SOME ASPECTS OF THE BEHAVIOUR AND PHYSIOLOGY OF SEXUAL ACTIVITY IN *TRYPODENDRON LINEATUM* OLIVIER (COLEOPTERA: SCOLYTIDAE)

by

Catherine Elizabeth Fockler

B. Sc., Simon Fraser University, 1970

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

February, 1972

APPROVAL

Name: Catherine Elizabeth Fockler

Degree: Master of Science

Title of Thesis: Some Aspects of the Behaviour and Physiology of Sexual Activity in *Trypodendron lineatum* Olivier (Coleoptera: Scolytidae)

Examining Committee:

Chairman: Dr. G. H. Geen

J. H. Borden
Supervisor

K. K. Nair

H. L. Speer

John A. Chapman
External Examiner
Research Scientist
Canadian Forestry Service
Pacific Forest Research Centre, Victoria, B. C.

Date Approved: 21 Feb. 1972

ABSTRACT

Mating frequency and behaviour of five color-coded adults of each sex of T. lineatum were observed for one hour periods in petri dish arenas. A mating was arbitrarily defined as assumption of a "precopulatory" position for a minimum of ten seconds. Newly emerged brood adults mated with very low frequency. The mating activity of revived overwintering beetles increased gradually until February, when there was a sudden rise in activity to an intensity approaching that of sexually active "spring" populations of March and April. This pattern, as well as the recovery of locomotion upon warming throughout the winter, provides evidence that overwintering populations undergo a reproductive diapause, or hibernation. Mating activity of excavating parents fell to one half that of "spring" beetles within two weeks of host invasion and declined further after four weeks. Parents that had recently emerged from brood logs had negligible mating activity. Flight is not an essential prerequisite to mating. There is no prolonged delay between initial contact of the sexes and mounting, and no observable courtship. Many observations

indicated that, behaviourally, the male is the aggressive partner; the female is entirely passive and nonresisting. Multiple matings by both sexes, with the same or different partners, and mounting of mating couples and other males by males were recorded. There was some behavioural evidence that males identify females through chemoreceptive means, but antennectomies did not reduce mating drastically; palpi ablations were inconclusive. Mating was not diminished in red light. The average duration of mating was 1.75 minutes and when copulation occurred, it increased to 3.35 minutes; dissections showed that this period was sufficient for insemination. Partial satiation of the mounting and copulation drives occurred within 30 to 40 minutes of initial contact with the opposite sex. Mating frequency depended primarily on the maturity and vigor of the male, although the maturity of females had a slight effect. There was evidence that the population aggregating and/or sex pheromone produced by excavating females is not directly associated with sexual activity.

Topical application of 50 μg of a juvenile hormone analog, 10-epoxy-2-cis/trans, 6 trans-farnesenic acid ethyl ester (EFA) in 1/2 λ acetone solution to both

sexes, totally repressed mating within one hour of treatment. Mating activity increased gradually with time and was slightly higher than the controls by 48 hours. The repressive effects of the hormone appeared to be primarily associated with the male, although the activity of the female also decreased somewhat. Sexual activity of treated females increased after 24 hours. A lower dose (0.05 μg) of EFA accelerated mating activity after one hour. Application of 50, but not 0.05, μg EFA to virgin females induced growth and maturation of the ovaries.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

I thank Drs. J. A. Chapman, K. K. Nair, H. L. Speer, Miss Eveline Stokkink, Mr. Ron Long and Mrs. Jean Gott for their advice and assistance.

Special appreciation goes to Dr. John H. Borden, who provided abundant intellectual and emotional support throughout the study.

INTRODUCTION

Sexual Activity of *Trypodendron lineatum*

Little information is available about the sexual behaviour of *Trypodendron lineatum* (Olivier). In an extensive examination of the species, Hadorn (1933) made some observations of the mating behaviour of specimens invading host logs in the spring. He noted that males removed from overwintering sites and placed manually on a log surface did not mate, presumably because they had not yet flown. Sexually active males approached females on the log surface, and followed and circled with vibrating antennae before attempting to mount; copulation generally occurred on the log surface during initial excavation of the entrance tunnel by the female. The female appeared to be uninterested in mating. The couple remained on the surface of the log for approximately one day with intermittent copulation until the depth of penetration of the female into the log prevented mounting; the male followed the female into the gallery. Hadorn hypothesized that most, if not all, mating occurred on the log surface during initial host

invasion. Numerous beetles have been observed copulating on log surfaces in the field during attack flights in the spring (Chapman 1954, 1962). Rudinsky and Daterman (1964a, b) and Chapman (1966) showed that excavating female T. lineatum produce a pheromone that has both population aggregating and sex attractant properties, although its effect on sexual activity has not been determined.

Life History

In the spring, when maximum daily temperatures have risen to approximately 15.5°C (60°F), reproductively active "spring" populations fly from overwintering sites and attack suitable hosts (Chapman and Kinghorn 1958, Rudinsky and Daterman 1964a, Daterman, Rudinsky and Nagel 1965). Within two weeks of host invasion, parent females commence oviposition (Prebble and Graham 1957). Brood development takes approximately 40 days and "brood" adults feed on ambrosia fungus in the gallery for several days before leaving the host (Hadorn 1933, Chapman 1954). Throughout the summer, there are sporadic flights consisting of both brood adults and emerged parents (Chapman 1958a;

Daterman, Rudinsky and Nagel 1965). It has been established that attacks on new hosts during the summer are initiated only by emerged parents (Hadorn 1933; Chapman 1955, 1958a; Schneider and Rudinsky 1969a); emerging brood fly directly to overwintering sites, burrowing into litter or constructing shallow galleries in trees within neighbouring forests (Chapman 1958b, Kinghorn and Chapman 1959, Dyer and Kinghorn 1961) without mating (Hadorn 1933). Eventually, surviving parents overwinter with the brood; parents have been recovered in overwintering populations (Chapman 1955, 1958a; Chapman and Nijholt 1965), and flying "spring" populations of the following year (Chapman 1955, 1958a).

Role of Juvenile Hormone in Insect Reproductive Activity

The corpora allata have been implicated in the regulation of several processes associated with reproduction in the adult insect. Juvenile hormone promotes the maturation and secretory activity of the accessory sex glands in males (Loher 1960, Wigglesworth 1964) and in females of several species (Gilbert 1964, de Wilde 1964). The hormone stimulates growth and maturation of the ovaries, as well as

advanced development of oocytes through regulation of yolk deposition by the follicle cells in the vitellogenesis phase (de Wilde 1964, Highnam 1964, Engelmann 1968, Wigglesworth 1970). Extremely reduced corpora allata secretion prevents oocyte maturation and diminishes general metabolic activity (de Wilde 1964) and juvenile hormone deficiency has been found to induce imaginal diapause in several insect species (de Wilde 1959, 1964; Danilevskii 1965; Bowers and Blickenstaff 1966). Termination of diapause, through secretion of juvenile hormone, occurs in most species upon re-exposure of the diapausing individual to conditions suitable for normal development, following a critical period of suboptimal conditions (Danilevskii 1965, Beck 1968).

Juvenile hormone may also be involved in processes associated with mating activity, such as pheromone production (Loher 1960; Barth 1962, 1965, 1968; Borden, Nair and Slater 1969; Menon 1970), courtship sound production (Loher 1966, Loher and Huber 1964), and sexual receptivity of females (Engelmann 1960a, Manning 1967, Adams and Hintz 1969) and males (Pener 1967, Walj and Pener 1970).

There appears to be a relationship between the hemolymph concentration of juvenile hormone (or secretory activity of the corpora allata) and the type of reproductive phenomena elicited. In certain species, the quantities of hormone that are necessary to initiate gonad maturation, the development of oocytes up to the initial stages of yolk deposition and mating activity are much lower than those required for the completion of egg development (i.e. vitellogenesis) (Johansson 1955, Engelmann 1960b, Lea 1968). In other species, the oocytes do not complete development until the sexually mature female is exposed to specific stimuli received from mating (Engelmann 1958, 1960b; Davey 1967; Barth 1968; Rohdendorf and Watson 1969), or feeding (Mellanby 1939, Clements 1956, Gillet 1956, Orr 1964), flying (Highnam and Haskell 1964) and male pheromone (Highnam and Lusis 1962), often in conjunction with mating stimuli. Increased activity of the corpora allata has been observed to follow these "trigger" stimuli (Engelmann 1959, Rohdendorf and Watson 1969) and has been correlated with terminal egg maturation (Scharrer and von Harnack 1959, Cassier and Papillon 1968, Rohdendorf and Watson 1969). A reduction of sexual activity often accompanies the final stages of egg

development (Engelmann 1960b, Roth and Dateo 1966, Loher 1966).

Objectives

The objectives of this research were: 1) to describe the sexual behaviour and seasonal mating activity of T. lineatum, attempting to elucidate some of the natural regulators of activity, and 2) to investigate the possible role of juvenile hormone in the reproduction of T. lineatum by artificially augmenting internal supplies through the administration of a juvenile hormone analog, 10-epoxy-2-cis/trans, 6 trans-farnesenic acid ethyl ester (EFA).[1] Farnesol derivatives have been shown to have juvenile hormone activity (c.f. Gilbert 1964, Wigglesworth 1970) and certain of these compounds have been shown to induce phenomena associated with reproduction in scolytids, including pheromone production (Borden, Nair and Slater 1969), flight muscle degeneration (Borden and Slater 1968), and ovary development (Sahota, Chapman and Nijholt 1970).

---

1. Supplied by F. Hoffman-La Roche and Co., Ltd., Basle, Switzerland

METHODS

Specimens

With few exceptions, overwintering T. lineatum were collected from litter on the floor of screened outdoor cages that had been filled the previous summer with naturally infested Douglas fir and hemlock collected from the University of British Columbia (U.B.C.) Research Forest, Maple Ridge, B.C. This ensured both a relatively uniform experimental population and a concentrated supply of beetles, permitting rapid collection of specimens prior to a test. However, for some of the tests examining the effect on mating of hormone treatment to either sex, and for all tests comparing the mating activity of "spring" beetles and newly emerged brood adults, specimens of "spring" populations were acquired from litter collected from Port Renfrew, B. C. Excavating parents were excised from their galleries in naturally infested Douglas fir collected from U.B.C. Forest. Emerged parents and brood adults were collected as they evacuated naturally infested Douglas fir logs from U.B.C. Forest. Observations revealed that parents begin to emerge before brood have matured and that the commencement of brood emergence, approxi-

mately two weeks later, is heralded by the appearance of callow adults. Without dissection, darkened brood beetles could not be distinguished from emerged parents. Therefore, only beetles which could be identified as callow were designated as brood, and darkened beetles emerging before and during the early period of callow emergence were considered emerged parents. To prevent mating as much as possible prior to testing, most beetles were collected within minutes of emergence. However, in contrast to "spring" populations, mating was never observed during collections of these populations and such precautions may have been unnecessary.

All duff samples were removed from the field before natural emergence of the insects, and stored at approximately 0°C. Beetles were revived from cold-immobilization just prior to testing. Specimens were collected individually as they emerged to the surface of a portion of litter placed in a shallow enamel pan heated from beneath. They were segregated immediately by sex to prevent mating, and cooled to a stuporous state in petri dishes resting on ice to prevent extensive metabolic change or flight. As soon as a sufficient number of beetles were collected (usually within one half hour), they were removed from the cold dishes and held at 24

± 1°C for a defined period prior to testing. Beetles used within five hours of collection were incubated in 100 x 15 mm disposable petri dishes with moistened filter paper flooring. Beetles held for a longer period were confined to eight or sixteen ounce, screw-cap jars containing moistened, crumpled paper tissue to prevent dehydration and reduce contact with other individuals. Excavating parents were segregated by sex and held at room temperature for approximately two hours prior to testing, due to a delay while control populations from the duff were conditioned to room temperature. Emerged parents and brood were segregated by sex and stored at 4°C in eight or sixteen ounce jars containing moistened paper tissue. Prior to testing, they were warmed to room temperature for one to two hours in disposable petri dishes.

Mating Tests

A mating test was conducted in a closed 100 x 15 mm disposable petri dish divided equally by a vertical partition and containing a paper toweling floor to facilitate locomotion. The partition prevented beetles from continually circling the container and reduced the arena to a size that

allowed greater contact between individuals. Each test comprised the continuous observation of the mating activity of five specimens of each sex for one hour. All insects were individually coded by applying a small dot of model paint (Testor's "pla") or nailpolish to the dorsum of the thorax or elytra. Specimens were never used in more than one mating test. The commencement time, participants and, whenever possible, the duration of each mating session were noted. By arbitrary definition, a mating occurred if a male remained mounted on a female for at least ten seconds and assumed the "precopulatory" position (Fig. 1) at least once. Copulation was recorded whenever observed. Most tests were performed in 1971 in a semicontrolled environment: relative humidity 40 to 60%, 24 $\pm$ 1°C, and constant fluorescent lighting. All tests performed in 1970 and those on emerged parents and brood beetles in 1971 were performed in a windowed laboratory with constant fluorescent lighting and at room temperature (23 to 29°C). Generally, the test chamber remained closed, but occasionally the lid was removed to replace a beetle that had strayed over the partition or to disperse a prolonged immobile aggregation of beetles.

Hormone Treatments

Hormone treatments were administered topically as acetone solutions of EFA to the abdominal venter of cold-immobilized insects. The majority of treatments were applied with a 25 µl Hamilton microliter syringe (No. 702-N), adapted to release 0.5 µl solution per application with a Hamilton repeating dispenser (P B600-1). In preliminary tests, solutions were applied with a nonadapted 10 µl Hamilton microliter syringe (7005-N). A treatment consisted of one of five doses of EFA, 50, 5, 0.5, 0.05, or 0.005 µg. Control samples were of two kinds: untreated, and treated with 0.5 µl acetone. Following preparation, all hormone solutions were stored at -50°C in one ml serum vials covered with serum caps. Samples were removed by puncturing the serum cap with the syringe and withdrawing the desired quantity of fluid. After treatment, beetles were segregated by sex and treatment and held at room temperature for a defined period until testing.

Dissections and Microscopic Examination

After mating tests or hormone treatments, representative cold-immobilized females were secured for dissection by inserting them, ventral side down, in warmed black wax, which provided color contrast with internal structures. Specimens were covered immediately with ice-cold saline (0.75% NaCl) and dissected under a dissecting microscope. An effort was made to determine the age of female insects, utilizing criteria established by Chapman (1958a). An emerged parent could be distinguished from a brood adult by the presence of pigmented oenocytes in the fat body and "corpora lutea" in the ovarioles. The reproductive tract was teased free, excised, placed on a glass slide at 0°C, and covered with two drops of saline. Specimens were held in this condition until five were prepared for observation, and then examined under a compound microscope. Tracings of all intact ovarioles were made with the aid of a drawing tube (Treffenberg type) attached to the microscope. The enclosed areas of the plane surface drawings were determined with a planimeter (Filotechnica Salmoiraghi Sp. A. Milano) and the values used as an index of relative size. This technique has been used

previously with apparent effectiveness (de Wilde and de Boer 1961). Finally, the reproductive tract was ruptured by pressure and examined for the presence and location of spermatozoa.

