REPRODUCTIVE ISOLATION AND CIRCADIAN RELEASE OF SEX PHEROMONES IN EUXOA (LEPIDOPTERA:NOCTUIDAE)

By

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"Most people would rather die than think; and that is why they are so ready to escape the necessity by adopting empiricism or Baconianism, which sometimes appears in modern dress as a substitution for thought. But in scientific research there is no substitute for thought."

Sir Vincent Wigglesworth. 1976.

For my mother, the late Margaret Louise Ferguson-Teal, and father, Wilfred Teal, without whose support and guidance I could never have come so far. Thank you!

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Abstract

The nocturnal calling behavior of three closely related, interfertile species of cutworm moths, Euxoa declarata, E. campestris, and E. rockburnei, was studied in the laboratory at four different temperatures. The temporal pattern of calling activity by virgin females was found to differ for each species, with peak periods of pheromone release by each species remaining distinct at each of the experimental temperatures. The calling period of declarata was found to be exclusive from that rockburnei and nearly exclusive from that of campestris at all temperatures. An overlap between the calling periods of campestris and rockburnei was found to occur, however the initial and peak phases, which under natural conditions with males present, would be by far the most important, were found to be separated by a minimum of 2 hours. Decrease in dark phase temperature was found to cause a shift to earlier calling by females of all three species, however such shifts did not eliminate the differences in the peak periods of release of pheromones occurring among the three species. Although differences in the periods of pheromone release may not be the only mechanism involved in the reproductive isolation of the species belonging to the declarata group, differences in the calling periods are sufficient to account for the strong conspecific mating tendency exhibited by these species in laboratory tests and are certainly major components of reproductive isolation.

The calling period of E. declarata ♀ X E. rockburnei ♂ (F_1) hybrid females was found to correspond almost exactly with that of the maternal declarata stock. The calling period of the reciprocal cross

was found to be extended, intersecting the calling periods of both parental species, however, the major activity peak occurred early in the scotophase.

The female terminalia, including abdominal segments 8 and 9 + 10 of Euxoa were found to be similar to the typical type of terminalia described for higher Lepidoptera by Mutuura (1972) and have evolved to enable the females to oviposit in the soil. Structural modifications adapted for pre-oviposition digging include: the heavily sclerotized 8^{th} tergite, anterior position of the copulatory opening on the 8^{th} segment, the reduced 8^{th} sternite, the elongate and highly flexible cuticle of the 8^{th} intersegmental membrane, the heavily sclerotized dorsal valves, the eversible ovipositor, and extensive musculature throughout the ovipositor. The functional aspects of these features in oviposition are discussed and a description of the mechanics of oviposition extension, digging and retraction is given.

Epidermal gland cells of the ovipositor were found in two specialized areas. One area is located in the ventral intersegmental membrane between segments 8 and 9 + 10. The "Class I" epidermal gland cells of this area were found to undergo progressive vacuolation for several days after adult emergence and comprise the sex pheromone gland. Glandular cells were also found to occur on the dorsal and lateral surfaces of the dorsal valves. These gland cells are typical trichogenous cells communicating with tubular setae. The function of large "droplets" within these cells and their presumed secretion is unknown at present.

RESUME

Le comportement nocturne de trois espèces interfertiles, Euxoa declarata, E. campestris, et E. rockburnei a été étudié à quatre températures au laboratoire. On a découvert que le mécanisme temporel de la libération de la phéromone est différent pour chaque espèce, et aussi que les périodes maxima d'appel sont distinctes à chaque température expérimentale. E. declarata a une période d'appel qui est exclusive de celle de rockburnei, et presque exclusive de celle de campestris.

Il existe un chevauchement entre les périodes d'appel de campestris et rockburnei; cependant les phases initiales et maximales, qui sous des conditions naturelles, et en presence des mâles, seraient les plus importantes, étaient séparées par un intervalle minimum de 2 heures.

Une baisse de température au cours de la scotophase a provoqué un déplacement de la période d'appel des femeles des trois espèces, mais ces déplacements n'ont pas éliminé les différences dans les périodes maximales d'émission de phéromone chez les trois espèces. Bien que les différences dans les périodes d'émission de phéromone ne soient pas le seul mécanisme impliqué dans l'isolement reproductif des espèces appartenant au groupe declarata, des différences dans les périodes d'appel sont suffisantes pour expliquer la forte tendance conspécifique d'accouplement démontrée par ces espèces dans des expériences de laboratoire et sont certainement des éléments majeurs de l'isolement reproductif.

La période d'appel des femelles de E. declarata X E. rockburnei (F_1) a été presque exactement conforme à la génération maternelle

declarata. La période d'apel des femelles de l'hybride réciproque est prolongée, et elle entrecoupe les périodes d'appel des deux espèces parentales, mais la plus grande phase de l'activité se situe dans la première partie de la scotophase.

Les segments abdominaux, 8 et 9 + 10, qui composent les segments reproducteurs des femelles de Euxoa sont similaires au type des Lepidoptères tel que décrit par Mutuura (1972) et sont développés pour l'oviposition dans le sol. Les modifications de structure adaptées pour cette activité fouisseuse comprennent: le 8ième tergite très sclérotisé, la position antérieure, l'orifice copulateur dans le 8ième segment, la cuticule tres flexible et allongé de la 8ième membrane intersegmentale, les papilles anales très sclerotisées, l'ovipore réversible, et une musculature étendue. Les aspects fonctionnels de ces caractères sont discutés, et une description des mécanismes d'extension, de fouissement, et rétraction de l'ovipositeur est donnée.

On a trouvé des cellules glandulaires épidermiques dans deux régions spéciales. Une de ces régions est située dans la 8ième membrane intersegmentale. Ces cellules de "Class I" qui sont glandulaires et épidermiques ont passé par un vacuolization progressive durant quelques jours après l'émergence de l'adulte, et comprennent la glande qui produit la phéromone sexuelle. On a trouvé aussi des cellules glandulaires dans les régions dorsales et latérales des papilles anales. Ces cellules glandulaires sont trichogènes et elles communiquent avec des soies tubulaires. La fonction des inclusions de ces cellules et leurs présumées sécrétions est encore inconnue.

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INTRODUCTION:

Species are defined as groups of interbreeding individuals which are reproductively isolated from other closely related groups. However, the description of species is, in the main, the result of studies on preserved specimens and therefore elucidation of traits involved in reproductive isolation are usually impossible. The problems associated with species description without adequate knowledge of mechanisms of reproductive isolation are particularly evident among species of Lepidoptera and many are coming to light as a result of studies into biological methods of pest control. An excellent example of a pair of sibling species which are still considered a single species group are the (E) and (Z) pheromone populations of Ostrinia nubilalis (Hubner), which have been shown to maintain high degrees of reproductive isolation in areas of sympatricity (Carde et al, 1978). Conversely, there is considerable question as to the validity of the individual species status of Diparopsis castanea (Hmps.) and D. watersi (Roths.) which are cross attractive and appear to exhibit no definite mechanisms of reproductive isolation (Beevor et al, 1973). Similarly there is a great deal of uncertainty as to the number of species in the genus Choristoneura (Sanders 1971a, b).

The genus Euxoa (Lepidoptera:Noctuidae) is composed of about 180 species in North America which exhibit high degrees of intraspecific variability (Hardwick 1970). The genus appears to have diversified relatively recently and some species groups are still in the process of developing adequate mechanisms of genetic isola-

tion (Hardwick 1970). Hence the member species of the genus provide an excellent opportunity to study the development of mechanisms involved in reproductive isolation and for the development of procedures useful in experimental taxonomy (Hudson 1973).

The declarata group of the genus Euxoa (Lepidoptera:Noctuidae), composed of E. declarata (Walker), E. campestris (Grote), and E. rockburnei (Hardwick) is one of the most difficult groups to interpret both morphologically and taxonomically (Hardwick 1973). In nature declarata is broadly sympatric with both campestris and rockburnei, whereas the latter 2 species are evidently parapatric (Hardwick and Lefkovitch 1973, Byers and Hinks 1978). The 3 species have very similar genitalia (Hardwick 1973, Hardwick and Lefkovitch 1973) and, in the laboratory, produce vigorous fertile hybrids from all parental combinations. However, in mating-choice tests all 3 species exhibit strong conspecific mating preference which is probably sufficient to maintain the genetic integrity of sympatric populations in nature (Byers and Hinks 1978), and is undoubtedly the result of the development of effective premating mechanisms of reproductive isolation.

The release of sex pheromones by female moths and the accompanying behavior (calling) is usually a diel activity characteristic for each species (Kettlewell 1946, Roelofs and Carde 1974a, Shorey 1974) and appears to be part of a general circadian activity rhythm subject to phase shifting by changes in temperature (Carde et al 1975a, Comeau et al 1976, Dreisig 1976). Such temporal differences in mating activity appear to be quite commonly involved in reproductive isolation between closely related, co-occurring species

(Roelofs and Carde 1974a, Shorey 1974).

Objectives

Although the 3 species of the declarata group do have differences in physiology, morphology, and enzyme structure, which support the validity of their species status, little evidence of actual mechanisms of reproductive isolation was known at the beginning of this research.

Knowing that all 3 species do have areas of sympatricity, occupy the same ecological niche, have overlapping flight periods and are fully interfertile, the following hypothesis was put forward: the circadian periodicity of sex pheromone release may function in reproductive isolation among the 3 species of the declarata group. Testing this hypothesis called for the following objectives: (1) description of the site of the sex pheromone gland and morphology of the ovipositor of the 3 species; (2) description of the behaviours involved in sex pheromone release by females of these species; and (3) the description of differences in the circadian periodicity of sex pheromone release among the 3 species at temperatures of 20^{o}, 15^{o}, 10^{o}, and 5^{o}C.

LITERATURE REVIEW:

Species and species concepts

Species are defined as groups, or taxa, of individuals sharing common characters, and therefore designate a rank in the hierarchic classification of an organism (Mayr 1970). The actual role of species, in the evolution and continued survival of organisms, has been under discussion for many years (see Dodson and Dodson 1976). Early evolutionists, notably Bessey (1908) advocated a "Nominalistic Species Concept" (Mayr 1970) in which the species did not exist in nature, the only unit of evolution and continuance being the individual. A second thesis was proposed, as it became increasingly evident that species were, in fact, reproductively isolated from other such taxa. This is the "Biological Species Concept" which defines species as natural groups having a defined geographical distribution, being self perpetuating, morphologically and physiologically distinct from other such groups, and being reproductively isolated from other taxa (Huxley 1940, Dobzhansky 1951, Mayr 1970, Bush 1975). This view is supported by the majority of evolutionists, although recently some workers have emphasized the role of the population as a distinct unit of evolution (Ehrlich and Raven 1969, Dodson and Dodson 1976). The evident pitfall in the consideration of either the population or the individual as a distinct unit of evolution is that both are fully interfertile with other such groups, heterozygosity (outcrossing) being the rule rather than the exception at these levels. Thus, among sexually reproducing organisms, the species is the most important unit of evolution, it's

most important property being the continuance of species epistatic relationships, which give rise to internal cohesion within each species (Mayr 1970).

Speciation and reproductive isolation

Speciation, the formation of new species (Mayr 1970, Dodson and Dodson 1976), is an adaptive process involving development of intrinsic barriers to gene flow between closely related populations (Bush 1975). The potential modes of speciation have been summarized by Mayr (1970), Scudder (1974), and Bush (1975). Of these the most prominant methods are: (1) Allopatric speciation by subdivision (Bush 1975) (gradual speciation of Bigelow 1965) in which a large continuous population is subdivided by the development of an extrinsic barrier, genetic barriers evolving slowly as the result of fortuitous adaptive genetic change; (2) Allopatric speciation by a small founder colony, now generally termed quantum speciation (Grant 1963, 1977, Bush 1975); (3) Parapatric speciation which occurs when continuous populations speciate along a continuous cline without spatial isolation (Bush 1975); and (4) Sympatric speciation which is, according to Mayr (1970), "the origin of isolating mechanisms within the dispersal area of the offspring of a single deme". The essential differences between these forms of speciation are well described by Bush (1975) and are summarized in Table 1.

As stated above the necessary event for the acquisition of species status by a population is the development of reproductive isolation (Dobzhansky 1970, Mayr 1970, Byers and Hinks 1978). This

means development of mechanisms which prevent hybridization among actually or potentially sympatric sibling species, thereby increasing mating efficiency and protecting the genetic integrity of each species (Dobzhansky 1950, Mayr 1970, Tamaki 1972, Dodson and Dodson 1976). Mechanisms involved in reproductive isolation act either prior to mating (premating mechanisms), or after copulation has occurred (postmating mechanisms). Premating mechanisms are highly succeptible to improvement via natural selection (Mayr 1970) because they prevent the wastage of gametes and reproductive (parental) investment. Such mechanisms include: (1) habitat isolation through differences in niche preferences, (2) seasonal and/or diurnal differences in mating cycles, (3) mechanical differences in the organs of reproduction, and (4) ethological isolation, (Mayr 1970, Tamaki 1972, Dodson and Dodson 1976). Postmating mechanisms including death of gametes prior to fertilization, death of the F_1 hybrid zygote, inviability of the F_1 hybrid, and infertility of the F_1 or F_2 hybrid generations (Mayr 1970, Tamaki 1972, Dodson and Dodson 1976), are improved only indirectly by natural selection, (Mayr 1970) as they are incidental by-products of genetic divergence (Dobhansky 1970, Kaneshiro 1976).

