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THE DISTRIBUTION OF THE EGGS OF MAMMALIAN LICE ON THEIR HOSTS

II. ANALYSIS OF THE OVIPOSITION BEHAVIOUR OF DAMALINIA OVIS (L.)

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Summary

The behaviour pattern of *Damalinia ovis* (L.) is adapted to the physical features of the environment in which the louse lives.

In stage 1, the louse was attracted to temperatures between 35 and 40°C and this temperature zone was necessary for oviposition to proceed satisfactorily. Optimum temperature conditions were between 37 and 39°C. In stage 2, the louse orientated itself so that its head was directed towards the warm end of a temperature gradient or towards the saturated end of a humidity gradient, but when these gradients were antagonistic the orientation to temperature dominated. At the commencement of stage 3, the louse reversed its orientation to both temperature and humidity gradients but again the attraction to temperature was dominant. Before egg laying commenced, a fibre of suitable diameter had to be caught by a gonopod and held next to the abdomen. The resulting tactile stimulus was critical and its absence inhibited oviposition. Other factors which influenced oviposition were the depressant effect of high humidities, the orientation to light, and the attraction to other ovipositing lice and eggs.

I. INTRODUCTION

The oviposition behaviour of some mammalian lice and the division of the behaviour pattern into three stages has been described in Part I of this series (Murray 1957). It was found that when female *Damalinia ovis* (L.), *Linognathus stenopsis* (Burm.), *Haematopinus eurysternus* (Nitz.), and a *Boopia* sp. were placed, together with wool or hair from their host, in a temperature gradient which simulated that found in the host's hair coat, the distribution of the eggs laid was similar to that found naturally. This suggested that the behaviour patterns were adapted to the common physical characteristics of the environments of the lice. In these experiments, external stimuli which might influence the oviposition behaviour could have originated from the fibres, from variations in temperature, humidity, and light, or from the presence of other lice.

This paper presents the results of a study of the influence of these factors on the oviposition behaviour of *D. ovis*.

II. THE INFLUENCE OF THE FIBRE

(*a*) *The Nature of the Fibre*

It was found that *D. ovis* would attach eggs readily to glass wool or 1½-denier "Nylon". To determine whether these materials were suitable for oviposition

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experiments, two collections, each of approximately 1500 lice,* were divided into three groups. One group was placed in a glass tube with glass wool, another with wool which had been scoured with ether and water to remove all traces of sweat and sebaceous gland secretions, and the third with natural wool from the sheep. The wool and the lice were removed from the same sheep. All groups were exposed to 37·5°C and the humidity was maintained at 60 per cent. R.H. by means of sulphuric acid solutions (Solomon 1951). The number of eggs was counted after 36 hr