Frass Bioassays

Olfactory orientation to frass was determined as an arrestment response on an olfactometer described by Borden, Brownlee and Silverstein (1968). The stimulus consisted of approximately 250 mg of frass, produced during the preceding summer by females excavating in logs in the laboratory, and stored at approximately -50°C prior to testing. The stimulus was replaced on alternate tests. Ten beetles of the same sex were tested simultaneously. An individual responded positively if it executed at least three right angle turns over the stimulus site, or if it made a single right angle turn over the stimulus site and completely traversed the width of the stimulus site, or if it reversed its direction after crossing the stimulus site, but before crossing a line three cm beyond the stimulus site and returned to the stimulus without crossing a line three cm before the stimulus site. An individual that was exposed to the stimulus but did not respond

positively, was considered a negative responder. Individuals that were not exposed to the stimulus, due to photonegative response, immobility, escape or flight to the light, were discounted. A test lasted for five minutes, or until all beetles had responded.

RESULTS AND DISCUSSION

Qualitative Observations

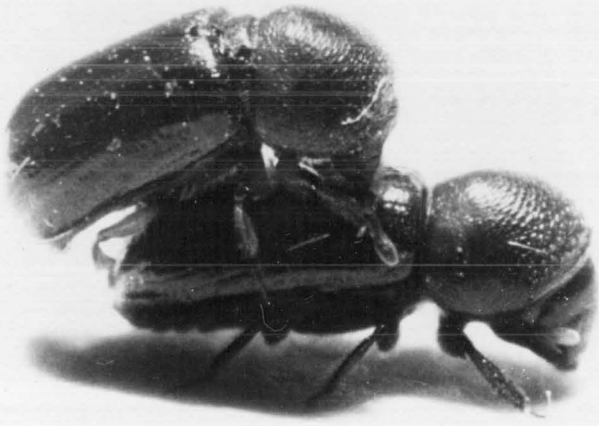
Neither sex became noticeably excited upon exposure to the opposite sex. Generally, individuals appeared to interact randomly, with mating occurring upon some encounters. Occasionally, males were observed to follow females, in contact, or up to two millimeters behind. This activity led at times to an attempt to mount the female, but more often the male spontaneously terminated the "chase" or the female moved out of range. On occasion, the trailing male successfully navigated several alterations in the direction of the moving female, caught up to the female, and then walked right past her!

There was no conspicuous courtship prior to mounting. Although a male sometimes caught and mounted a rapidly moving female with a burst of activity, males usually mounted stationary or slow moving females. The male mounted the back of the female from any direction, but most commonly from the rear. Even as a male mounted, he often curved the abdomen ventrally, and, with aedeagus extended, probed the surface of the female. The male manoeuvred upon the back into a

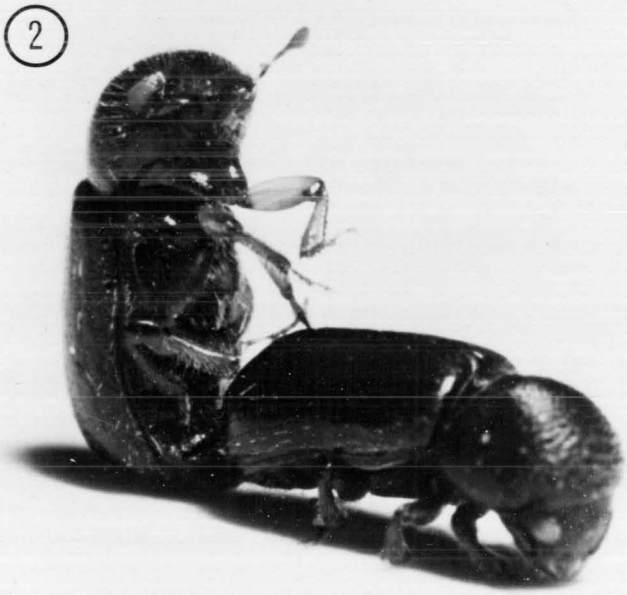
"precopulatory" position, male venter against female dorsum, both individuals facing in the same direction, male slightly caudad to the female (Fig. 1). Occasionally, there were long delays before a male oriented into the "precopulatory" position and infrequently, males failed to manoeuvre into position even after prolonged mounts. A male was noticeably agile on the female's back and would sometimes turn a 360° circle or rapidly dismount and remount. Infrequently, males reversed their position on the back, paused with the head over the caudal region of the female, and then returned to the original position. Generally, the aedeagus was retracted during these manoeuvres. In the mating position, the male usually clutched the elytra of the female with the prothoracic and mesothoracic legs. The metathoracic legs were occasionally used for support but generally remained free and were often seen rhythmically brushing the caudal portions of the elytra of the female. Whether this activity served a courtship function is unclear. However, males were occasionally observed to brush females with the metathoracic legs while in copulo if the female remained active, and to cease when she became immobile, suggesting that this action may have had a pacifying effect. Similar activity was observed in

Figs. 1 - 2 Mating *T. lineatum*: Fig. 1, Beetles in "precopulatory" position; Fig. 2, Beetles *in copulo*.

①



②



lucanid beetles more frequently with unreceptive females and when a female became immobile (Mathieu 1969), indicating a courtship function. Tactile stimuli are used by males of several species in order to quieten and reduce the mobility of courted females (Engelmann 1970). Infrequently, the mounted male stroked the female with the prothoracic and mesothoracic legs, employing the metathoracic legs for support. In the "precopulatory" position, the orientation of the male varied considerably, from one high on the female's back and at the same angle to the substrate to one much more caudad and at a much steeper angle. The antennae of the male sometimes "quivered" during mating but did not "pat" the female, as in the alfalfa weevil (Le Cato and Pienkowski 1970) and others (Engelmann 1970).

The mounted female did not respond noticeably to the male's presence; she appeared neither to cooperate with nor reject the male. Occasionally, a female fell to her side and struggled during mating, but it could not be determined whether she was rejecting the male or was simply burdened by his activities. These observations support those of Hadorn (1933), that females appeared to ignore the presence of the male and continued to search for excavation sites and/or to

construct entrance tunnels during mating. They also suggest that the sexual pattern of T. lineatum is similar to that of many species in which the aggressive male must persuade the "coy" female to copulate (Richards 1927). The low incidence of copulation (5.3% of 398 matings in a sexually active sample) suggests that these females are highly unreceptive to the advances of the males and may have some covert rejection mechanisms. Mayer and Brazzel (1963) and Le Catc and Pienkowski (1970) observed that female weevils prevented intromission by raising the abdominal apex under the elytra. This mechanism could not be observed in T. lineatum females because of their small size.

In order to insert the aedeagus, males had to move to a more vertical position so that the abdominal tip was sufficiently ventral to the vulva of the female to permit entry. In copulo, the male assumed the "male vertical pose" (Richards 1927) and was often almost perpendicular to the female (Fig. 2). The prothoracic and mesothoracic legs either rested on the female's back or were held free. Support and balance were maintained with the metathoracic legs and particularly, the aedeagus, which was lodged firmly within the vagina. During copulation, the male remained practically

motionless with only occasional twitching of the antennae or legs; the female became motionless or continued to walk slowly.

Increased movements of the legs and antennae by the male signalled the termination of copulation. The female began to walk if she had been immobile previously. The male gradually retracted the aedeagus before dismounting, sometimes with the aid of pushes of the hind legs, and he left the female's back immediately.

The average duration of mating was 1.75 minutes (range, 0.45 to 7.00 minutes; n = 145). When copulation occurred, mating duration increased to 3.35 minutes (range, 2.00 to 6.25 minutes; n = 18). Dissections indicated that, in many cases, this period was sufficient to permit sperm transfer.

Multiple mounting with the same and with different partners and very rarely, multiple copulations, were observed in both sexes. Two females that had copulated once each with the same male during a test had evidence of fresh insemination, indicating that males are capable of inseminating more than one female within a few minutes.

The mating activity of males was noticeably aggressive. Males were seen to mount mating couples, either on the anterior of the female or on the back of the mounted male. They sometimes disturbed mating couples by shoving them or forcing the head between the two bodies. Occasionally, one male would displace another on a female. On three occasions, males were observed to mate very actively with recently crushed, deformed females and on several occasions, to mount and assume the "precopulatory" position for prolonged periods on other males in a mixed group. "Homosexual" activity among males has been observed in other insects, including the alfalfa weevil, Hypera postica (Le Cato and Pienkowski 1970), two species of Callosobruchus (Nakamura 1969) and the grasshopper, Aulocara elliotti (Ferkovich, Wellso and Wilson 1967). Sexual attraction to mating couples has been noted in males of several species, including the white pine weevil and alfalfa weevil (c.f., Le Cato and Pienkowski 1970).

Contrary to the claim that male T. lineatum require a period of flight before becoming sexually active (Hadorn 1933), males in these experiments mated without previous flight exercise, indicating that mating may occur before beetles leave the overwintering site. Chapman (1955)

observed that many spring females taken in flight were already fertilized. Although he assumed that they had mated on host logs and then taken flight again, some mating could have occurred as beetles emerged from overwintering sites. Additional support comes from the finding that beetles emerge from bark and litter, and are locomotory, at temperatures below those conducive for flight (Daterman, Rudinsky and Nagel 1965).

The trailing behaviour of certain males provides evidence that the male recognizes the female through chemoreception. The occasional mounting of mating couples, males, and recently injured, deformed females (wings spread, head and prothorax crushed) indicate that vision is not a precise means of identifying a female. On the other hand, the release of chemical stimuli by injured or sexually active females, contamination of males in close proximity to females, or a state of general excitement produced by high levels of odour in a confined area could explain these activities. However, in an unreplicated experiment with late spring populations in 1970, antennectomies reduced mating activity by only 17% and 37% when males and females were antennectomized, respectively. These reductions are not the total cessation

of activity that would occur if the antennae were the only organs used for recognition of the other sex. Mating was extremely reduced by antennectomy as well as palpi cauterization in experiments with early "spring" populations in 1971, but control injuries (perforation of the lateral edges of the prothorax) also eliminated mating activity, making it impossible to separate the effects of injury from the experimental operations.

A single test showed that mating activity was not hindered, and may have been enhanced, by a drastic reduction in light intensity. In red light, a wavelength poorly or not perceived by most insect species (Burkhardt 1964), beetles mated nineteen times with three successful copulations, compared with a control group which mated sixteen times in bright light, with three successful copulations. However, it is not known whether these conditions were optically deficient, since this species spends a large portion of its active state in a dark gallery system and may be able to perceive the longer wavelengths, as do a few species (Burkhardt 1964).

Quantitative Observations

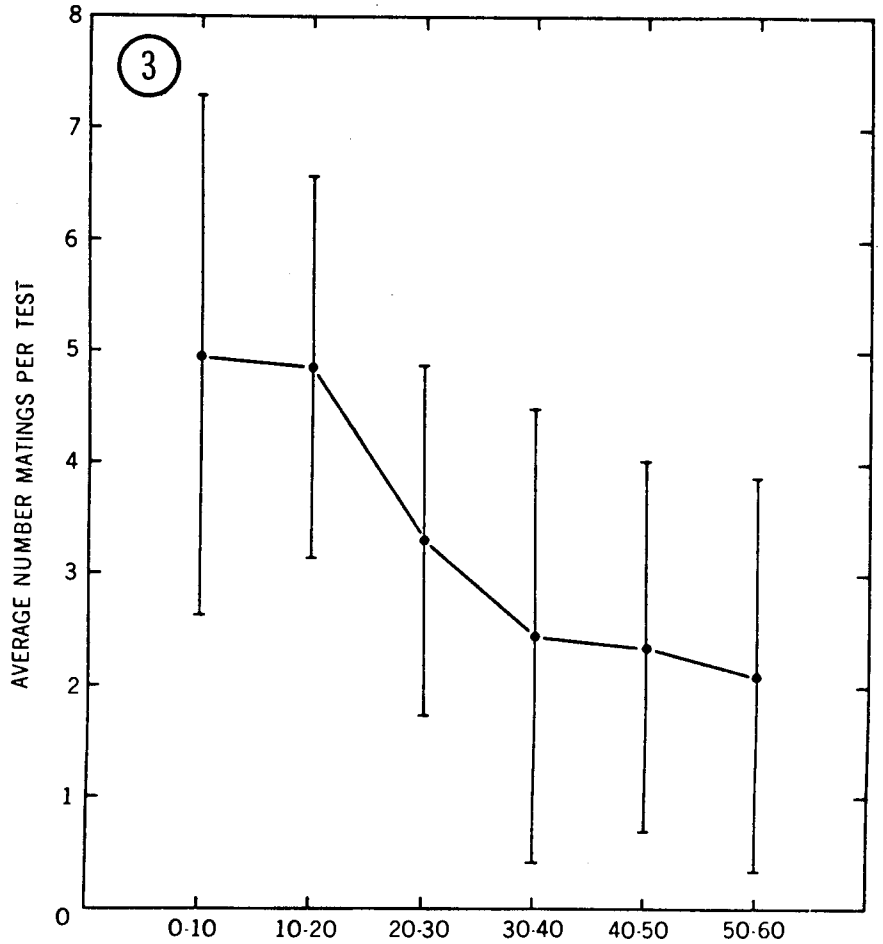
Weather Conditions

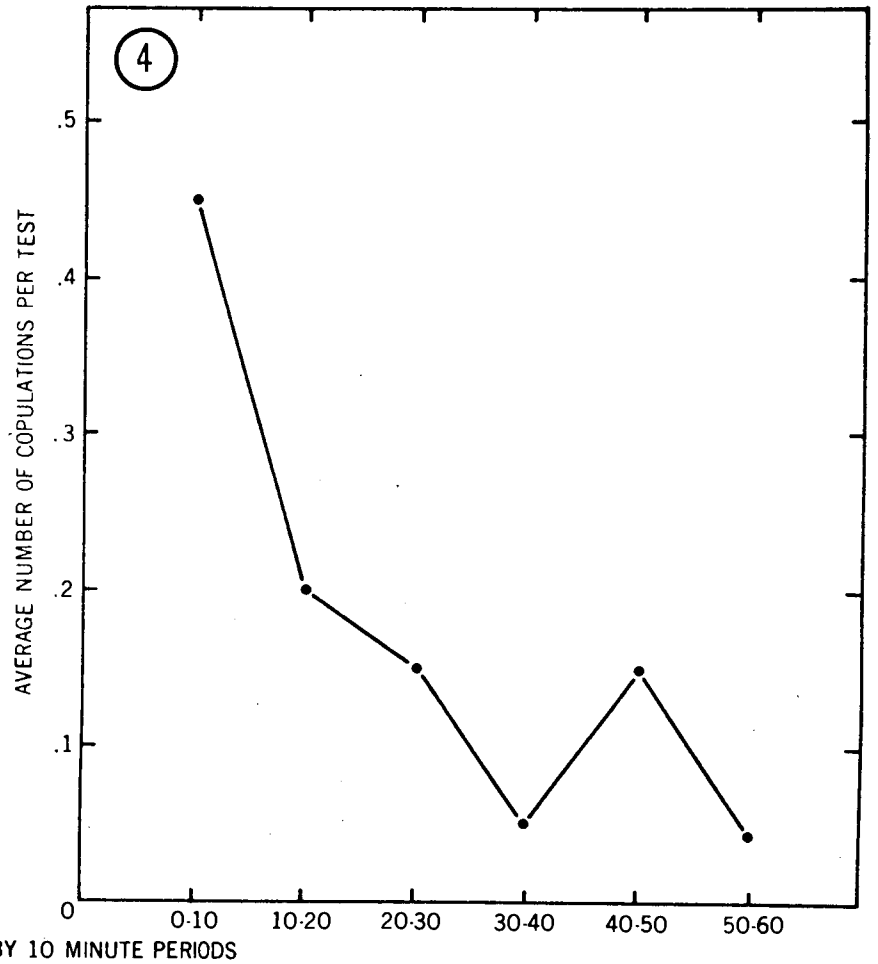
Although all tests were performed under semi-controlled conditions, the mating frequency of sexually active specimens was examined in relation to the state of the weather, since individuals could not be isolated from atmospheric pressure and extremely high relative humidities. No correlation was discovered with weather conditions occurring at the time of testing.

