56 ± 4,11 (n = 40)	72,95 ± 5,09 (n = 42)	91,44 ± 2,26 (n = 54)	0 (n = 1)	0 (n = 1)	67,71 ± 8,75 (n = 7)	
4	30,60 ± 7,49 (n = 15)	36,10 ± 6,02 (n = 30)	88,19 ± 2,87 (n = 47)	0 (n = 1)	0 (n = 1)	75 ± 25 (n = 2)	
5	1,29 ± 0,89 (n = 7)	0,60 ± 0,37 (n = 7)	83,83 ± 4,98 (n = 24)				
6	0 (n = 6)	4,13 ± 1,91 (n = 8)	75,93 ± 8,54 (n = 15)				
7	0 (n = 2)	0 (n = 1)	73,33 ± 24,69 (n = 3)				
8	33 ± 33 (n = 3)	43,33 ± 13,02 (n = 9)	0 (n = 0)				

viabilities do not follow a normal distribution, the t-test could not be used to prove that difference, but it is clear enough from Figure 3. The mean difference between sample *c* and the other two samples *a* and *b* consists in a rapid reduction of egg viability in the latter two ; this reduction starts from the fourth oviposition and reaches almost zero at the fifth oviposition. Meanwhile viability remains much higher in sample *c*, where mating is repeated frequently. The values for the eighth oviposition are less reliable, because of the low numbers (e.g. for sample *a* : one nymph I from 3 eggs = 33 % !). The overall viability of all the eggs layed by the ticks (see Table I) correlates with the statements above : the value for sample *c* is also higher than those for the samples *a* or *b*.