Of these 2 groups of isolating mechanisms, the development of premating mechanisms is seen to be of prime importance in the isolation of vagile sympatric species because such mechanisms limit energy loss through futile mating attempts (Mayr 1970, Tamaki 1972, Bush 1975) and are expected to operate more often between sympatric than allopatric species (Kaneshiro 1976, Richmond and Dobzhansky 1976). The supportive evidence for the above lies in

the existence of many sympatric sibling species, which are potentially hybridizable (producing viable F_1 and F_2 hybrid generations in laboratory studies), but are rarely, if ever, found to hybridize in nature (Dobzhanski et al 1969, Mayr 1970, Burkeholder and Lanier 1974, Roelofs and Carde, 1974a, Bush 1975, Tamaki & Honma, 1976, Kaneshiro 1976, Kaneshiro and Val 1977).

Although all of the afore-mentioned premating mechanisms may be involved in the reproductive isolation of closely related species, one mechanism is generally dominant (Mayr 1970). Among insect species either habitat isolation or behavioral isolation appears to play this dominant role in the majority of cases (Bush 1975). Habitat isolation is described as being of major importance in the reproductive isolation of highly host specific species, e.g. parasites and parasitoids. For these species the host organism provides a rendezvous point for mating, and mate selection is, therefore, dependent upon host selection alone (Bush 1975). The ethological, or behavioral, barrier, is far more prominent among insect species (Mayr 1970) due to the insects' high vagility, broad distributions, and their ability to adapt to changing environments and food sources. The scope of the behavioral barrier in reproductive isolation is very broad, and, as the key factors involved are the production and reception of stimuli by opposite sexes, encompass all sense organs (Mayr 1970). Examples of the stimuli involved in the attraction of conspecific mates are virtually limitless among the animal phyla, but, as this work deals with Insecta I shall limit my examples to these organisms.

The action of visual stimuli in behavioral isolation is exem-

plified by the species specific lengths and colours of light flashes used in mate attraction by male fireflies (Coleoptera:Lampyridae) (Lloyd 1966), and by the species specific swarming behaviors of many species of Diptera (Downes 1969). The use of auditory stimuli in behavioral isolation is best developed in the species specific songs of crickets (Orthoptera:Gryllidae) (Alexander and Thomas 1959, Ulagaraj and Walker 1973) and the buzzing of the 13 and 17 year cicadas (Homoptera:Cicadidae) (Alexander and Moore 1972). Tactile signals appear to be of great importance in the sexual stimulation of female phycitid moths, the correct physical contact by the male being necessary to elicit copulation (Grant and Brady 1974). Lastly differences in chemical signals have been shown to play very extensive roles in the reproductive isolation of insects particularly Hymenoptera, Coleoptera (Burkeholder and Lanier 1974) and Lepidoptera, among which both males and females may release chemical messengers, or sex pheromones (Tamaki 1972, Roelofs and Carde 1974a, Shorey 1976a). The function of sex pheromones in the reproductive isolation of Lepidoptera is reviewed in the next section.

It must be emphasized that, although one form of stimulus may be solely responsible for ethological isolation, often several factors function together, each factor being necessary to begin a successive step in courtship. Figure 1 summarizes the events occurring during the courtship of Plodia interpunctella (Hubner) as documented by Grant and Brady (1974). If the sequence is not followed in the exact order mating will not occur.

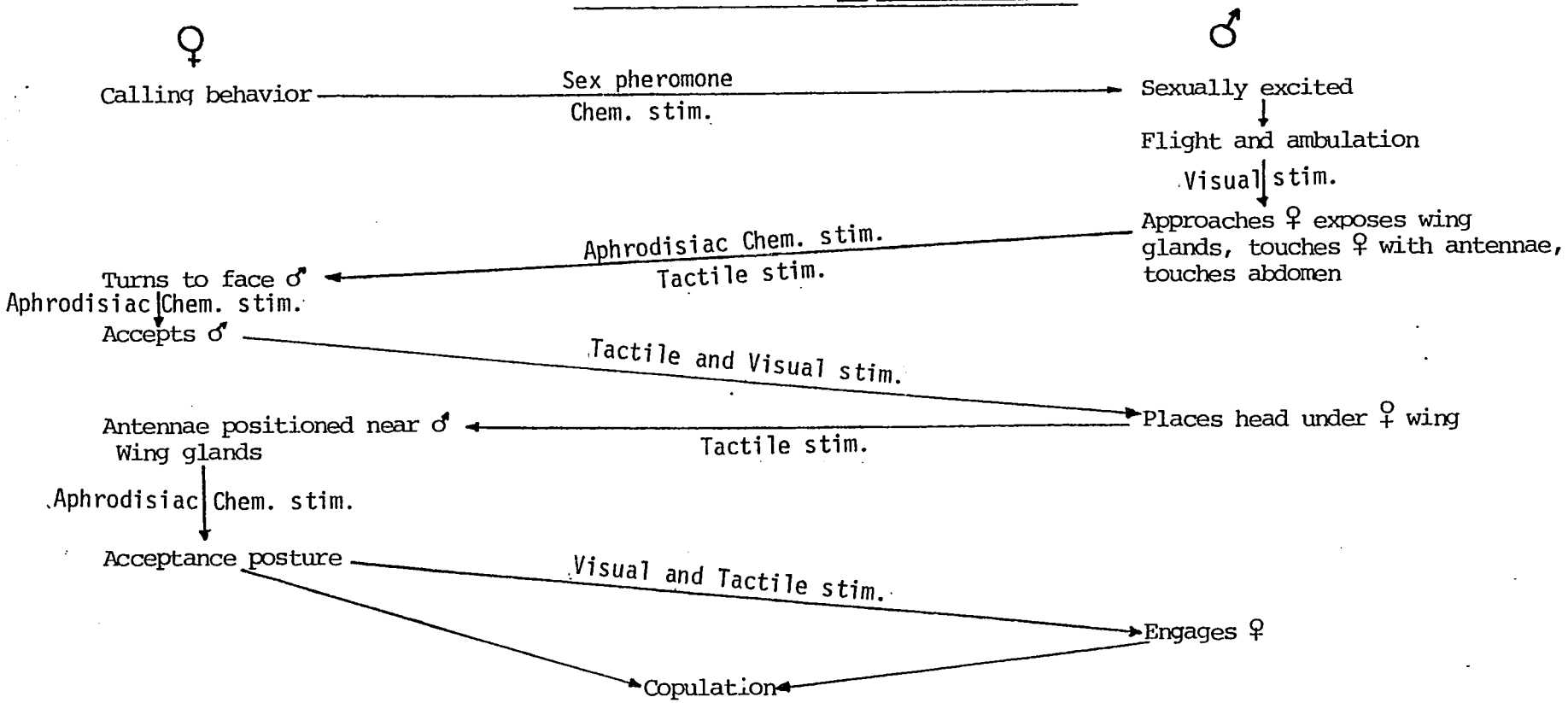
Type of Speciation (Table 1)*

	Property	Allopatric (1)	Allopatric (2)	Parapatric (3)	Sympatric (4)
1	Reproductive Strategy	low Rate, late maturity	high Rate, early maturity	Same as 2	Same as 2
2	Mobility	high	high	low	variable (niche limited)
3	Population Size	large	small	small	variable
4	Feeding habits	oligophagous	variable	specialized	specialized
5	Niche change	speciation without niche change	1 or 3	Change to new niche	change to new niche
6	Mate selection	not niche dependent	Same as 1	1 or 4	niche dependent
7	Breeding Scheme	heterozygous	homozygous	homozygous	homozygous
8	Chromosome rearrangement	not associated with speciation	may be associated with speciation	frequently associated with speciation	same as 2
9	Genetic Revolutions	Speciation due to summation of adaptive changes of structural and regulatory genes	may be as in 1	speciation associated with alterations of regulatory systems, minor structural gene mutation may occur	speciation results from minor structural and regulatory gene changes
10	Evolution of mechanisms of reproductive isolation (MRI)	Premating MRI limited to areas of sympatricity Postmating MRI are fortuitous	Same as 1 but genetic changes occur rapidly MRI extend through whole population	Pre- and post mechanisms selected for during shift to new niche, widespread	premating MRI selected for before shift to new niche
11	Gene flow	none	none	some initially	some initially
12	Distribution of sibling spp.	Allopatric or high competition in areas of Sympatricity	Allopatric or broadly Sympatric, competition may be high	Parapatric, interspecific competition moderate	Usually Sympatric niche requirements difficult no competition
13	Speciation rate	slow	rapid	rapid	rapid
14	Example	true dogs (canids)	Hawaiian Drosophila	certain snails	Hymenopteran parasites

* Modified from Bush 1975

Figure 1

Mating System among P. interpunctella*



* Modified from Grant and Brady 1975

Pheromones: (1) General

Pheromones are chemicals emitted and secreted by an individual and received by another individual of the same species, in which they cause a species specific reaction (Karlson and Lusher 1959). Because chemical methods were probably the first forms of interorganismal communication (Haldane 1955), the extensive use of pheromones by certain fungi, algae, and virtually all animal phyla (Birch 1974a, Comfort 1974, Shorey 1976a), including man is not unexpected. Reviews on pheromonally-induced behaviors among animals have been given by Wilson (1970), Birch (1974a), and Shorey (1976a).

By far the greatest variety of pheromone-mediated systems occur among the insects. The hexapod's extensive use of pheromone communication is expected because insects rely heavily on chemical stimuli for continued survival (Mayr 1970, Inscoe 1977), and undoubtedly stems from the development of chemosensory organs and cells early in it's evolutionary history, perhaps even before the development of light-sensitive organs (Snodgrass 1926). Although all insect orders use pheromones in communication, the highly social Hymenoptera and Isoptera have developed pheromone usage to it's highest degree (see Table II). In fact, Blum (1974) suggests a strong evolutionary relationship between the development of insect societies and diversification of pheromone communication. Among subsocial insects, pheromones have been shown to play major roles in: (1) the initiation of gregarious behavior during group oviposition among certain mosquitoes (Hudson and Mclintock 1967) and the desert locust Schistocerca gregaria (Forskal) (Norris 1963); (2) the formation of aggregations

at food sites, particularly among scolytid beetles (Burkholder and Lanier 1974); (3) dispersal behavior among generally gregarious species during predator attack (Blum 1974, Shorey 1976b) (4) the synchronization of gamete maturity among species exhibiting aggregative behaviors (Blum 1974); and (5) mate attraction among species which maintain a solitary life style, particularly Lepidoptera.

(2) Sex Pheromones of Lepidoptera

The principal uses of pheromones by Lepidoptera are in the attraction and reproductive stimulation of conspecific mates (Roelofs and Carde 1974). Sex attractants are generally released by females (Birch 1970, Weatherston and Percy 1977), and may act as male copulatory stimulants, when present in high concentration (Shorey and Gaston 1970, Shorey 1976a). Other copulatory stimulants, aphrodisiacs (Birch 1974b), are released by the males of many species, when in close proximity to a calling female, and cause the female to assume a receptive posture (Grant 1970, Birch 1974b).

According to Inscoe (1977), conspecific attractancy among Lepidoptera species has been known since 1690, when John Ray reported several male Biston betularia (L) flying around a caged female. This knowledge, of the attractive capacity of female Lepidoptera, was also used by such great naturalists as Fabre for collection of rare specimens; the procedure used was essentially the same as that of Ray (Kettlewell 1946, Inscoe 1977). The use of live females for population monitoring of the gypsy moth, Lymantria dispar (L), began in 1914, but by 1920 the females had been

replaced by crude abdomen tip extracts, which remained active for longer periods than females (Collins and Potts 1932). Attempts to isolate the chemical components of lepidopteran sex attractants also began in the 1920's. Unfortunately the methods then available for chemical analysis were not adequate, requiring large sample quantities and necessitating continuous rearing of large numbers of insects, therefore little headway was made (see Kettlewell 1946, Inscoe 1977). The first sex pheromone identified was that of Bombyx mori (L), the silkworm moth, by Butenandt et al in 1959. The elucidation of the chemical composition of bombykol ((E,Z)-10, 12-hexadecadien-I-ol), as it was called, had taken 20 years and 500,000 female abdomens. With the development of extraction techniques using highly purified solvents, techniques for absorption of volatiles on "Porapak", chemical analyses using nano- and microgram quantities in gas-liquid chromatography and mass spectrophotometry, and bioassay techniques using electroantennograms (EAG), the determination of sex pheromone composition has become greatly simplified and extremely accurate (Inscoe 1977, Weatherston and Percy 1977). In the 16 years following the discovery of bombykol the composition of the sex attractants of at least 200 species, chiefly those of economic importance, have been elucidated (see Tamaki 1972, Roelofs and Carde 1974a, Inscoe and Beroza 1976).

Most lepidopteran sex attractants, for which the chemical composition is known, are long chain (10-20 carbons) alcohols, acetates, ketones and aldehydes (Roelofs and Comeau 1970, Tamaki 1972, Roelofs and Carde 1974a) which are not directly assimilated from the larval host plant (Miller et al 1976). Most are unsaturated

having 1 or 2 double bonds and assuming the cis (E) or trans (Z) configuration depending upon the species, known exceptions include the hydrocarbons used by Arctiidae (Roelofs and Carde 1974b) and the epoxide of the gypsy moth (Bierl et al 1970).

