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Physiological Changes Induced by Mating and their Effect on Oviposition and Oviposition Site Selection in the Eastern Spruce Budworm, Choristoneura fumiferana Clem. (Lepidoptera: Tortricidae).

Marie-Pascale Rivet

A Thesis

in

the Department

of

Biology

Presented in Partial Fullfillment of the Requirements
for the Degree of Master of Science at
Concordia University
Montréal, Québec, Canada

March 1991

ISBN 0-315-64659-4

ABSTRACT

Physiological Changes Induced by Mating and their Effect on Oviposition and Oviposition Site Selection in the Eastern Spruce Budworm, Choristoneura fumiferana Clem. (Lepidoptera: Tortricidae)

Marie-Pascale Rivet

This study looks at the effects of physiological changes induced by mating on oviposition site selection and total oviposition to determine what events trigger those behaviors and possibly how information about mating status proceeds to reach the brain. Previous studies on spruce budworm, Choristoneura fumiferana, showed that mated females laid significantly more eggs than virgin females. Mated females also laid their eggs at specific oviposition sites whereas virgin females seemed indiscriminate.

Oviposition is not stimulated by the injection of testis extract but is partially stimulated by the injection of hemolymph from mated females. The hemolymph of mated females has stimulating properties from 2 to 12 hours after separation of the mating pair, but loses this ability between 12 and 24 hours after separation. Abdominal ligation somewhat interferes with oviposition but mating still stimulates oviposition in ligated females, suggesting a kind of local action.

Oviposition site selection is induced by both injections of testis extract and hemolymph from mated females. Again, the active factor in hemolymph is present 2 to 12 hours after separation of the mating pair after which time the hemolymph loses its stimulatory effect. Abdominal ligation abolishes discrimination between oviposition sites in mated females, suggesting involvement of the brain.

In spruce budworm, a substance of testicular origin is transferred from the male to the female. This substance seems capable of passing through the wall of the female reproductive tract. A time-dependent hemolymph factor also seems involved. However, these substances do not appear to affect all aspects of oviposition in the same way.

ACKNOWLEDGEMENTS

I would like to thank the following people for their help with different aspects of this thesis.

Ms. Sue Parisella for her help with the rearing and her support throughout the entire project.

Mr. Claude Guertin for his help with the statistics.

Mr. Rémy Deslandes for his assistance in printing the thesis.

Dr. N. Kapoor and Dr. D. Fairbairn, my thesis committee, for reviewing this thesis.

My friends (especially Yves) and my family for their constant support.

A special thanks to Dr. Paul Albert, my supervisor, who's help made the realization of this thesis possible.

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INTRODUCTION

It is generally accepted that oviposition is regulated by various internal and external stimuli (Raabe, 1986; Davey, 1983). These stimuli ensure that the energy the female invests in reproduction will not be wasted, and that her offspring will have a maximal chance of survival. A female is not likely to waste energy searching for an appropriate oviposition site if she does not carry mature oocytes and/or has not been fertilized. If she has been fertilized and is ready to lay eggs, she will search for a site which will provide food and the best chance of survival for her offsprings. Therefore a female should display a normal oviposition behavior if she carries mature oocytes, is fertilized and has found a suitable oviposition site. Many researchers have looked at these aspects separately, but very few have been concerned with how they interact. Studying those interactions is essential for understanding the way in which information about both the internal and the external milieus are integrated and result in a specific behavior. It is also important to know what information is relevant to a particular behavior and how this information reaches the "integration center". The present study deals with how information about mating status reaches a "decision center". This is done by assessing the effect of mating on oviposition site selection and total oviposition. Preliminary studies on spruce budworm (Choristoneura fumiferana) indicated that

mating triggers the search for an oviposition site as well as an increase in total oviposition (Rivet and Albert, 1990). The same species will be used in this study.

Spruce budworm, Choristoneura fumiferana (Clem.), is a serious pest which does considerable damage to eastern forests of Canada and the United States. Although this project was not executed for pest management purposes, knowing more about the biology of the insect can only be helpful in controlling it. Several authors have already studied the reproductive behavior of spruce budworm. The females start calling (eversion of the pheromone gland) 13.5 hours after sunrise (Sanders and Lucuik, 1972) and mating lasts an average of 4 hours (Outram, 1971). Females lay their eggs between 12:00 and 18:00, the highest percentage being laid on the 2nd day after mating (Sanders and Lucuik, 1975). The females oviposit on white spruce (Picea glauca), balsam fir (Abies balsamea), red spruce (Picea rubens) and black spruce (Picea mariana), in order of preference (Jayens and Speers, 1949). The epicuticular waxes, perceived by the proboscis, and volatiles such as D-α-pinene and L-β-pinene, have been shown to stimulate oviposition (Rivet and Albert, 1990 and Städler, 1974). Städler (1974) showed that texture and leaf shape were also important.

Several studies have considered physiological events following mating in lepidopterans as well as in other order

of insects. Although other means of observing the effects of mating are used, comparing the number of eggs laid by females having received different treatments to controls, is a common way of assaying these effects, since mating has been shown to induce or increase egg production in several insects.