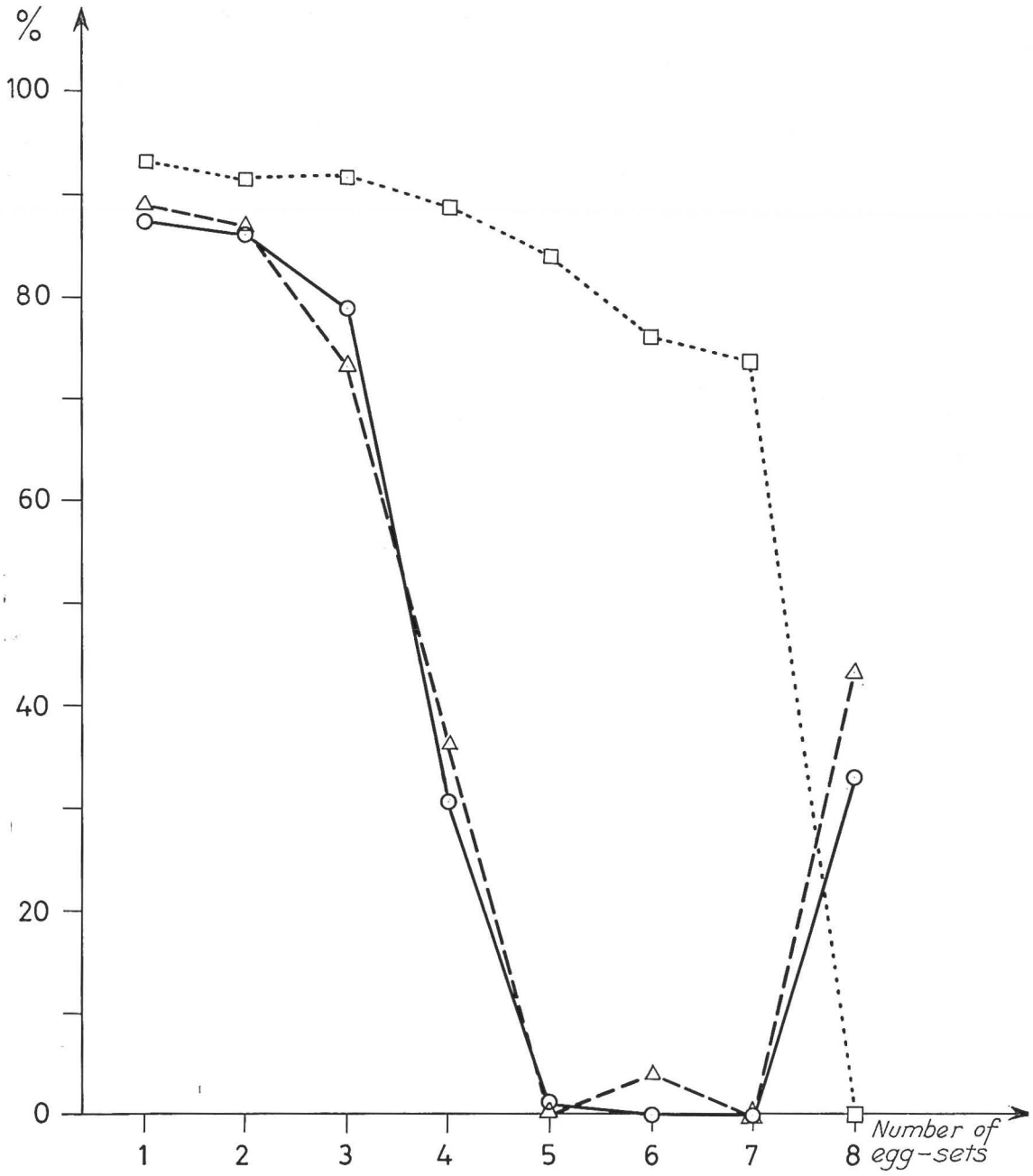

FIG. 3. *Viability of the eggs layed* : percentage of eggs from which viable nymphs I could be obtained ;
o———o sample *a* one single mating after first ; blood meal
△- - - -△ sample *b* 3 to 4 matings after first blood meal ;
□. . . . .□ sample *c* matings after each blood meal.

3.4. *Pre-oviposition period.*

TABLE IV.
Duration of pre-oviposition period.

number of egg- set	Duration (in days) *a*	*b*	*c*	values of t comparing *a* and *b*
1	11,57 ± 0,24 (n = 45)	10 ± 3 (n = 47)	10,04 ± 0,18 (n = 55)	0,510 P = 0,4
2	11,39 ± 0,48 (n = 43)	10,43 ± 0,24 (n = 46)	10,13 ± 0,14 (n = 55)	1,814 P = 0,5
3	12,58 ± 0,72 (n = 40)	11,64 ± 0,56 (n = 42)	10,50 ± 0,18 (n = 54)	1,013 P = 02
4	31,13 ± 11,20 (n = 15)	21,50 ± 1,50 (n = 30)	11,89 ± 0,41 (n = 47)	1,187 P = 0,2
5	36,86 ± 8,14 (n = 7)	30,42 ± 6,76 (n = 7)	15,04 ± 0,68 (n = 24)	0,608 P = 0,3
6	21,83 ± 2,63 (n = 6)	30,25 ± 11,5 (n = 8)	13,00 ± 0,34 (n = 15)	0,988 P = 0,2
7	36 (n = 1)	31 (n = 1)	15,00 ± 2,08 (n = 3)	×
8	23 ± 0 (n = 3)	22,22 ± 1,75 (n = 9)	×	0,249 P > 0,4

The duration of vitellogenesis and of the time-lapse between blood-meal and egg-laying (i.e. the pre-oviposition period) are normal after the three first blood meals, i.e. about 8 to 15 days. In sample *c*, the pre-oviposition period remains normal up to the end as shown in Table IV and Figure 4. In both samples *a* and *b* the mean pre-oviposition period becomes longer, from the fourth oviposition on (twice or three time the values of sample *c*). A very prolonged period of pre-oviposition (30 to 90 or eventually 120 days) may be found in several females of sample *a* or *b*. Such females either lay no more eggs after another blood meal although they are still alive, or do lay eggs after an interval, e.g. after the fourth and the sixth, but not after the fifth blood meal.