Satiation of Mating Activity

Although there was considerable variation, sexually active beetles became at least partially satiated during the hour of testing (Figs. 3, 4). Mating was most frequent during the first twenty minutes, declined to approximately one half this level between thirty and forty minutes, and remained at the lower level for the remaining twenty minutes of the test. Almost one half of the copulations occurred within the first ten minutes of the test (Fig. 4) indicating that, unlike many species (Richards 1927), there may be little delay between initial contact and copulation in this species.

Figs. 3 - 4 Mean frequency of mating (Fig. 3) and copulation (Fig. 4) of sexually active T. lineatum (five hours postrevival) during twenty, hour-long tests. Vertical bars in Fig. 3 represent ± 1 standard deviation. Numbers in Fig. 4 too low to make similar calculations.

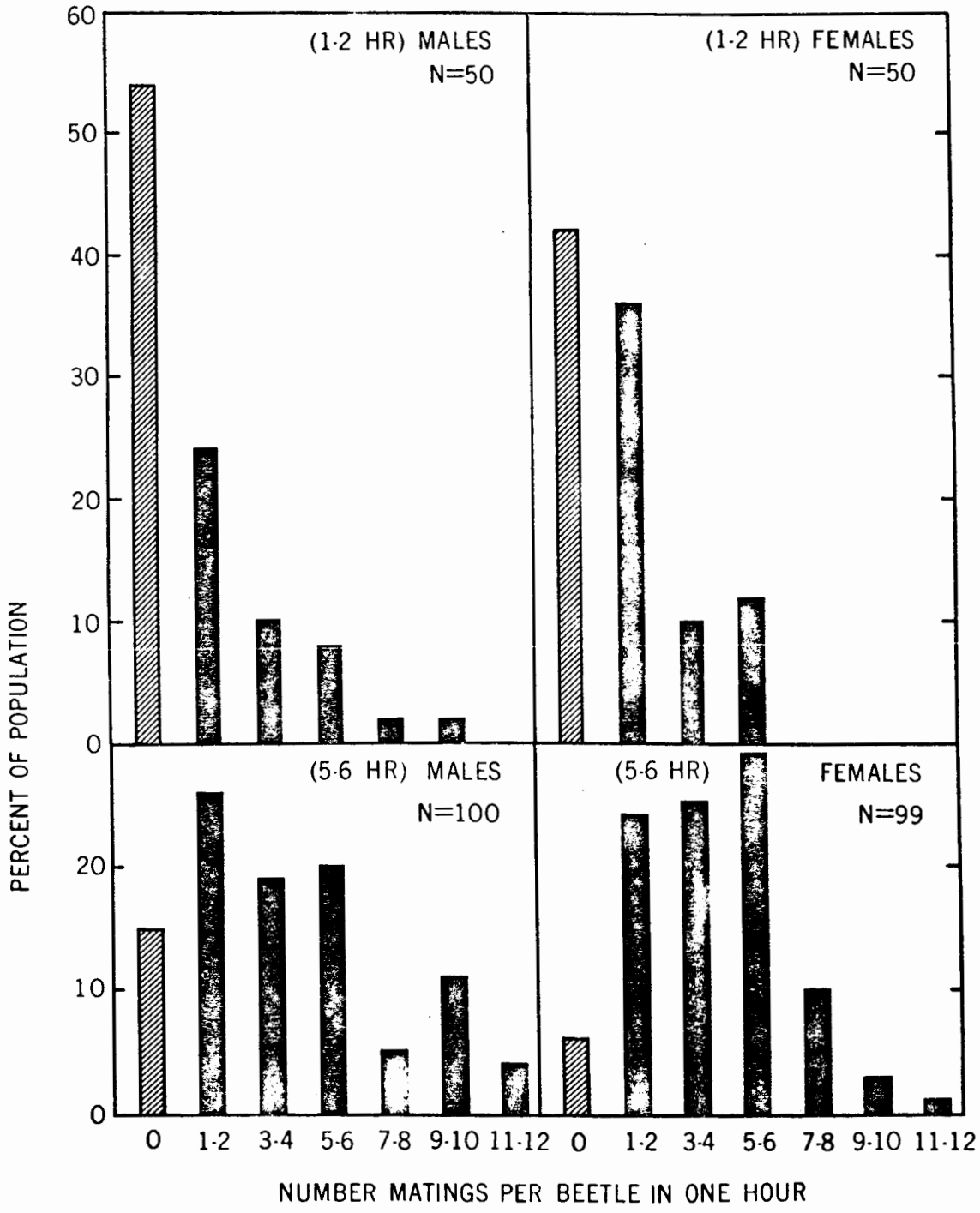

3
AVERAGE NUMBER MATINGS PER TEST
0
1
2
3
4
5
6
7
8
0-10
10-20
20-30
30-40
40-50
50-60


4
AVERAGE NUMBER OF COPULATIONS PER TEST
0
.1
.2
.3
.4
.5
0-10
10-20
20-30
30-40
40-50
50-60
TIME INTERVAL BY 10 MINUTE PERIODS

Comparison of Male and Female Activity

There was a noticeable difference between the character of the mating activity of males and females (Fig. 5). Males tended to mate several times or not at all, whereas females tended to mate at least once and a moderate number of times. For example, in tests conducted one to two hours after revival of the insects, 58% of the females mated at least once, whereas only 46% of the males mated. This pattern persisted at five to six hours after revival, 96% of the females and 85% of the males mating at least once. Moreover, 2% and 15% of the males mated more than eight times per hour at one to two and five to six hours, respectively, whereas 0% and 4% of the females mated more than eight times during the same periods. Thus, there is some tendency for a few active males to select females by chance. These data indicate that there is a delay of sexual maturation in some males, whereas females are more universally susceptible to mating in a shorter period of time.

Fig. 5 Comparison of mating activity of male and female T. lineatum at 1-2 hours (top) and 5-6 hours (bottom) postrevival. N refers to number of specimens.

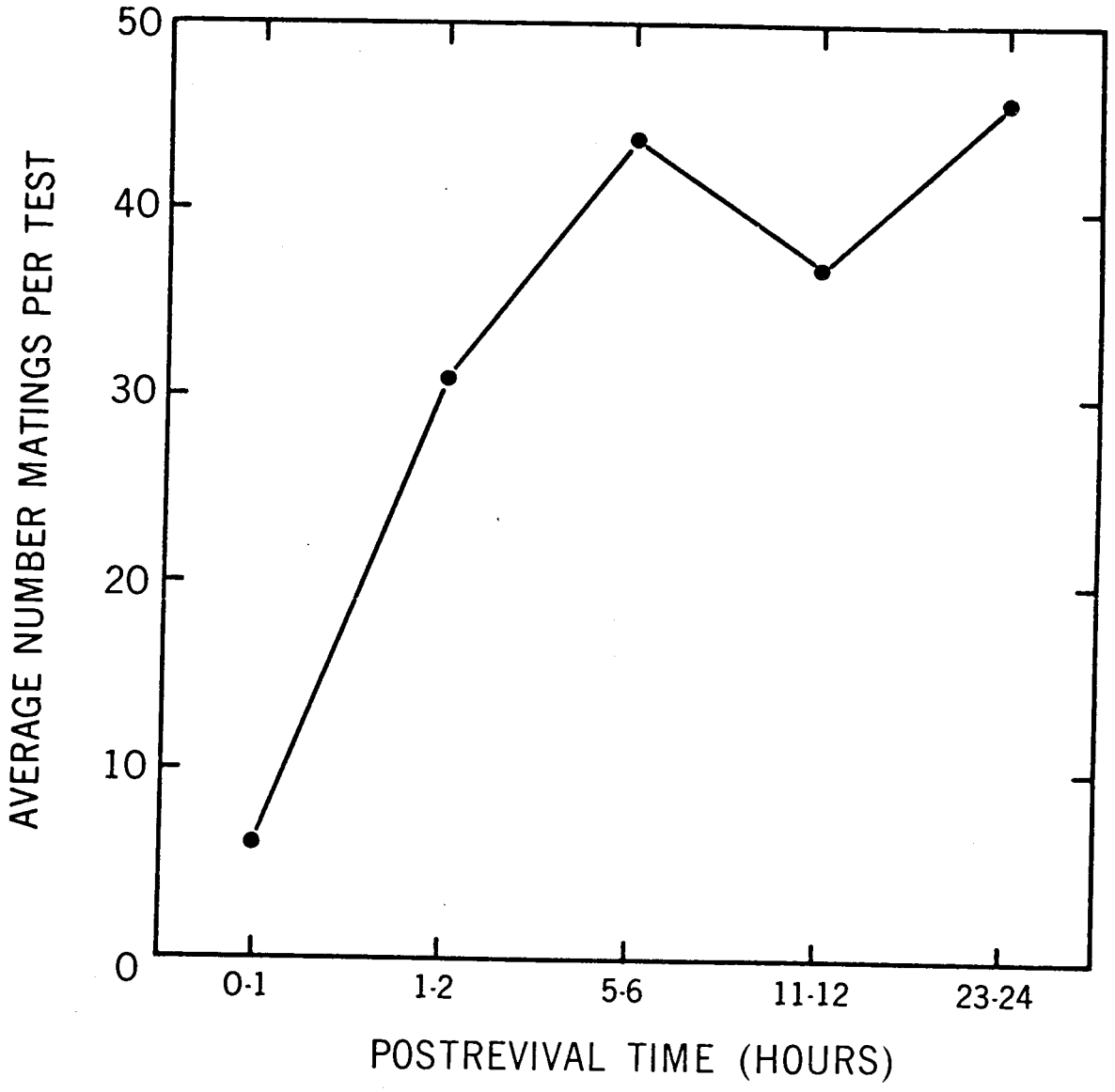

(1-2 HR) MALES
N=50
(1-2 HR) FEMALES
N=50
(5-6 HR) MALES
N=100
(5-6 HR) FEMALES
N=99
PERCENT OF POPULATION
60
50
40
30
20
10
0
20
10
0
0 1-2 3-4 5-6 7-8 9-10 11-12 0 1-2 3-4 5-6 7-8 9-10 11-12
NUMBER MATINGS PER BEETLE IN ONE HOUR

Delay Before Maximum Mating Intensity

Immediately after revival from the overwintering condition, beetles mated very seldom; maximum activity occurred after a delay of between two and five hours (Fig. 6). Thus, a period of exposure to temperatures conducive to normal activity is necessary before beetles that are removed directly from chilling temperatures become sexually active. This maturation or "activation" period is probably associated with the secretion of essential hormones and/or perhaps pheromones, processes which commence only upon exposure of the reactivated individual to temperatures that are stimulatory to metabolic activity (Gilbert 1964). The phenomenon is probably not obvious in nature since field populations are exposed to increasing temperatures which revive metabolic activity before emergence threshold temperatures are reached. The maturation lag period observed in the laboratory tests is probably an experimental artifact produced by the immediate exposure of a chilled overwintering population to temperatures which induce emergence.

The maturation lag suggested a means by which certain details of maturation could be investigated. When

Fig. 6 Effect of length of activation on the mating frequency of T. lineatum (two tests, with fresh beetles, for each period). Tests performed March, 1970.


50
40
30
20
10
0
AVERAGE NUMBER MATINGS PER TEST
0-1
1-2
5-6
11-12
23-24
POSTREVIVAL TIME (HOURS)

one sex was permitted only one hour exposure to room temperature and the other, five hours, there was a sex-dependent effect on mating activity (Fig. 7). After one hour exposure, males did not mate actively, even with females that had been warmed for five hours, but males warmed for five hours were very active, even with females warmed for only one hour. Therefore, maturation of males accounts for the majority of the delay in maximum mating activity.