The stimulation provided by mating can be mechanical or humoral. In Diploptera punctata, the mating stimulus is thought to be the mechanical stimulation of receptors in the bursa copulatrix (Roth and Stay, 1961). A similar mechanism has been suggested for Tenebrio molitor by Gerber (1967). The presence of a male factor has been demonstrated in several species. An extract of whole male reproductive tract stimulated oviposition in Bombyx mori virgin females (Yamaoka and Hirao, 1977). In Hyalophora cecropia, mating to castrated males failed to increase the rate of oviposition (Truman and Riddiford, 1971). This shows that a substance transferred from the fertile males is essential for the oviposition rate increase. The authors conclude that the male factor is viable sperm or a substance present in associated fluids; the same conclusion was reached for other lepidoptera: Zeiraphera diniana (Benz, 1969), Manduca sexta (Sasaki and Riddiford, 1984), Trichoplusia ni (Karpenko and North, 1973). Sugawara (1986) also suggested that the mating factor was of testicular origin since the number of eggs laid by females mated to castrated males was not significantly different than that laid by untreated virgin females, in Teleogryllus commodus. The hypothesis that the male factor is of

testicular origin will be tested on budworm. The experimental design will also give a few clues as to the identity of the "male factor". The information available on this last subject was obtained from research on crickets and is summarized in the following paragraph.

In crickets, the substance transferred from male to female is thought to be prostaglandin synthetase. In Acheta domesticus, the enzyme prostaglandin synthetase is found in the testis, vesicle seminalis and spermatophore of males; it is also found in the bursa copulatrix, spermatheca and oviducts of mated females, but it is undetected in virgin females (Raabe, 1986). Loher et al. (1981) found that prostaglandin synthetase transforms arachidonic acid into prostaglandin E2 in the female spermatheca of Teleogryllus commodus. However, transplants of spermatheca from mated females to virgin females did not increase oviposition (Sugawara, 1986). Further experiments show that both cutting the spermathecal duct and denervating the base of the duct inhibit oviposition in mated females (Sugawara, 1986). These results suggest that the substance (prostaglandin?) does not stimulate oviposition via the hemolymph but rather directly in the genital chamber where the substance's presence would be sensed by nerves.

Experimental evidence points to the bursa copulatrix or the spermatheca as the site of action of the male factor. However, in most cases, it is not known whether the factor diffuses out of the organ or stimulates the release of

another substance. Riddiford and Ashenhurst (1973) showed, with bursa implants, that the bursa copulatrix of a mated female emits a factor which triggers an increase in oviposition rate in Hyalophora cecropia. In Rhodnius prolixus, the role of the spermatheca in reproduction has been established (Davey, 1965) and the existence of a spermathecal hormone has been confirmed (Davey, 1983). The hypothesis that mating has an effect on oviposition via the hemolymph (as in Hyalophora cecropia) is preferred over the neural hypothesis (as in Teleogryllus commodus) for spruce budworm for phylogenetic reasons.

The male factor has been shown to act on the last abdominal ganglion in Bombyx mori. An increase in spontaneous discharge was recorded from the last abdominal ganglion of virgin females when the preparation was bathed with a male reproductive tract extract (Yamaoka and Hirao, 1977). This, of course, would suggest that the male factor can pass through the walls of the female genital tract. The capacity of a factor to stimulate oviposition at the abdominal level will be tested on spruce budworm by performing an abdominal ligature. Alternatively, in other insects, the male factor acts on the neurosecretory cells.

The present study does not investigate physiological events beyond this point, but a summary of those events may be useful for interpretation of the data.

The stimulation of the neurosecretory cells may be neural or hormonal. In Diploptera punctata, the information from the bursa receptors is relayed to the brain through a neural route (Roth and Stay, 1961). Other experiments suggest that information about mating is transmitted to the brain by a humoral factor rather than by a neural route. This was demonstrated in the desert locust, Schistocerca gregaria, by Okelo (1971). Hemolymph taken from ovipositing females and injected into virgin females, which had ovulated, induced oviposition within hours. The hemolymph had no effect on virgin females which had not ovulated. Injected virgin females, which had ovulated and had previously undergone brain extirpation, also failed to oviposit. This suggests that a substance present in the hemolymph of mated females probably acts on the brain to induce oviposition.

The neurosecretory cells of the pars intercerebralis induce oviposition by secreting a myotropic factor which is released at various sites throughout the nervous system. Brain extracts of fertilized females induce oviposition in normal and decapitated virgin Clitumnus extradentatus females, in normal virgin Carausius morosus females (Thomas and Mesnier, 1973) and in normal and decapitated virgin Galleria mellonella females (Mesnier, 1972). In Rhodnius prolixus, extracts of 10 large neurosecretory cells of the pars intercerebralis stimulate contraction of the ovary muscles. The extract also stimulates oviposition when injected into virgin females (Davey, 1983). Furthermore,

action potentials can be recorded from the large neurosecretory cells of the pars intercerebralis and from the corpora cardiaca of mated Rhodnius females but not from virgins (Ruegg et al., 1982). Neurohormones are usually released from the corpora cardiaca and the perisympathetic organs along the ventral nerve cord (Raabe, 1986). It has been shown that an injection of extract of perisympathetic organs stimulates oviposition in Galleria mellonella (Mesnier et Provensal, 1975). Other organs could also be involved. In both Clitumnus extradentatus and Carausius morosus, an extract from thoracic ganglia also stimulates oviposition (Thomas and Mesnier, 1973). Neuroheamal areas are observed in the transverse, segmental and link nerves. Mesnier (1985) showed that extracts of thoracic and abdominal neuroheamal areas stimulate oviposition in Clitumnus extradentatus, however the stimulatory activity of the extract cycles. The activity of the neuroheamal area extract progressively increases during the photophase and decreases during the scotophase. It is most active at the beginning of the scotophase, when the females lay their eggs (Mesnier, 1985). The activity of the abdominal and thoracic ganglia extract increases during the photophase and decreases during the scotophase. The author proposes that the brain and ganglia synthesize OSH during the night, that it is transferred to neuroheamal areas during the day and that it is released from those areas during the night (Mesnier, 1985). OSH seems to act directly on the oviducts, oviductal sphincter, valvae and

ovipositor (Raabe, 1986). Sefiani (1986) tested the oviduct's reaction to several possible compounds, in vitro. Those experiments, performed on Gryllus bimaculatus, show that acetylcholine, GABA and dopamine did not induce contractions, but proctolin, glutamate, serotonin and octopamine did. However, none of the contractions induced by those compounds had the characteristics of the contractions induced by a central nervous system extract. The identity of the OSH remains unknown.

