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SOME REQUIREMENTS FOR MATING IN THE RABBIT FLEA, *SPILOPSYLLUS CUNICULI* (DALE)

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INTRODUCTION

Whilst most species of fleas breed in the nests of their hosts, few appear to be as dependent on the sexual condition of their host as is the European rabbit flea *Spilopsyllus cuniculi* (Dale). Mead-Briggs & Rudge (1960) reported that maturation of the ovaries occurred only in fleas which were allowed to feed on rabbits in the final stages of pregnancy. It was postulated that such rabbits provided a 'factor' which was required before vitellogenesis could occur in the fleas. It was found subsequently (Mead-Briggs, 1964*a*) that nestlings up to at least 7 days of age also provided the 'factor' and Rothschild & Ford (1964*a*, *b*) demonstrated that hormones of the host were involved.

This mechanism ensures that fleas have mature ovaries whenever the female rabbit prepares and uses a maternal nest, which is the only nest made by wild rabbits. Most of the fleas on the doe detach themselves at the time of parturition and pass into the nest. Copulation of the virgin fleas then occurs in the nest, typically whilst the female fleas are feeding avidly on the hindquarters of the new-born nestlings. The Hon. Miriam Rothschild had realized that it was important to determine whether male fleas also underwent maturation on the pregnant host before they could mate. She suggested studying whether copulation would occur in the nest between gravid female fleas and males taken from non-pregnant or male hosts. The results of experiments to investigate this question are reported here, with some observations on the reproductive system of the male flea and on mating behaviour.

MATERIALS AND TECHNIQUES

The basic experimental procedure was to put small numbers of virgin male and female fleas together in a mating arena, a warmed box which would also accommodate a recently born nestling rabbit if required, for at least 6 hr. Subsequently the spermatheca (receptaculum seminis) of each female flea was excised and examined for the presence of spermatozoa as the criterion of successful mating and insemination. The undamaged spermatheca was readily teased from the posterior portion of a flea which had been divided, in a drop of saline on a glass slide, by a cut directed obliquely through tergites 7–8 and sternites 4–5. An estimate of the number of spermatozoa present was made on a scale of none, 1–2, 3–10, 11–100, and more than 100. The bulga of the spermatheca can be partially opaque and if only a few spermatozoa are present they

may be obscured and the specimen incorrectly classified as non-impregnated. To ensure that this did not occur the bulga was always ruptured, by removing saline from beneath the coverslip with blotting-paper until sufficient pressure was exerted, when any spermatozoa present flowed out.

Initially the fleas were taken from cultures shortly after they emerged from their cocoons and the sexes were separated, roughly by eye and then checked under CO_2 anaesthesia with a low-power dissecting microscope. Batches of 100–200 fleas of the same sex were then released on individually caged rabbits, and allowed to remain for up to 30 days. For the male fleas the hosts selected were male, non-pregnant, and pregnant female domestic rabbits so that fleas given different maintenance régimes would be available. In some cases these fleas were put for a further 24 hr. with a new-born rabbit. Gravid female fleas were usually required and were obtained by releasing females on recently mated does and recovering them at the time of parturition, when they all had mature ovaries.

Small brown rabbits, from a stock which had been backcrossed occasionally with a wild buck, were used in the first three series of experiments. In subsequent series New Zealand Whites were used as the mortality of fleas on them was lower, their pregnancies were more reliable, and their litters larger. This change of rabbit stock had no apparent effect on the results. The sex of the nestlings used in the preliminary maintenance régime and in the mating arena was recorded in all the series of experiments in which New Zealand Whites were used as hosts. Ten of these nestlings were used in maintenance and thirty-nine in the arenas. As we were unable reliably to sex new-born rabbits by external characters they were either killed and their urino-genital systems dissected, or they were marked and re-examined when 3–4 weeks old.

The boxes used as mating arenas were glass-topped, cardboard museum (display) boxes sized $6 \times 4 \times 2$ in. Although boxes with well-fitting lids were always selected one or two fleas occasionally escaped. During an experiment the boxes were maintained in the dark in an incubator at 24–25° C., 70% R.H. A young nestling rabbit could be kept in such conditions for up to 24 hr. without becoming chilled and if returned to its nest was always accepted by the doe.

It was necessary to prevent certain batches of fleas from feeding on the nestling rabbit which was frequently present in the mating arena. The method used was to amputate part of the piercing mouthparts. To accomplish this the flea was lightly anaesthetized with CO_2 and orientated on a slide on the stage of a dissecting microscope so that the head was to the right and the mouthparts pointed away from the operator. The flea could be held in this position by light pressure from a fine, bent, mounted needle laid across the pronotal comb and behind coxa 1. The laciniae and epipharynx were then gently drawn forwards, clear of the other appendages, using the back of a Borradaile needle, and when clear were pressed firmly against the slide and the terminal 50 μ, or so, cleanly removed with the blade. Particular care was taken to ensure that both labial palps, which are very thin and transparent, were free from the laciniae.

Some direct observations were made of the behaviour of small numbers of male and female fleas put together in an arena with a nestling. At first all the fleas were virgin, but mature, and taken from different pregnant rabbits at the time of parturition. For these observations the glass-topped museum boxes used as arenas were kept on a

warmed surface on the laboratory bench. In some cases the fleas were individually marked with spots of differently coloured cellulose paints.