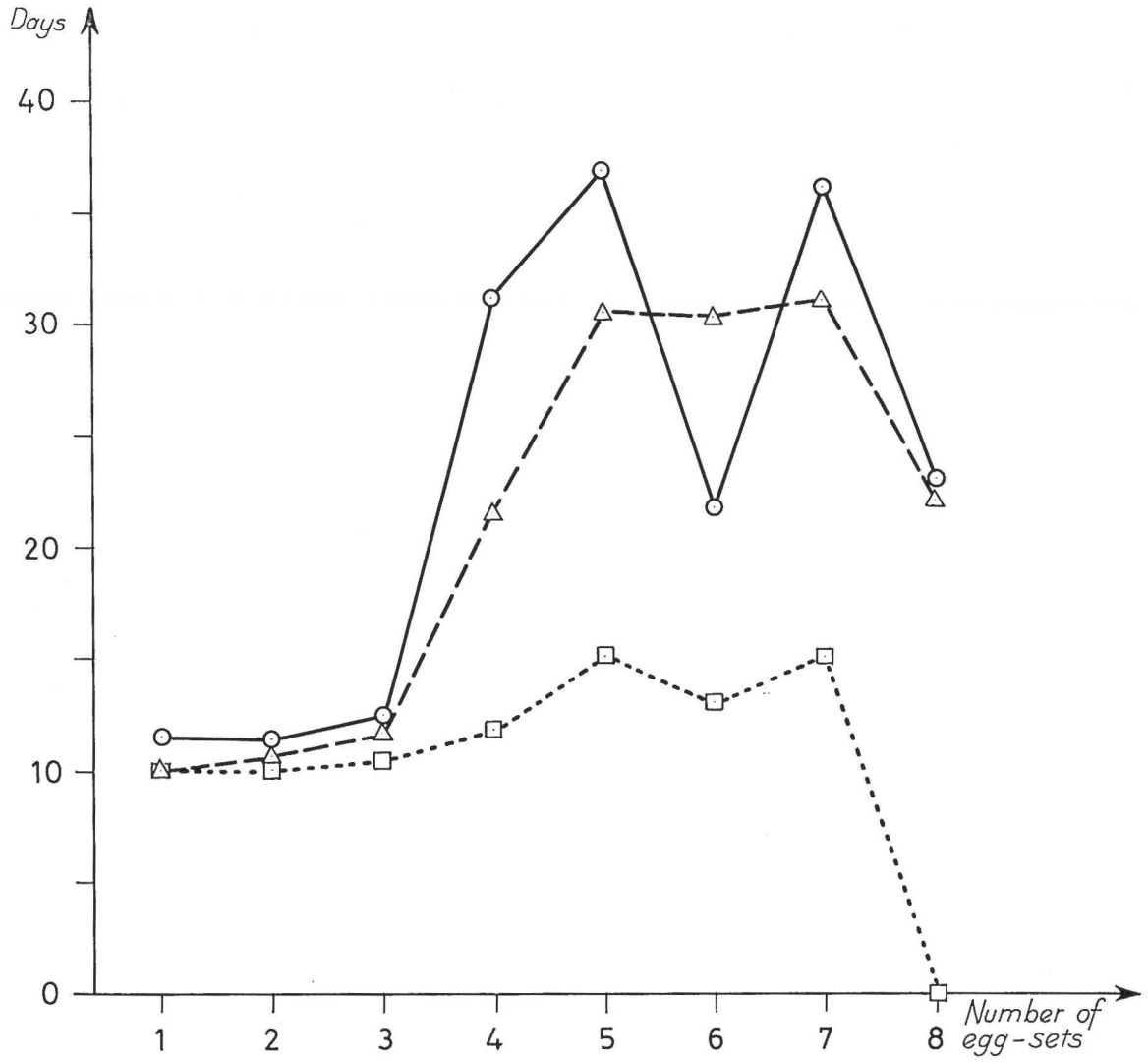

FIG. 4. *Mean duration for the pre-oviposition period in days* ;
o ——— o sample *a* one single mating after first blood meal ;
△- - - -△ sample *b* 3 to 4 matings after first blood meal ;
□. . . . .□ sample *c* matings after each blood meal.

Assuming a normal distribution of the time lapse the use of the Student t-test leads to the conclusion, that the differences occuring between the samples *a* and *b* are not significant.

3.5. *Survival of the females.*

As shown in Figure 5, death of the females occurs mainly after the fourth oviposition and onwards, at a gradually increasing rate. It is interesting to observe that the females which have mated after each blood meal (sample *c*) die earlier than those which mated only after the first blood meal (samples *a* and *b*). There is a slight difference between the two latter samples. Half of the females of sample *a* are still alive after the eighth oviposition (Figure 5), but very few of them still produce eggs (Figure 1). Only one fifth of the females of sample *b* was able

to take in its eighth blood meal. These females were again induced to mate, with resulting oviposition (see Table II and Figure 1). None of the females of samples *c* were able to take another blood-meal, because they all died after the seventh oviposition.