It has been postulated that mating is necessary but insufficient to induce OSH release. In Rhodnius, action potentials characteristic of release, are recorded from the corpora cardiaca 1 to 2 hours after ovarectomized mated females have been injected with ecdysterone (Ruegg et al., 1982). Recordings from the corpora cardiaca of ovarectomized mated females, which have not been injected with ecdysterone, do not show the characteristic action potentials. Action potentials can also be recorded from the heads of mated females incubated in saline solution containing ecdysterone but not from heads of virgin females incubated in the same medium (Ruegg et al., 1982). The same hypothesis has been put forward for Glossina austeni, by Ejezie and Davey (1977).

The purpose of the following experiments is to gain some insight into how mating affects oviposition site selection and total oviposition in the spruce budworm. This study is divided into 3 sets of experiments, each testing a

step in the transmission of information about mating status by assaying 2 aspects of oviposition. The injections of testis extracts are performed to confirm the origin of the male factor. Two extracts obtained by the same procedure are injected into 2 groups of females. Two groups are injected as a means of control for the extraction procedure itself. Another control consists of females injected with insect Ringer (in which the extract is dissolved). In the event the extract should have an effect on oviposition, clues about its identity would be revealed due to the type of extraction used. This would also suggest that the "male factor" is somehow capable of going through the wall of the female reproductive tract. The purpose of the hemolymph injections is to indicate the presence or absence of any humoral regulation after mating. Hemolymph is sampled from mated females at different times after separation of the mating pair and injected into virgin females. Hemolymph is taken at different times to test its stimulatory activity through time since time-dependent activity has been observed in spontaneous discharge of the last abdominal ganglion (GIX) in Bombyx mori (Yamaoka and Hirao, 1973). As a control, a group of females is also injected with hemolymph from virgin females. A modification in the oviposition behavior or increase in oviposition of virgin females injected with hemolymph from mated females would suggest the involvement of a stimulatory hemolymph factor, implying a humoral regulation of the process. The abdominal ligature is performed to

determine whether or not the head is necessary for oviposition. In Bombyx mori, Yamaoka and Hirao (1977) recorded an increase in spontaneous discharges from isolated abdomens in response to a male reproductive tract extract. The ligature performed on female budworms will isolate the abdomen. If no input for the head is required, that is if integration occurs in the abdomen (presumably GIX), an increase in oviposition should be observed and probably a discrimination of oviposition site based on mechanical cues (leaf shape, texture, etc...).

MATERIALS AND METHODS

Animals

The unfed 2nd instar larvae were received from the Forest Pest Management Institute (Sault-Ste-Marie, Ontario). They were reared on artificial diet (Grisdale, 1984) in an incubator maintained at 27°C and 40% humidity, under a photoperiod of 15L:9D until pupation. The pupae were sexed and transferred to dishes placed in wire-mesh cages (35 x 35 x 20 cm) at room temperature, under a 15L:9D photoperiod, until the moths emerged. If mated females were needed, males and females were put in the same cage and allowed to mate. Mating pairs were removed from the cage and placed in plastic petri dishes until separation, the females were then used in the bioassay. If the experiment called for unfertilized females, virgin females were taken from the cage within 12 hours of ecdysis and operated or isolated. Only moths with no noticeable abnormalities were used.

Bioassay

The containers used were 11.5 x 8.0 x 11.5 cm clear plastic dishes. Two balsam fir twigs (one treated and one control) were set in plastic cups (Solo P075S) filled with water. A styrofoam plate, the size of the container, was placed on top of the plastic cups. This provided a more leveled topography. Females were placed in the containers as soon as possible after separation of the mating pair or

recovery from anesthesia. During the test, the containers were kept in an incubator maintained at 27°C and under a 15L:9D photoperiod. Whether or not a female had been fertilized was determined by recording egg hatch, or the presence of a spermatophore in the bursa copulatrix of the female.

Foliage

Healthy, mature twigs of balsam fir were obtained from the Ministère Energie et Ressources (Québec, Québec). The twigs were collected in September 1988 and September 1989 and were kept frozen at -20°C until needed. The twigs used were 7 cm high. The treated twigs were dewaxed by dipping them in hexane for 1 minute (Maloney et al., 1988). Special care was taken to use 2 twigs with similar needle length and needle density in the same container.

Testis extraction procedure and injection

The testes of 167 males were dissected. It should be noted that spruce budworm males have a single, spherical testis which is heavily pigmented (Retnakaran, 1970). Those testes were deep-frozen (-80°C), and finally freeze-dried. The frozen testes were ground with sand, in water. The mixture was centrifuged and the supernatant collected. This operation was repeated three times. The supernatants collected each time were combined and evaporated under a flow of nitrogen or using a rotatory evaporator. The extract was

dissolved in 0.5ml of water and the undissolved particles were filtered using an ultra filter. Two ml of water were used for washing the filter. The filtrate was dried under a flow of nitrogen. The dry filtrate was dissolved in 0.5 ml of insect Ringer solution. The insect Ringer was made of 7.7g of NaCl, 0.36g of KCl and 0.24g $CaCl_2$ in 1 liter of distilled water (Blum, 1985). The concentration of these extracts were 9 mg/ml, which means each female was injected with 27µg of material dissolved in 3µl of Ringer solution. This was equivalent to one testis per female. The extract was injected dorsally into CO2 anesthetized 1 day old females, using a 50µl Hamilton syringe with a 33-gauge needle. Three µl was the largest dose the insect could be injected with, without causing any visible distension of the abdomen. This was done to minimize possible stimuli due to an increase in pressure in the abdomen. This procedure was replicated and the concentration of the second extract was 11.3 mg/ml, or 34 µg of material in each 3 µl injection.