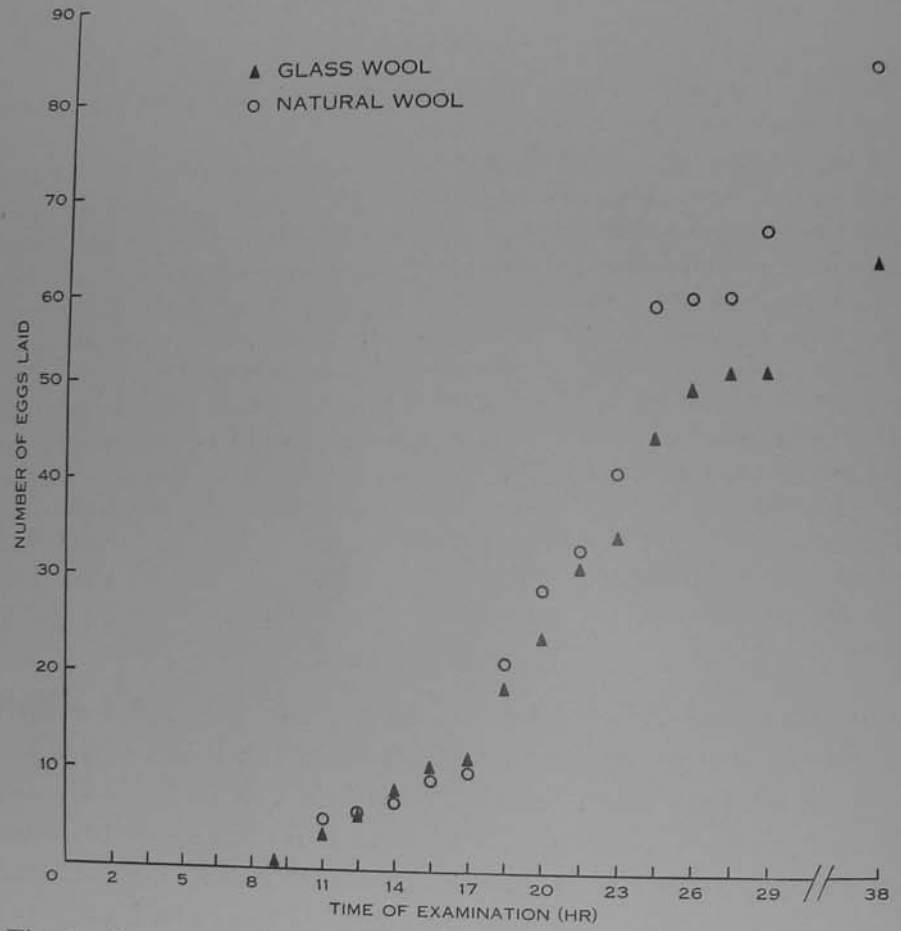

Fig. 1.—Comparison of rate of oviposition of *D. ovis* on glass wool and on wool from sheep when exposed to 37·5°C.

and again after 60 hr. In the tubes containing scoured wool 13 per cent. of the lice laid eggs and 23 per cent. of the lice laid eggs in each of the other tubes. However, after another 24 hr, 37 per cent. of the lice on natural wool had laid eggs whereas no more eggs had been laid on the scoured wool or on glass wool. It was observed that after this period the majority of the lice on scoured and glass wool were dead.

In a further experiment, about 600 lice were randomly divided into six groups. Three of these groups were placed in cells with wool of the same sheep from which they were collected and the other three groups were placed in cells with glass wool. All groups were exposed to 37·5°C and examined at 1½-hr intervals. As may be seen in Figure 1, no eggs were laid for the first 9 hr. For the next 12 hr eggs were laid at a similar rate in each group, but for the remaining 17 hr more eggs were laid in the cells containing natural wool. Over this period there was again a greater

* Throughout this paper the term "lice" will refer to female lice.

mortality of lice in the cells containing glass wool. After 29 hr, 24 per cent. of the lice on glass wool were dead and on the natural wool 20 per cent. were dead. This mortality had risen to 56 per cent. on glass wool and 36 per cent. on natural wool, when the experiment concluded 9 hr later.

(*b*) *The Presence of a Fibre*

Two collections of lice were each divided into two groups. One group was placed in a glass tube with no fibre material and the other group in a similar tube with glass wool. Both groups were exposed to 37·5°C and the relative humidity was maintained at 60 per cent. After 36 hr, the number of eggs laid was counted. In the first experiment, 380 lice were placed in the tube without glass wool and one egg was laid whereas 62 eggs were obtained from 381 lice in the tube with glass wool. In the second experiment, three eggs were laid by 506 lice in the tube without glass wool and 161 eggs by 589 lice in the tube with glass wool. Examination of the lice in the tubes without fibres revealed that the egg within many of the lice had developed to maturity (see Section III(*b*)). A population of lice which contained a high percentage of females ready to oviposit was frequently required in subsequent experiments and was obtained by witholding fibres from the lice for 24 hr prior to the experiment.

(i) *Influence on Stage* 2 *of the Behaviour Pattern.*—Lice were placed in four cells along which a temperature gradient from 20 to 40°C was established. In cell 1, there were no fibres present; in cell 2, glass wool fibres were placed so that a louse could move freely on one fibre along the gradient but at no time was it possible for its abdomen to come into contact with any other fibre; in cell 3, the number of glass wool fibres was such that the abdomen of the louse was at all times in contact with several fibres; and in cell 4, no fibres were present but the roof of the cell was lowered so that the abdomen of the louse was at all times in contact with it and the floor. In all cells the lice moved to the warm end and the only difference observed in stage 2 was that the abdominal movements of the lice in cells 1 and 2 were not as vigorous as those of the lice in cells 3 and 4. Only the lice in cells 2 and 3 laid eggs and there was no difference in the duration of stage 2 between the lice in these two cells. When glass wool was added to cells 1 and 2 so as to make the density of the fibres in these cells similar to that in cell 3, vigorous abdominal movement commenced immediately.