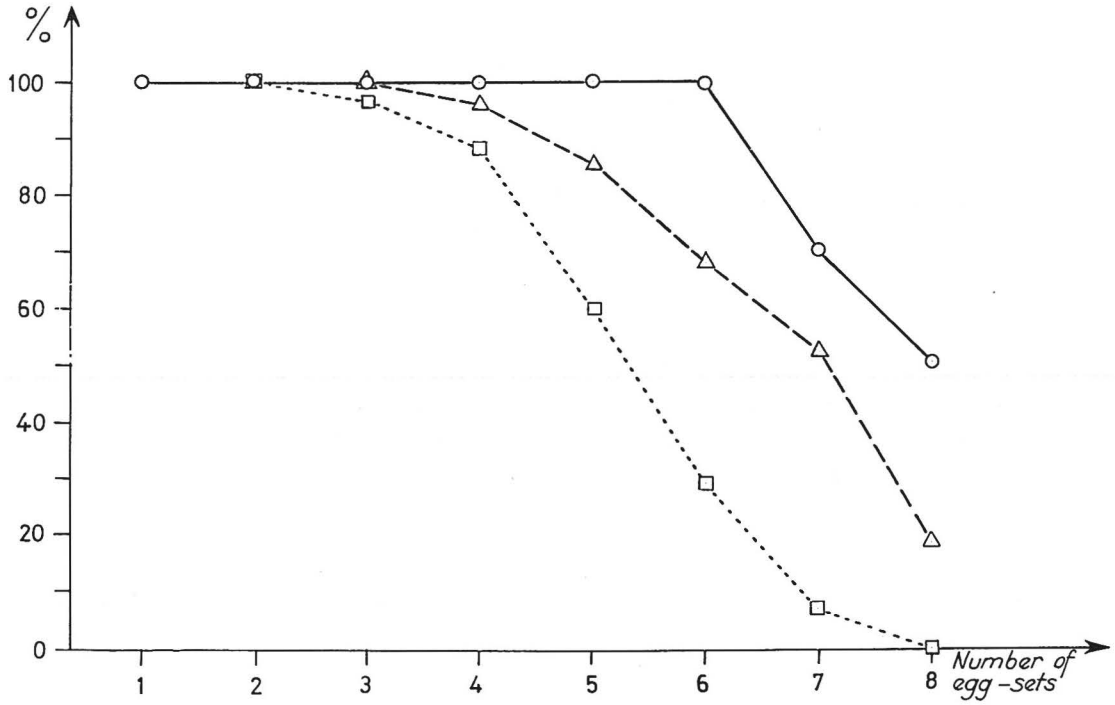

FIG. 5. *Number of surviving females.*
o——o sample *a* one single mating after first blood meal ;
△- - -△ sample *b* 3 to 4 matings after first blood meal ;
□.....□ sample *c* matings after each blood meal.

4. CONCLUSIONS.

1) *O. moubata* very rarely lays eggs more than eight times under laboratory conditions. Young females are able to lay eggs a second or even a third time without a previous blood meal. The pre-oviposition period of these " *repeated ovipositions* " lasts for about two months. We do not yet know whether this is due to a slow vitellogenesis starting with the initial blood meal, or whether there occurs a second rapid vitellogenesis beginning immediately before a second oviposition (" repeated oviposition "). This no longer occurs after the fifth blood meal.

2) Very wide biological variation may be found in different tick samples of the same strain : e.g. difference in the number of eggs laid by females of sample *c* and *b*.

3) The number of matings, i.e. the number of endospermatophores (and therefore of spermiophores) has no influence on fecundity in *O. moubata*. It is known that both, a blood meal and the presence of spermiophores in the female, are necessary to initiate normal vitellogenesis (AESCHLIMANN, 1968 ; AESCHLIMANN & GRANDJEAN, 1973 ; GERMOND & AESCHLIMANN, in preparation). Thus ticks of the samples which mated only after the first blood meal (*a* and *b*) demonstrate that the spermiophores present after a single mating are sufficient to ensure several

viable ovipositions, throughout the life of the females in our experiment. Matings repeated after each blood meal (sample *c*) did assure a higher viability of the offspring, but reduced the longevity of the tick. During the pre-oviposition period, i.e. after a blood meal and/or after mating, digestive processes linked to vitellogenesis become more important. The metabolic turnover of a mated female is higher than that of a virgin female (AESCHLIMANN & GRANDJEAN, 1973 ; GRANDJEAN & AESCHLIMANN, 1973). From a hypothetical point of view, we could assume that the females activated in their metabolism by the repeated arrival of spermiophores would be exhausted earlier and therefore would also die earlier.