Hemolymph sampling and injection

The donors received an injection of insect Ringer solution in the thorax to increase the internal pressure. The abdomen was punctured and the blood was removed with a capillary tube. Fertilization of the donors was assessed by hatching of the eggs laid before and/or after the sampling of hemolymph. Donor females, which did not lay eggs, were dissected and the presence of a normal spermatophore in the

bursa was used to confirm that the females were indeed fertilized. Recipient females received 3 µl injections using the same procedure as for extract injections. The hemolymph was sampled from mated females 2, 12 or 24 hrs after separation of the mating pairs. For the control experiment, hemolymph was taken from virgin females which had been treated in the same way and were the same age as the mated 2h donor females. The recipients were taken from the emergence cages at the same time as were the donors; therefore, all females used were the same age.

Ligation procedure

The females were anesthetized with CO_2 and placed, ventral side up, on a plasticine bed. They were ligated between the 2nd and 3rd abdominal segments, with a cotton thread, 24 to 48 hours after ecdysis. The females mated more readily if they were ligated on the 2nd day after ecdysis than if they were ligated on the first day (personal observation). If the ligation was more anterior, almost all females died within 24h; if it was more posterior, the bursa copulatrix was consistently anterior to the ligature as well as part of the eggs. After the operation, the females were allowed to mate and placed in the bioassay container after separation or as a mating pair. At the end of the experiment, the females were dissected to verify mating status as well as the location of the bursa relative to the ligature.

Statistical Analysis

The data were not normally distributed, therefore the use of non-parametric tests was required. The data relating to site selection was analyzed by a Wilcoxon paired-sample test. A paired design was used because the data from the 2 samples (the eggs on the 2 twigs in the same container) were not independent, the insect had to make a choice. The Mann-Whitney or the Kruskal-Wallis tests were used to compare results from 2 or more tests pertaining to oviposition. If the Kruskal-Wallis test showed a significant difference among groups, a non-parametric Tukey-type multiple comparisons was performed to determine which group(s) differed. (Zar, 1984).

RESULTS

Injection of testis extract

Females injected with 3 µl of either testes extract (9 mg/ml and 11.3 mg/ml) preferred twigs to other substrates, and the untreated twigs to the dewaxed twigs (Table 1). These results show that the injection of an aqueous extract from male testis of concentration 9 to 11.3 mg/ml will elicit the search for an oviposition site. Since the extract is diluted in insect Ringer solution, virgin females were injected with 3µl of insect Ringer solution as a control. Ringer-injected females did not show any preference for a substrate or for one of the twigs (Table 1). This behavior is similar to that of untreated virgin females.

The number of eggs laid by Ringer-injected and extract-injected females were not significantly different from each other ($p>0.50$). All Ringer- or extract- injected females laid significantly less eggs than mated females ($p<0.05$) but laid a number of eggs similar to that laid by untreated virgin females ($p>0.50$) (Figure 1). The extract, therefore, did not seem to stimulate oviposition.

Injection of hemolymph

Hemolymph taken from mated females at various intervals after separation was injected into virgin females. The results are shown in Table 2. Virgin females injected with hemolymph taken from mated females 2 hours after

Table 1. Oviposition site preference of virgin females injected with testis extracts or Ringer solution.

Treatment	mean number of eggs	number of females	p
	Ringer solution		
Untreated	30.54	11	n.s.
Dewaxed	20.45		
Twigs	29.53	19	n.s.
Other substrates	48.37		
	Extract #1		
Untreated	41.93	29	<0.0025
Dewaxed	20.83		
Twigs	60.67	30	<0.0005
Other substrates	13.10		
	Extract #2		
Untreated	62.05	20	<0.0005
Dewaxed	15.45		
Twigs	70.45	22	<0.0025
Other substrates	14.36		