A certain degree of maturation of the female also occurs since both one- and five-hour males mated more frequently with females that had been warmed for five hours than with females that had been warmed for one hour, although the effect in both cases was statistically insignificant. When females warmed for one hour were replaced by females warmed for five hours, the increased mating frequency of males was due to increased mating intensity of active individuals, not to the activation of nonmating invididuals (Table I). This indicates that females of a more prolonged activation period enhance the mating frequency of sexually mature males, presumably through the development of some form of excitatory stimulation. That the males become more generally excited at a later stage of activation is suggested by the

Fig. 7 Effect of difference in duration of activation time of either sex on mating frequency in *T. lineatum* (t-test probability levels for bars numbered from the left are:

bar 1 versus bar 2, $p < .05$;

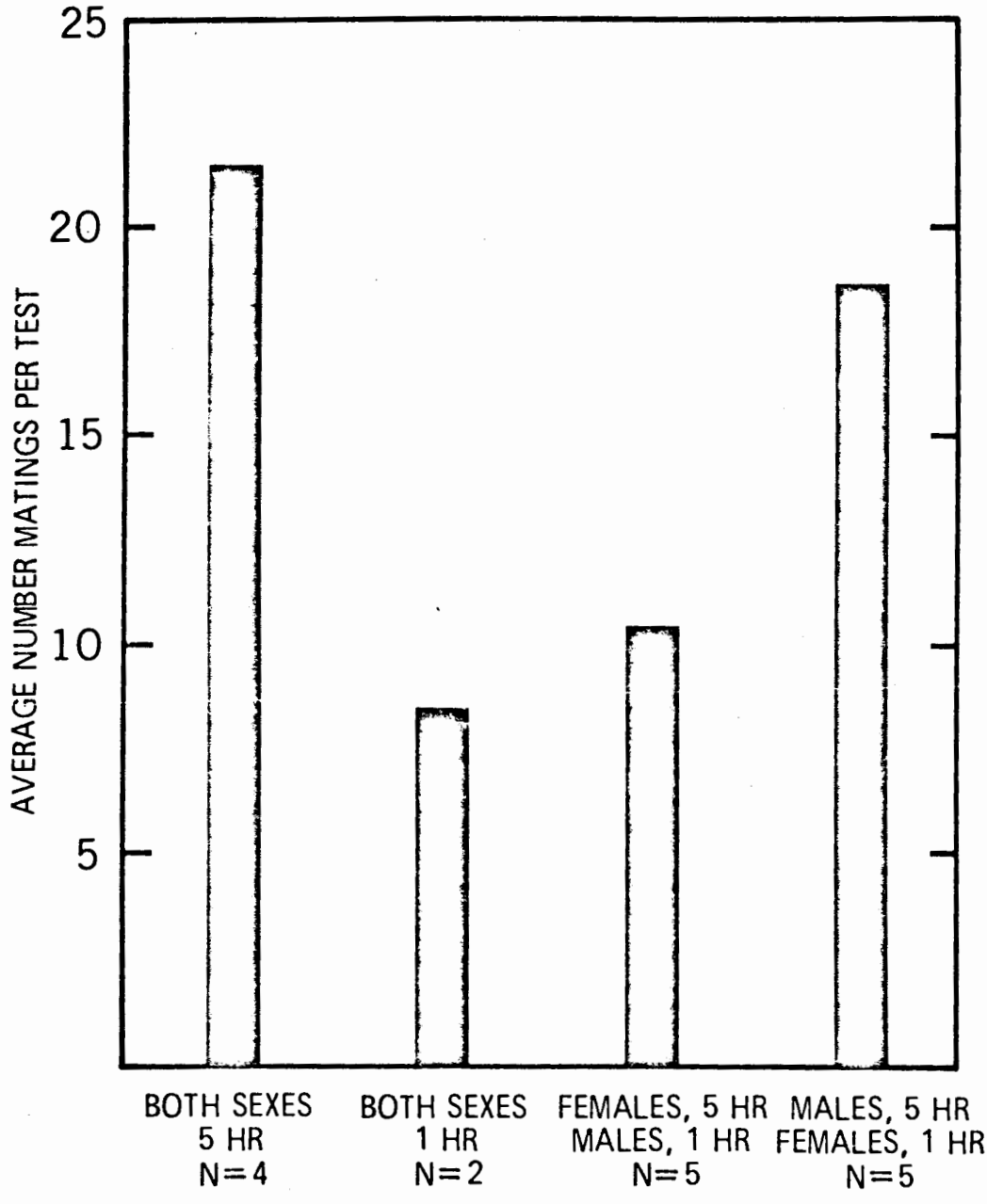
bar 1 versus bar 3, $p < .025$;

bar 1 versus bar 4, $p < .50$;

bar 2 versus bar 3, $p > .50$;

bar 2 versus bar 4, $p < .10$;

bar 3 versus bar 4, $p < .05$).


25
20
15
10
5
AVERAGE NUMBER MATINGS PER TEST
BOTH SEXES 5 HR N=4
BOTH SEXES 1 HR N=2
FEMALES, 5 HR MALES, 1 HR N=5
MALES, 5 HR FEMALES, 1 HR N=5
POSTREVIVAL TIME (HOURS) AND NUMBER OF REPLICATES

observation that the occurrence of copulation as a percent of mating attempts decreased from 13.4% of 67 matings in the one hour activation sample to 5.3% of 398 matings in the six hour sample. This indicates that the male becomes more excited and mounts more often with older females.

Seasonal Activity

Brood Adults

Dissections confirmed previous findings that, in brood female T. lineatum, freshly emerged from logs in which they matured, the ovaries appear mature but do not contain developing oocytes (Chapman 1955). Mating activity was negligible (1.3 matings per test) (Fig. 8). However, two of the six observed matings terminated in copulation. No dissections were made to confirm insemination. However, Chapman (1954) observed that the testes of emerged brood males were of full size and contained mobile sperm, suggesting that males may be capable of inseminating females at this time. In many species, males produce sperm and are sexually active prior to diapause (Beck 1968).

Table I Effect of postrevival time on mating activity, by sex, in *T. lineatum*. Number of replicates same as in Fig. 7.

Postrevival time prior to test	Number of individuals tested		Percent of individuals mating		Number of matings per mating individual	
	♂	♀	♂	♀	♂	♀
Both sexes, 5 hr	20	20	74	94	5.4	4.5
Both sexes, 1 hr	10	10	60	86	2.8	1.9
Females, 5 hr, Males, 1 hr	25	25	48	84	4.3	2.5
Males, 5 hr, Females, 1 hr	25	25	84	88	4.4	4.2

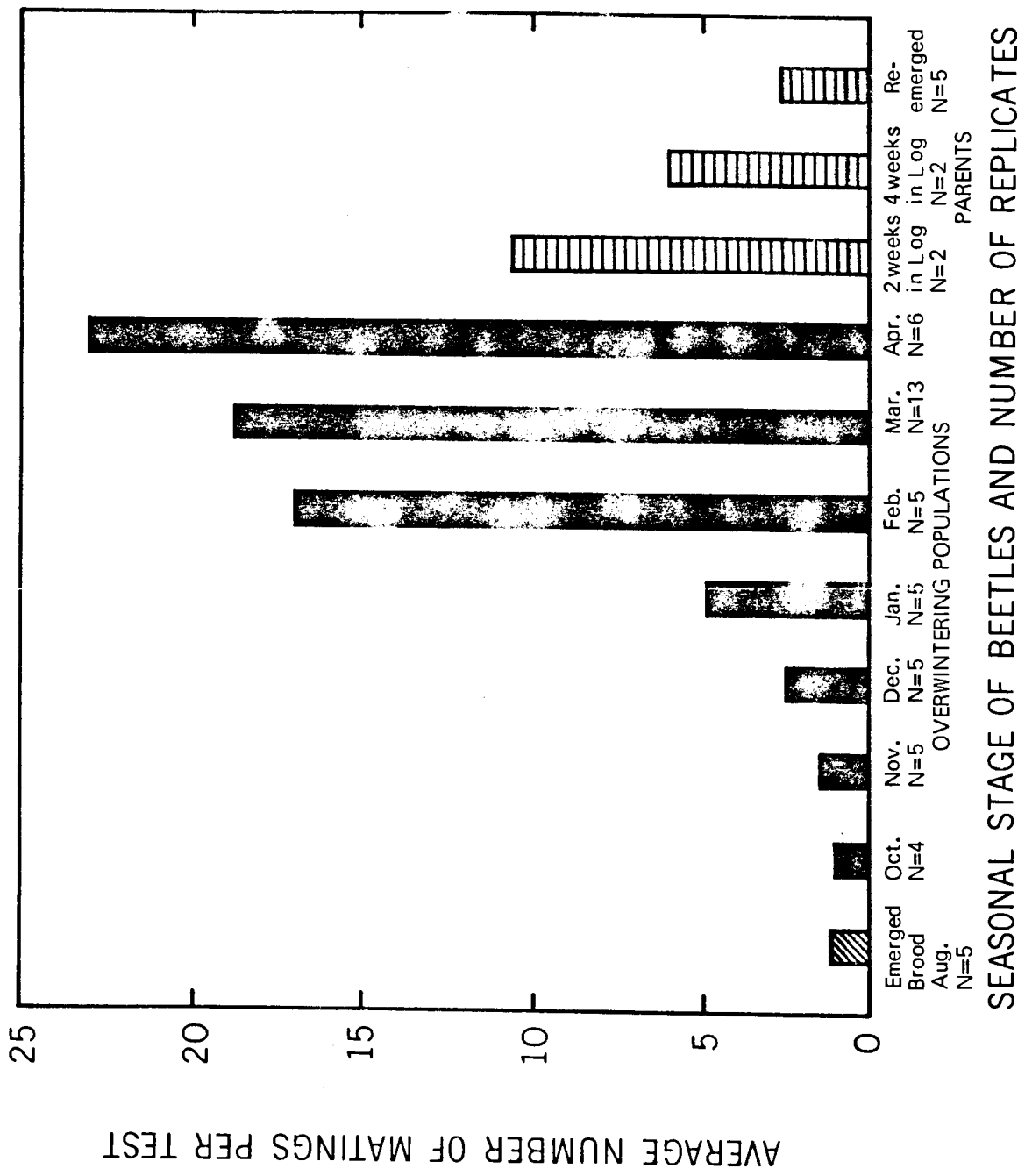
Overwintering Populations

When samples of an overwintering population of T. lineatum were warmed at monthly intervals to temperatures conducive to locomotion and tested for sexual activity after five hours at 24 ± 1°C, an initial absence of sexual activity became apparent (Fig. 8). Mating frequency was very low in early fall and increased gradually throughout the winter until February, when there was an abrupt increase to levels approaching those of normally active "spring" populations. Increasing mating activity was due to both increasing numbers of participating individuals and increasing mating frequency of active individuals (Table II).

Overwintering T. lineatum become mobile when warmed, a response that is similar to that of a number of insect species that overwinter in the adult stage (Patton 1963), and which suggests that if T. lineatum undergoes a true diapause, it is a shallow form of diapause termed "hibernation" (Danilevskii 1965). The principal feature of such an adult diapause is the arrest of sexual maturation (de Wilde 1964, Danilevskii 1965).

A diapausing insect is not physiologically inert,

Fig. 8 Mating activity of T. lineatum at different stages of adult life.



SEASONAL STAGE OF BEETLES AND NUMBER OF REPLICATES

Table II Changes in seasonal mating activity, by sex, in T. lineatum. Number of replicates same as in Fig. 8.

Seasonal stage of beetles	Number of individuals tested		Percent of individuals mating		Number of matings per mating individual	
	♂	♀	♂	♀	♂	♀
Emerged brood	25	25	22	22	1.2	1.2
Overwintering populations						
Oct.	20	20	20	25	1.0	0.8
Nov.	25	25	20	24	1.4	1.2
Dec.	25	25	28	36	1.7	1.3
Jan.	25	25	32	64	3.0	1.5
Feb.	25	25	84	92	4.0	3.7
Mar.	65	65	84	88	4.3	4.1
Apr.	30	30	93	90	4.6	4.7
Parents						
2 weeks in log	10	10	70	80	3.0	2.6
4 weeks in log	10	10	70	50	1.7	2.4
Emerged	25	25	20	30	2.0	1.3

but undergoes "diapause development" or "reactivation" processes which, when complete, permit normal maturation to recommence when the individual is re-exposed to temperatures conducive to normal development (Danilevskii 1965). Cold-diapausing insects often require a period of exposure to critical low temperatures (0-10°C) before reactivation can occur (de Wilde 1964). A generally accepted hypothesis (Gilbert 1964) is that of Williams (1946), supported by the studies of Highnam (1958), that the exposure of a diapausing brain to low temperatures converts it to a "competent" brain, in which material has accumulated in the median neurosecretory cells (mnc) of the pars intercerebralis. This substance is required to stimulate the corpora allata to synthesize juvenile hormone (de Wilde 1964). Exposure of the reactivated individual to high temperatures induces the release of material from both the mnc and corpora allata, thus increasing the hemolymph concentration of juvenile hormone, resulting in diapause termination (de Wilde 1964, Gilbert 1964).

Because of genetic and environmental variations, the reactivation times of individuals vary, but the proportion

of reactivated insects tends to increase gradually throughout the winter until spring, when essentially the entire diapausing population is reactivated and individuals resume development concurrently following an increase in environmental temperatures to values above the threshold for normal activity (Danilevskii 1965). In nature, reactivation trends are not evident since both reactivated and nonreactivated insects are immobilized by cold. Artificial warming of overwintering T. lineatum revealed reactivation trends in reproductive activity, confirming previous evidence of hibernation in this species. Increasing mating frequency in reactivated individuals later in the winter (Table II), demonstrates that there is increasing sexual tension in reactivated individuals, under prolonged storage. These tests also show that diapause is "broken" in the majority of the population, two to three months before beetles resume activity in the field.

Before February, there was no evidence of insemination during mating, either through observation of copulation or the diagnostic presence of masses of sperm in the lower reproductive tract of the female. However, in females dissected after fall and early winter tests, nine of thirty-

two females which had been mounted during a test, and four of twenty-six nonmounted females contained sperm within the spermatheca and associated "gland". Of twenty females removed from November and December diapause populations, seven (35%) appeared, upon dissection, to be emerged parents. Two parents and one brood female contained live sperm in the spermatheca. These dissections support the findings that some parents hibernate a second year (Chapman 1955, 1958a; Chapman and Nijholt 1965). Chapman (1958a) found that 52% of the females of an autumn litter sample and 37% of a spring litter sample were emerged parents. These results also show that brood females may be inseminated before or during confinement to overwintering sites, indicating that the matings that were observed among brood insects (Fig. 8) are capable of insemination and occur naturally.

"Spring" Adults and Excavating Parents

The mating activity of beetles removed from stored duff in March and April averaged 18.7 and 23.0 matings per test, respectively (Fig. 8). These data are considered to be typical of sexually active "spring" populations in nature,

since overwintering populations have been known to emerge from hibernation and initiate host attacks and sexual activity during these months. Following host invasion, mating frequency declined considerably, falling to approximately one half the intensity of the "spring" populations two weeks after log entry and declining further to 6.0 matings per test after four weeks (Fig. 8).

Emerged Parents

The mating activity of parents that had left the host was almost negligible (2.6 matings per test) (Fig. 8). This finding is elucidated by the observations of Hadorn (1933) that seven of ten galleries constructed by reattacking females contained no male partners, and that the spermathecae of these females contained residual supplies of live sperm, suggesting that sexual activity and reinsemination are unnecessary during host reinvasion.