(ii) *Influence on Stage* 3 *of the Behaviour Pattern.*—Another collection of lice was divided into three groups. They were placed in similar glass tubes and exposed to 37·5°C and a relative humidity of 60 per cent. In one tube was placed glass wool, the fibres of which were approximately 0·02 mm in diameter, in another, glass fibres approximately 0·1 mm in diameter, and in the other, glass fibres ranging from 0·2 to 0·35 mm. On 0·02-mm glass wool 200 lice laid 22 eggs, on the medium-sized fibres 255 lice laid two eggs, and 407 lice on the widest fibres laid four eggs. Similar results were obtained on repetition. It was observed that the louse was unable to hold the larger fibres between the gonopod and abdomen.

Thus the presence of a fibre which could be held next to the abdomen by a gonopod was necessary for egg laying to take place.

III. The Influence of Temperature

(a) *On the Number of Eggs Laid*

Five collections, each of 8000–10,000 lice, were made and all the lice of each collection were exposed to one of the following temperatures: 32·5, 35, 37·5, 40, or 42°C. Another collection of 20,000 lice was divided into four groups and each group was exposed to either 32·5, 35, 37·5, or 40°C. The relative humidity was maintained at 65 per cent. and the number of eggs laid was counted after 48 hr.

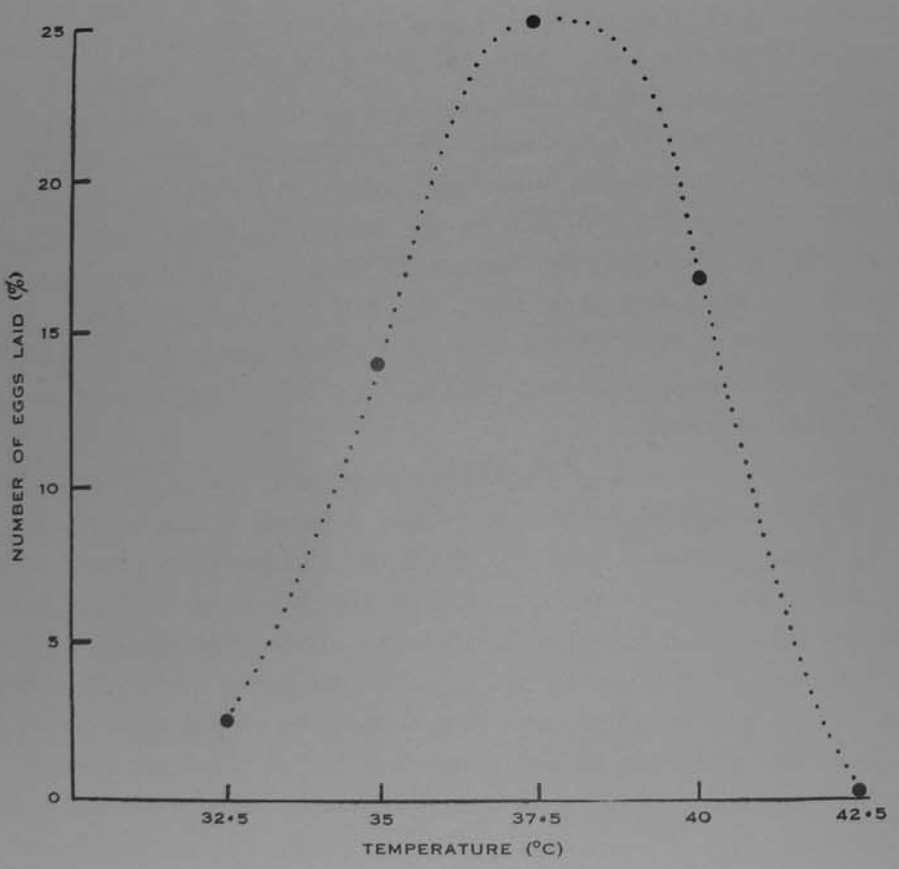

Fig. 2.—Influence of temperature on the number of eggs laid by *D. ovis*.