It was apparent from the results of the mating experiments that the sexual condition of the rabbit on which the male fleas were maintained prior to being put with the female fleas partly determined whether copulation and impregnation occurred. As a consequence, the reproductive systems of male fleas which had been kept for different lengths of time on male, non-pregnant and pregnant female rabbits were examined for any gross differences in appearance. The reproductive system of the male flea is relatively simple and easily dissected from a freshly killed specimen (Mead-Briggs, 1962). Each of the paired pyriform testes contains, within a thin contractile outer connective tissue sheath, many bundles of spermatozoa, and also proximally (posteriad) a convoluted tube of much greater diameter than the vas deferens. This tube has been referred to as the epididymis (Minchin, 1915) and may function as a vesicula seminalis. There are two pairs of tubular accessory glands, those of each side opening by separate pores close to where the vas deferens and ductus ejaculatorius unite. At first there are paired ducti ejaculatorii bound together in a common muscular sheath but where they enter the penis they unite into a common duct terminating at the gonopore. The phallosome, or intromittent organ, is a complex structure and as it was of no concern in the present observations it was dissected away from the penis proper before a cover slip was put over the preparation.

Previous experience with the male system had indicated that there was little variation in the overall size of the testes of different virgin fleas. Some of the most important criteria of variation between different males concern the location of spermatozoa in the carefully removed system, and the possibility of forcing spermatozoa through the ducts to the exterior. The procedure for applying pressure was to remove saline very gradually from beneath the coverslip with blotting-paper, if necessary until the sheaths of the testes finally ruptured.

The midgut epithelium was also examined since a correlation between its height and ovarian development has been reported in the case of the female flea (Mead-Briggs, 1964*b*). Measurements of the heights of the epithelial cells were necessarily very approximate, and were obtained from fresh, unfixed preparations in which the epithelium had been everted by contraction of the circular muscle fibres after the muscular gut wall has been slit with a fine mounted needle.

RESULTS, OBSERVATIONS AND INFERENCES

(1) *The influence on mating success of different maintenance régimes for the male flea*

The scheme of experiments and summary of results are indicated in Table 1; the results are given in a more detailed form in Table 2. There were eighteen basic experiments each repeated two or more times to give a total of sixty-seven individual experiments. Each experiment was started by placing ten gravid female and eight male fleas in a mating arena and terminated 6–24 hr. later when the spermatheca of every female recovered was examined for the presence of spermatozoa. Seventeen of the 670 females either escaped during the experiments, or their spermathecae were lost during dissection, leaving 653 definite records.

The male fleas could be grouped into six categories according to their preliminary maintenance régimes, viz. kept for 26–30 days on male, non-pregnant and pregnant female rabbits and similar fleas subsequently kept 24 hr. alone with a new-born rabbit. The fleas maintained on pregnant rabbits were always collected within a few hours of the parturition of their host and before they had moved into the nest.

Table 1. *Summary of the proportions of gravid female fleas impregnated during confinement in an arena with male fleas that had been given various preliminary maintenance régimes and in the presence or absence of a new-born rabbit*

(In one group of experiments the mouthparts of the male fleas were damaged to prevent their feeding on the nestling in the arena.)

Maintenance régime for ♂ fleas prior to experiment	Proportion of gravid ♀ fleas impregnated during experiment		
	No nestling in arena	Nestling in arena	
		♂ fleas intact	♂ fleas with damaged mouthparts
On pregnant rabbit up to parturition	0/18*	59/60	20/39
Ditto and then 24 hr. on nestling	0/18	46/50	30/40
On male rabbit	0/19	34/60	0/40
Ditto and then 24 hr. on nestling	0/20	25/27	15/50
On non-pregnant female rabbit	0/18	33/60	0/49
Ditto and then 24 hr. on nestling	0/20	27/28	8/37

* 0/18 indicates none out of eighteen females was impregnated.

In most experiments a new-born nestling was placed in the arena immediately before the fleas were released, but in one group of experiments the nestlings were absent. The results show that, whatever type of male flea was used, there was no successful mating when there was no nestling even though several of the experiments were run for 24 hr. By contrast, when a nestling was present mating and sperm transfer occurred in many cases. These experiments fell into two groups, those in which the male fleas were intact and thus able to probe and feed at will on the nestling in the arena and parallel ones in which the mouthparts had been damaged, immediately prior to release in the arena, to prevent their piercing the skin of the nestling.

The results in Table 1 show that a very high proportion of female fleas (59/60) was impregnated when intact male fleas from pregnant hosts were used. The partial amputation of the laciniae and epipharynx of similar male fleas reduced the proportion of females impregnated (20/39) but clearly did not so upset the males as to prevent all mating. A higher impregnation rate (30/40) was recorded when the males had had a preliminary 24 hr. on new-born rabbits before their mouthparts were damaged.

The results with male fleas prepared on male rabbits and on non-pregnant female rabbits were remarkably similar to one another, and yet differed in several respects

from those just described. Consider first the cases in which males with damaged mouthparts were used. There was no successful mating (0/40 and 0/49) if the males had not had contact with a nestling whilst still intact, whereas there was some mating (15/50 and 8/37) when they had. There was some copulation by each category of undamaged male but the proportion of females impregnated was very much higher (25/27 and 27/28 compared with 34/60 and 33/60) if the males had had preliminary access to a nestling.