Production of attractant pheromones takes place in specialized gland cells found in the intersegmental membrane between segments 8 and 9 of the abdomen (Shorey and Gaston 1965, Jefferson et al 1966, Waku and Suminoto 1969, Percy and Weatherstone 1974). An exception to this is Estigmene acrea (Drury) where the gland is located on the ninth segment and dorsal valves (Macfarlane and Earle 1970). During calling (ie. pheromone release) the female extends the ovipositor via increased hydrostatic pressure and/or muscle contraction (Jefferson et al 1966, 1968, Jefferson and Rubin 1970, Weatherston and Percy 1974) and exposes the cuticle overlying the pheromone gland to the air. It is hypothesized that sex pheromone, released from the gland cells, moves up epicuticular filaments extending through the endocuticle and exocuticle and diffuses through the epicuticle to the surface where it evaporates (Weatherston and Percy 1974).

The differentiation of pheromone gland cells occurs during the final stages of adult development with rapid production beginning about 2 days after adult eclosion in many cases (Shorey et al 1968, Waku and Summimoto 1969, Miller and Roelofs 1977, Smithwick and Brady 1977). Pheromone production is also dependent upon the mating history of the female (Shorey 1976b). For example, the amount of pheromone decreases rapidly after mating among species which mate only once (Collins and Potts 1932, Perez and Long 1964). Among species which

mate more than once, although the amount of pheromone within the gland remains high (Shorey and Gaston 1965, Shorey et al 1968), it is often not released for several days after mating (Raulston and Graham 1975 and Marks 1976), or is released for much shorter periods (Shorey 1976a).

The release of female sex pheromones by Lepidoptera is a species specific diel activity (Kettlewell 1946, Roelofs and Carde 1974a, Shorey 1974), and appears to be part of a general circadian activity rhythm which includes flight, feeding, and male response periods (Carde et al 1975a, Driesig 1976). This circadian rhythmicity is controlled by both intrinsic and extrinsic factors. Intrinsic variables include: (1) Age-pheromone release does not generally reach it's maximum until gametes are mature (Shorey et al 1968, Shorey 1974) and is maintained at high levels until senescence or death (Shorey et al 1968, Brady and Smithwick 1968, Lawrence and Bartell 1972, Miller and Roelofs 1977); and (2) Mating history-mated females generally exhibit a refractory period after mating and on resumption of calling, pheromone is released at lower levels, conversely virgin females extend their calling periods with increasing age (Hirano and Muramoto 1976, Marks 1976, Swier et al 1977) to a certain maximum after which the length of the calling period diminishes. The major environmental, or extrinsic, variables influencing calling periodicity include light intensity, temperature and air velocity.

Light intensity is both the most pronounced and predictable of the environmental variables. It appears that the biological clock governing pheromone release and mating is set by a certain critical

light intensity (Shorey 1974, 1976b) above which, as is the case of Trichoplusia ni (Hubner) (Shorey and Gaston 1964, Sower et al 1970), or below which, in diurnal species (Shorey 1974, Gorsuch et al 1975), no calling will occur. Further, it has been shown that the time of the dusk or dawn periods, in a 24 hr. cycle, govern the initiation of pheromone release behaviors (Sower et al 1970, Carde and Roelofs 1973, Carde et al 1975a).

The temperature ranges, in which pheromone communication can occur, are related to the geographic distribution and lifestyle of each species. For example tropical and temperate diurnal species release sex pheromones at warmer temperatures than do nocturnal and arctic species (Shorey 1976b). Within certain maxima, above and below which calling does not occur (Roelofs and Carde 1975), temperature depressions cause calling periods to be shifted to earlier periods (Sower et al 1970, Sanders and Lucuik 1972, Carde and Roelofs 1973, Carde et al 1975a, Marks 1976). This effect is of such magnigude that it can override the influence of dusk or dawn on the induction of pheromone release (Roelofs and Carde 1975), thereby insuring that mating will occur before the temperature drops below the threshold for mating (Marks 1977).

The role of air velocities in the control of pheromone-mediated behaviors is chiefly due to effects on pheromone dispersal. Very low air speeds inhibit response because (1) the pheromone may not be volatilized or carried far from it's source before settling on surrounding foliage (Shorey 1974, Nakamura 1976), and (2) the pheromone plume may become disrupted to the extent that males cannot

follow it (Shorey 1974). High wind speeds prevent responses by inhibiting flight activity (Shorey 1974, Nakamura 1976, Marks 1977), and disrupting the pheromone plume (Shorey 1974). In response to air velocities of low magnitude female moths may move to the tops of foliage and fan their wings (Shorey 1974). At high wind velocities the opposite occurs, males tending to move through the foliage, females calling within the foliage, and exhibiting no fanning behavior (Shorey 1974, Marks 1977). Another response, exhibited by female T. ni (Kaae and Shorey 1972), is that less time is spent in pheromone release during periods when wind velocities are unfavourable, thereby limiting pheromone release.

(3) Sex Pheromones and Reproductive Isolation:

According to Tamaki (1972) mating behavior is species specific. Sex pheromones - which initiate reproductive behaviors - are of principal importance in maintaining the species specific nature of mating behavior among Lepidoptera, particularly among co-occurring sibling species (Tamaki 1972, Roelofs and Carde 1974a). Because of the many intrinsic, genetically-controlled factors governing the production and release of sex attractants, there is an almost infinite number of chemicals, chemical combinations, and differences in release periodicity that can account for the species specific nature of sex pheromones. The following is a brief description of the mechanisms which give rise to the specificity; more comprehensive reviews are given by Tamaki (1972), and Roelofs and Carde (1974a).

The most obvious mechanism of pheromonal isolation among spe-

cies is the use of different chemicals (Roelofs and Comeau 1971, 1973, Tamaki 1972, Roelofs and Carde 1974a). This is accomplished by: (a) differences in the functional moiety (ie. alcohols, acetates, ketones and aldehydes); (b) addition of carbon atoms to the basic chain; (c) changes in position of a double bond or addition of double bonds; and (d) changes in configuration from cis to trans (Roelofs and Comeau 1971, 1973, Tamaki 1972, Roelofs and Carde 1974a).

Although simple pheromones are composed of only one chemical, the sex pheromones of many species, particularly sibling species, are now being found to be composed of 2, 3, or even 4 chemical components (Tamaki 1972, Roelofs and Comeau 1973, Roelofs and Carde 1974a, Tamaki and Honma 1976, Carde et al 1977) thus increasing evidence for Wright's thesis (1964), that most insects use more than one attractant chemical. Recently these components have been shown to induce the different behaviors involved in mating (Carde et al 1975b, 1977, Baker et al 1976, Nakamura 1976). Specificity can also be caused by the addition of a second chemical, usually released in small quantities, which acts synergistically with the principal attractant in promoting maximal male responses among species using the same principal attractant (Tamaki 1972, Roelofs and Carde 1974a). The addition of a second chemical can also act as an inhibitor among co-occurring species using the same principal attractant (Ganyard and Brady 1971, Steck et al 1977). Also the proportion of each pheromonal component chemical has been shown to be important in reproductive isolation, particularly among many species of Tortricidae (Carde et al 1977). Lastly there is some evidence that among some species pheromone specificity may be due

to the chirality of the component chemicals (Klimetzek et al 1977).

Even though there would seem to be an almost infinite number of chemicals and groups of chemicals which function in the maintenance of pheromonal isolation, cross-attraction occurs and has been documented between some sympatric species (Kettlewell 1946, Ganyard and Brady 1971, Sanders 1971a, Tamaki 1972, Kaae et al 1973a, Roelofs and Carde 1974a, Tamaki and Honma 1976). In these cases species have developed non chemical mediated mechanisms of pheromonally induced isolation. There are many behaviors associated with pheromone release which could function in isolation: the stance assumed by calling females, the type of vegetation on which the female calls, variable degrees of wing fanning (thus setting up different air currents and vibrations), the rate of pheromone release, and different circadian periods of pheromone release. Only the last 2 have been documented as playing major roles in reproductive isolation. In the numerous cases reported in which release rates function in isolation (see Roelofs and Carde 1974a) the classic example occurs between the closely related sympatric species T. ni and Autographa californica (Speyer). In these 2 species the males of the former are attracted by release rates of the mutual pheromone (E-7-dooecenyl acetate) which are 10 to 100 times greater than the most attractive rates for the A. californica male (Kaae et al 1973a). The occurrence and functioning of difference in the circadian periodicity of pheromone release in isolation has been discussed by Roelofs and Carde (1974a). It appears that temporal differences in release function more often in conjunction with other isolating mechanisms (Sanders 1971b, Kaae et al 1973b, Roelofs and Carde 1974,

Tamaki and Honma 1976, Carde et al 1977, Grant 1977), although cases are documented, including the present work, in which periodicity differences do function as major components of reproductive isolation.

Table 2

Pheromones of Social Insects*

Chemical Stimulus	Response
Reproductive-caste inhibitory pheromone	Inhibition of gamete maturity and caste determination
Queen substance	Ovarian inhibition, Inhibition or stimulation of queen cell construction, Queen identification by workers
Solicitation pheromone	Regurgitation and exchange of solid food
Trail and alarm pheromone	Recruitment, migration, alarm behavior
Attractant pheromone	Recruitment and migration
Surface pheromone	Oral grooming
Aggregation pheromone	Clustering behavior
Territorial and sex attractant pheromones	Attraction of winged males
Queen-recognition pheromone	Queen identification by workers
Flight induction pheromone	Flight induction of sexually mature adults
Alarm pheromone	Repellency
Thermoregulatory pheromone	Abdominal pumping

* Adapted From Blum (1974)

The Genus Euxoa

The genus Euxoa (Lepidoptera: Noctuidae) is a large homogeneous group of cutworm moths, represented by some 180 species in North America (Hardwick 1970, Hardwick and Lefkovitch 1970). The species of this genus have been placed into six subgenera by Hardwick (1970): Orosagrotis, Longivesica, Chorizagrotis, Pleonectopoda, Crassivesica, Euxoa. The first five subgenera contain only about 1/4 of the total number of described species, the remaining 75% being found in the subgenus Euxoa (Hardwick 1970), including members of the declarata group (Hardwick 1973) used in this study.

The genus, as a whole, is broadly distributed throughout North America, with species being found from the low Arctic to Central America, and from east to west coasts. Most species are found in xerophytic, middle temperate areas of western North America (Hardwick and Lefkovitch 1973), with the largest numbers found in the high plains and Great Basin. Of the 30 species occurring in eastern North America only 7 are endemic to the east, the remaining 23 being found throughout the continent (Hardwick 1970).

About 10% of the described species have been reported as pest species and several of these are considered to be of major economic importance. The most prevalent, and hence best documented, of these are: (1) Euxoa messoria (Harris), the dark-sided cutworm, which attacks tobacco and orchard crops in southern Ontario, (2) E. scandens (Riley), the white cutworm, which attacks surface crops, fruit trees and nursery stock in eastern Canada; and (3) E. orchrogaster (Guenee), the redbacked cutworm,of western and central North

America, which feeds on many types of cereal crops (Beirne 1972).

The economic importance of the large number of species endemic to the high plains and range land of western North America is unknown (Hardwick 1970). However, because larvae are general feeders and the highest concentrations of adults in North America are found in these areas, their impact on available cattle forage is obviously negative.

Hardwick (1970) stated that adults of the genus Euxoa may exhibit a high degree of intra-specific variability and often a lack of distinctive inter-specific structural and morphological characters. Further, common patterns of macular variation have developed, with similar forms duplicated in species which are not closely related. This lack of inter-specific characters is also in evidence to such high degrees, during larval stages, that Crumb (1959) could not find sufficient data to develop a satisfactory larval key. An excellent example of adult intra-specific variability is seen in the several colour morphs of E. ochrogaster, all of which may occur in siblings resulting from a singly mated female (Hardwick 1965). The lack of species specific morphological characters is typified by the female genitalia of members of the declarata group (Hardwick 1973, Hardwick and Lefkovitch 1973). Hardwick (1970) also states that the existence of inter-specific similarities is the result of the genus, as a whole, being both successful and adaptable, and in a stage of great evolutionary mutability. The substantiation of this is the strong presumptive evidence for natural inter-specific hybridization among closely related co-occurring species. This is further supported by the success of inter-specific hybridization

experiments under laboratory conditions (Byers and Hinks 1978).

The declarata group

The 3 sibling species of the declarata group (fig. 2) - Euxoa declarata (Walker), E. campestris (Grote) and E. rockburnei Hardwick - are difficult to interpret both morphologically and taxonomically (Hardwick and Lefkovitch 1973). E. declarata is of medium size, exhibiting a broad range of colouration, from light mauve-grey in dry areas to dark purplish-brown in moist climates (Hardwick and Lefkovitch 1973). E. campestris is generally the smallest and darkest of the group, however dark forms of declarata are often indistinguishable from campestris on the basis of colouration or musculature. E. rockburnei, the largest of the group, is generally of intermediate colouration between declarata and campestris (Hardwick 1973). The male genitalia of declarata and campestris are generally distinguishable, while that of rockburnei is quite distinct. Female genitalia are less discrete, those of declarata and campestris being indistinguishable from one another, but generally distinct from rockburnei (Hardwick 1973, Hardwick and Lefkovitch 1973).

Although campestris is normally of slightly higher altitudinal distribution and earlier flight period than declarata, both species co-occur in many areas and are considered broadly sympatric (Hardwick 1973, Hardwick and Lefkovitch 1973, Byers and Hinks 1978). Ranges extend from north eastern North America to central British Columbia and southward, in the Rocky Mountain system, to New Mexico. Rockburnei inhabits lowland and mid-montane areas of the Great

Basin. This species is parapatric with campestris and sympatric with declarata in the latter species' southwestern range (Fig. 3) (Byers and Hinks 1978).