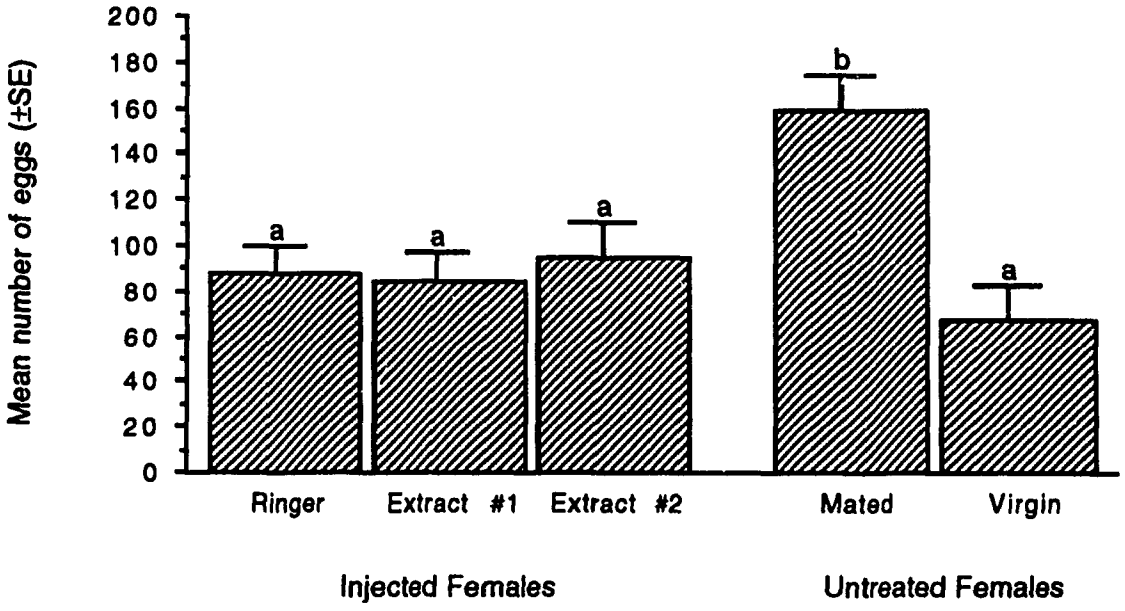

Figure 1. Number of eggs laid by females injected with testes extract or ringer solution. Same letters represent groups that do not significantly differ.

Table 2. Oviposition site preference of virgin females injected with hemolymph from different donors.

Treatment	mean number of eggs	number of females	p
Donors: Virgin females			
Untreated	40.76	21	n.s.
Dewaxed	23.76		
Twigs	54.20	25	n.s.
Other substrates	30.88		
Donors: Mated females 2h after separation			
Untreated	53.52	23	<0.0025
Dewaxed	23.48		
Twigs	76.52	23	<0.01
Other substrates	27.30		
Donors: Mated females 12h after separation			
Untreated	63.72	25	<0.01
Dewaxed	26.08		
Twigs	89.80	25	<0.0005
Other substrates	14.08		
Donors: Mated females 24h after separation			
Untreated	13.52	25	n.s.
Dewaxed	12.76		
Twigs	19.73	33	<0.001
Other substrates	50.06		

separation, showed a preference for untreated twigs over dewaxed twigs and also preferred twigs over other substrates. Females injected with hemolymph taken 24 hours after separation did not show a preference for untreated twigs; however they laid significantly more eggs on the container and styrofoam. This is probably due to the large amount of time spent on these substrates since the females were not searching for an oviposition site. In an attempt to narrow down the duration of the activity of the hemolymph factor, virgin females were injected with hemolymph from mated females taken 12 hours after separation. The females preferred twigs to other substrates and untreated twigs to dewaxed twigs. Therefore, there seems to be a hemolymph factor capable of triggering the active search for an oviposition site. Furthermore, this factor seems to be active for up to 12 hours but is inactive 24 hours after separation of the mating pair. However, there is no evidence that the release of this factor is related to photoperiod (Figure 2). As a control, virgin females were injected with hemolymph taken from other virgin females. Those females did not show any preferences.

The number of eggs laid by virgin females injected with hemolymph from virgin females did not differ significantly ($Q=1.45$, $k=6$, $p>0.50$) from the number of eggs laid by untreated virgin females (Figure 3). The injection in itself, therefore, seems to have no effect on total oviposition. The number of eggs laid by females injected with

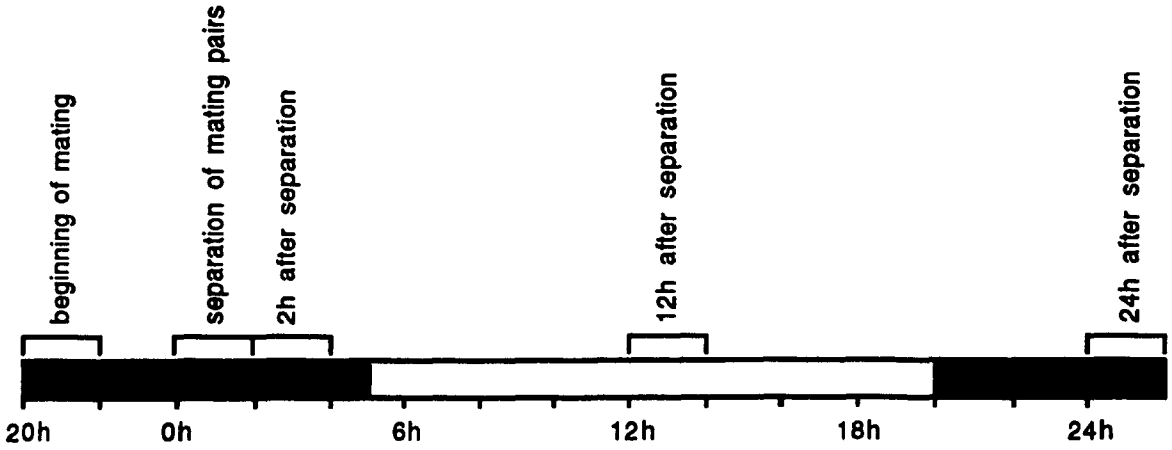

Figure 2. Relationship between photoperiod and time at which hemolymph was taken from mated females and injected into virgin females.