Some additional facts are illustrated by reference to the more detailed results in Table 2. On two occasions the same groups of males were used for two experiments (a1, a2 and b1, b2). There was no impregnation of the females within 8 hr. in the absence of a nestling but when the males were placed with similar females in the presence of a nestling successful mating (6/8 and 8/8) occurred during 6 hr. It could be that the female flea refuses to mate until she had fed on, or been in contact with, a new-born rabbit. In three experiments (c1, c2 and c3) the mouthparts of gravid females were damaged to prevent their feeding on the nestlings during the experimental exposure and yet 29/30 were impregnated. In two other experiments (d1, d2) the intact, gravid females were left with a nestling for 24 hr. before being put, in the absence of a nestling, with males that had been prepared on pregnant rabbits and nestlings. None of the females was impregnated. It thus appears that feeding on a nestling by the already gravid female fleas is not the essential preliminary to copulation. However, immediate contact with a nestling may be vital for the female and/or the male in the chain of events culminating in successful mating. In one experiment (e) the skin of the nestling was washed in water, to remove water-soluble components left from the amniotic fluid, before the nestling was used in the mating arena. There was no indication that this treatment affected the mating of the fleas. However, there was no mating in a parallel experiment in which a similar, but freshly killed nestling was used.

Although the proportion of females impregnated in the different experiments appears very variable a pattern can be distinguished. As a hypothesis let us assume that before a male flea will mate it requires to be 'primed' by probing, and presumably feeding on, a pregnant rabbit or a new-born nestling, and that male or non-pregnant rabbits do not provide the necessary 'priming'. In those experiments with intact males and a nestling in the arena there was always an opportunity for the males to probe a nestling. With each category of male there was some successful mating but the proportion of females impregnated was clearly much higher when the fleas had been prepared on a pregnant host and/or on a nestling than merely on a male or non-pregnant rabbit.

The hypothesis is more clearly supported by the experiments in which the males had their mouthparts damaged before release in the arena. Although this method of preventing the fleas from piercing the skin of the nestling was far from ideal, e.g. it did unknown damage to the sensory receptor system and led to a loss of haemolymph which reduced the survival of most individuals to under 8 hr., it did not in itself entirely preclude mating. No female was impregnated by males straight from the male or non-pregnant hosts, but some were when the males had been 'primed' on a nestling for 24 hr. A higher rate of impregnation was found with males 'primed' on the pregnant rabbits and the highest of all when a nestling was also included in the régime.

Table 2. *Details and results of all the individual experiments that are summarized in Table* 1

Maintenance régime for ♂ fleas prior to experiment. (No. of days on initial host and sex of nestling if known)			Expt. series no.	Proportion of gravid ♀ fleas impregnated during expt. and its duration (hr.)							
				No nestling in arena		Nestling in arena (and its sex if known)					
						♂ fleas intact			♂ fleas with damaged mouthparts		
	Days	Nestling			Hr.		Hr.	Nestling		Hr.	Nestling
On pregnant rabbit up to parturition	26	—	3	0/8	23	10/10	15	—	3/10	17	—
	30	—	4	0/10	24	10/10	7	♂	8/10	7	♀♀
	27	—	5	—	—	10/10	18	♂♂	6/9	18	♀♀
	27	—	5	—	—	10/10 (e)	18	♂♂	—	—	—
	28	—	6	—	—	9/10	6	♂♂	3/10	6	♂
	28	—	9	—	—	10/10 (c1)	7	♀	—	—	—
Ditto and then 24 hr. on nestling	26	—	1	—	—	7/10	10	—	—	—	—
	26	—	1	—	—	10/10	10	—	—	—	—
	26	—	1	—	—	9/10 (c 2)	8	—	—	—	—
	26	—	3	0/8	8	—	—	—	6/10	8	—
	30	♀	4	0/10	24	10/10	7	♂♀	10/10	7	♀
	30	♀♀	4	—	—	10/10 (c3)	7	♂♀	—	—	—
	30	♂♂	7	—	—	—	—	—	7/10	7	♂♀
	30	♂♂	7	—	—	—	—	—	7/10	7	♂♀
	29	♂♀	8	0/10 (d1)	7	—	—	—	—	—	—
	28	♂♀	9	0/10 (d2)	24	—	—	—	—	—	—
On male rabbit	26	—	1	—	—	3/10	10	—	—	—	—
	26	—	2	0/9	17	—	—	—	0/10	17	—
	29	—	4	0/10	24	0/10	6	♂	0/10	6	♀
	30	—	5	—	—	8/10	6	♂♂	—	—	—
	30	—	5	—	—	10/10	15	♂♂	—	—	—
	30	—	5	—	—	9/10	24	♂♀	0/10	24	♂♀
	28	—	6	—	—	4/10	6	♂	0/10	6	—
Ditto and then 24 hr. on nestling	26	—	2	0/10 (a1)	8	6/8 (a2)	6	—	4/10	7	—
	29	♂♀	4	0/10	24	10/10	7	♂♀	3/10	7	♂♂
	28	♀♀	7	—	—	9/9	7	♂♀	3/10	7	♂♀
	28	♀♀	7	—	—	—	—	—	2/10	9	♀♂
	30	♀	7	—	—	—	—	—	3/10	7	♂