The results are shown in Figure 2 from which it will be seen that temperature had a profound influence on the number of eggs laid. The number of eggs laid increased as the temperature rose from 32·5 to 37·5°C and there was a decline in the number laid at 40°C. At 42°C no eggs were laid and all the lice died within 18 hr.

A further collection of lice was divided into five groups and each group was exposed to 42°C at either 20, 40, 60, 80, or 100 per cent. R.H. Again all the lice died within 18 hr without an egg being laid.

The failure to lay eggs at 42°C was, therefore, due to the lethal effect of this temperature. The variation in the number of eggs laid in the other groups may have been due to an effect of the temperature on the development of the egg within the louse or to an effect on the act of oviposition.

(b) *On the Development of the Egg within the Female*

Only one egg develops at a time in *D. ovis*. Its length can be measured by placing the louse between two glass slides and examining the abdomen under the

microscope. The weight of a slide does not injure the louse but flattens the abdomen sufficiently to enable the egg to be seen when light is transmitted through the abdomen. The earliest stage which can be detected measures 0·2 mm and a fully developed egg 0·85 mm.

The length of the developing egg within 163 lice was measured. The lice were then divided into four groups, the first contained 34 lice in which no developing egg was visible; the second, 62 lice with an egg 0·2–0·48 mm in length; the third, 44 lice with an egg 0·49–0·72 mm in length; and the fourth, 23 lice with an egg longer than 0·72 mm. All lice were kept on glass wool at 37·5°C until egg laying

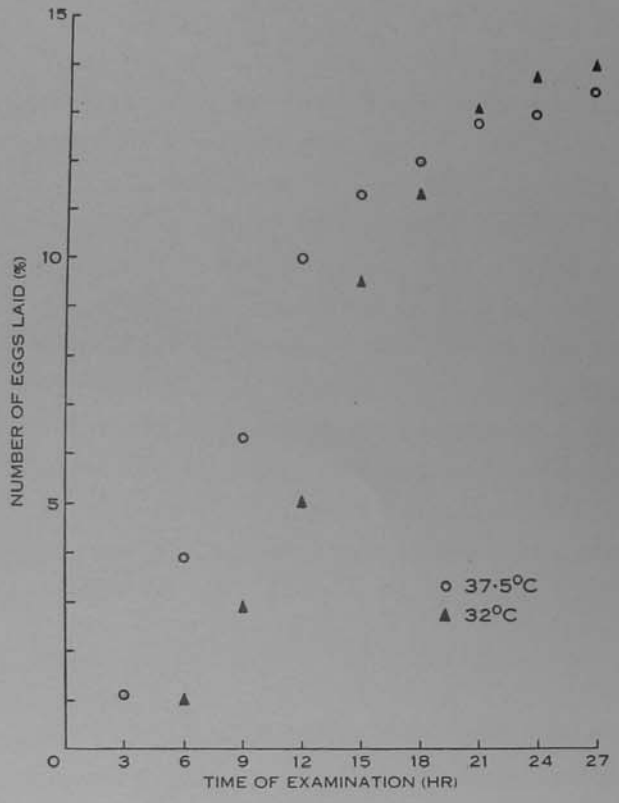

Fig. 3

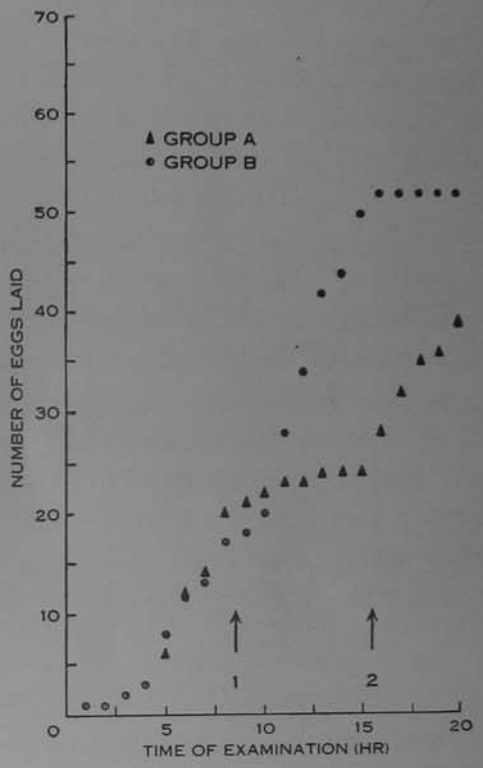

Fig. 4

Fig. 3.—Influence of temperature on the development of the egg within *D. ovis*. Both groups prevented from ovipositing for 24 hr by witholding fibres during which time group ○ kept at 37·5°C and group ▲ kept at 32°C. Both groups then presented with fibres and exposed to 37·5°C.