Cross Matings Between Brood and 'Spring' Adults

In order to observe the effect upon mating intensity of a naturally active sex partner, brood adults were

exposed to "spring" adults of the opposite sex. Brood males were only slightly stimulated by sexually active "spring" females whereas "spring" males were not appreciably deterred by the youth of their brood partners (Fig. 9), revealing that the lack of sexual activity in brood beetles is due primarily to the immaturity of the male. However, inhibition of mating is partially female-dependent as well. Both brood and "spring" males mated slightly (although insignificantly) more frequently with "spring" females than brood females. Further analysis revealed that exposure of males to females of a different life stage altered the mating frequency of active males but did not affect the percent of male participants (Table III). These results are notably similar to those that occurred when males and females of sexually active "spring" samples were allowed different maturation times (Fig. 7, Table I). Thus, similar to the conclusions drawn from the maturation tests, it appears that if a male has, through intrinsic processes, reached a state whereby he is capable of recognizing and/or responding to a female, the maturity of the female has some effect on his mating intensity.

Fig. 9 Effect of the adult life stage of either sex on mating frequency in *T. lineatum*.

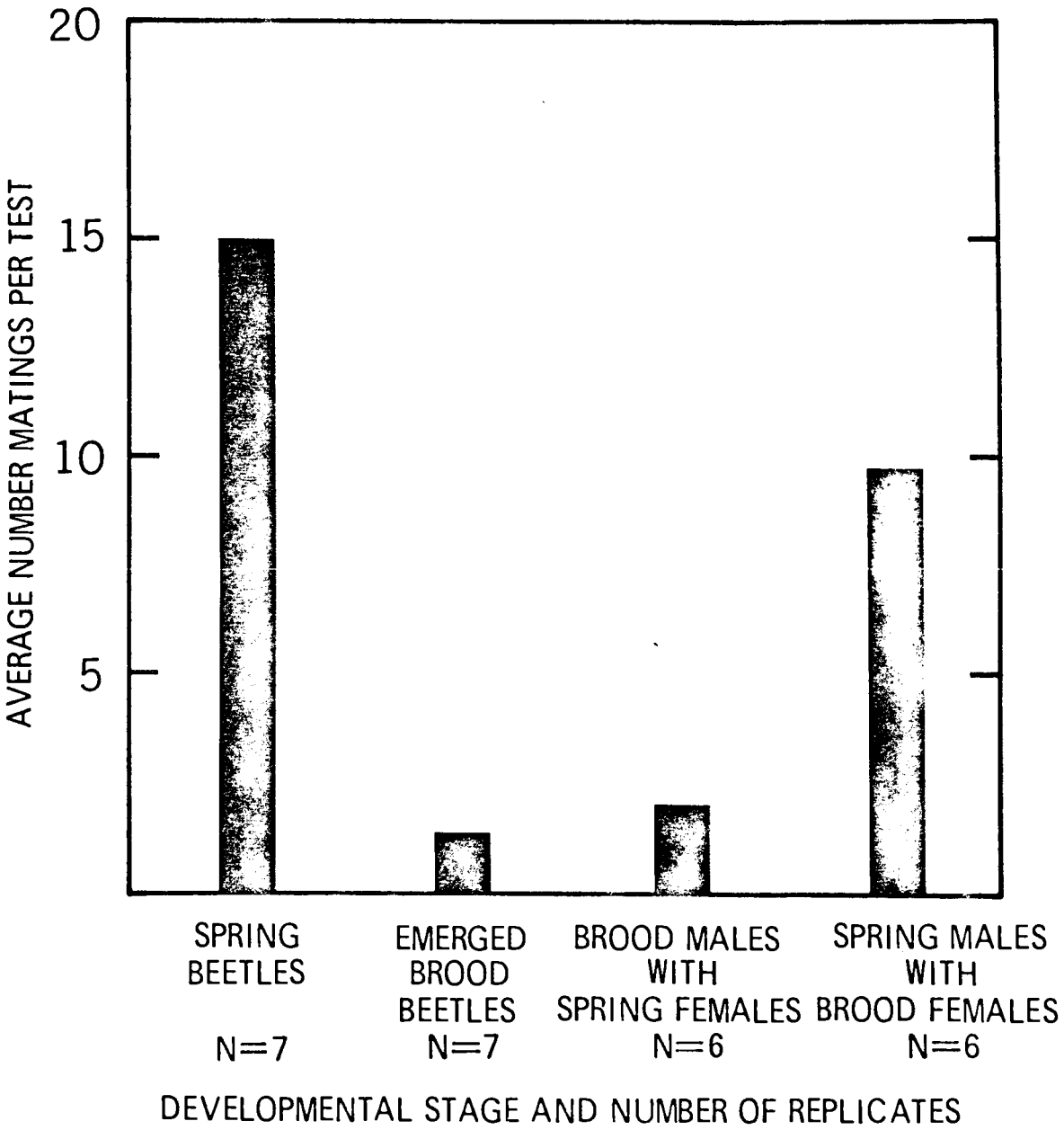

20
15
10
5
AVERAGE NUMBER MATINGS PER TEST
SPRING BEETLES N=7
EMERGED BROOD BEETLES N=7
BROOD MALES WITH SPRING FEMALES N=6
SPRING MALES WITH BROOD FEMALES N=6
DEVELOPMENTAL STAGE AND NUMBER OF REPLICATES

Table III Effect of adult life stage on mating activity, by sex, in *T. lineatum*. Number of replicates same as in Fig. 9.

Developmental stages of test beetles	Number of individuals tested		Percent of individuals mating		Number of matings per mating individual	
	♂	♀	♂	♀	♂	♀
Spring beetles	35	35	60	88	5.0	3.4
Emerged brood beetles	35	35	22	22	1.2	1.2
Brood males with spring females	30	30	20	22	1.9	1.7
Spring males with brood females	30	30	60	80	3.2	2.4

Frass Bioassays

After one hour of activation, 11.7% of "spring" females and 45.7% of males oriented to attractive frass; after five hours, 31.9% of females and 53.2% of males responded. Between one and five hours, the response of females increased 172%; of males, only 16%. Notably, males responded to frass at almost peak intensity while still sexually immature but females required a period of maturation before response was marked. Hibernating males (December populations) also responded quite strongly to frass (31.1%), while hibernating females showed a negligible response (4.7%) (E. Stokkink, personal communication).[2]

The results suggest that the frass response of a male is not directly related to sexual activity. Frass response did not follow the pattern of mating activity; both hibernating and immature "spring" males responded almost as strongly to frass odour as mature "spring" males.

---

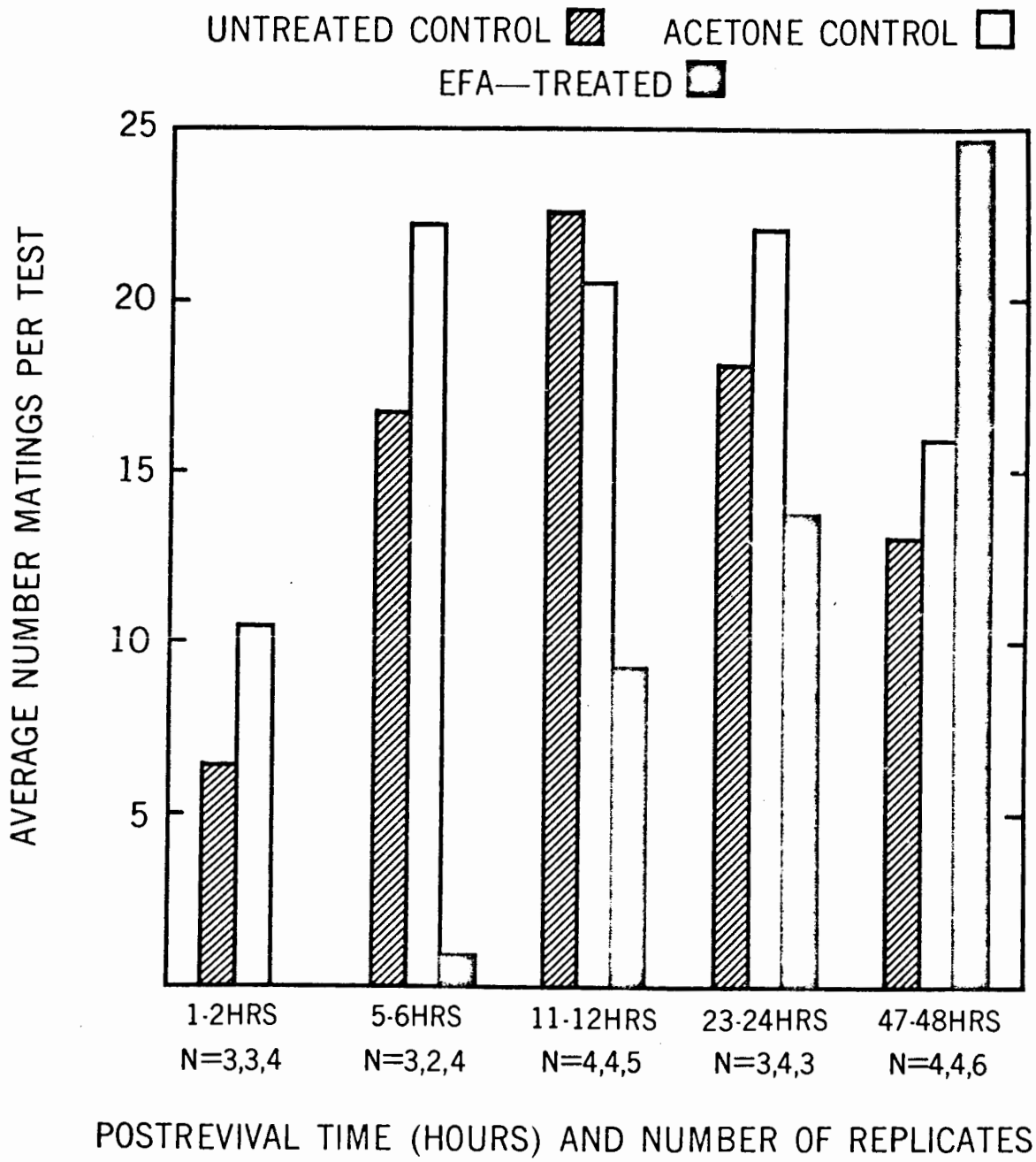
2. Department of Biological Sciences, Simon Fraser University

Juvenile Hormone Tests

Treatment of Both Sexes with 50 µg of EFA

Application of 50 µg of EFA to both sexes of a "spring" population sample caused complete inhibition of sexual activity within one hour of treatment (Fig. 10). This effect is extremely rapid compared to most known effects of hormone treatment, and is the first demonstration of induction of mating inhibition in insects by treatment with juvenile hormone. Mating frequency of treated beetles increased gradually with time, but remained significantly depressed from the acetone-treated and untreated controls (t-test, $p < 0.10$) until 48 hours posttreatment, when EFA-treated specimens mated at a level slightly higher than the controls. This apparent stimulation of mating resulted from extremely intense activity in two of the six replicates (47 and 53 matings in the hour test). Until 48 hours, increasing mating activity was caused by both increasing numbers of participants and increasing mating frequency of active beetles but, between 24 and 48 hours, the rapid rise in mating activity was produced primarily by the higher frequency of mating by treated beetles (most notably males) as compared to the controls, even

Fig. 10 Effect of treatment of both sexes of T. lineatum with 50 μg EFA on mating activity at different times posttreatment. Mating activity of EFA-treated individuals significantly lower than untreated or acetone controls (t-test, $p < .01$, .005; $p < .005$, .005; $p < .10$, .025; $p < .01$, .01 for acetone and untreated controls at 1-2, 5-6, 11-12 and 23-24 hours, respectively) except at 47 to 48 hours. Higher activity of EFA-treated individuals at 47 to 48 hours not significantly (t-test, $p < .50$) different from untreated and acetone controls, respectively.


UNTREATED CONTROL
ACETONE CONTROL
EFA—TREATED
AVERAGE NUMBER MATINGS PER TEST
25
20
15
10
5
1-2HRS
N=3,3,4
5-6HRS
N=3,2,4
11-12HRS
N=4,4,5
23-24HRS
N=3,4,3
47-48HRS
N=4,4,6
POSTREVIVAL TIME (HOURS) AND NUMBER OF REPLICATES

though a large number of treated males remained inactive (Table IV). The trend indicates that the dramatic rise in mating activity in some tests at 48 hours is not due to the same factors as were the previous increases, but that active males are suddenly stimulated to mate very abundantly. Although this change could be due to factors intrinsic in the males, previous observations of mating data (Tables I, III) suggest that these males were excited by the treated females. Further evidence will be presented later.