Table 2 *cont.*

Maintenance régime for ♂ fleas prior to experiment. (No. of days on initial host and sex of nestling if known)			Expt. series no.	Proportion of gravid ♀ fleas impregnated during expt. and its duration (hr.)								
				No nestling in arena		Nestling in arena (and its sex if known)						
						♂ fleas intact			♂ fleas with damaged mouthparts			
	Days	Nestling			Hr.		Hr.	Nestling		Hr.	Nestling
On non-pregnant female rabbit	26	—	1	—	—	3/10	10	—	0/9	6	—
	26	—	2	0/9	17	—	—	—	0/10	17	—
	29	—	4	0/9	24	0/10	6	♂	0/10	6	♂
	30	—	5	—	—	7/10	6	♂♂	—	—	—
	30	—	5	—	—	9/10	15	♂♀	—	—	—
	30	—	5	—	—	10/10	24	♂	0/10	24	♀
	28	—	6	—	—	4/10	6	♂	0/10	6	♀
Ditto and then 24 hr. on nestling	26	—	2	0/10 (b1)	8	8/8 (b2)	6	—	1/10	7	—
	26	—	3	—	—	—	—	—	1/8	9	—
	29	♀	4	0/10	24	9/10	7	♀	0/10	7	♀
	30	♂	5	—	—	10/10	6	♀	6/9	6	♂

Notes. The male fleas used in Expts. a1 and b1 were subsequently used in a2 and b2. The females used in c1, c2 and c3 had their mouthparts damaged before release in the arena to prevent their probing the nestling. The females used in d1 and d2 were allowed to feed on a new-born rabbit for 24 hr. prior to the experiment. Owing to this difference in preparation of the females these two experiments are not included in Table 1. In e the nestling was washed with water before the experiment.

The series of experiments were done as follows: 1, July 1963; 2, August 1963; 3, September 1963; 4, February 1965; 5, April 1965; 6, July 1965; 7, October 1965; 8, November 1965; 9, September 1966.

Three experiments were carried out to determine whether the male fleas had to remain on the pregnant host until the litter was born, or whether they were 'primed' some days pre-partum. Groups of eight male fleas were taken from a pregnant rabbit 5, 3½–4 and 2½ days pre-partum, their mouthparts were damaged, and then the males were placed with ten gravid females and a nestling in an arena. Impregnation (5/10) occurred only in the case in which the males had remained until 2½ days pre-partum.

It was also found that long maintenance régimes on the pregnant host were unnecessary. Male fleas were released on a pregnant rabbit only 2 days pre-partum, recovered at parturition and then put for 24 hr. with a nestling. Eight intact males impregnated 6/8 females and eight damaged males impregnated 5/9 females. However, access for 1 day to a new-born nestling was apparently inadequate to 'prime' previously unfed male fleas. Thus no gravid females were impregnated during two 8 hr. experiments in which such males were used.

Table 3. *The proportions of immature female fleas impregnated during experimental exposures with male fleas which had had various preliminary maintenance régimes*

Maintenance régime for ♂ fleas prior to experiment	♀ fleas from non-pregnant rabbit		♀ fleas from male rabbit	
	No nestling in arena (24 hr.)	Nestling in arena (7 hr.)	No nestling in arena (24 hr.)	Nestling in arena (7 hr.)
On pregnant rabbit up to parturition, 30 days	0/8	1/9 0/8	0/10	0/10 0/5
On male rabbit, 29 days	0/10	0/9	0/10	0/10
On non-pregnant female rabbit, 29 days	0/10	0/8	0/10	0/10

Attempts to 'prime' intact male fleas by keeping them for 24 hr. close to, but not in probing contact with, a new-born rabbit failed. The fleas, which had previously spent 26–28 days on a male rabbit, were placed in an arena on one side of a double-layered gauze partition through which they could not reach a nestling on the other side. At the end of the 24 hr. the nestling was changed and gravid female fleas were put with the males. None out of 30 females was impregnated in three experiments. The mouthparts of two groups of males were then damaged and these fleas were put with females into the nestling compartment but again there was no successful mating. It was concluded that male fleas could not be 'primed' by such odour contact with a nestling.

In all the experiments so far reported gravid female fleas were used. This would approximate to the situation in nature, since most of the female fleas taken to the nest by the parturient doe will have mature ovaries. It was, however, of interest to determine whether well-fed female fleas with undeveloped ovaries mated under our experimental conditions. Two groups of experiments were carried out, one with female fleas that had been maintained on a non-pregnant female rabbit for 31 days and the other with females from a male rabbit after a similar period. The experimental scheme and the results are summarized in Table 3. As anticipated there was no mating in the absence of a nestling but only one flea was impregnated, out of a possible sixty-nine, when nestlings were in the arenas. This flea received many (over 100) spermatozoa

and had been with males that had been 'primed' on a pregnant host. It may be concluded that fleas with undeveloped ovaries are rarely inseminated.

The results for mating success of the fleas were examined according to the sex of nestling used but no likely effect of sex was indicated, although the data were sparse.

(2) *The number of spermatozoa transferred*

An estimate was made of the number of spermatozoa in each spermatheca and the results are summarized in Table 4. These results show a general similarity in pattern to those found for overall mating success. The concept that the male requires to be 'primed' by probing contact with a pregnant rabbit or a nestling is supported, and this 'priming' appears to be quantitative.