All 3 species are univoltine, overwintering as fully developed 1st instar larvae in the egg, and in diapause. Under normal conditions there are 6 larval instars with a short prepupal aestivation occurring in the last. Larvae are polyphagous, feeding chiefly on field and vegetable crops. Pupation occurs in mid-summer and the duration of pupal period is variable in length, depending upon ambient temperature, but is generally 3-4 weeks in length. Adult flight periods extend from late July until early October. A more detailed description of their life cycles and that of 33 other species of Euxoa has been given by Hinks and Byers (1976).

As stated previously, the member species of the declarata group are not easily distinguished by methods commonly used in systematics. However studies into the physiology, genetics, morphology, life stages, and ethology of the 3 species have provided material useful for adequate taxonomic descriptions and development of phylogenetic relationships. These investigations have shown that: (1) the fusiform plasmocytes of 5th instar larval rockburnei are more similar in size to declarata than campestris (Arnold and Hinks 1975, Arnold 1976); (2) the overwintering first instar larvae of campestris have an intense diapause, while those of declarata and rockburnei have a diapause of moderate length (Hinks and Byers 1976); (3) larval aestivation periods are longer in declarata and rockburnei than in campestris (Hinks and Byers 1976); (4) esterase isozymes are different among the 3 species, those of campestris

being more similar to rockburnei than to declarata (Hudson and Jui 1976); (5) differences in geographic distributions do exist between campestris and declarata (Hardwick 1973 and Byers and Hinks 1978) (6) sex pheromone cross-attractancy does occur among the 3 species (Byers and Hinks 1978) (7) the ease of inter-specific hybridization in the laboratory is greater among campestris and rockburnei than between declarata —— rockburnei and declarata —— campestris under no choice conditions (Byers and Hinks 1978); and (8) strong premating isolation does occur between all 3 species in mating selection tests (Byers and Hinks 1978).

Similarities in larval diapause, blood cell size, and aestivation periods led Arnold (1976) to suspect a closer phylogenetic relationship between declarata and rockburnei than between declarata and campestris. However the work of Byers and Hinks (1978), Hudson and Jui (1976), and Hudson (personal communication) have led these workers to consider a closer relationship between campestris and rockburnei, Byers and Hinks hypothesizing the development of rockburnei from a founding population of campestris during the Wisconsin Glaciation.

Figure 2: The three species of the declarata group of Euxoa (Lepidoptera:Noctuidae). A-F are E. declarata, G-H are E. campestris, I-J are E. rockburnei.

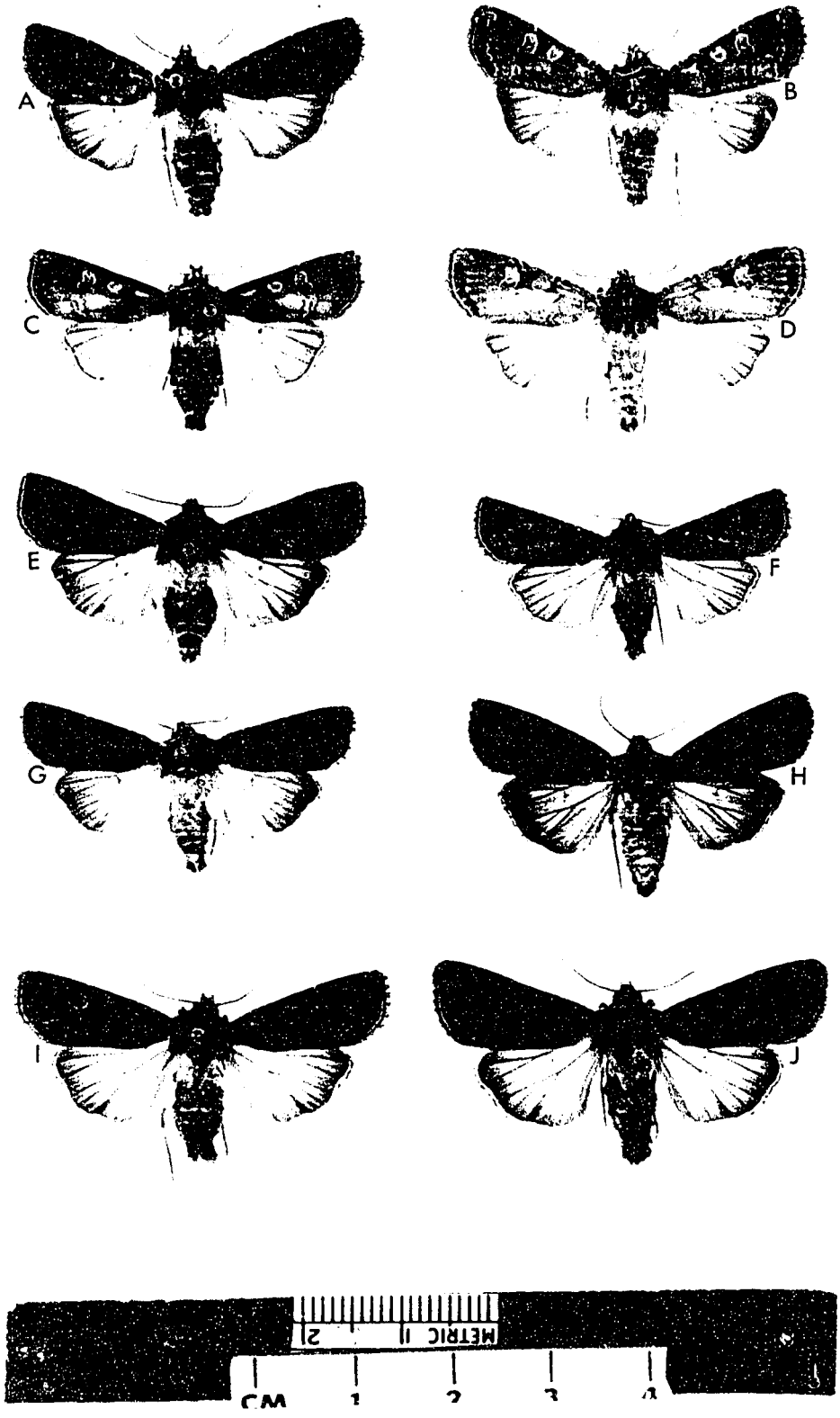
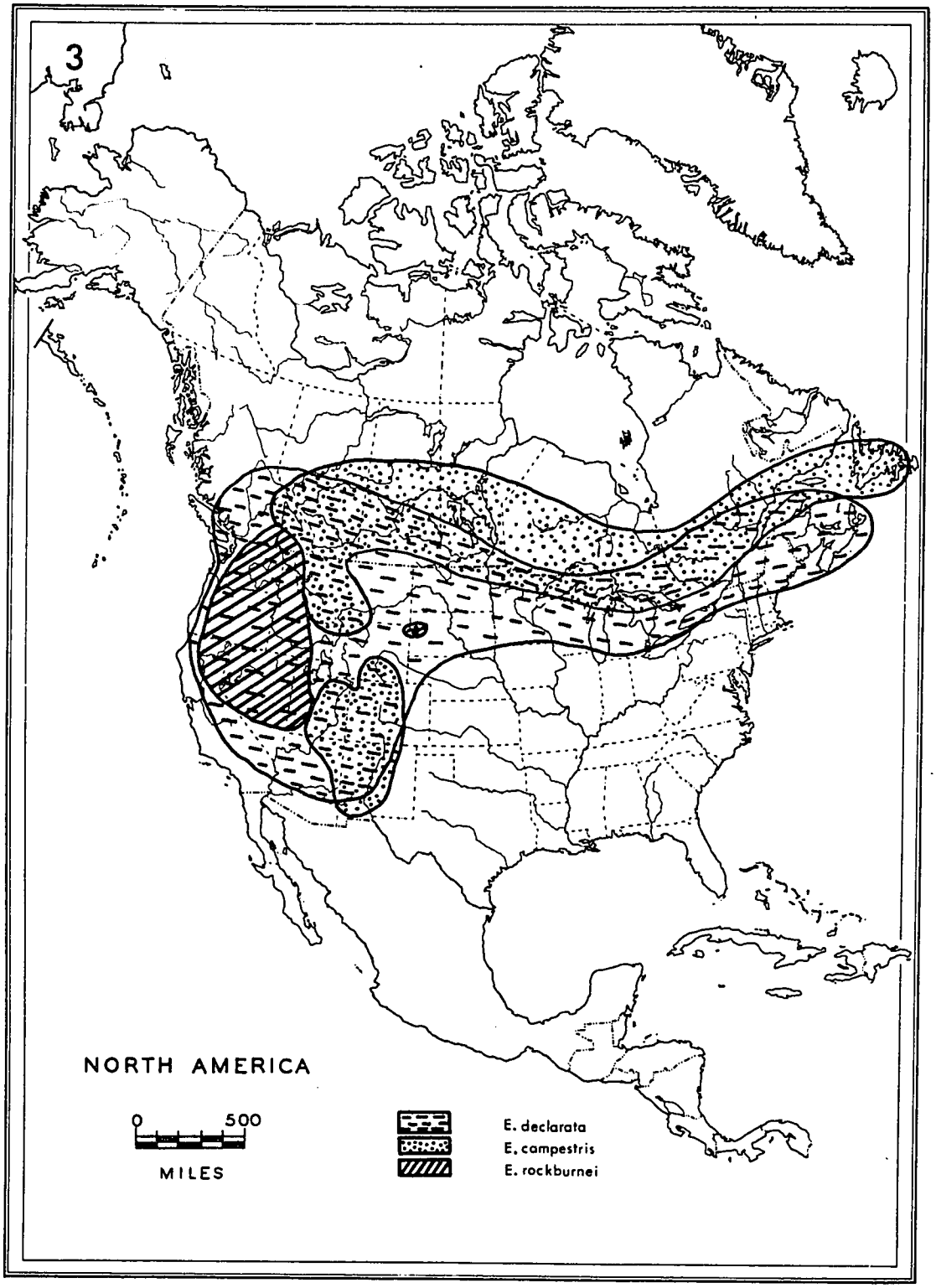

A
B
C
D
E
F
G
H
I
J
METRIC 1 2
CM 1 2 3 4

Figure 3: Distributions of E. declarata, E. campestris and E. rockburnei. Modified from Byers and Hinks 1978.


3
NORTH AMERICA
0
500
MILES
E. declarata
E. campestris
E. rockburnei

METHODS AND MATERIALS

1. Rearing Procedures

The insects used in this study, with the exception of the declarata-rockburnei hybrid stocks, were the first to third generation progeny of mated females collected in the field using a mercury vapour light trap or sugar bait (Hinks and Byers 1976). The hybrid stocks available, E. declarata X E. rockburnei and E. rockburnei X E. declarata, were F_1 progeny of laboratory reared parents. Insects of fourth and successive laboratory generations were not used because of the diminished vigour and fertility inherent among Euxoa species maintained as separate lines through successive generations (Hinks and Byers 1976). Stocks used were, whenever possible, chosen from available material originating from areas having broad differences in habitat and climate at an intraspecific level, and limited differences at an inter-specific level (Table 3). Unfortunately the 2 available stocks of E. rockburnei originated from the same collecting site in California.

Larvae were reared in a manner adapted from that of Hinks and Byers (1976). The artificial diet used was similar to that used by Shorey and Hale (1965) but contained wheat germ and twice the concentration of agar (Table 4). Photoperiod and temperature were held constant at 16 hrs. and 21°C respectively, throughout the immature stages.

After hatching from cold stored eggs (2°C) groups of about 50 first-instar larvae were placed in 2.5 cm. diameter polystyrene vials containing a 0.5 cm. layer of coarse silica sand and strips

Table 3

Origins of Stocks

Species	Stock	Location of Collection
E. declarata	1	Bridgeford Sask.
	2	Lethbridge Alta.
	3	Lake Louise Alta.
	4	Drumheller Alta.
E. campestris	5	Elkwater Alta.
	6	Elkwater Alta.
	7	Fort Steele B.C.
E. rockburnei	8	Mount Shasta Calif.
	9	Mount Shasta Calif.

Table 4

Artificial Diet*

Pea beans (hydrated)	854g
Ascorbic Acid	13g
Brewer's yeast	128g
Wheat germ	200g
Methyl-p-hydroxybenzoate	8g
Sorbic acid	4g
Formaldehyde	8ml
Agar	100g
Distilled H_2O	2560ml

*Developed by Hinks and Byers (1976).

of diet separated by pieces of absorptive filter paper. The vials were then covered by a layer of fine plastic screen and capped with vial tops which had a 0.5 cm. hole, to allow air exchange. The vials, diet, sand and paper were changed twice a week. The number of larvae per vial was decreased as larval size increased, until, in the third instar, each vial contained about 10 larvae. Groups of 25 late third and early fourth-instar larvae were transferred to glass jars having a diameter of 10 cm., height of 13 cm., a 1 cm. layer of sand, and several cubes of diet. After transfer several strips of pleated paper towel were placed over the larvae and the jars covered by fine plastic mesh tops. As in earlier stages larvae were transferred to new jars biweekly and dead or diseased larvae were discarded. Larvae were maintained in jars until entering the prepupal period without noticeable loss resulting from cannibalism (see Hinks and Byers 1976).

Prepupae were removed from the jars and placed in 17.5 cm X 8.5 cm. X 4.5 cm. polystyrene boxes containing 3 cm. of moist peat moss. The boxes were covered and the larvae were allowed to pupate. After the pupal cuticle had hardened pupae were sexed and placed in fresh boxes (ca. 75 pupae of one sex per box). Shortly before the first adult emergence was anticipated the boxes were placed in flight cages and the box lids removed, to ensure adequate space for wing inflation and hardening after eclosion.