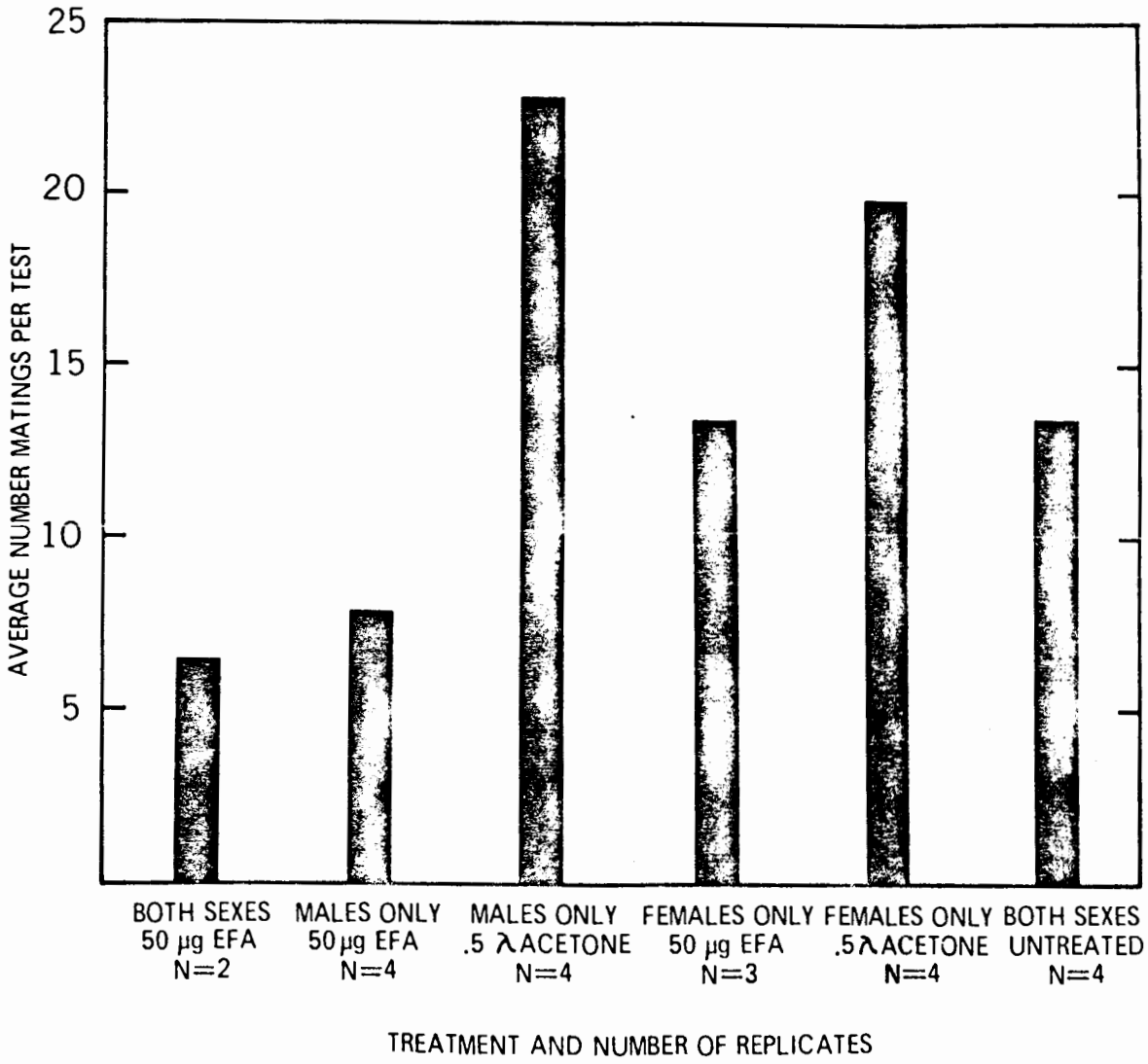
Treatment of Either Sex with 50 μg EFA

In order to elucidate the source of mating inhibition, sexes were treated individually with 50 μg of EFA and exposed to untreated specimens of the opposite sex. When males alone were treated (Fig. 11), mating activity was very similar to that when both sexes were treated. When females alone were treated, mating activity was somewhat greater than after treatment of both sexes (t-test, $p < .50$). Both segregated treatments were significantly different from their acetone controls (t-test, $p < .01$ and $p < .10$ for males and females, respectively), but neither was significantly different (t-test, $p < .50$ and $p > .50$ for males and females, respectively) from the untreated controls which performed at

Table IV Mating activity by sex at various times following 50 μg EFA treatment to both sexes of *T. lineatum*. Number of replicates same as in Fig. 10.

Time of test	Treatment	Number of individuals tested		Percent of individuals mating		Number of matings per mating individual	
		♂	♀	♂	♀	♂	♀
1-2 hr	EFA	15	15	0	0	0	0
	Acetone	15	15	66	74	3.1	2.8
	Untreated	20	20	26	54	4.8	2.3
5-6 hr	EFA	15	15	10	16	1.6	1.0
	Acetone	10	10	80	80	5.5	5.5
	Untreated	20	20	80	86	4.2	3.9
11-12 hr	EFA	20	20	36	60	5.1	3.1
	Acetone	20	20	86	90	4.8	4.6
	Untreated	25	25	90	90	5.0	5.0
23-24 hr	EFA	15	15	74	74	3.7	3.7
	Acetone	20	20	86	94	5.1	4.7
	Untreated	15	15	94	100	3.8	3.6
47-48 hr	EFA	20	20	76	90	6.5	5.5
	Acetone	20	20	80	86	4.0	3.7
	Untreated	30	30	66	80	3.9	3.3

Fig. 11 Effect on mating activity of treatment of either sex of *T. lineatum* with 50 µg EFA. Beetles tested 5-6 hours after treatment.


25
20
15
10
5
AVERAGE NUMBER MATINGS PER TEST
BOTH SEXES 50 µg EFA N=2
MALES ONLY 50 µg EFA N=4
MALES ONLY .5 λ ACETONE N=4
FEMALES ONLY 50 µg EFA N=3
FEMALES ONLY .5 λ ACETONE N=4
BOTH SEXES UNTREATED N=4
TREATMENT AND NUMBER OF REPLICATES

an unusually low level.

Reduction in mating frequency when males were treated with hormone resulted from a reduction in the number of mating individuals only; there was little effect on the mating frequency of active males (Table V). Repression of mating in the EFA-treated groups appears to be due to inhibiting physiological influences on the male and, to a lesser degree, the female. However, observation of some mortality in treated male specimens raises the possibility that the healthy-appearing individuals used in the tests may have been pharmacologically incapacitated. Nevertheless, the effect on mating was much more prominent when males were treated.

The acceleration of mating of hormone-treated specimens at 48 hours (Fig. 10) was probably due to increased sexual activity of the females. Two tests in which females were treated with 50 μg EFA and held for 24 hours before being exposed to untreated males held for six hours, revealed consistently greater mating activity than control females of the same activation period (35.5, 15.5 and 7.0 matings per test after EFA, acetone and no treatment, respectively). One can assume that treated males do not undergo a similar

Table V Mating activity by sex following treatment of either sex of *T. lineatum* with 50 µg EFA. Number of replicates same as in Fig. 11.

Treatment	Number of individuals tested		Percent of individuals mating		Number of matings per mating individual	
	♂	♀	♂	♀	♂	♀
Both sexes 50 µg EFA	10	10	30	50	4.3	2.6
Males only 50 µg EFA	20	20	36	66	4.3	2.4
Males only .5 λ Acetone	20	20	90	96	5.1	4.8
Females only 50 µg EFA	15	15	60	86	4.4	3.1
Females only .5 λ Acetone	20	20	80	90	4.6	4.4
Both sexes Untreated	20	20	70	100	3.9	2.7

trend since mating activity when both sexes were treated was lower than the controls at 24 hours (Fig. 10). Since treatment of females only did not result in accelerated activity at five to six hours posttreatment (Fig. 11), it appears that their activity also increases with time. One hypothesis which would explain the trend of activity displayed in Figure 10 is that decreasing concentrations of hormone, through metabolization and/or excretion, resulted in diminishing inhibition of the male, and, to a lesser extent, the female, as well as increasing sexual activity of the female.

Treatment of Both Sexes with Doses of EFA Lower than 50 µg

Since there is evidence that juvenile hormone is necessary to augment mating activity, an effort was made to find concentrations of EFA lower than 50 µg that would enhance mating after one hour of activation to intensities approaching those at five to six hours, by obviating the time delay required for natural secretion. Although the 50 µg dose induced the predicted inhibition, with a single exception there was no stimulation of mating activity by lower doses (Fig. 12) and none were able to accelerate mating fre-

Fig. 12 Mating intensity of T. lineatum 1 and 5 hours after application of different concentrations of EFA to both sexes.

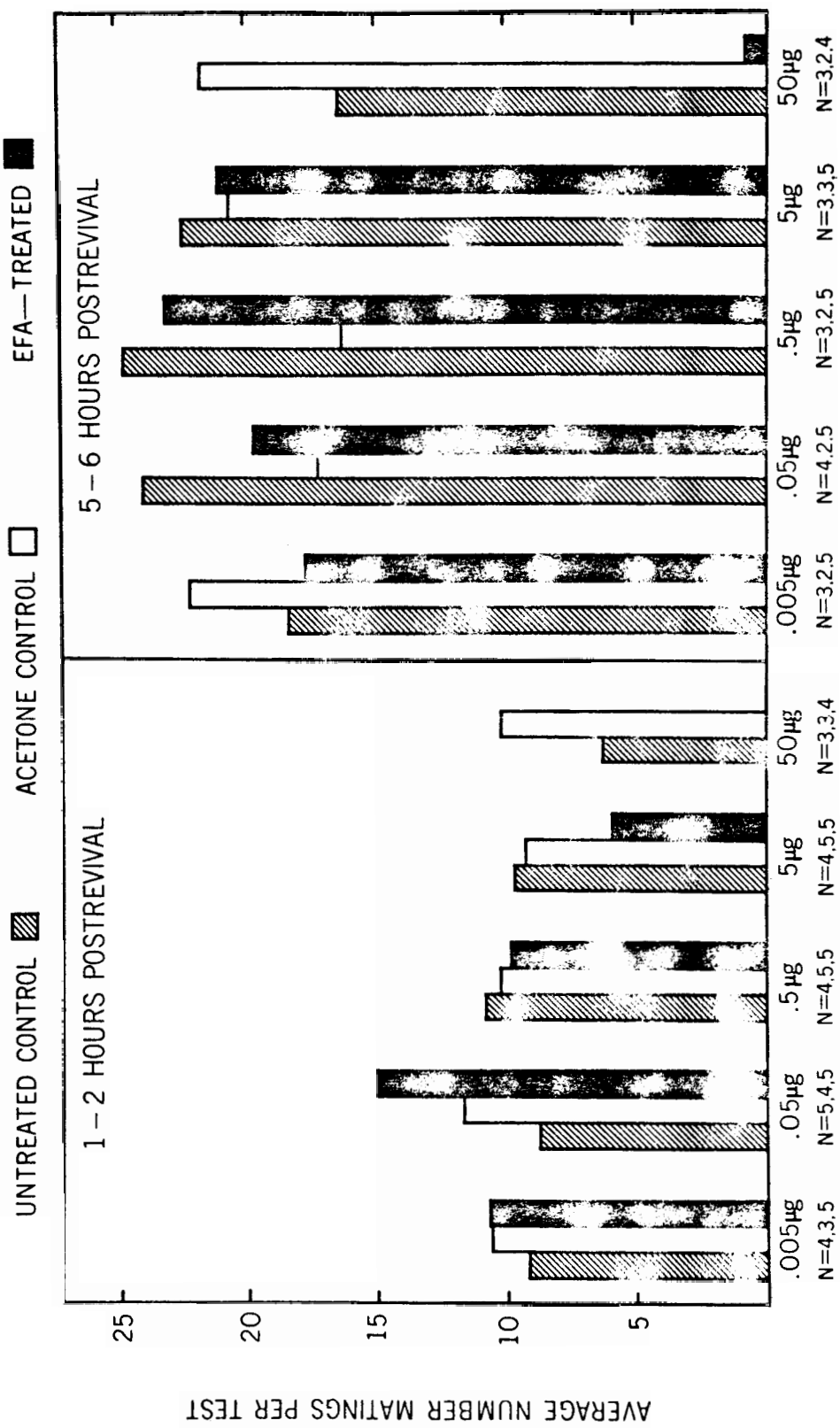

UNTREATED CONTROL
ACETONE CONTROL
EFA—TREATED
1 – 2 HOURS POSTREVIVAL
5 – 6 HOURS POSTREVIVAL
AVERAGE NUMBER MATINGS PER TEST
25
20
15
10
5
.005µg
.05µg
.5µg
5µg
50µg
N=4,3,5
N=5,4,5
N=4,5,5
N=4,5,5
N=3,3,4
N=3,2,5
N=4,2,5
N=3,2,5
N=3,3,5
N=3,2,4
EFA DOSE AND NUMBER OF REPLICATES

Table VI Mating activity by sex after treatment of both sexes of T. lineatum with different doses of EFA. Number of replicates same as in Fig. 12.

Time of test	EFA Dose (μg)	Number of individuals tested		Percent of individuals mating		Number of matings per mating individual	
		♂	♀	♂	♀	♂	♀
1-2 hr	0.005	25	25	64	68	3.4	3.2
	0.05	25	25	64	80	4.8	3.8
	0.5	25	25	56	60	3.6	3.3
	5.0	25	25	44	64	2.7	1.9
	50.0	20	20	0	0	0.0	0.0
$\bar{x}$ of Acetone controls		30	30	76	80	3.1	3.0
$\bar{x}$ of Untreated controls		30	30	64	64	2.8	2.8
5-6 hr	0.005	25	25	92	92	3.9	3.9
	0.05	**25**	**25**	**92**	**92**	**4.3**	**4.3**
	0.5	25	25	96	100	5.1	4.7
	5.0	25	25	76	92	5.6	4.7
	50.0	20	20	10	16	1.6	1.0
$\bar{x}$ of Acetone controls		30	30	86	92	4.1	3.9
$\bar{x}$ of Untreated controls		30	30	84	92	4.7	4.2

quency to levels comparable to those at five to six hours. The sexual activity of beetles treated with 0.05 μg EFA was greater (t-test, $p < 0.10$) than that of untreated controls at one hour posttreatment, although not different from the acetone controls. The enhanced mating activity was due primarily to increased mating frequency of active individuals and activation of the population to a participation level characteristic of more mature individuals at 5-6 hours did not occur (Table VI). The effect, although very weak, is still considered valid due to the consistent trend of dose effect at 1-2 hours.

Effect of EFA on Ovary Development

Topical application of 50 μg EFA induced typical ovary maturation (Fig. 13). Within 48 hours, ovarioles of treated females were significantly larger than those of both controls (t-test, $p < .10$). Increased size was due to both elongation of the ovariole and the production and growth of oocytes (Fig. 14). Yolk deposition, characterized by increased density of the oocytes (Chapman 1955), was evident in developed ovarioles. The appearance of the ovarioles of

Fig. 13 Effect of application of 50 µg EFA to female *T. lineatum* on ovary size at different times posttreatment. Each replicate represents the average ovariole size of one female.