Table 4. *The estimated number of spermatozoa in the spermathecae of gravid female fleas after they had been confined with males that had been given various preliminary maintenance régimes*

(In one group the males were damaged to prevent their feeding on the new-born rabbit also present in the arena).

Maintenance régime for ♂ fleas prior to experiment	No. of spermathecae containing						% ♀♀ impregnated
	0 S.*	1–2 S.	3–10 S.	11–100 S.	> 100 S.	Total	
			♂ fleas intact				
On pregnant rabbit up to parturition	1	0	1	4	54	60	98
Ditto and then 24 hr. on nestling	4	0	2	5	39	50	92
On male rabbit	26	2	8	14	10	60	57
Ditto and then 24 hr. on nestling	2	1	2	8	14	27	93
On non-pregnant female rabbit	27	5	11	9	8	60	55
Ditto and then 24 hr. on nestling	1	1	4	8	14	28	97
Total	61	9	28	48	139	285	79
			♂ fleas with mouthparts damaged				
On pregnant rabbit up to parturition	19	4	9	6	1	39	51
Ditto and then 24 hr. on nestling	10	3	6	14	7	40	75
On male rabbit	40	0	0	0	0	40	0
Ditto and then 24 hr. on nestling	35	4	4	7	0	50	30
On non-pregnant female rabbit	49	0	0	0	0	49	0
Ditto and then 24 hr. on nestling	29	1	1	6	0	37	22
Total	182	12	20	33	8	255	29

* S. = Spermatoza

In the experiments with intact males the proportion of females receiving large quantities of sperm was highest when the males had come from a pregnant host and lowest when they were from male or non-pregnant hosts and their only contact with

a nestling was in the mating arena. Preliminary exposure to a nestling for 24 hr. of fleas from male and non-pregnant hosts clearly increased the overall quantity of sperm transferred.

The practice of damaging the mouthparts of some males to prevent their successfully probing the nestling during the experiment reduced not only the percentage of females impregnated but also the relative number of spermatozoa transferred; nevertheless, the pattern was similar. The only females to receive large numbers of spermatozoa were those put with males given preliminary access to a pregnant rabbit and a nestling.

Table 5. *To show that the number of spermatozoa transferred to the spermathecae of gravid female fleas by male fleas previously maintained on male or non-pregnant female rabbits increased with time for up to at least 24 hr.*

Source and condition of ♂ fleas		Duration of exposure (hr.)	No. of spermathecae containing				
			0 S.*	1–2 S.	3–10 S.	11–100 S.	> 100 S.
Male rabbit, 30 days	Mouthparts intact	6	2	1	3	4	0
		15	0	1	1	4	4
		24	1	0	0	4	5
	Mouthparts damaged	24	10	0	0	0	0
Non-pregnant female rabbit, 30 days	Mouthparts intact	6	3	3	4	0	0
		15	1	0	2	3	4
		24	0	0	1	5	4
	Mouthparts damaged	24	10	0	0	0	0

* S. = Spermatozoa

The results of all experiments are incorporated in Table 4 irrespective of the length of time the sexes were together; this varied from 6 to 24 hr. (Table 2). Consequently all the data are not strictly comparable but the only important differences concern the categories of intact male fleas from male and non-pregnant female rabbits and whose only access to a nestling was in the arena. In one series of experiments groups of similar fleas were left together for 6, 15 and 24 hr. The numbers of spermatozoa found in the spermathecae were greater the longer the experiment (Table 5). These results support the concept of a quantitative 'priming' of male fleas whilst they are in probing contact with a new-born nestling. The influence of the duration of the experiment was relatively unimportant in the case of fleas 'primed' on a pregnant rabbit since nearly all the females received a large number of spermatozoa, approaching the maximum spermathecal capacity, within 6 hr. It was also unimportant with damaged males since the operation impaired their survival and few were fully active beyond 6–8 hr.

When the experiments were planned it was considered impracticable to use only one male flea per box. Almost certainly very many more replicate experiments would have been required and sufficient new-born rabbits were not readily available. A six-replicate experiment was carried out to obtain some idea of what a single, fully 'primed' male could achieve under conditions similar to those already described. One male flea maintained on a pregnant doe for 28–29 days up to parturition, ten gravid

females and a new-born nestling were put together in an arena for 7 hr. The results were as follows:

Sex of nestling in arena	Number of spermathecae containing				
	0 S.*	1–2 S.	3–10 S.	11–100 S.	>100 S.
♂	3	1	1	3	2
♂	2	0	1	4	3
♀	10	0	0	0	0
♀	3	0	0	4	3
♀	3	0	3	3	1
♀	8	0	1	1	0

* S. = Spermatozoa.