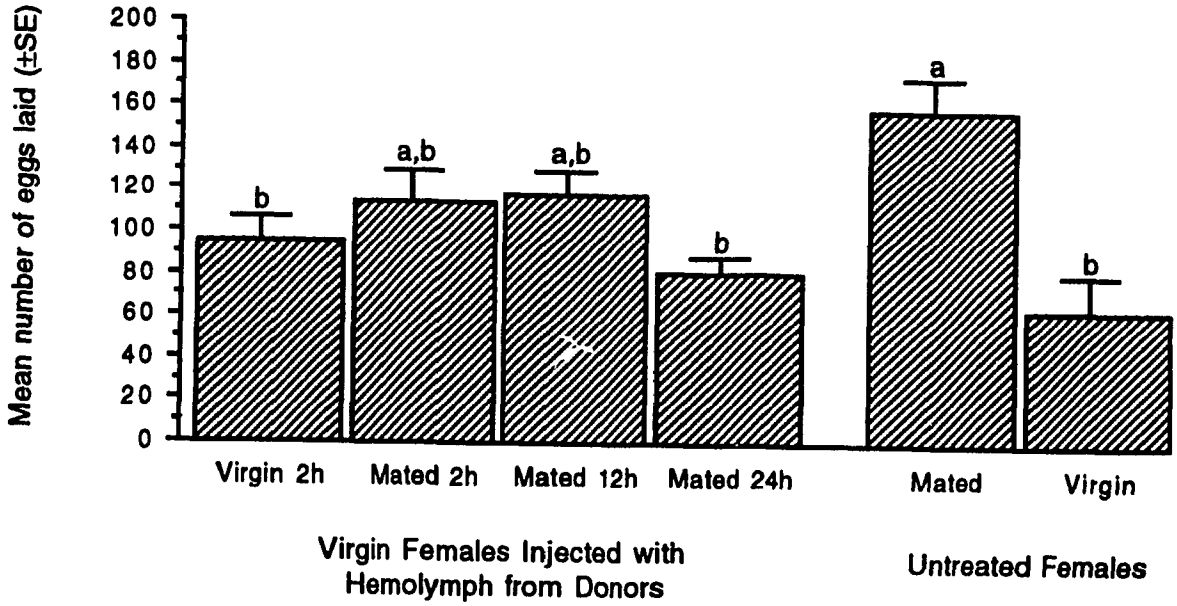

Figure 3. Number of eggs laid by females injected with hemolymph. Same letters represent groups that do not significantly differ.

hemolymph from 2h or 12h mated females did not significantly differ from the number of eggs laid by virgin female ($Q=2.16$, $k=6$, $p>0.20$ and $Q=2.54$, $k=6$, $p>0.10$, respectively) or from untreated mated females ($Q=2.04$ and 1.67, $k=6$, $p>0.50$). This could suggest a partial stimulation of oviposition by an hemolymph factor. The number of eggs laid by females injected with 24h mated hemolymph was significantly different from the number of eggs laid by mated females ($Q=3.84$, $k=6$, $p<0.002$) but not significantly different than that laid by virgin females ($Q=0.70$, $k=6$, $p>0.50$). Hemolymph does not seem to stimulate oviposition 24 hours after separation of the mating pair.

Abdominal ligation

Mated ligated females did not prefer twigs to other substrates, or untreated twigs to dewaxed twigs (Table 3). These females, of course, were not expected to discriminate between twigs since it has been suggested that the proboscis perceives waxes (Rivet et Albert, 1990). It would be impossible for the information from the receptors on the proboscis to reach the nervous system posterior to the ligature or any other posterior organ. Virgin ligated females lay significantly more eggs on other substrates than on twigs, in fact, they did not lay enough eggs on twigs to permit an analysis of chemical preference. This unexpected apparent preference of virgin ligated females for substrates other than twigs, may be related to the amount of time the

Table 3. Oviposition site preference of control and ligated females.

Treatment	mean number of eggs	number of females	p
	Mated females*		
Untreated	107.13	15	<0.0005
Dewaxed	38.33		
Twigs	218.20	10	<0.0005
Other substrates	5.50		
	Virgin females*		
Untreated	46.87	15	n.s.
Dewaxed	33.47		
Twigs	14.31	16	n.s.
Other substrates	32.31		
	Ligated mated females		
Untreated	31.75	8	n.s.
Dewaxed	21.12		
Twigs	31.91	11	n.s.
Other substrates	11.55		
	Ligated Virgin females		
Twigs	0.08	13	<0.0005
Other substrates	17.38		

* Data from Rivet and Albert, 1990.

females spend on those substrates rather than by a preference. Communication (neural or hormonal) with an organ anterior to the ligature therefore seems needed to induce the search for an oviposition site.

The number of eggs laid by mated ligated females was significantly higher than the number of eggs laid by virgin ligated females ($U=117$, $p<0.005$) as was the case for untreated mated and virgin females ($U=168$, $p<0.0005$) (Figure 4). Mating seems to increase oviposition in ligated females, although the procedure seems to affect the total number of eggs laid by operated females.