Fig. 4.—Influence of temperature on oviposition by *D. ovis*. Both groups exposed initially to 37·5°C. At *1*, group A exposed to 32°C whilst group B was kept at 37·5°C. At *2*, group A returned to 37·5°C and group B exposed to 32°C.

had ceased. No eggs were laid in the first two groups, 28 eggs were laid in the third group, and 10 in the fourth. From these results it may be concluded that when lice are starved, as in these experiments, only those which contain an egg longer than 0·48 mm when collected subsequently lay an egg.

From another collection were obtained 52 lice, each containing an egg 0·46–0·72 mm in length. These lice were randomly divided into group A, the mean egg length of which was 0·57 mm, and group B, with a mean egg length of 0·58 mm. Fibres were not provided for either group. Group A was exposed to 37·5°C and group B to 32°C at 60 per cent. R.H. The egg lengths were measured after 24 hr when the mean

egg length of group A had increased to 0·80 mm and that of group B to 0·78 mm. Thus the lower temperature did not materially affect the development of the egg.

A further collection of about 1000 lice was divided into two groups. One group was exposed to 37·5°C without fibres and the other to 32°C, also without fibres. After 24 hr each group was given glass wool and placed at 37·5°C. Examinations were made every 3 hr and the number of eggs laid was recorded. The results are presented in Figure 3 from which it is apparent that exposure to 32°C delayed egg laying slightly.

(*c*) *On Oviposition*

Another collection of 800 lice was kept at 37·5°C for 30 hr without fibres and was then divided into five groups. Each group was given glass wool and exposed to either 32·5, 35, 37·5, 40, or 42°C for 12 hr after which the eggs laid were counted. The experiment was repeated with another 600 lice and the results were combined. At 32·5°C, 3·7 per cent. of the lice laid eggs; at 35°C, 14·7 per cent.; at 37·5°C, 26 per cent.; at 40°C, 21 per cent.; and at 42°C, 5·5 per cent. laid eggs.

From another collection, 240 lice with a developing egg of 0·72 mm or over were selected. These were randomly divided into two groups, A and B. After a period of 24 hr at 37·5°C without fibres, glass wool was inserted. Both groups were still exposed to 37·5°C and examinations were made hourly to count the number of eggs laid. When oviposition had been in progress 8 hr, group A was transferred to 32°C while group B was kept at 37·5°C. After another 8 hr group B was transferred to 32°C and group A returned to 37·5°C. The results are shown in Figure 4 from which it may be seen that the rate of egg laying decreased as soon as the lice were exposed to 32°C and was restored when they were returned to 37·5°C. Similar results were obtained when the experiment was repeated.

(*d*) *On the Distribution and Alignment of the Eggs*

Lice were placed in cells with glass wool and exposed to a constant temperature of 37·5°C. Others were placed in similar cells with glass wool and exposed to temperature gradients of either 32–40°C, 19–43°C, 19–47°C, or 12–56°C. As may be seen in Figure 5(*a*) the eggs of the lice exposed to the constant temperature were randomly distributed on the fibres in the cell whereas those on the temperature gradients were laid in the zone 35 to 42°C (Figs. 5(*b*)–5(*e*)). In addition, of 254 eggs laid in the cells exposed to the constant temperature, 126 were aligned in one direction and 128 in the other, whereas in each of the gradients, over 78 per cent. of the eggs were aligned with the end of attachment towards the warm end.

(*e*) *On the Behaviour Pattern*

Lice were placed on a temperature gradient of 20–40°C. Those preparing to oviposit moved to the warm end but were prevented from proceeding beyond the 30°C zone. They remained there with their heads towards the warm end but there was no abdominal movement. After 5 hr, they were allowed to move to the 40°C zone and normal abdominal movements soon commenced. No eggs were laid during the first hour in the 40°C zone.