Table 6. *To show that changes occur in the mobility of spermatozoa within the genital ducts of male fleas when maintained on pregnant rabbits but not when kept on male or non-pregnant female rabbits*

Maintenance régime for ♂ fleas		Proportion of preparations with spermatozoa out of testes and at various positions in system				
		No pressure applied		Slight pressure applied to preparation		
		In epid.	In vasa def.*	In epid.	In vasa def.*	Ex gonopore
Unfed (straight from culture)		0/3	0/3	1/3	0/3	0/3
On male rabbit for	9 days	0/3	0/3	3/3	0/3	0/3
	14 days	0/3	0/3	3/3	0/3	0/3
	21 days	0/3	0/3	3/3	0/3	0/3
	28 days	0/3	0/3	3/3	0/3	0/3
On non-pregnant female rabbit for	9 days	0/3	0/3	3/3	0/3	0/3
	14 days	0/3	0/3	2/3	0/3	0/3
	21 days	0/3	0/3	3/3	0/3	0/3
	28 days	0/3	0/3	3/3	0/3	0/3
On pregnant rabbit for	4 days	1/3	0/3	1/3	½/3	0/3
	9 days	2/3	0/3	3/3	½/3	0/3
	14 days	2/3	0/3	3/3	1/3	0/3
	17 days	3/3	½/3	3/3	½/3	1/3
	19 days	3/3	0/3	3/3	½/3	0/3
	21 days	3/3	2½/3	3/3	3/3	0/3
	23 days	3/3	0/3	3/3	3/3	1/3
	25 days	3/3	½/3	3/3	2/3	2/3
	27 days	3/3	2/3	3/3	3/3	3/3
(Day of host's parturition)	29 days	3/3	½/3	3/3	2½/3	3/3

* 1/3 indicates spermatozoa were present, or forced into, both vasa deferentia of one individual out of three, ½/3 means into only one vas deferens of one individual and ½/3 into one vas deferens in each of two out of three individuals, etc.

(3) *The influence on the male reproductive system of different maintenance régimes*

A typical series of observations is summarized in Table 6. Unfed male fleas were released on male, non-pregnant and pregnant rabbits and samples of three fleas were removed at various intervals from each host and dissected. No difference was observed between the fleas from the male rabbit and the non-pregnant female. Before pressure

was applied the spermatozoa were entirely restricted to the testes proper, but with pressure some spermatozoa entered the epididymes but not the vasa deferentia. These observations were in marked contrast to those on males from the pregnant rabbit. In most of these spermatozoa were already present in the epididymes and in several fleas taken during the latter half of the gestation spermatozoa were present in one or both vasa deferentia. Gentle pressure frequently caused spermatozoa to pass into the vasa deferentia but at first they rarely penetrated beyond the level of the junction with the accessory glands and the ejaculatory ducts. With fleas taken when the host was only a few days pre-partum, however, spermatozoa could usually be made to pass right through the system and out of the gonopore into the surrounding saline. Thus spermatozoa in fleas kept on a pregnant rabbit exhibit much greater mobility within the genital ducts than do spermatozoa in fleas kept on male and non-pregnant female hosts.

As already mentioned there is little change in the overall size of the testes that can be correlated with the type of flea maintenance. This observation is probably consistent with the fact that spermatogenesis is largely completed by the time the adult flea emerges from its cocoon. The ranges of size found for the testis proper, i.e. the part of the capsule containing bundles of spermatozoa were as follows:

54 specimens from pregnant host	285–340 × 130–160 μ
24 specimens from non-pregnant host	280–330 × 135–155 μ
24 specimens from male host	290–320 × 135–155 μ

There were spaces between the bundles of spermatozoa in the testes of many unfed fleas and of fleas which had been on a host for less than 2–3 days. These spaces appeared to be filled by a fluid containing small granules. The heads of the spermatozoa of each bundle were typically embedded in a granular, gelatinous cap, but when the sheath of a testis was ruptured at least some freely motile spermatozoa were to be seen, irrespective of the feeding régime of the flea.

The size of the accessory glands increased progressively in fleas kept on a pregnant host, but did not do so in fleas on non-pregnant and male hosts. Data included in Table 7 show that the length and width of the lateral accessory glands increased by about 50%. These glands, which consist of a simple columnar epithelium, appeared to be in an active secretory phase in fleas taken from a host shortly pre-partum.

Table 7 also includes data on the heights of the epithelial cells lining the midgut. The results show that these epithelial cells were significantly larger in fleas taken from a pregnant host during the final week of gestation than from non-pregnant ones. A similar, but more marked, development of these cells was found in the case of female fleas kept on pregnant hosts (Mead-Briggs, 1964b; Rothschild, 1965a).

(4) *Mating behaviour*

In rabbit nests rabbit fleas may be found *in copula* both on the skin of the nestlings and amongst the nest material. Similarly, during the observations on the fleas in the cardboard boxes it was found that copulation could be initiated between fleas both on and off the body of the new-born nestling enclosed with them. However, no mating attempts were seen when there was no nestling in the box.

Twelve sets of fleas were watched, and details are given in Table 8 of the numbers

Table 7. *The mean length (height) of midgut epithelial cells (m.ep.c.), and the mean length and width of lateral accessory glands (a.gl.) of male rabbit fleas dissected after maintenance for various periods on male, and non-pregnant and pregnant female rabbits*

(Each mean is based on the dissection of a sample of three fleas.)