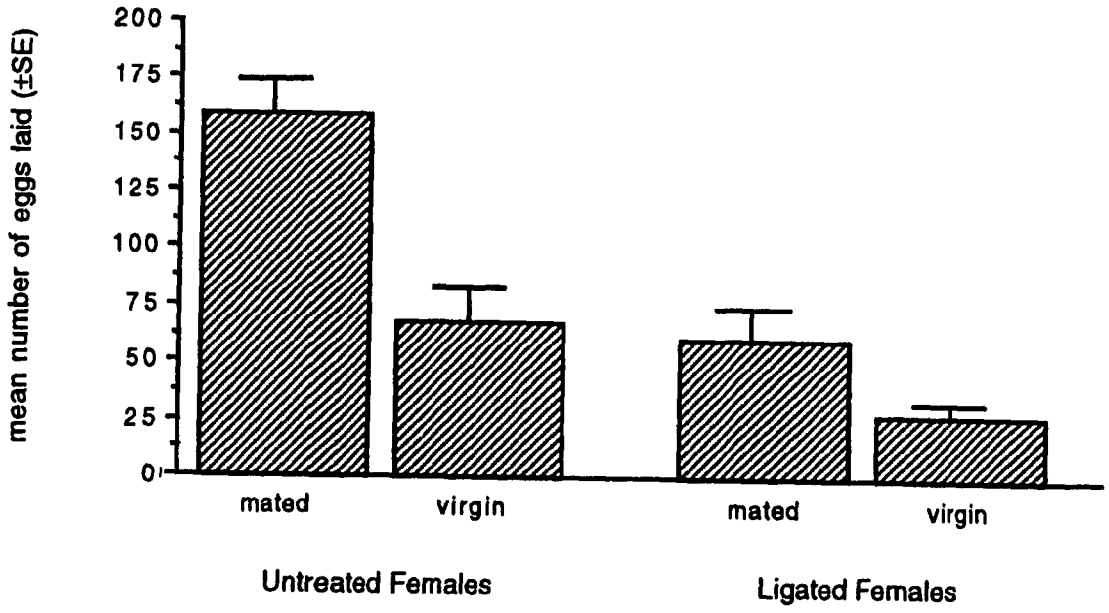

Figure 4: Number of eggs laid by untreated and abdomen ligated females.

DISCUSSION

In discussing the total oviposition results, it should be kept in mind that steps preceding oviposition, such as vitellogenesis or chorionization, could also be affected by the different treatments. It is possible that oviposition simply reflects egg maturation, that the number of eggs laid increases because egg maturation is stimulated. A male factor was shown to stimulate egg maturation in Drosophila melanogaster (Merle, 1968). In Heliothis virescens, mated females laid 98% of their mature eggs while virgin females laid 90% (Proshold et al., 1982), however mated females laid more eggs suggesting that mating stimulates egg maturation and possibly oviposition. It has been hypothesized that short-lived, non-feeding or feeding Lepidoptera mature their eggs at the pupal stage, and even the last larval stage (Raabe,1986). Those females, therefore, may not need a very strict regulation of vitellogenesis (Ramaswamy et al., 1990) since the female will emerge with ovaries already well developed and, in a few cases, mature oocytes. The spruce budworm is a short-lived moth which may or may not feed, therefore, the females would not be expected to have a very strictly regulated vitellogenesis. This is reinforced by the fact that budworm females are gravid upon emergence, they do mature some eggs prior to adult ecdysis. Since spruce budworm mature eggs before adult emergence, it is unlikely that any physiological event linked to oogenesis is responsible for a

change in the oviposition behavior. It is not likely that egg maturation is induced by mating in spruce budworm, but it is possible that it increases the rate of maturation. Whether mating increases the laying of eggs already mature or increases the rate of maturation was not determined in the present study, increase in oviposition may be due to either or both of these.

Virgin females injected with aqueous testis extracts at a concentration 9.0 mg/ml or 11.3 mg/ml oviposit the same number of eggs as untreated virgin females or Ringer injected females. The extract, therefore, does not seem to stimulate oviposition. In Bombyx mori, an aqueous extract of the male reproductive tract partially stimulates oviposition (Yamaoka and Hirao, 1977). It is possible that the male factor responsible for the stimulation of oviposition originates in an organ other than the testis, possibly the male accessory glands as suggested by Raabe (1986). It is also possible that the factor is not soluble in water or that the amount injected is not high enough to produce a response. Relating to this last hypothesis, it should be noted that the amount injected is the equivalent of one testis and is sufficient to induce another behavior: the search for an oviposition site.

The stimulation of oviposition in virgin females by an injection of hemolymph from mated females has been reported in Hyalophora cecropia (Riddiford and Ashenhurst,

1973). In spruce budworm, the injection of hemolymph taken from mated females 2h or 12h after separation of the mating pair, seems to partially stimulate oviposition. The number of eggs laid by those females does not significantly differ from that of untreated mated females, but the stimulation seems partial since the stimuli are not strong enough to induce an oviposition which significantly differ from that of untreated virgin females. It is possible that the concentration of the hemolymph factor is not high enough to fully induce oviposition or that the adequate stimulus must be sustained as the hemolymph factor may be released over a period of time. A similar occurrence has been reported in Drosophila where a sustained stimulation is needed from sperm present in the ventral seminal receptacle for the induction of normal mated oviposition (Boulétreau-Merle, 1977). Other stimuli such as the mechanical stimulation of copulation, storage of mature eggs or presence of an adequate oviposition site, may also combine with the hemolymph factor to fully induce oviposition. This kind of "step induction" was postulated in Zeiraphera diniana (Benz, 1969) and has the advantage of minimizing the energy spent by the females while maximizing the chances of survival of the offspring. In Drosophila, mating is shown to provide several different stimuli: genitalia coaptation, paragonial fluid and sperm (Boulétreau, 1974). These stimuli seem to act synergistically on oogenesis.

The stimulation provided by the hemolymph factor may be time dependent. If the hemolymph taken 2 hours or 12 hours after separation has some effect on oviposition, the hemolymph taken 24 hours after separation has none. It appears that after 12 hours, the concentration of the factor declines and that by 24 hours, the concentration is too low to induce a response. Such a time-dependent activity has been observed in Bombyx mori (Yamaoka and Hirao, 1973). Spontaneous discharges can be recorded immediately and 2 hours after mating, however 24 hours after mating the discharges were back to a virgin female's level. It is not likely that this time dependent activity is related to photoperiod since the 2 hours and 24 hours samples are taken at about the same time of the day; one stimulates oviposition, the other does not. The hemolymph factor may be responsible for "turning on" the oviposition behavior. However it is not likely that this is done by inducing the release of an oviposition stimulating hormone (OSH). If the hemolymph factor is the OSH (a myotropic factor) the injected females would start laying immediately, but there is a considerable delay between the injection and the beginning of egg laying. Furthermore, such females would not be expected to discriminate between oviposition sites since the OSH acts at the abdominal level, this means the brain would probably not receive proper stimuli.