Other lice were exposed to a temperature gradient, the warm end of which was 37°C. When three lice had commenced stage 2 and two lice had commenced stage 3, the temperature at the warm end was dropped from 37°C to 26°C. Eggs were not laid until the temperature had been restored to 37°C, 3 hr later. The lice which had commenced stage 2 laid their eggs within a ½–1 hr and those which had commenced stage 3 within 5 min.

Further lice were exposed to a gradient of 34–40°C distributed over 1 in. and when two had commenced stage 2 the gradient was reversed. The lice turned about and moved to the warm end. The gradient was reversed again and once more the lice turned about and moved to the warm end.

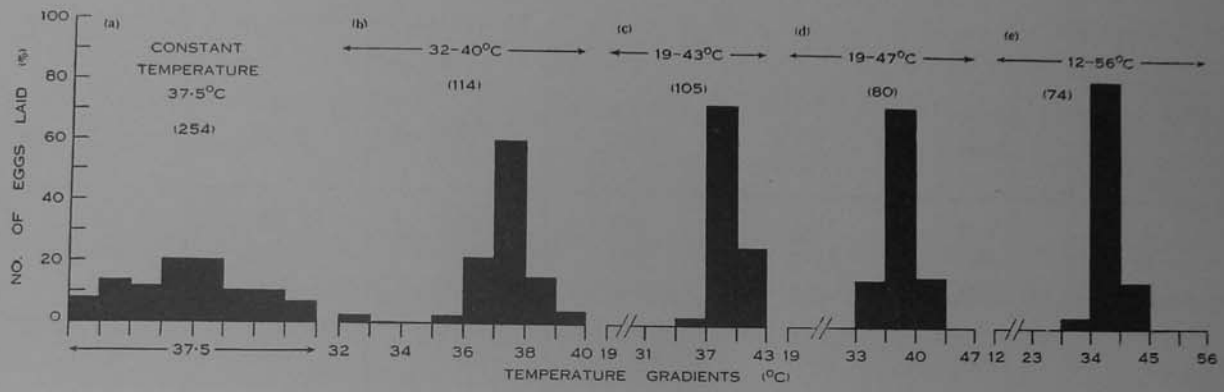

Fig. 5.—(*a*)–(*e*) Distribution of eggs of *D. ovis* on various temperature gradients. Number of eggs laid on each temperature gradient shown in parenthesis on the figure.

IV. Influence of Humidity

(*a*) *On the Number of Eggs Laid*

Three groups of lice on glass wool were exposed to relative humidities of 95, 60, or 20 per cent., and kept at 37·5°C. After 48 hr, the number of eggs laid was counted. The experiment was repeated twice. The results are presented in Table 1, from which it may be seen that, although there was a difference in the number of eggs laid by each collection, in each, more eggs were laid at 60 or 20 per cent. R.H. than at 95 per cent. R.H. Using the χ^2 test these results were shown to be significant. There were also significantly more eggs laid at 60 than at 20 per cent. R.H.

(*b*) *On the Distribution of the Eggs*

The circular, humidity-choice chambers used were basically similar to those used by Wigglesworth (1941). The arena in which the lice were placed was 1 mm in height and its floor was made of organdie. Humidity was controlled with saturated salt solutions and measured with cobalt thiocyanate papers (Solomon 1945). In all experiments the lice within the chambers were exposed to 37·5°C for 48 hr. In 16 experiments the air in one-half of the arena was kept over calcium chloride and was almost dry and the air in the other half was kept over wet sand and was nearly saturated. A total of 187 eggs was attached to the frayed fibres of the organdie in the almost dry atmosphere and 17 in the nearly saturated atmosphere.

Other lice were placed either in experimental chambers in which the relative humidity was 33 per cent. in one-half and 75 per cent. in the other, or in control

chambers in which the relative humidity was 75 per cent. throughout. Glass wool was spread evenly over the organdie floor of the arenas of the chambers. In the three control chambers a total of 93 eggs was laid, 51 in one half and 42 in the other half, whereas in the six experimental chambers a total of 212 eggs was laid, 148 in the 33 per cent. R.H. atmosphere and 64 in that of 75 per cent. R.H.