(1) Fleas from ♂ rabbit	No. of days on rabbit	9	14	21	28							
	Mean length m.ep.c. (μ)	15	15	16	16							
	Mean length a.gl. (μ)	61	61	60	61							
	Mean width a.gl. (μ)	13	13	12	12							
(2) Fleas from non-	No. days on rabbit	9	14	21	28							
	Mean length m.ep.c. (μ)	16	18	16	17							
pregnant ♀ rabbit	Mean length a.gl. (μ)	61	59	64	61							
	Mean width a.gl. (μ)	12	12	12	12							
(3*a*) Fleas from	No. days on rabbit	6	14	21	23	25	27	30				
pregnant ♀ rabbit	No. days pre-partum	24	16	9	7	5	3	0				
(*a*)	Mean length m.ep.c. (μ)	14	14	16	20	20	21	31				
	Mean length a.gl. (μ)	—	—	—	—	—	—	98				
	Mean width a.gl. (μ)	—	—	—	—	—	—	20				
(3*b*) Fleas from	No. days on rabbit	0	4	9	14	17	19	21	23	25	27	29
pregnant ♀ rabbit	No. days pre-partum	—	25	20	15	12	10	8	6	4	2	0
(*b*)	Mean length m.ep.c. (μ)	7	20	17	18	18	17	17	18	22	22	26
	Mean length a.gl. (μ)	39	69	81	83	81	79	86	93	83	90	99
	Mean width a.gl. (μ)	8	17	16	15	17	17	21	17	19	21	21

Table 8. *Details of the numbers of fleas which copulated, or attempted to do so, when male and female fleas, which had been maintained on pregnant rabbits up to parturition, were put together with a new-born rabbit in a glass-topped box on a warmed bench*

No. of fleas used		No. of attempted copulations	No. of successful copulations			Time to 1st copulation (min.)
♂	♀		In box	On nestling	Total	
3	5	3	2	1	3	130
3	5	0	0	0	0	—
4	10	0	0	1	1	100
4	8	2	1	2	3	9
4	8	0	1	1	2	45
4	8	7	1	1	2	120
4	8	2	0	0	0	—
6	7	0	0	0	0	—
5	10	9	1	3	4	50
5	7	0	0	1	1	45
5	7	1	0	1	1	10
10	20	1	1	0	1	30

of fleas which were used, and the numbers which copulated or attempted to do so. The number of matings or attempted matings observed was low, presumably because the conditions obtained on the bench were not so satisfactory as in the incubator used for the other experiments. Perhaps darkness, the usual situation in nature, is important. Although it was thought that all the fleas of the same sex would be in a similar physiological state, as they were similarly prepared, there was a wide range of reaction to individuals of the opposite sex. Thus, in some cases males and females encountered each other as they moved about, or they came to rest, or to feed, side by side and yet showed no signs of mating activity. Sometimes a male approached a female, ran around her and attempted to copulate but the female would not accept. As he at-

tempted to slip beneath her abdomen with his abdomen curved upwards and the terminalia raised she might either move away or, in the case of some feeding females, repel him by kicking with the hind leg. Using individually marked fleas additional situations involving unsuccessful mating attempts were noted. An individual male might attempt to copulate with several females, sometimes in quick succession, but be rejected each time. The male did not necessarily attempt to mate with every female encountered. Sometimes one or more males made repeated attempts at copulation with a particular female but were rejected each time. Some females might mate with one male and later reject another, or vice versa.

When a male was accepted and copulation successfully achieved the female might move about, dragging the male with her, or remain attached and feeding on the nestling. The male was not seen to feed whilst *in copula*. The duration of copulation varied widely from 30 sec. to 9 or 10 min. The actual mating 'behaviour' appeared to have a very simple pattern, with no preliminary 'courtship'. The male made a definite approach to certain females, and was either allowed to copulate or rejected. The 'characteristic zig-zag approach' of the male referred to by Rothschild (1965*a*) seemed to be a purely mechanical effect as the flea scrambled through loose fur or scuttled over the body of the nestling, since both sexes moved in an irregular path in this way.

DISCUSSION

The results described in the preceding sections indicate that several conditions must be satisfied before successful copulation can occur between male and female rabbit fleas. Thus, the male fleas, and probably the females, need to be in an appropriate 'physiological state', and there must be a live, young nestling in the vicinity of the fleas. Fleas which will mate soon after reaching an occupied nest never copulate whilst still on the adult host, even if it is pregnant or immediately post-partum. Although fleas were frequently found *in copula* off the body of the nestling in the boxes (and also in natural rabbit nests) and were sometimes seen to commence mating whilst away from the nestling they were never found to have mated successfully, using impregnation as the criterion, in the complete absence of a nestling. Groups of male fleas which failed to impregnate any females in an otherwise empty box mated effectively with similar females as soon as a nestling was included.

The present direct observations and the literature suggest that in many species of fleas the female determines whether copulation will be permitted. If she is not ready to accept copulation courting males may be repulsed by vigorous kicking with the hind legs. In several species the males are believed to be positively attracted to females when the latter are ready for copulation, and it has been suggested that the females may produce a pheromone which is detected at a distance by receptors on the maxillary palps of the male (Geigy & Suter, 1960). Humphries (1967) found that male *Ceratophyllus gallinae* (Schrank) do not locate females from a distance, but that mating behaviour is initiated after the male has accidentally collided with a female and a contact-chemical stimulus has been received by his maxillary palps.