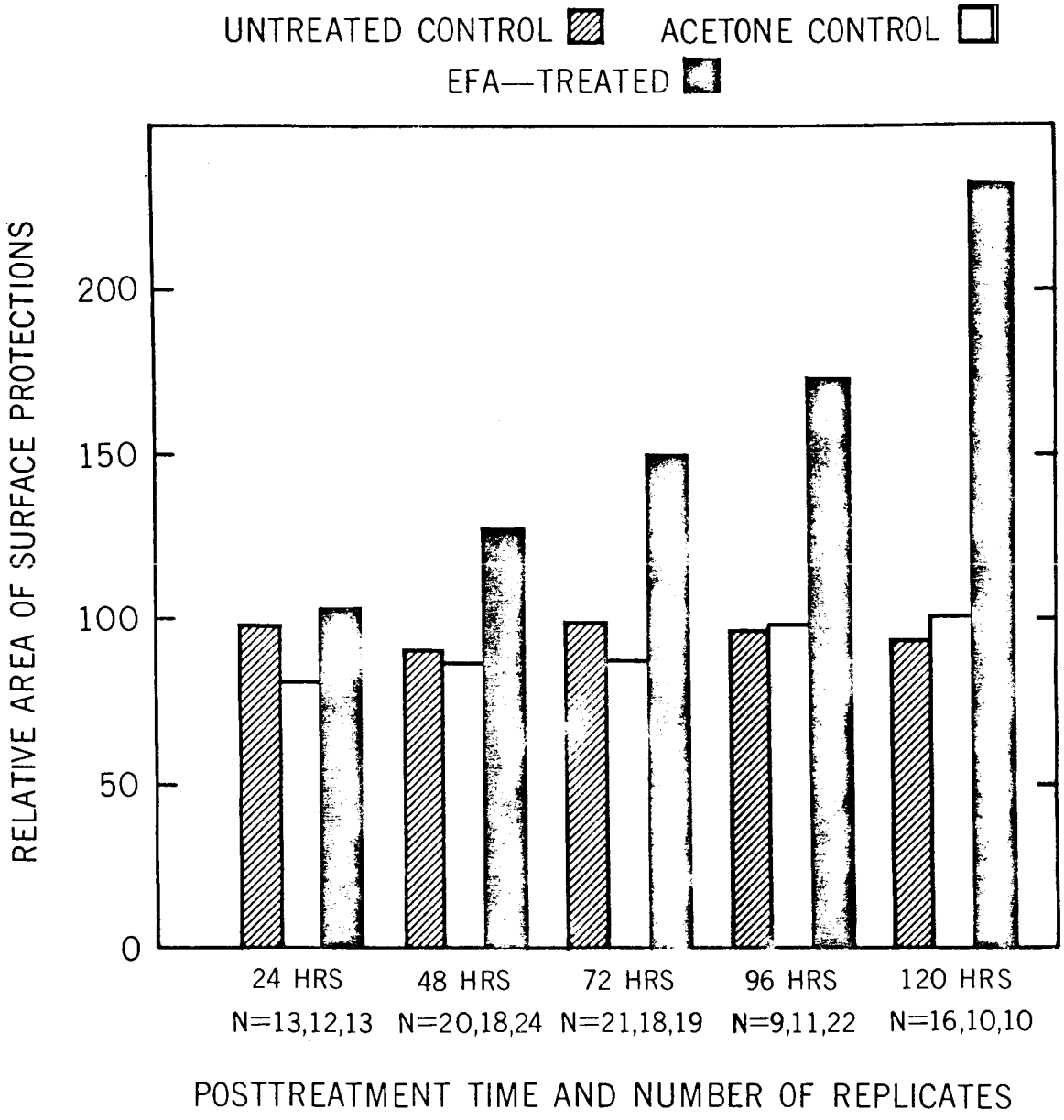
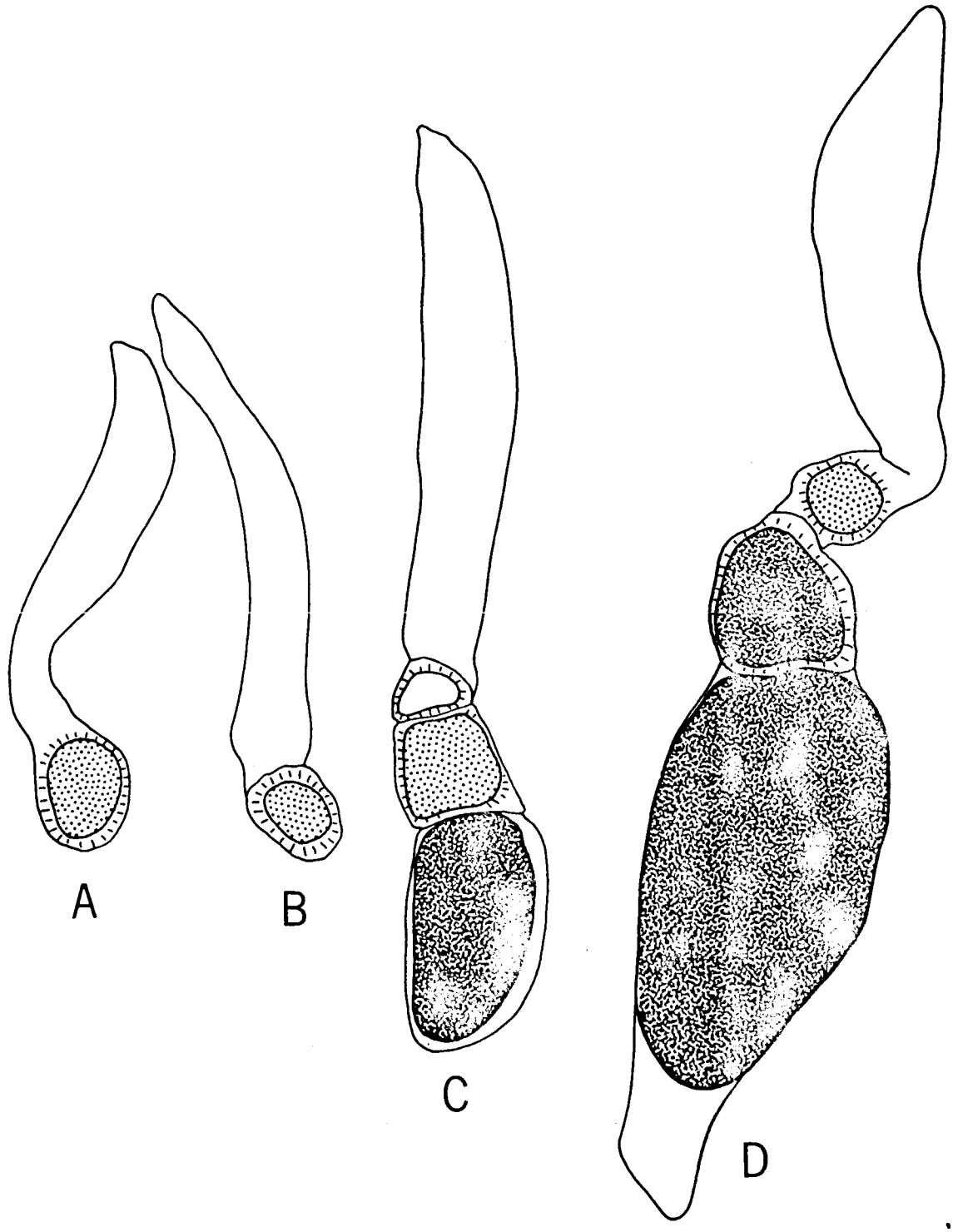

UNTREATED CONTROL
ACETONE CONTROL
EFA—TREATED
RELATIVE AREA OF SURFACE PROTECTIONS
0
50
100
150
200
24 HRS
N=13,12,13
48 HRS
N=20,18,24
72 HRS
N=21,18,19
96 HRS
N=9,11,22
120 HRS
N=16,10,10
POSTTREATMENT TIME AND NUMBER OF REPLICATES

Fig. 14 Plane surface projections of typical ovarioles excised from female T. lineatum following different treatments.

A-C., No, acetone, and EFA treatments, respectively, 120 hours posttreatment;

D., Egg-laying female, 2 weeks in log in the field.


A
B
C
D

treated females, 120 hours following application, was very similar to that of egg-laying females removed from logs two to four weeks after invasion (Fig. 14). These observations support the hypothesis that egg maturation is regulated by the corpora allata in this species.

Previous observations led to the hypothesis that the internal concentration of EFA declines with time (Fig. 10) and one would expect that very little, if any, of the applied hormone remains in the system by 120 hours. However, as suggested by Sahota (1970), perhaps only an initial quantity of hormone of sufficient titer is necessary to "activate" yolk deposition processes. Application of 0.05 μg EFA did not induce egg maturation by 120 hours, although the same dose was stimulatory to mating at one to two hours. This dose was probably not of sufficient titer to "activate" yolk deposition.

Concluding Discussion

The hindguts and frass of excavating female T. lineatum contain a pheromone with both population aggregating and sex attractant properties (Rudinsky and Daterman 1964a,b; Chapman 1966). Since sex attractants are often closely

associated with mating stimulation (Karlson and Butenandt 1954, Jacobsen 1965), pheromone produced by females was hypothesized as the major regulator of sexual activity in T. lineatum (J. H. Borden, personal communication).[3] However, it can be concluded that the predominant regulation of mating activity in T. lineatum is not through alterations in the attractivity of the female. The exposure of immature to mature members of the opposite sex of a "spring" population (Fig. 7, Table I), the exposure of brood to "spring" beetles of the opposite sex (Fig. 9, Table III), and, the treatment of either sex with inhibiting doses of EFA (Fig. 11, Table V) showed that the physiological state of the male has a much more profound effect on mating than that of the female. Since mating activity is very close to maximum levels at five to six hours postrevival (Fig. 6; Fig. 10, untreated controls), the activity of the male probably determines mating intensity throughout the period of natural sexual activity.

---

3. Department of Biological Sciences, Simon Fraser University

The mysterious acceleration of the mating activity of females 24 hours following treatment with 50 μg EFA was not observed as a natural phenomenon, even when untreated, virgin females were held for 120 hours and then exposed to males that had been held for six hours (120-hour females, 12.0; six-hour females, 16.5; n = 2). The mating activity of virgin females producing attractive frass (63.3% response by 49 males) removed from the log five days after log entry was also not accelerated (19.5 matings, as compared to 19.5 for six-hour control females; n = 2, both tests). It is concluded that this hormone-produced acceleration does not occur in nature.

Although some behavioural observations indicate that some form of chemostimulation by the females is likely in T. lineatum, the sex attractant released by excavating females is probably not intimately associated with sexual activity. Males that were in a stage unresponsive to sexually mature females were highly responsive to pheromone containing frass, indicating that the stimuli involved in each activity may be different. The pheromone is released

by both mated and virgin females excavating in wood (Rudinsky and Daterman 1964a,b; Castek, Barbour and Rudinsky 1967; Schneider and Rudinsky 1969a,b; Borden and Slater 1969; Nijholt 1970). Excavating beetles show little interest in sexual activity (Fig. 8). After a period of excavation, a female does not emerge to the log surface to mate (Chapman 1966). Mating within a tunnel is physically impossible (Hadorn 1933) and the likelihood of mating at a tunnel bifurcation is slight, since the female is uncooperative sexually. In addition, most mating occurs on the log surface during the initial attack (Hadorn 1933) or upon emergence from overwintering sites. Therefore, the excavating female is not attracting males for mating. However, the presence of a male may be beneficial in other ways since Chapman (1959) noted that more offspring are produced when a male is present in the gallery. The pheromone probably serves the primary function of attracting both sexes to a suitable host, since hosts are relatively rare in nature (see Atkins 1966). The apparent sex attractancy of the pheromone may be due to a stronger selection pressure for the male to respond to the aggregating pheromone because of additional mating opportunities when he

arrives at a source concurrently with other females.

Multiple mating is a common occurrence in insects (Davey 1965). T. lineatum displayed a considerable number of mating attempts (Fig. 3, 5, 6) as well as both polygyny and polyandry. However, in the field, males and females have been observed to remain together for the entire duration of sexual activity without multiple matings or interference by other males (Hadorn 1933). Therefore, many of the phenomena that occurred under the crowded conditions of these experiments may be rare in nature, although the tests probably illustrate maximum sexual activity under optimum conditions for contact. A high degree of sexual tension may expose the population to several dangers, including attraction of predators and wasted sperm (Richards 1927). However, if the female does not mate once she has entered the host, considerable activity would ensure that most females would be mated during the brief period prior to log invasion, an advantage probably outweighing the hazards.

There is considerable evidence that the reproductive development and mating activity of T. lineatum is regulated by the corpora allata. Just after eclosion and prior to hibernation, when juvenile hormone concentrations would

be expected to be extremely low, brood adults mate very little (Fig. 8, Table II). The ovaries, although fully developed, rarely show signs of oogenesis. Throughout hibernation, there is an early gradual, and later abrupt, increase in the proportion of insects that are sexually active upon warming, including virtually the entire population by February (Fig. 8, Table II). This trend indicates that "reactivation" does occur (i.e., diapause is broken).

Following activation from hibernation in the spring (April) when moderate quantities of juvenile hormone are expected to be released, beetles mate abundantly in the laboratory (Fig. 8, Table II) and, in nature, on the log surfaces (Chapman 1954, 1962). Moreover, low doses of EFA stimulated mating activity in immature specimens (Fig. 12, Table VI). The ovaries of virgin females often contain mature oocytes in the primary stages of yolk deposition (Chapman 1955). Females in the early stages of gallery construction, when internal juvenile hormone concentrations are theoretically higher, have greatly enlarged ovaries (Fig. 14) (Chapman 1958a). Similar development was induced by the application to nonexcavating, virgin females,

of 50 μg of EFA (Fig. 13, 14), but not 0.05 μg EFA. Parents removed from logs two and four weeks after natural entry, have greatly diminished mating activity (Fig. 8, Table II) coinciding with the egg laying activity of the females. It is hypothesized that high doses of juvenile hormone analog (Fig. 10, Table IV) may temporarily create a physiological condition similar to that of excavating parents.

BIBLIOGRAPHY

1. Adams, T. S. and A. M. Hintz. 1969. Relationship of age, ovarian development, and the corpus allatum to mating in the housefly, Musca domestica. J. Insect Physiol. 15: 201-15.

2. Atkins, M. D. 1966. Behavioural variation among scolytids in relation to their habitat. Can. Entomol. 98: 285-8.

3. Barth, R. H., Jr. 1962. The endocrine control of mating behaviour in the cockroach, Byrsotria fumigata (Guerin). Gen. Comp. Endocrinol. 2: 53-69.

4. Barth, R. H., Jr. 1965. Insect mating behaviour: endocrine control of a chemical communication system. Science 149: 882-3.

5. Barth, R. H., Jr. 1968. The comparative physiology of reproductive processes in cockroaches. I. Mating behaviour and its endocrine control. In, Advances in Reproductive Physiology VIII (ed., A. McLaren): 168-207.

6. Beck, S. D. 1968. Insect Photoperiodism. Acad. Press, New York and London. 288 p.

7. Borden, J. H. and C. E. Slater. 1968. Induction of flight muscle degeneration by synthetic juvenile hormone in Ips confusus (Coleoptera: Scolytidae). Z. Vergl. Physiol. 61: 366-8.

8. Borden, J. H., R. G. Brownlee and R. M. Silverstein. 1968. Sex pheromone of Trypodendron lineatum (Coleoptera: Scolytidae): production, bio-assay and partial isolation. Can. Entomol. 100: 629-36.

9. Borden, J. H., K. K. Nair and C. E. Slater. 1969. Synthetic juvenile hormone: induction of sex pheromone production in Ips confusus. Science (Washington) 166: 1926-7.