Although ligation of the abdomen interferes with oviposition, mating still seems to stimulate oviposition in ligated females. The present data do not support the conclusion that ligation leads to partial stimulation. Although the low number of eggs laid by ligated mated females when compared to the number of eggs laid by unoperated mated females appears to support such a conclusion, the relationship between ligated and unoperated virgin females is exactly the same. These data suggest that part of the stimulation induced by mating in ligated females is local; the stimulus acts directly on an abdominal organ. Local action has been reported in Bombyx mori (Yamaoka and Hirao, 1977) and Teleogryllus commodus (Sugawara, 1986). The fact that the ligation in itself somewhat inhibits oviposition suggests that communication with an anterior organ, presumably the brain, is necessary for some steps in the oviposition sequence which both mated and virgin females undergo. For example, the ligation may interfere with some input from the brain needed for movement of the ovipositor. It is also possible that other stimuli are needed to attain the levels of untreated females. Neural or hormonal input from the brain may be required to complement the local stimulation. Dissection of the female abdomens show that most eggs are posterior to the ligature. However, it is possible that the anterior part of the ovarioles, the germinarium, is anterior to the ligature. This means that the female would

not be able to mature more eggs once its complement of mature eggs had been laid.

Dissection of some of the ligated females led to some interesting observations. Females that laid fertilized eggs, had a spermatophore stored in their corpus bursa, posterior to the ligature. This suggests that the spermatophore has to be stored in the corpus bursa for the female to lay fertilized eggs. Females that had been fertilized but had their bursa anterior to the ligature (spermatophore stored in ductus) laid unfertilized eggs. The proper storage of the spermatophore in the bursa copulatrix seems essential for the laying of fertilized eggs.

Virgin females injected with either one of the testis extracts were able to discriminate between oviposition sites to the same degree as untreated mated females. This suggests that the search for an oviposition site can be induced by a water soluble substance present in the testis of the male. Viable sperm has been suggested by several authors as an oviposition stimulant (Truman and Riddiford, 1971; Karpenko and North, 1973). Viable sperm do not appear to be involved in "turning on" the search for a site in this case since no sperm were alive or even intact after the extraction procedure. The testes extract injected into the hemocoel induced the search for a site. This suggests that the male factor is able to pass through the wall of the female genital tract to induce the search for an oviposition site.

Alternatively, the "male factor" may be able to stimulate the release of a second factor by a female reproductive organ even if it is present in the hemolymph rather than inside the genital tract.

A factor which induces the search for an oviposition site seems to appear in the hemolymph of mated females, 2 hours to 12 hours after separation of the mating pair and the activity disappears after 12 to 24 hours. It is possible that the hemolymph factor is in fact the male factor which would stimulate OSH release before being inactivated 12 to 24 hours after its transfer. As is the case for total oviposition, the activity of the factor is independent of photoperiod.

Ligated females are not able to discriminate between oviposition sites. As already mentioned, discrimination between untreated and dewaxed twigs is not expected. Discrimination on the basis of texture (twigs vs other substrates) is expected if the "decision center" is located in the abdomen. The best candidate for the "decision center" is the last abdominal ganglion which has the motor control of most of the reproductive organs. It has been suggested that in species which require more input about an oviposition site than is provided by the ovipositor, the information collected by the ovipositor is sent to the brain via the last abdominal ganglion and the ventral nerve cord (Raabe, 1986). In the brain, the input from the ovipositor is integrated with other inputs from various senses and structures, such as temperature, time of day, odors, taste, etc. The command to

lay eggs is sent down the ventral nerve cord to the last abdominal ganglion. If the "decision center" is in the brain, the ligated females are not expected to discriminate between oviposition sites as seems to be the case for spruce budworm.

These results show that the stimuli needed to trigger the search of an oviposition site and the stimuli needed to increase oviposition are different. Two completely different factors may be involved, but more likely, the same factor acts on 2 different target organs or even, the same organ in different ways. For example, the search behavior may be "turned on" by a transient stimulation of the brain by the hemolymph factor (which may be the male factor), while an increase in oviposition may require a sustained stimulation of the neurosecretory cells to maintain production of OSH. The results obtained fit this hypothesis quite well. The females are injected with 3 µl of testis extract, which is probably less than is transferred by the male, spermatophores contain more than 3 µl. Furthermore, the bursa may regulate the release of the factor over time so that the neurosecretory cells would receive a continuous input. In the present experiment, the extract was injected directly in the hemocoel, making any regulation by the bursa impossible. If this was the case, the stimulation induced by the male factor would be sufficient to trigger the search for an oviposition site but the increase in oviposition would be ephemeral and would probably have very little effect on total oviposition.

CONCLUSION

As can be seen, the same treatment seems to have different effects on oviposition and site selection. The injection of testis extract stimulates the search for an oviposition site but not oviposition itself. It is possible that different stimuli affect site selection and oviposition or that the same factor stimulate site selection and oviposition differently. Hemolymph from mated females taken 2h or 12h after separation induces the search for an oviposition site as well as oviposition although only partially. Hemolymph taken 24 hours after separation has no effect on either oviposition or site selection. Since the time frame during which the hemolymph has a stimulatory effect is the same for both site selection and oviposition, there is probably only one factor involved. Other stimuli or a sustained supply of the factor may be needed for full stimulation of oviposition, as has already been suggested. Ligation inhibits discrimination between oviposition sites but stimulates oviposition in mated females. The same factor may act both locally and on the brain or two different stimuli may be involved, one of which is able to act locally. Further investigation would be necessary to determine if different factors are involved in the regulation of the two processes or if the same factor is acting differently on the two processes.

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