Virgin female moths were collected on the day of emergence and placed, 5 per cage, in 30 cm. X 30 cm. X 30 cm. wood frame cages (Fig. 7) having a wooden floor, clear plastic top and front, and fine plastic screen sides and back, Only moths in good condition were used and if fewer than 5 emerged on the same day they were

stored at 5°C for up to 1 week or until 5 females had been accumulated (no differences in calling periodicity was observed among females stored at 5°C). Nutriment consisted of 10% sucrose supplied from plastic capped vials fitted with a dental roll wick. Initially cages were housed at 60-70% relative humidty and 19-20°C for a period of 3 days to ensure sexual maturity and acclimation to the photoperiod. Incandescent lighting was controlled to provide a reverse photoperiod with a photophase of 11.5 hrs. at 5,800 lux, a scotophase (dark period) of 11.5 hrs. at 0.35 lux and 0.5 hr. of simulated dusk and dawn.

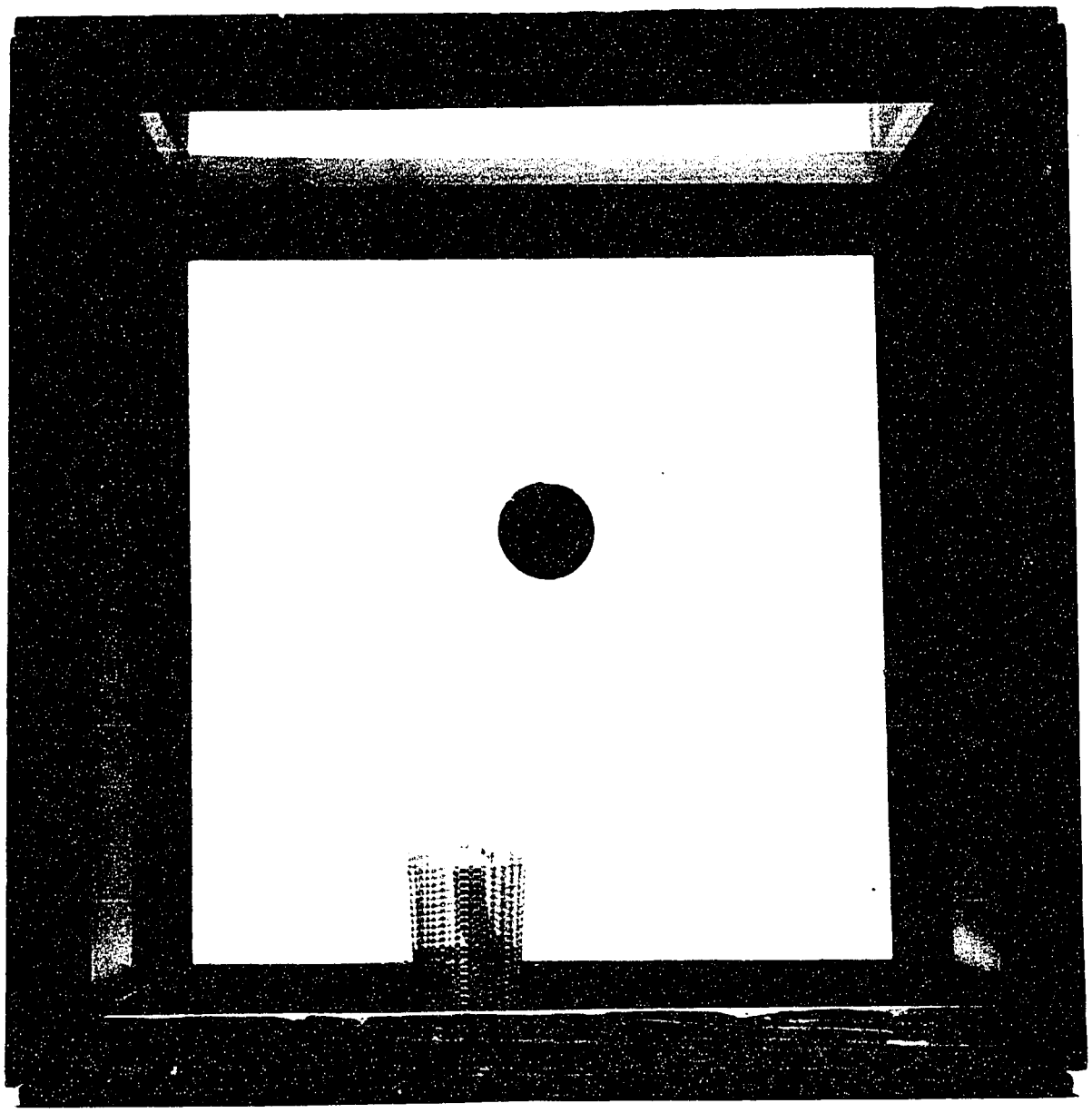
(II) Observation of Calling Periodicities

Initial observations on calling periodicities were made at the constant temperature of 20°C in order to establish if the 3 species were temporally isolated. After having established that differences in calling periodicities do occur among the species, experiments designed to monitor the effects of temperature depression on calling periodicity were set up. Observations on the release periods of the hybrid stocks were carried out at 20°C only.

a) Experiments at 20°C

Initial observations were made at 30 min. intervals from the beginning of dusk until the end of dawn for each stock. After the calling period had been approximately established, further observations were made for 3 consecutive days from about 1 hour before calling would begin, and continued until 1 hour after calling had ceased. Observations were made using a flashlight dimmed with 2

Figure 4: Cage used in observation of calling periodicities.



layers of kleenex. The number of females calling at each 30 min. interval were recorded and the time of maximum calling (T) was calculated, by a method adapted from Comeau et al (1976) as follows:

$$T = \frac{\Sigma[(hr)X(\#\text{ females calling at hr.})]}{\Sigma\text{ Females calling per night}}$$

Although flight behavior and feeding sometimes began in the last half of the dusk phase, time 0, was considered to be the beginning of the scotophase (i.e. when light intensity reached 0.35 lux).

b) Temperature Depression Experiments

Observations on the effect of temperature depression on calling were begun during the third scotophase of the acclimation period to insure that the females had assumed their species specific calling periods.

Temperature changes were initiated at the termination of the dusk phase of the fourth day, with experimental cage temperatures of 15°C, 10°C, 5°C being reached at 20 min., 1 hr., and 1.5 hrs. respectively. Moths were held at these temperatures throughout the remaining dark period.

In experiments at 10°C and 5°C cages were covered with 2 black sheets at the end of the dusk phase and quickly transferred to chambers calibrated to have the appropriate temperature, but having the same photoperiod, relative humidity, and equivalent illumination during the scotophase, as 20°C chambers. Once inside the chambers the sheets were removed and observations made at 30 min. intervals. At the termination of the scotophase cages were again covered with

black sheets and quickly returned to the 20°C chamber. The covering was then removed.

Each group underwent 2 dark cycles at each temperature and a 24 hr. refractory period between each temperature. Experiments were conducted on a rotational basis (Fig. 9) thereby minimizing any effects brought about by increasing age. Data for each species group were pooled and"T" values for each species were calculated as above. These data were compaired with those of the initial experiments at 20°C.

(III) Morphological Studies:

Insects used in studies on the structural morphology of the ovipositor and pheromone gland were reared as described above. After emergence females were allowed to mature under the same conditions as those used in observations on the circadian periodicity. On the fourth day after emergence females were removed and killed by alcohol injection (95% EtOH), fast freezing, or thoracic crushing. Whole abdomens were then separated from the thorax and either fixed or immediately dissected.

Unfixed abdomens were dissected, with the ovipositors in either the extended or retracted positions, in Lepidoptera Ringer's solution, using microscissors and stainless steel "watchmaker's" forceps. Ovipositors were extended by application of pressure to the anterior 6 abdominal segments and then the seventh clamped using a surgical clamp. The extended papillae anales (dorsal valves) were then pinned in a dissecting dish. Additional pins were applied to the posterior seventh, fifth, and third abdominal segments and the surgical clamp

removed. Abdomens having retracted ovipositors, were pinned in the same manner. Midline incisions were made through dorsal, ventral, or lateral surfaces and the edges of the incisions folded back and pinned. Tracheae, fat body, and ovarioles were carefully removed.

Material used in the observation of abdominal segmentation and the internal cuticular structure of the ovipositor, was prepared using the method described by Hardwick (1950). The genitalia were not mounted, but viewed in a petri dish containing 95% EtOH so that dorsal, ventral, and lateral aspects could be observed. Permanent mounts, from the Canadian National Insect Collection, were also used in these observations.

Ovipositors used in scanning electron microscopy (SEM) were extended in the manner described above. After the seventh abdominal segment had been clamped, the terminal 4 segments including intersegment 6/7 were removed. The clamp and specimens were then plunged into liquid nitrogen and fast frozen. After freezing, the terminal segments were positioned on filter paper and placed in a prechilled dessicator (-15°C). The latter was then connected to a freeze dryer (New Brunswick Scientific)* and the specimens freeze-dried for 18 hrs. The material was then mounted on SEM stubs using silver conductive paint and evaporation-coated with gold (thickness approximately 200 Å) using a Speedy Vac Coating Unit.** SEM observations were made using either a Cambridge Stereoscan Mark IIA or an AMR 1000A.

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* New Brunswick Scientific Co. International, New Brunswick N.J. USA.

** Edwards High Vacuum Ltd. Sussex Engl.

Ovipositors used in semi-thin and thick sections for light microscopy were extended and removed from the anterior portion of the abdomen in the same manner as for SEM observations. After removal, extended ovipositors were put in a dissecting dish, flooded with 2% gluteraldehyde fixative and pricked several times on the dorsal and lateral surfaces with a minuten pin. The ovipositors were sufficiently fixed to remain extended on removal of the surgical clamp after about 10 min. Further steps involved in fixation, dehydration, and embedding were followed as outlined in table 5. Some specimes were not post-fixed in OsO_4 in which cases steps 3 and 4 were omitted.

The material was sectioned at 0.5 to 2.5µ with a Reichert OmU2 ultramicrotome and a glass knife. Sections were stained on a hot plate (90°C) using either methylene blue in 1% borax or polychrome stain (Alsop 1974). Methylene blue was used for general tissue and cuticular components, while polychrome stain was used for differentiation of cuticular components. Microscopic observation and photomicrography was carried out using a Reichert Zetopan microscope.

Thin sections used in transmission electron microscopy (T.E.M.) were cut using a glass knife as above. Gold-blue coloured sections were collected on 200 mesh copper E.M. grids and stained with uranyl acetate in 50% ethanol, and lead citrate. T.E.M. observations were made using a Philips E.M. 300 electron microscope.

Fig. 5: Rotational System of temperature depression experiments showing the temperature at which each group entered the experiments.

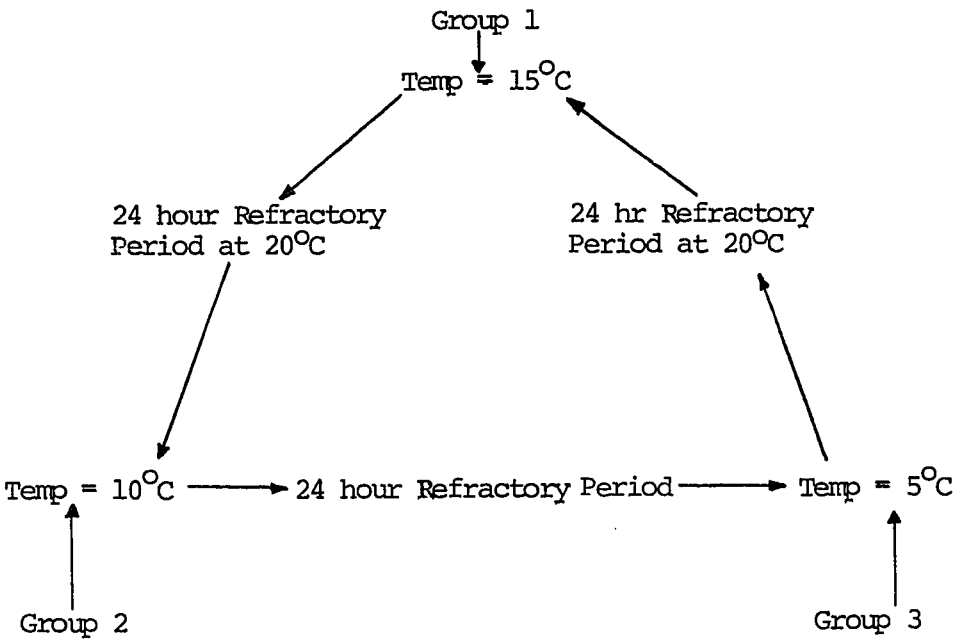


Table 5

Procedure followed in Dehydration and Infiltration

1) 2% Gluteraldehyde in 0.2M phsophate buffer - 18 hrs.
2) Phosphate wash buffer (pH = 7.2) - 10 min
3) OsO_4 in 0.2M phosphate buffer - 5 hrs.
4) Phosphate wash buffer - 10 min.
5) 70% Ethanol - overnight.
6) 95% Ethanol - 30 min.
7) Absolute Ethanol - 30 min.
8) Absolute Ethanol - 1 hr.
9) Propylene Oxide - 15 min.
10) Propylene Oxide - 30 min.
11) Propylene Oxide: Spurr's Resin (1:1) - 1 hr.
12) Spurr's Resin - overnight.
13) Spurr's Resin - overnight.
14) Embed in Spurr's resin - 60°C overnight.