10. Bowers, W. S. and C. C. Blickenstaff. 1966. Hormonal termination of diapause in the alfalfa weevil. Science (Washington) 154: 1673-4.

11. Burkhardt, D. 1964. Colour discrimination in insects. In, Advances in Insect Physiology II (ed., J. W. L. Beaumont, J. E. Treherne, V. B. Wigglesworth). Acad. Press, New York and London. 131-73.

12. Cassier, P. and M. Papillon. 1968. Effects of the implantation of corpora allata on the female reproductive groups of Schistocerca gregaria (Forsk) and on the polymorphism of their descendents. C. R. Hebd. Seances Acad. Sci. Ser. D Sci Natur (Paris) 266: 1048-51.

13. Castek, K. L., J. F. Barbour and J. A. Rudinsky. 1967. Isolation and purification of the attractant of the striped ambrosia beetle. J. Econ. Entomol. 60: 658-60.

14. Chapman, J. A. 1954. Physiological and biological studies on the ambrosia beetle, Trypodendron lineatum (Oliv.) and the Douglas fir beetle, Dendroctonus pseudotsugae Hopk. Unpublished Report, Forest Biology Laboratory, Victoria, B. C.

15. Chapman, J. A. 1955. Interpretation of adult history in the ambrosia beetle, Trypodendron. Can. Dep. Forest Div., Forest Biol. Bi-mon. Progr. Rep. 11(6): 3-4.

16. Chapman, J. A. 1958a. Studies on the physiology of the ambrosia beetle Trypodendron in relation to its ecology. Tenth Int. Congr. Entomol. Proc. 4: 375-80.

17. Chapman, J. A. 1958b. Response of Trypodendron to forest litter. Can. Dep. Agr., Forest Biol. Div., Bi-mon. Progr. Rep. 14: 3.

18. Chapman, J. A. 1959. Forced attacks by the ambrosia beetle, Trypodendron. Can. Dep. Agr., Forest Biol. Div., Bi-mon. Progr. Rep. 15(5): 3.

19. Chapman, J. A. 1962. Field studies on attack flight and log selection by the ambrosia beetle Trypodendron lineatum (Oliv.) (Coleoptera: Scolytidae). Can. Entomol. 94: 74-92.

20. Chapman, J. A. 1966. The effect of attack by the ambrosia beetle Trypodendron lineatum (Oliv.) on log attractiveness. Can. Entomol. 98: 50-9.

21. Chapman, J. A. and J. M. Kinghorn. 1958. Studies of flight and attack activity of the ambrosia beetle, Trypodendron lineatum (Oliv.) and other scolytids. Can. Entomol. 90: 362-72.

22. Chapman, J. A. and W. W. Nijholt. 1965. Proportion of old and young adults in an overwintering population of the ambrosia beetle, Trypodendron lineatum (Oliv.). Can. Dep. Forest Entomol. Pathol., Bi-mon. Progr. Rep. 21: 3-4.

23. Clements, A. N. 1956. Hormonal control of ovary development in mosquitoes. J. Exp. Biol. 33: 211-23.

24. Danilevskii, A. S. 1965. Photoperiodism and Seasonal Development in Insects. Oliver and Boyd, Edinburgh and London. 283 p.

25. Daterman, G. E., J. A. Rudinsky and W. P. Nagel. 1965. Flight patterns of bark and timber beetles associated with coniferous forests of Western Oregon. Agr. Exp. Station, Oregon St. Univ., Tech. Bull. 87: 3-46.

26. Davey, K. G. 1965. Reproduction in the Insects. W. H. Freeman, San Francisco. 96 p.

27. Davey, K. G. 1967. Some consequences of copulation in Rhodnius prolixus. J. Insect Physiol. 13: 1629-36.

28. Dyer, E. D. A. and J. M. Kinghorn. 1961. Factors influencing the distribution of overwintering ambrosia beetles, Trypodendron lineatum (Oliv.). Can. Entomol. 93: 746-59.

29. Engelmann, F. 1958. The stimulation of the corpora allata in Diploptera punctata (Blattaria). Anat. Rec. 132: 432-3.

30. Engelmann, F. 1959. The control of reproduction in Diploptera punctata (Blattaria). Biol. Bull. 116: 406-19.

31. Engelmann, F. 1960a. Hormonal control of mating behaviour in an insect. Experientia 16: 69-70.

32. Engelmann, F. 1960b. Mechanisms controlling reproduction in two viviparous cockroaches (Blattaria). Ann. N. Y. Acad. Sci. 89: 516-36.

33. Engelmann, F. 1968. Endocrine control of reproduction in insects. Annu. Rev. Entomol. 13: 1-26.

34. Engelmann, F. 1970. The Physiology of Insect Reproduction. Pergamon Press Inc. 307 p.

35. Ferkovich, S. M., S. G. Wellso and W. T. Wilson. 1967. Mating behaviour of the big-headed grasshopper, Aulocara elliotti (Orthoptera: Acrididae) under caged conditions in the greenhouse and outdoors. Ann. Entomol. Soc. Amer. 60: 972-5.

36. Gilbert, L. I. 1964. Physiology of growth and development: endocrine aspects. In, The Physiology of Insecta I (ed., M. Rockstein). Acad. Press, New York and London. 149-225.

37. Gillett, J. D. 1956. Initiation and promotion of ovarian development in the mosquito Aedes (Stegomyia) aegypti (Linnaeus). Ann. Trop. Med. Parasitol. 50: 375-80.

38. Hadorn, C. 1933. Recherches sur la morphologie, les stades évolutifs et l'hivernage du bostryche liseré (Xyloterus lineatus Oliv.). Supplaux. org. de la Soc. forest. Suisse, Bern (11): 120 pp.

39. Highnam, K. C. 1958. Activity of the brain/corpora cardiaca system during pupal diapause "break" in Mimas tiliae (Lepidoptera). Quart. J. Micr. Sci. 99: 73-88.

40. Highnam, K. C. 1964. Endocrine relationships in insect reproduction. Roy. Entomol. Soc. Symp. 2: 26-42.

41. Highnam, K. C. and O. Lusis. 1962. The influence of mature males on the neurosecretory control of ovarian development in the desert locust. Quart. J. Micr. Sci. 103: 73-83.

42. Highnam, K. C. and P. T. Haskell. 1964. The endocrine systems of isolated and crowded Locusta and Schistocerca in relation to oocyte growth and the effects of flying upon maturation. J. Insect Physiol. 10: 844-64.

43. Jacobsen, M. 1965. Insect Sex Attractants. Interscience Publishers, John Wiley and Sons Inc. 154 p.

44. Johansson, A. S. 1955. The relationship between corpora allata and reproductive organs in starved female Leucophaea maderae (Blattaria). Biol. Bull. 108: 40-4.

45. Karlson, P. and A. Butenandt. 1954. Pheromones (ectohormones) in insects. Ann. Rev. Entomol. 4: 39-58

46. Kinghorn, J. M. and J. A. Chapman. 1959. The overwintering of the ambrosia beetle Trypodendron lineatum (Oliv.). Forest Sci. 5: 81-92.

47. Lea, A. O. 1968. Mating without insemination in virgin Aedes aegypti. J. Insect Physiol. 14: 305-8.

48. LeCato, L. G. and R. L. Pienkowski. 1970. Laboratory mating behaviour of the alfalfa weevil, Hypera postica. Ann. Entomol. Soc. Amer. 63: 1000-7.

49. Loher, W. 1960. The chemical acceleration of the maturation process and its hormonal control in the male of the desert locust. Proc. Roy. Entomol. Soc. London Ser. B 153: 380-97.

50. Loher, W. 1966. Die Steuerung sexueller Verhaltensweisen und der Oocyten entwicklung bei Gomphocerus rufus L. (English summary by author). Z. Vergl. Physiol. 53: 277-316.

51. Loher, W. and F. Huber. 1964. Nervous and endocrine control of sexual behaviour in a grasshopper (Gomphocerus rufus L., Acridinae). Symp. Soc. Exp. Biol. 20: 381-400.

52. Manning, A. 1967. The control of sexual receptivity in female Drosophila. Anim. Behav. 15: 239-50.

53. Mathieu, J. M. 1969. Mating behaviour of five species of Lucanidae (Coleoptera: Insecta). Can. Entomol. 101: 1054-62.

54. Mayer, M. S. and J. R. Brazzel. 1963. The mating behaviour of the boll weevil, Anthonomus grandis. J. Econ. Entomol. 56: 605-9.

55. Mellanby, K. 1939. Fertilization and egg production in the bed-bug Cimex lectularius L. Parasitology 31: 193-9.

56. Menon, M. 1970. Hormone-pheromone relationships in the beetle, Tenebrio molitor. J. Insect Physiol. 16: 1123-39.

57. Nakamura, H. 1969. Comparative studies on the mating behaviour of two species of Callosobruchus (Coleoptera: Bruchidae). Jap. J. Ecol. 19: 20-6.

58. Nijholt, W. W. 1970. The effect of mating and the presence of the male ambrosia beetle, Trypodendron lineatum, on "secondary attraction". Can. Ent. 102(7): 894-7.

59. Orr, C. W. M. 1964. The influence of nutritional and hormonal factors on egg development in the blowfly, Phormia regina (Meig). J. Insect Physiol. 10: 53-64.

60. Patton, R. L. 1963. Introductory Insect Physiology. W. B. Saunders Co., Philadelphia and London. 245 p.

61. Pener, M. P. 1967. Effects of allatectomy and sectioning of the nerves of the corpora allata on oocyte growth, male sexual behaviour and color change in adults of Schistocerca gregaria. J. Insect Physiol. 13: 665-84.

62. Prebble, M. L. and K. Graham. 1957. Studies of attack by ambrosia beetles in softwood logs on Vancouver Island, British Columbia. Forest Sci. 3: 90-112.

63. Richards, O. W. 1927. Sexual selection and allied problems in insects. Biol. Rev. 2: 298-364.

64. Rohdendorf, E. B. and J. A. L. Watson. 1969. The control of reproduction cycles in the female firebrat, Lepismodes inquilinus. J. Insect Physiol. 15: 2085-2101.

65. Roth, L. M. and G. P. Dateo. 1966. A sex pheromone produced by males of the cockroach, Nauphoeta cinerea. J. Insect Physiol. 12: 255-65.

66. Rudinsky, J. A. and G. E. Daterman. 1964a. Field studies on flight patterns and olfactory responses of ambrosia beetles in Douglas fir forests of Western Oregon. Can. Entomol. 96: 1339-52.

67. Rudinsky, J. A. and G. E. Daterman. 1964b. Response of the ambrosia beetle Trypodendron lineatum (Oliv.) to a female-produced pheromone. Z. Angew. Entomol. 54: 300-3.

68. Sahota, T. S. 1970. Haemolymph and ovarial proteins in the bark beetle, Dendroctonus pseudotsugae in relation to ovarian development. Can. J. Zool. 48: 1307-12.

69. Sahota, T. S., J. A. Chapman and W. W. Nijholt. 1970. Ovary development in a scolytid beetle Dendroctonus pseudotsugae (Coleoptera: Scolytidae): effect of farnesyl methyl ether. Can. Entomol. 102: 1424-8.

70. Scharrer, B. and M. von Harnack. 1958. Histophysiological studies on the corpora allata of Leucophaea maderae. I. Normal life cycle in male and female adults. Biol. Bull. 115: 508-20.

71. Schneider, I. and J. A. Rudinsky. 1969a. Anatomical and histological changes in internal organs of adult Trypodendron lineatum, Gnanthotrichus retusus and G. sulcatus (Coleoptera:

Scolytidae). Ann. Entomol. Soc. Amer. 62: 995-1003.

72. Schneider, I. and J. A. Rudinsky. 1969b. The site of pheromone production in Trypodendron lineatum (Coleoptera: Scolytidae): Bioassay and histological studies of the hindgut. Can. Entomol. 101: 1181-6.

73. Walj, E. and M. P. Pener. 1970. The effect of the corpora allata on the mating behaviour of the male migratory locust, Locusta migratoria migratorioides. Israel J. Zool. 18: 179-92.

74. Wigglesworth, V. B. 1964. The hormonal regulation of growth and reproduction in insects. In, Advances in Insect Physiology II (ed., J. Beament, J. E. Treherne, V. B. Wigglesworth). Acad. Press, New York. 248-336.

75. Wigglesworth, V. B. 1970. Insect Hormones. Oliver and Boyd, Edinburgh. 159 p.

76. deWilde, J. 1959. Endocrine influence on behavior and reproduction in insects. Acta. Physiol. Pharmacol. Neer. 8: 106-7.

77. deWilde, J. 1964. Reproduction--endocrine control. In, The Physiology of Insecta I (ed., M. Rockstein). Acad. Press, New York. 67-90.

78. deWilde, J. and J. A. de Boer. 1961. Physiology of diapause in the adult Colorado beetle. II. Diapause as a case of pseudo-allatectomy. J. Insect Physiol. 6: 152-61.

79. Williams, C. M. 1946. Physiology of insect diapause: the role of the brain in the production and the termination of pupal dormancy in the giant silkworm, Platysamia cecropia. Biol. Bull. 90: 234-43.

CURRICULUM VITAE

Name:	Catherine Elizabeth Fockler (nee Slater).
Place and Year of Birth:	Vancouver, British Columbia, 1948.
Education:	Graduate, Windsor Secondary School, North Vancouver, British Columbia, 1966.
	Simon Fraser University, B.Sc., Biology, 1970.
	Simon Fraser University, Biological Sciences, Graduate Studies, 1970-1972.
Experience:	Research Assistant (summer employment), Simon Fraser University, 1966-1969.
	Teaching Assistant, Simon Fraser University, summer 1971.
Awards:	North Vancouver Centennial Scholarship, 1966.
	B. C. Government First Class Scholarships; 1966, 1969.
	National Research Council Scholarships; 1970, 1971.

Publications:

Borden, J. H., K. K. Nair and C. E. Slater. 1969. Synthetic juvenile hormone: induction of sex pheromone production in Ips confusus. Science 166: 1626-7.

Borden, J. H. and C. E. Slater. 1969. Sex pheromone of Trypodendron lineatum: production in the female hindgut-malpighian tubule region. Ann. Entomol. Soc. Amer. 62(2): 454-5.

Borden, J. H. and C. E. Slater. 1969. Flight muscle volume change in Ips confusus (Coleoptera: Scolytidae). Can. J. Zool. 47: 29-32.

Borden, J. H. and C. E. Slater. 1968. Induction of flight muscle degeneration by synthetic juvenile hormone in Ips confusus (Coleoptera: Scolytidae). Z. Vergl. Physiol. 61: 366.