It would be interesting if this difference between short-distance and contact attractiveness of the female could be correlated with the need or otherwise for blood meals by the female prior to mating. There is evidence that the females of several

species of fleas do require to feed before they mate. Geigy (1953) observed that copulation occurs in *Tunga penetrans* (Linn.) only after the female has bored into the skin and various organs have undergone hypertrophy. Female *Megabothris c. clantoni* (Hubbard) need access to a suitable host for at least one day before they will mate (Poole & Underhill, 1953). Geigy & Suter (1960) showed that before copulation female *Echidnophaga gallinacea* (Westwood) must feed for 2–3 days, during which time their ovaries reach a certain, but undefined, stage of maturity. Suter (1964) implied that the females of all species of fleas must undergo a period of maturation before they are ready for copulation and become attractive to males. However, the females of many ceratophyllids do not require a blood meal prior to copulation although feeding will doubtless be necessary for egg production. Examples include the bird fleas *Ceratophyllus farreni* Roths. (Waterston, 1910), *C. niger* Fox, *C. idius* Jordan & Roths. and *C. riparius* Roths. (Holland, 1955) and *C. gallinae* (Schrank) (Rothschild, 1965*a*; Humphries, 1967) and the grey squirrel flea *Orchopeas h. howardi* (Baker) (R. J. C. Page, unpublished).

Unfed female rabbit fleas do not mate. Even fleas which have been allowed to feed for 30 days on male or non-pregnant rabbits, but of course still have immature ovaries, mate only rarely when put in an arena with fecund males and a nestling. By contrast, in a similar situation nearly every female with mature ovaries would be impregnated. A systematic study to determine whether the propensity to mate can be correlated with the precise degree of ovarian development has not been undertaken. (Adams & Mulla (1968) have demonstrated that mating in the eye gnat *Hippelates collusor* occurs when ovarian development is in oogenetic stages 6–9, with a peak in stage 7. This can be correlated with production of a pheromone sex attractant from stage 6 to stage 9, after which the females become repellent during stage 10, the stage of mature eggs.) Indeed such a study in the rabbit flea would be difficult because ovarian development is very rapid, passing from immaturity to near maturity in 24 hr., when the female flea can feed on a nestling rabbit, and it is essential to provide a nestling if mating is to be possible. Presumably fleas would have to be taken from pregnant rabbits when their ovaries were at different stages of development and then prevented from feeding further, on the nestlings, by damage to their mouthparts or some similar technique.

Mating occurred in all three of the present experiments in which the mouthparts of gravid females taken directly from an adult host were deliberately damaged to prevent their feeding on the nestling in the arena. This appears to conflict with the belief of Rothschild (1965*a*, *b*) that the female flea, even if mature, needs to feed on a young rabbit before she will accept copulation. Under natural circumstances feeding would occur on the nestling in any case to provide nourishment for continued oocyte maturation. Rothschild (1965*a*) and Rothschild & Ford (1966) have shown experimentally that different hormones of the host are involved in ovarian development and the acceptance of copulation in the fleas. Apparently the 'copulatory factor' can produce its releaser effect without being imbibed.

Other workers have found that the males of most species of fleas do not require to feed before they can copulate successfully. This is the case for all those ceratophyllid species in which the females do not need preliminary meals, and for *T. penetrans*, *E. gallinacea* and *Xenopsylla cheopis* (Roths.) which do (Geigy & Suter, 1960). However, males of *M. c. clantoni* fed on rat blood are insufficiently aggressive to copulate

satisfactorily, by contrast with ones fed on mice (or voles) (Poole & Underhill, 1953). Our experiments indicated that the male rabbit flea must be in the correct 'physiological state' before it can mate successfully. This state is usually reached when the flea has been in probing contact with a pregnant rabbit not more than 2–3 days prepartum, or a new-born rabbit. The contact with a nestling needs to be for only a few hours, particularly if the fleas have already fed for some days on an adult rabbit. The nestling contact has to be one that allows probing, and presumably feeding. Males which have been only in odour contact or did not probe because of damage to their mouthparts fail to mate. It is possible that the accessory glands of the reproductive system of the male rabbit flea have to be brought into good secretory activity before the spermatozoa can pass readily through the genital ducts.

It is generally considered that reproductive processes in the male insect are little affected by nutrition although mating behaviour may be elicited by many kinds of stimuli, including various types of food. It has not been possible to deduce whether the 'factor' needed by the male rabbit flea to bring it into the correct 'physiological state' for mating, and obtained from the pregnant host and/or the new-born rabbit, is a nutritional requirement or a sensory trigger. The same difficulty was found when considering the 'factor' needed by the female flea for initiation and maintenance of vitellogenesis (Mead-Briggs, 1964*a*). These two factors may well be one and the same, since their distribution appears to be identical, i.e. present during the final stages of a rabbit's pregnancy, and at high level in the new-born, but waning to zero during the first 7 days, or so, of life.

SUMMARY

1. Rabbit fleas *Spilopsyllus cuniculi* (Dale) do not mate while on adult rabbits; copulation is usually initiated when the fleas are on the body of a new-born rabbit and at least the close proximity of such a nestling is essential. The female probably becomes receptive only after close contact, but not necessarily probing contact, with a young nestling.

2. Fleas with mature ovaries mate much more readily than immature fleas.

3. Male fleas must be in a suitable 'physiological state' before they can mate and inseminate females. This state is reached by probing contact with rabbits in the final stages of pregnancy or, for a relatively shorter period, with new-born rabbits. Spermatozoa of such fleas show much greater mobility within the genital ducts than those from fleas kept on male or non-pregnant rabbits.

It is a pleasure to record the help given by Mr R. J. C. Page in the earlier stages of this work. Many of our colleagues throughout England and Wales assisted by sending us flea-infested wild rabbit nests.

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