RESULTS

I Calling Postures

The key component of the calling posture among noctuid females is extension of the terminal abdominal segments to expose the cuticle overlying the sex pheromone gland (Fig. 6, 7). In Euxoa species calling normally occurs with the female on a vertical surface with it's head upwards. In the initial phases of pheromone release the ovipositor is held extended for periods of up to 3 min. and then withdrawn for between 1 and 3 seconds. After several bouts of this behaviour the terminal segments are extended and remain so for prolonged periods after which no further calling occurs (the longest observed extended period was 2.25 hrs.). During extended periods, calling moths are capable of ambulatory and flight behaviors without retraction of the ovipositor.

The only differences in the calling postures of the three species occurred at 20°C and were characterized by wing positioning. Declarata females held their wings flat and only slightly raised over the abdomen (Fig. 6), while females of both campestris and rockburnei raised their wings to an angle of about 45°C, tilting them in such a manner that the upper surfaces formed a "V" (Fig. 7). At temperatures below 15°C the majority of the campestris and rockburnei females did not raise their wings and were indistinguishable from declarata females. At 15°C varying degrees of wing elevation were observed among both campestris and rockburnei.

Calling Periodicities:

The temporal periodicities of calling by females of each species at temperatures of 20, 15, 10, and 5°C are listed in tables 6, 7, 8, and shown graphically in figures 8, 9, 10, 11, respectively. The initial and maximum phases of pheromone release behaviours, which in the natural situation, with males present, would be by far the most significant, are temporally discrete for each species, at all temperatures. The mean times of maximum calling (T) for each species, listed in table 9 show shifts in pheromone release periods resulting from temperature depression in the dusk and early scotophase, with major shifts occurring below 10°C. A linear regression of the T values at each temperature is given in figure 12 and Table 10. Populations of each species originating from different geographical areas are not significantly different from either the species as a whole or other populations when monitored at 20°C (Table 11).

An examination of the calling periodicities of each of the hybrid stocks (Table 12, and fig. 13) indicates that in the declarata ♀ x rockburnei ♂ F_1 hybrid the calling period was the same as that of the declarata females. The calling period of the rockburnei ♀ x declarata F_1 hybrid stock was extended and intermediate between those of the parental species.

Figure 6: Calling posture of declarata female.

Figure 7: Calling posture of campestris female.

6

7

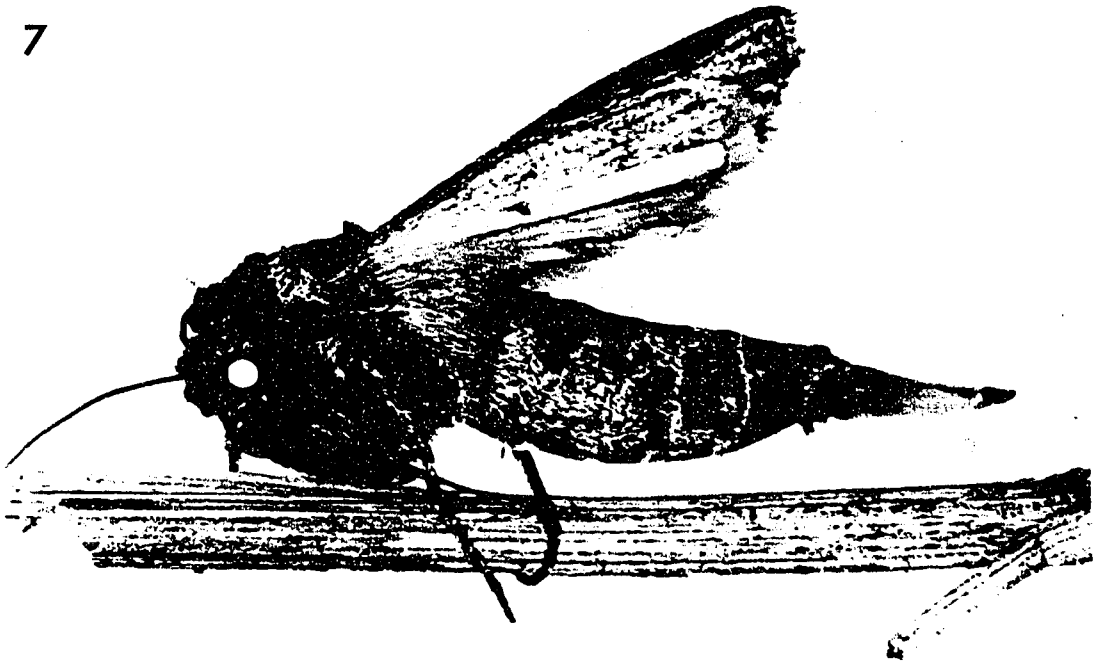


Table 6

% Calling at different temperatures for E. declarata

Time in Scotophase	T=20°C (n=204)	T=15°C (n=38)	T=10°C (n=45)	T=5°C (n=53)
Dusk	0	0	0	0
0	0	0	0	0
0.5	1.0	2.9	15.6	19
1	29.1	60.5	68.9	73.6
1.5	47.1	71.1	80.0	69.8
2	64.7	81.6	77.8	56.6
2.5	45.6	76.3	48.9	28.3
3	32.4	55.3	20.0	7.5
3.5	18.6	39.5	2.2	0
4	9.3	34.2	0	0
4.5	2.9	7.9	0	0
5	0.5	5.3	0	0
5.5	0	5.3	0	0
6	0	0	0	0
6.5				
7				
7.5				
8				
8.5				
9				
9.5				
10				
10.5				
11				
11.5				
Dawn				

Table 7

% Calling at different temperatures for *E. campestris*

Time in Scotophase	T=20 (N=231)	T=15 (N=40)	T=10 (N=34)	T=5 (N=52)
Dusk	0	0	0	0
0	0	0	0	0
0.5	0	0	0	0
1	0	0	0	0
1.5	0	0	0	0
2	0	0	0	0
2.5	0	0	0	0
3	0	0	0	0
3.5	0	0	0	0
4	1.3	0	0	0
4.5	1.7	0	0	5.8
5	2.6	0	0	40.4
5.5	2.6	2.5	14.7	55.8
6	24.2	12.5	29.4	71.2
6.5	63.6	27.5	61.8	50.6
7	79.7	37.5	41.2	46.2
7.5	89.6	50.0	20.6	19.2
8	61.9	55.0	5.9	9.6
8.5	54.5	47.5	2.9	1.9
9	50.2	37.5	0	0
9.5	20.3	32.5	0	0
10	14.3	22.5	0	0
10.5	12.6	7.5	0	0
11	0.4	5.0	0	0
11.5	0	0	0	0
Dawn	0	0	0	0

Table 8

% Calling at different temperatures for E. rockburnei

Time in Scotophase	T=20 (N=114)	T=15 (N=24)	T=10 (N=28)	T=5 (N=24)
Dusk	0	0	0	0
0	0	0	0	0
	0	0	0	0
1	0	0	0	0
	0	0	0	0
2	0	0	0	0
	0	0	0	0
3	0	0	0	0
	0	0	0	0
4	0	0	0	0
	0	0	0	0
5	0	0	0	0
	0	0	0	0
6	0	0	0	4.2
	0	0	0	20.8
7	0	0	0	37.5
	0	0	0	45.8
8	0	0	28.6	54.2
	7.0	25.0	53.6	62.5
9	7.0	37.5	67.9	41.7
	28.1	50.0	53.6	37.5
10	49.1	50.0	25.0	16.7
	66.7	33.3	7.1	0
11	59.6	16.7	0	0
	9.6	12.5	0	0
Dawn	0	0	0	0

Figure 8: Calling periodicities of E. declarata, E. campestris and E. rockburnei et 20°C.

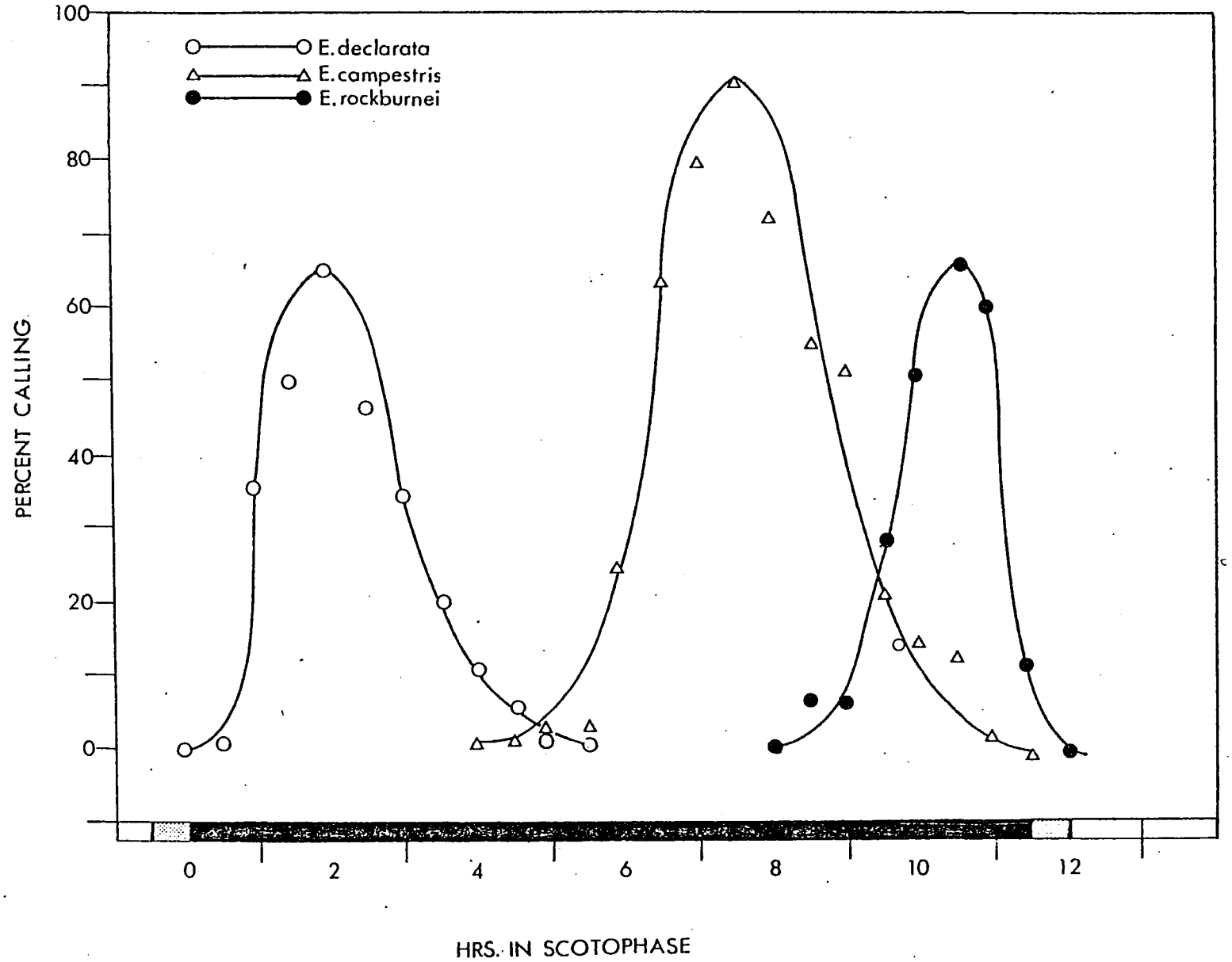

E. declarata
E. campestris
E. rockburnei
PERCENT CALLING
100
80
60
40
20
0
0
2
4
6
8
10
12
HRS. IN SCOTOPHASE

Figure 9: Calling periodicities of the members of the declarata group at 15°C.