4) Further copulations seem to have a positive influence on the appearance of " *repeated ovipositions* " (see point 1). After the second blood-meal, still one third of the recently mated ticks of sample *c* lay eggs twice (see Table II), whereas only one tenth of the females of sample *b* do so. The number of the spermiophores is about the same in the two samples (several 3-4 matings for *b*, as well as for *c*), but the ticks of the sample *c* do contain fresher spermiophores.

With respect to " repeated ovipositions ", the number of the spermiophores present could also play a rôle : only one of over 40 ticks of sample *a* did repeat oviposition, whereas five ticks of sample *b* did so (see Table I). The age of the spermiophores is the same in both cases (mating occuring just after the first blood-meal), but their number is higher in the females of sample *b* (3-4 matings instead of only one in sample *a*).

5) Further copulations after each blood meal enable vitellogenesis to occur within the normal pre-oviposition period (8-15 days). In the absence of further copulations (sample *a* and *b*, mating only after first blood meal), this pre-oviposition period is prolonged.

6) The conclusions mentionned above do somehow modify the idea about the biological cycle in *O. moubata*. The possibility of successive oviposition after further blood-meals favours dispersion of the species. We have shown (AESCHLIMANN, 1968 ; AESCHLIMANN & GRANDJEAN, 1973) that mating may induce normal egg-laying even if it occurs several months after the last blood meal. These two facts, as also the ability of ticks to starve during a very long period (up to several years), enable female ticks to produce offspring during a very long time without being strictly dependent on blood meals.

In nature *O. moubata* females probably live much longer as they do not feed as often as in the laboratory. The above remarks are not lacking in importance for the epidemiology of relapsing fever. It is well know that *Borrelia duttoni*, the pathogenic agent of relapsing fever, may be transmitted transovarially to the offspring of an infected tick (BURGDORFER, 1951 ; AESCHLIMANN, 1958 ; GEIGY & AESCHLIMANN, 1964). Infected females may therefore produce infected nymphs several months (or even years) after the females themselves became infected. Even in the absence of sick people, *O. moubata* may therefore be a reservoir for *B. duttoni* and assure for years the survival of endemic foci of Spirochaetes.

RÉSUMÉ.

Dans le présent travail, les auteurs étudient les rapports existant entre le nombre des copulations (c'est-à-dire la quantité de spermiophores transmis aux femelles) et le nombre des pontes, le nombre des œufs pondus et la viabilité de ces œufs.

Pour ce faire, trois lots de femelles ont été utilisés :

lot a : une seule copulation après le premier repas sanguin ;
lot b : plusieurs copulations (3 à 4) après le premier repas sanguin ;
lot c : plusieurs copulations après chacun des repas sanguins.

Les femelles des trois lots sont nourries tous les 3 mois.
Les résultats de ces expériences en laboratoire sont les suivants :

1) Dans aucun cas il n'y a eu plus de huit pontes, soit parce que les tiques meurent, soit parce qu'elles refusent de prendre un neuvième repas sanguin.

2) La fécondité (nombre d'œufs pondus) ne dépend pas du nombre de copulations, c'est-à-dire de la quantité de spermiophores présents dans la tique (cf. en particulier les lots *a* et *b*). La viabilité des œufs pondus (pourcentage de nymphes I obtenues) ne reste élevée que lorsqu'il y a régulièrement un apport nouveau de spermiophores (lot *c*).

3) Les auteurs ont observé des « pontes répétées », c'est-à-dire des pontes non précédées d'un nouveau repas sanguin ni de nouvelles copulations. L'importance de ces « pontes répétées » semble être favorisée par le nombre et la fraîcheur « des spermiophores présents dans le tractus génital de la femelle.

4) La durée de la préoviposition (le temps entre le repas sanguin et la ponte) s'allonge lorsqu'il n'y a pas d'apport de spermiophores frais (lots *a* et *b*).

5) Le présent travail permet d'envisager sous un nouvel angle certains aspects de l'épidémiologie de la fièvre récurrente africaine dont l'agent pathogène, le spirochète *Borrelia duttoni*, est transmis par *Ornithodorus moubata*.

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