TABLE 1

INFLUENCE OF HUMIDITY ON THE NUMBER OF EGGS LAID BY D. OVIS

Collection	95% R.H.		60% R.H.		20% R.H.	
	No. of Lice	No. of Eggs (%)	No. of Lice	No. of Eggs (%)	No. of Lice	No. of Eggs (%)
A	700	17·3	481	25·6	590	21·2
B	587	11·2	573	25·5	882	26·6
C	656	3·8	400	7·5	934	6·4
Total	1943	10·9	1454	20·6	2406	17·5

Humidity-choice chambers were then constructed on a copper sheet along which a temperature gradient was established so that one-half of the arena was in the temperature zone of 20–30°C and the other half in the zone 30–40°C. In one-half of the arena the air was saturated with water vapour, in the other half the air was dry. In each experiment, two chambers were used and the humidities were reversed. Thus, in one the saturated atmosphere and in the other the dry atmosphere was at the warm end of the gradient. The oviposition behaviour was normal under both experimental conditions. Eggs were laid only at the warm end and all of the 110 eggs laid were aligned with end of attachment towards the warm end. The distribution of the eggs was determined by temperature preference and not by humidity preference.

(c) *On the Alignment of the Eggs*

The dividing partitions of the humidity-choice chambers were increased from 1 to 8 mm in width so as to increase the length of the humidity gradient in this region. Parallel "Nylon" fibres were attached to the floor in this region so as to lie along the gradient. In the experimental chambers, calcium chloride was placed in one half and sand saturated with water in the other. The relative humidity within the control chambers was 33 per cent. The lice within the chambers were exposed to 37·5°C for 48 hr. In the four control chambers, 136 eggs were laid and the end of attachment of 67 was in one direction and that of 69 in the other. A total of 205 eggs was laid in 16 experimental chambers and 187 of these were aligned with the end of attachment towards the saturated end. The results as depicted by these totals were consistent in all chambers.

A louse preparing to lay an egg rested with its head towards the saturated end of the gradient but shortly before the egg was laid it turned about so that its head was towards the dry end of the gradient.

In another cell, along which there was a temperature gradient of 20–40°C, a humidity gradient was established so that the humidity was low at 40°C and high at 35°C. "Nylon" fibres, which ran parallel to the gradients, and a small strip of cobalt thiocyanate paper to demonstrate the presence of the gradient, were placed in this region of the cell. Lice were kept at 37·5°C without a fibre for 24 hr before they were placed in the cell. In the first experiment, 15 eggs were attached to the fibres with the attachment towards the warm end and eight with the attachment towards the cool end; in the second experiment, the end of attachment of 40 of the 45 eggs laid was towards the warm end; and in the third 34 of the 48 eggs laid were attached in this manner. The lice orientated themselves to the temperature gradient rather than to the humidity gradient.

V. Influence of Light

Lice were placed in cells each of which had one half shaded and the other half exposed to daylight. The temperature within the cells was kept at 37·5°C. Of the 168 eggs laid, 122 were laid in the shaded areas.

Along similar cells was established a temperature gradient of 20–50°C so that the temperature zone 20–30°C was shaded and the zone 30–50°C exposed to strong daylight directed along the gradient from the warm end. The oviposition behaviour was uninfluenced and, of the 36 eggs laid in this experiment, 33 were laid in the temperature zone 41–35°C in the lighted area and the ends of attachment were towards the warm end of the gradient.

VI. Influence of the Presence of Other Lice and Eggs

Lice kept singly in cells on a temperature gradient of 20–40°C behaved normally and laid their eggs at the warm end with the end of attachment towards the warm end. When other lice were present it was observed that lice in stage 2 frequently congregated in groups before any eggs had been laid. When some eggs had been laid there was a tendency for others to be laid next to them.

VII. Discussion

In the initial experiments it was shown that *D. ovis* would attach eggs readily to synthetic fibres. There was no difference between the time of commencement or the rate of oviposition between two groups of lice one of which was offered glass wool and the other wool from the sheep, so the influence of the fibres was associated with their physical rather than with their chemical characteristics.