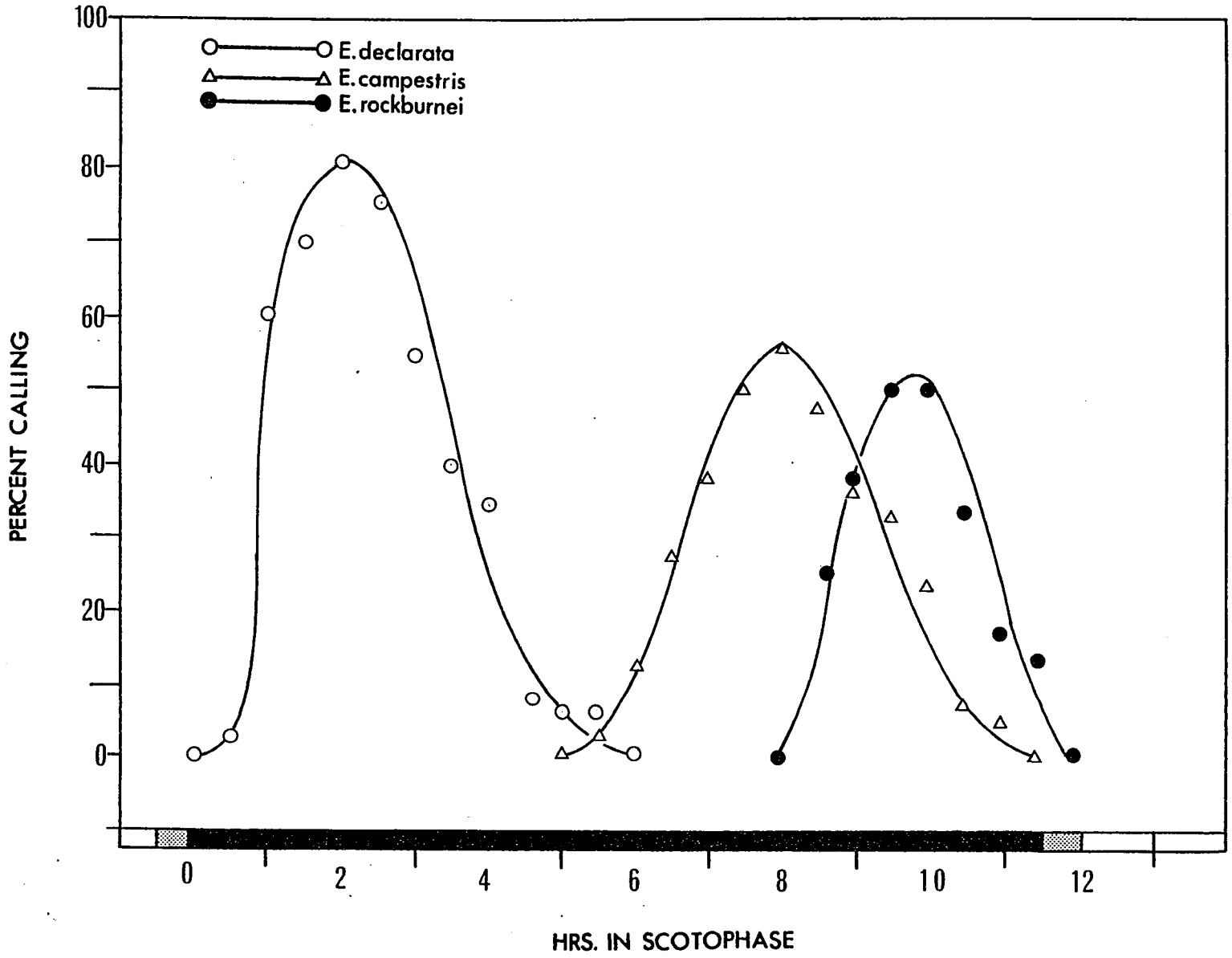

E. declarata
E. campestris
E. rockburnei
PERCENT CALLING
100
80
60
40
20
0
0
2
4
6
8
10
12
HRS. IN SCOTOPHASE

Figure 10: Calling periodicities of *declarata*, *campestris* and *rockburnei* at 10°C.

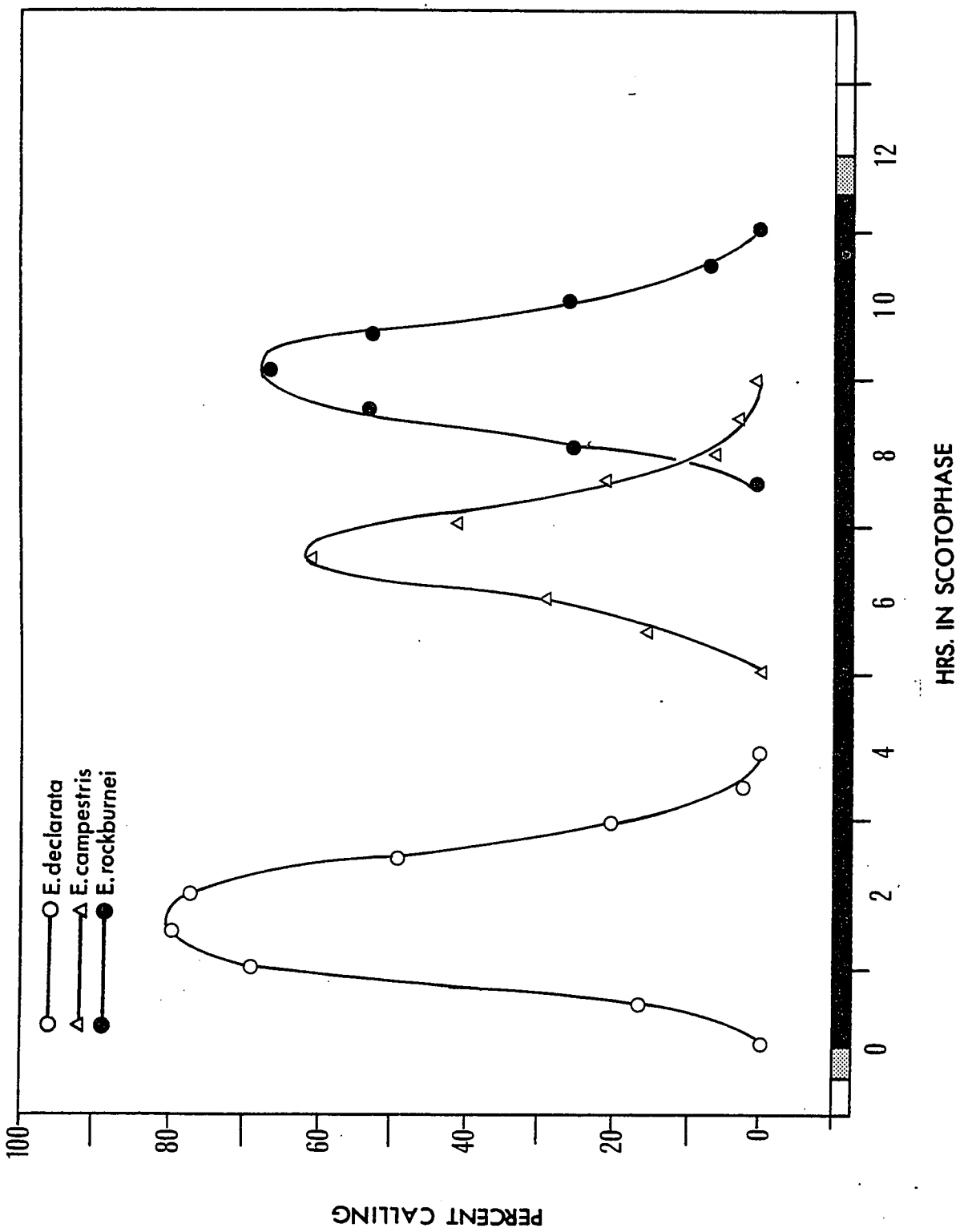

E. declarata
E. campestris
E. rockburnei
PERCENT CALLING
100
80
60
40
20
0
0
2
4
6
8
10
12
HRS. IN SCOTOPHASE

Figure 11: Calling periods of the species of the declarata group at 5°C.

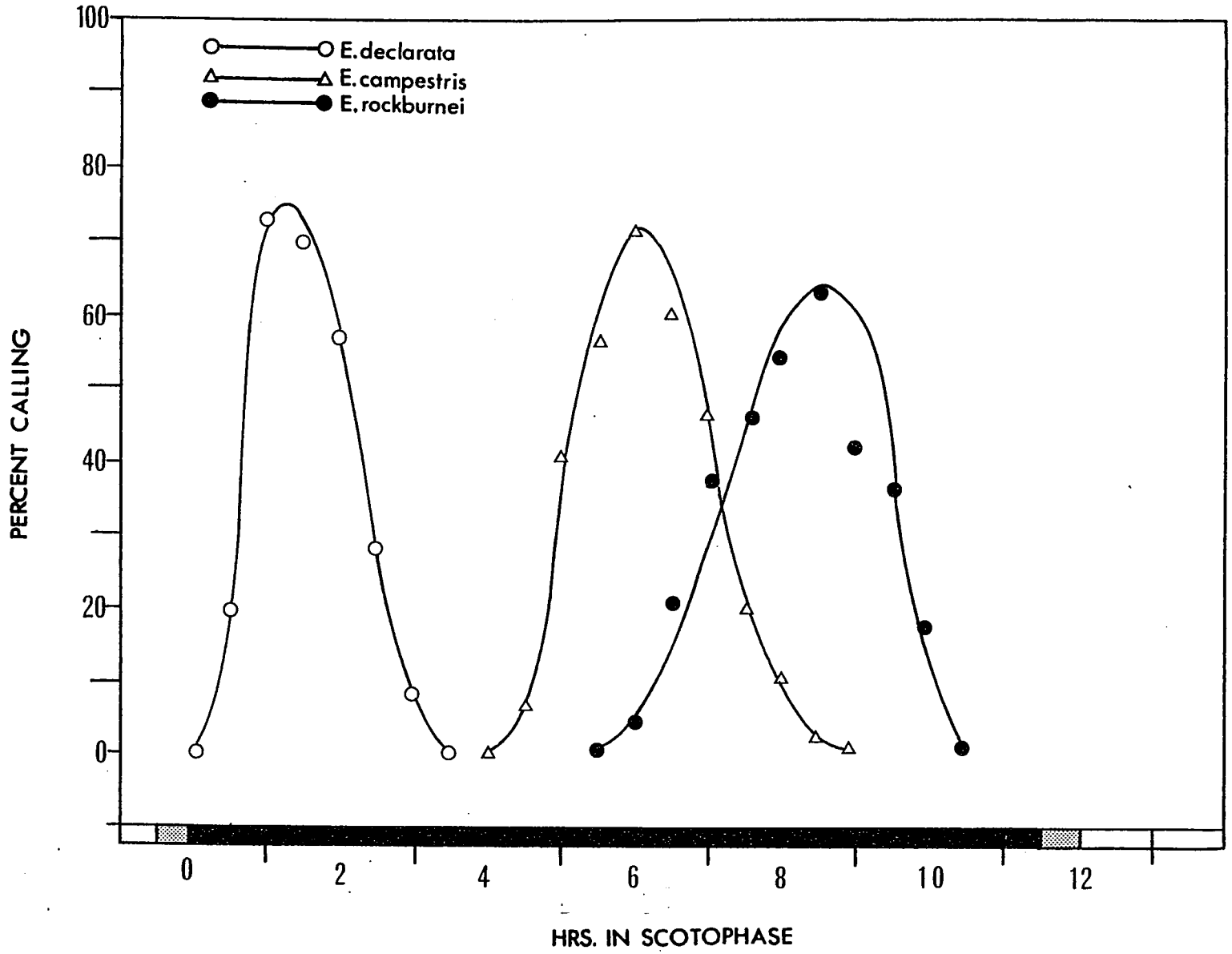

E. declarata
E. campestris
E. rockburnei
PERCENT CALLING
100
80
60
40
20
0
0
2
4
6
8
10
12
HRS. IN SCOTOPHASE

Table 9:

Values of T*

Temp	declarata	campestris	rockburnei
20	2.3	7.7	10.3
15	2.3	7.3	9.8
10	1.7	6.7	9.0
5	1.5	6.1	8.1

* $T = \frac{\Sigma[\#\ \text{Calling at hr}) \times (\text{hr})]}{\Sigma\#\ \text{Calling per night}}$

Table 10

Regression equations of "T" with respect to temperature

Species	Regression equation	Correlation coefficient
declarata	Y = 1.22 + 0.06X	r = 0.92
campestris	Y = 5.6 + 0.11X	r = 0.99
rockburnei	Y = 7.48 + 0.15X	r = 0.85

Figure 12: Linear regression of the mean maximum times of calling as a function of temperature for the species of the declarata group.