(*a*) *Stage* 1

Lice were exposed to a constant temperature and humidity and eggs were randomly distributed on the fibres within the cell. When a choice was given between

air that was nearly saturated with water vapour and air that contained hardly any water vapour, or between air at 75 per cent. R.H. and air at 33 per cent. R.H., most eggs were laid in the drier atmosphere. However, eggs were laid at the warm end of a temperature gradient of 20–40°C, regardless of the prevailing humidity. Strong daylight, directed along the gradient from the warm end, did not influence this movement towards the warm end. In stage 1, therefore, the lice were attracted to warmth and in particular to temperatures between 35 and 40°C.

(*b*) *Stage* 2

Orientation to any particular direction was not seen when lice were exposed to constant temperature and humidity. However, orientation was apparent when they were exposed to either a temperature gradient without a humidity gradient or to a humidity gradient without a temperature gradient. In the presence of antagonistic temperature and humidity gradients, the orientation to temperature dominated. When lice were in stage 2, and the gradient was reversed, they turned about to rest again with their heads towards the warm end. Orientation to temperature also dominated the normal negative phototactic behaviour.

Exposure of lice to different constant temperatures influenced greatly the number of eggs laid and it was shown that at temperatures lower than 42°C, which was lethal, the effect was exerted at the time of oviposition. It may also be seen from Figure 4, that when lice were held at 32°C and were then restored to 37·5°C, the rate of oviposition was similar to that of lice kept at 37·5°C throughout. If stage 3 only had been affected all the eggs would have been laid within $\frac{1}{2}$ hr because only 5 min are required for stage 3 to be completed. When lice were placed on a gradient which only permitted them to reach a temperature of 30°C, no egg was laid until they were subsequently kept for 1 hr at 35–40°C. Again, when lice were in stage 2 and the temperature was dropped from 37·5 to 26°C, no eggs were laid until the temperature was restored to 37·5°C. Temperature, therefore, influenced stage 2.

Humidity influenced the number of eggs laid but only high humidities had a severe effect and in all experiments concerning temperature the relative humidity was kept at 60 per cent. or lower. Clearly, temperature was a critical factor and influenced, in all probability, the systems controlling the passage of the egg down the genital tract.

It is reasonable to assume that abdominal movements were associated with the passage of the egg as they ceased at temperatures of 30°C and lower. Fibre density influenced the vigour of abdominal movements. They were less obvious when fibres were sparse, but there was no evidence that stage 2 was prolonged as a consequence. When lice were placed in cells in which the roof was lowered so that the abdomen was always in contact with the floor and roof of the cell, the abdominal movements were of the usual vigorous type. It would appear, therefore, that the number of tactile stimuli received by the abdomen determined the vigour of the abdominal movements.

During this stage, lice were attracted to other ovipositing lice.

(c) *Stage* 3

For the satisfactory completion of stage 3, it was again necessary for temperatures to be in the region of 37·5°C. When lice were exposed to a constant temperature and humidity, eggs were aligned along the fibre in either direction. In a temperature gradient without a humidity gradient, eggs were aligned with the end of attachment towards the warm end, and in a humidity gradient without a temperature gradient, eggs were aligned towards the high humidity. However, in the presence of antagonistic temperature and humidity gradients, the orientation to temperature dominated.

Before egg laying commenced, one of the gonopods was raised from the abdomen and a fibre held between it and the abdomen. When a fibre was not present or the fibre was too great in diameter to be held egg laying was inhibited. A tactile stimulus was required before egg laying could proceed. This stimulus is probably similar to that required by the silkworm moth, *Bombyx mori* (L.), in which it has been shown to operate as a simple reflex through the posterior abdominal ganglion (McCracken 1907).

In the oviposition behaviour there were two distinct phases. In the first, stage 1 of the behaviour pattern, the louse sought a temperature zone in which oviposition could proceed. The second phase, stages 2 and 3, was concerned with the passage of the egg down the genital tract and its deposition. The two critical requirements during this phase were correct temperature conditions and the presence of a fibre of suitable diameter. These requirements being satisfied, other factors, such as orientation to temperature and humidity, orientation to light, attraction to other ovipositing lice and eggs, and the effect of high humidities, exerted their influences.

It will be apparent that these factors can influence both the distribution and the abundance of the eggs of *D. ovis*, but the ecological implications arising from the manner in which they vary in the natural environment will be discussed in a subsequent paper.

VIII. Acknowledgment

I wish to thank Dr. D. F. Stewart, Officer-in-Charge, McMaster Laboratory, for his many helpful suggestions during the preparation of this paper.

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