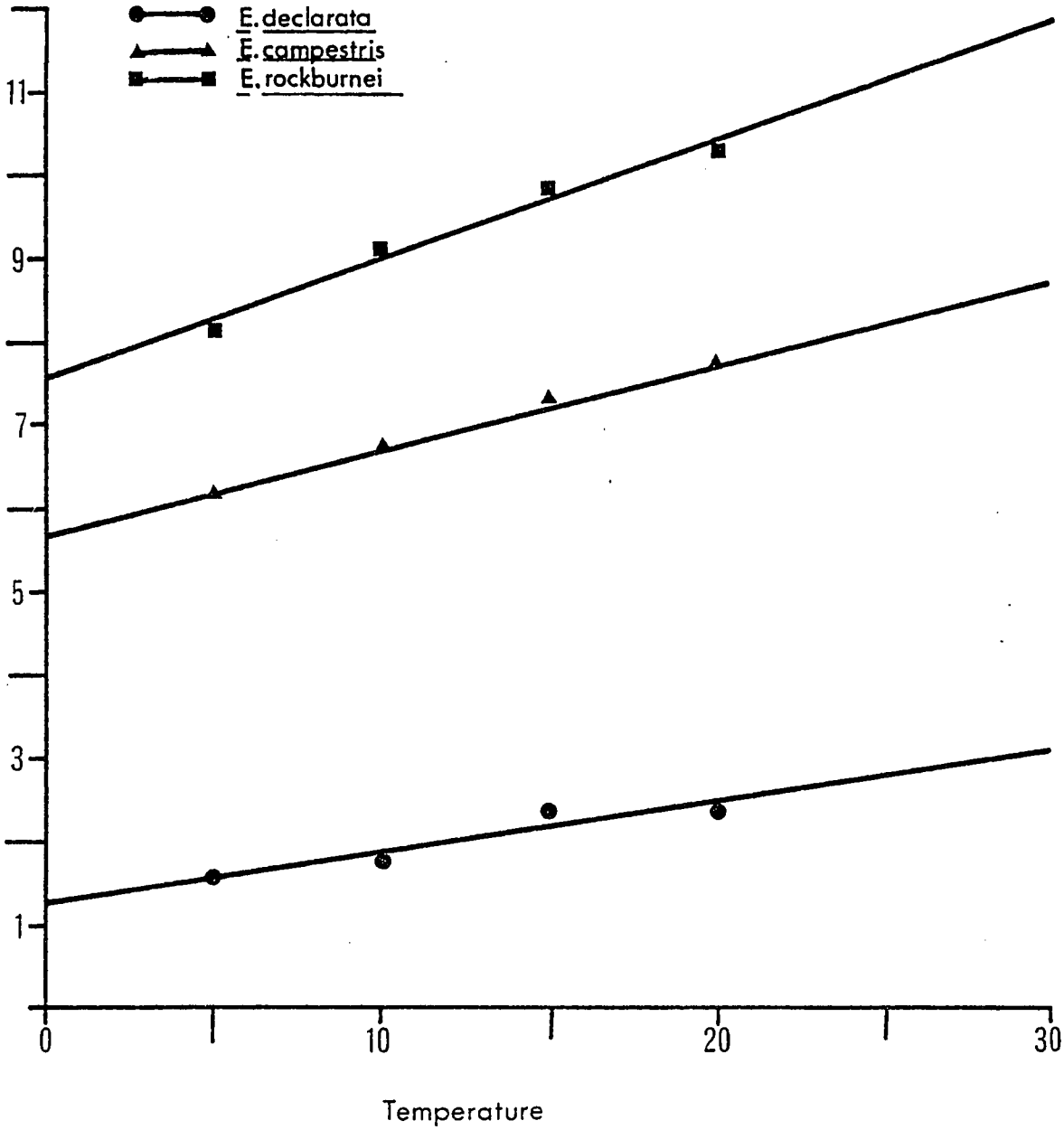

E. declarata
E. campestris
E. rockburnei
Mean Maximum Time of Calling (T)
11
9
7
5
3
1
0
10
20
30
Temperature

Table 11 Mean Percentage of Virgin Females Calling

3 days

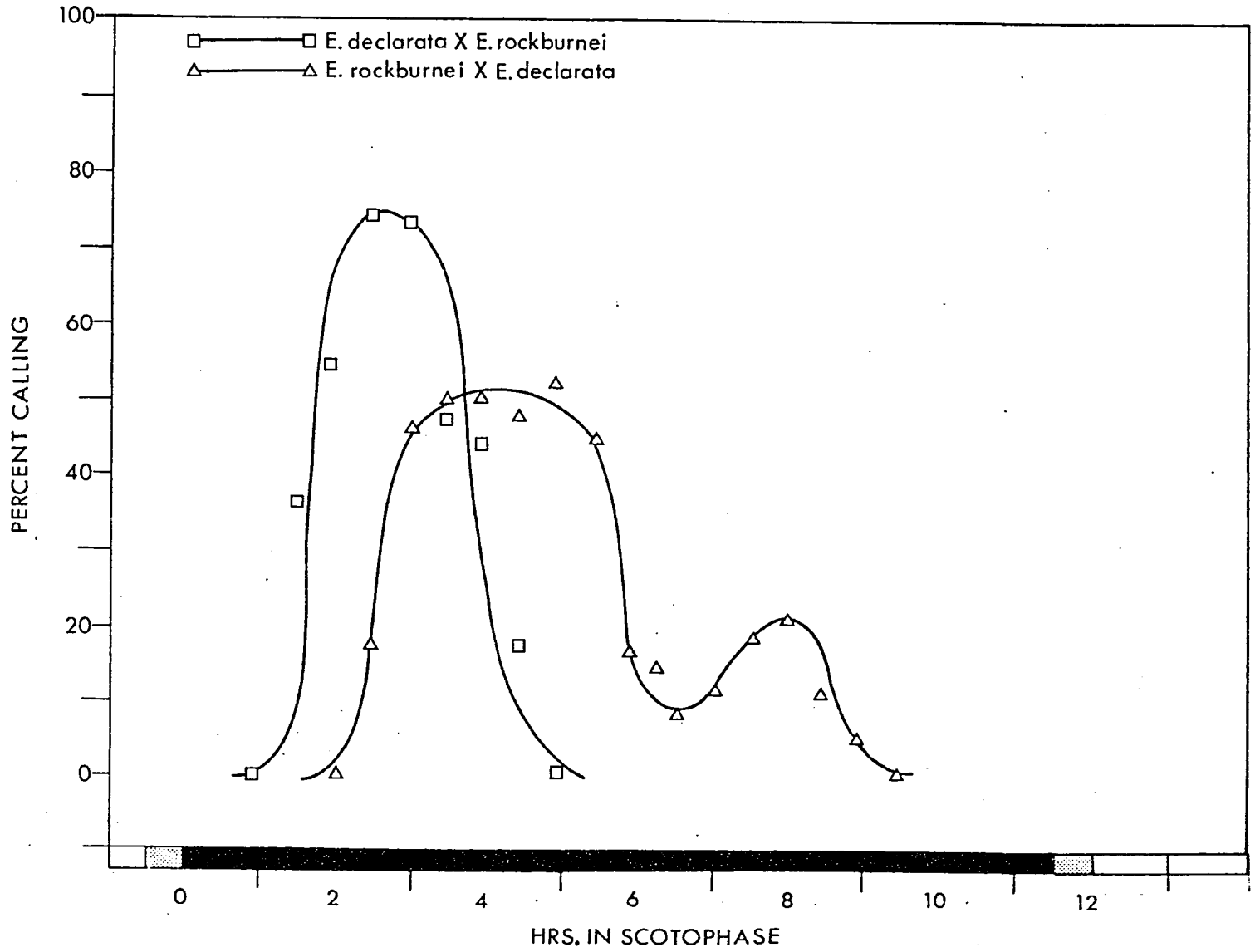
Time in Scotophase	*E. declarata*				*E. campestris*			*E. rockburnei*	
	Stock 1 N=60	Stock 2 N=84	Stock 3 N=30	Stock 4 N=30	Stock 1 N=93	Stock 2 N=90	Stock 3 N=48	Stock 1 N=54	Stock 2 N=60
Dusk									
0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	6.7	0	0	0	0	0
1.0	47.0	31.8	30.0	20.0	0	0	0	0	0
1.5	62.7	45.5	43.3	33.3	0	0	0	0	0
2.0	59.7	78.6	53.3	46.7	0	0	0	0	0
2.5	36.9	53.6	60.0	26.7	0	0	0	0	0
3.0	27.1	39.3	46.7	10.0	0	0	0	0	0
3.5	16.7	17.9	40.0	3.3	0	0	0	0	0
4.0	6.9	7.2	36.7	0	0	3.5	0	0	0
4.5	0	3.6	30.0	0	0	3.5	0	0	0
5.0	0	0	6.7	0	3.2	3.5	0	0	0
5.5	0	0	0	0	3.2	3.5	0	0	0
6.0	0	0	0	0	19.4	34.6	10.9	0	0
6.5	0	0	0	0	67.7	80.7	18.8	0	0
7.0	0	0	0	0	98.4	77.4	34.4	0	0
7.5	0	0	0	0	98.4	93.6	57.8	0	0
8.0	0	0	0	0	90.3	64.5	53.1	0	0
8.5	0	0	0	0	83.9	29.0	35.9	0	13.4
9.0	0	0	0	0	70.9	34.6	34.4	0	13.4
9.5	0	0	0	0	32.3	3.5	21.9	30	26.7
10.0	0	0	0	0	29.0	0	9.4	66.7	36.7
10.5	0	0	0	0	29.0	0	6.2	80.0	55.0
11.0	0	0	0	0	0	0	0	93.3	30.0
11.5	0	0	0	0	0	0	0	15.6	3.3
Dawn	0	0	0	0	0	0	0	0	0

Table 12

Mean Percent Calling By Hybrid Females

Time	E. rock X E. dec (N=135)	E. dec X E. rock (N=108)
0	0	0
0.5	0	0
1	0	0
1.5	0	35.9
2	0	54.4
2.5	0	73.9
3	16.4	72.2
3.5	45.7	46.1
4	50	44.5
4.5	50	17.8
5	47.8	0
5.5	52.2	0
6	44.9	0
6.5	17.9	0
7	15.7	0
7.5	6.6	0
8	10.9	0
8.5	17.9	0
9	20.2	0
9.5	6.5	0
10	4.4	0
10.5	0	0
11	0	0
11.5	0	0
12	0	0
12.5	0	0

Figure 13: Calling periods of E. declarata x E. rockburnei and E. rockburnei x E. declarata at 20°C.


E. declarata X E. rockburnei
E. rockburnei X E. declarata
PERCENT CALLING
100
80
60
40
20
0
0
2
4
6
8
10
12
HRS. IN SCOTOPHASE

II General Morphology

The posterior tip of the abdomen of Euxoa females is normally retracted, being held within the 7th segment, and is extended during sex pheromone release and egg laying. The integument of these segments is devoid of scales, but setae and microtrichie are present on the segments and intersegmental membranes (Figs. 16, 17).

The 2 terminal abdominal segments, 9 + 10, are fused with the paired dorsal valves (=papillae anales or supraanal lobes of Matsuda (1976)), extending from posterior dorso-lateral origins (Figs. 16, 17). The dorsal and lateral surfaces of the valves have numerous setae (see Mcdunnough 1950, Ryabov 1950, Hardwick 1970) which communicate with neuronal and glandular cells beneath. The posterior apophyses arise from the anterior lateral surfaces of the valves (Figs. 16, 17).

The anus and ovipore open between the dorsal valves, the ovipore being surrounded by an evagination of the terminal segment (Figs. 14, 19, 20). The positions of the anus and ovipore are similar to that described by Imms (1957) in ditrysian Lepidoptera, the anus opening dorsal to the ovipore (Fig. 14).

The copulatory opening is situated mid-ventrally in the anterior portion of the 8th segment (Mcdunnough 1950), being formed by a sclerotized subgenital plate and an arch of cuticle. This plate represents the modified 8th sternite extending into the ductus bursae and lies anterior to a mid-ventral groove of unsclerotized cuticle (Figs. 18, 19) which serves as a guide for insertion of the penis. The arch of sclerotized cuticle is formed by the

anterio-ventral fusion of the 8th tergite and is not continuous with the 8th sternite (Fig. 17). Paired anterior apophyses arise from mid-lateral extensions of the 8th tergite (Figs. 14, 19) and retractor muscles arising laterally in the anterior 7th segment are attached to the apophyses. The posterior apophyses extend through the 8th segment and terminate in the 7th, where they are inserted by another series of retractor muscles.

Cuticle

The cuticle of the ovipositor is composed of a uniformely thick epicuticular layer and endo- and exocuticular layers of variable thickness. The tergite and sternite of the 8th segment are heavily sclerotized, having a thick exocuticular component and thin endocuticle. The cuticle of the remaining areas of this segment is not heavily sclerotized, having exo- and endocuticular components of approximately equal thickness (Fig. 21). The intersegmental membrane, between segments 8 and 9 + 10, is wide and highly flexible, being composed of a thick endocuticular layer and a thin unsclerotized exocuticle (Figs. 14, 19, 23). Tergites of segments 9 + 10, found on the dorsal and lateral surfaces (Fig. 14, 22) are fused and heavily sclerotized with a thick exdocuticle and thin endocuticle. The cuticle of the ventral areas of these segments is similar to that of the unsclerotized areas of the ventral 8th segment, as endo- and exocuticular components are of similar thickness. The exocuticle of the dorsal valves is heavily sclerotized and very thick. Beneath this lies a very thin endocuticular layer.

Skeletal Musculature

Muscles originating from the anterior margins of segment 7 (Fig. 24) insert on the anterior margins of the 8th tergite and the anterior termini of the anterior apophyses. These muscles function, in conjunction with muscles extending from mid-lateral origins in the 7th segment to the anterior lateral margins of the 8th tergites, in the retraction of the 8th segment.

Muscles involved in retraction of the intersegmental membrane between segment 8 and segments 9 + 10 are of 2 types (Fig. 24). The first extend from origins on the mid-lateral anterior margin of segment 7 and insert on the anterior termini of the posterior apophyses. These muscles are similar to those inserting on the anterior termini of the anterior apophyses, both groups representing modified paradorsal muscles (see Snodgrass 1935). The second type extend from the anterior termini of the anterior apophyses and insert on dorsal, lateral, and ventral areas of the intersegmental membrane, including an insertion on the cuticle above the pheromone gland, the 9 + 10 segments, and the dorsal valves. During retraction these muscles function in unison.

Protractor muscles of the 8th segment have their origins on the mid-dorsal and mid-ventral posterior margin of the 7th segment and insert on the anterior termini of the anterior apophyses (Fig. 16). A far more extensive group of protractor muscles function in extension of the 9th + 10th segments. These muscles extend from lateral origins on the posterior margin of the 8th tergite and extend to the anterior termini of the posterior apophyses. In

addition to their function in extension of the terminal segments these muscles control upward and downward distortion of the ovipositor during pre-oviposition digging.

Oblique muscles of the 9^{th} + 10^{th} segments are divided into 2 groups. One group is made up of the dorso- and ventro-lateral compressor muscles (Fig. 25), extending from a mid-dorsal and /or mid-ventral apodeme and inserting on the origins of the posterior apophyses. Independent contraction of these muscles cause dorso- and ventro-lateral movements of the terminal segments during digging. The other group is composed of lateral compressor muscles (Fig. 25) which extend between the origins of the posterior apophyses, either above or below the oviduct. Independent contraction of these muscles cause either dorsal or ventral displacement of the dorsal valves and terminal segments during digging. Simultaneous contraction of both groups of compressor muscles probably aid the quasi-circular muscles inserted on the oviduct in squeezing eggs through the oviduct during oviposition.

Two groups of tergo-sternal compressor muscles are present at the origins of the dorsal valves (Fig. 26). The first group extend ventro-laterally from the relatively unsclerotized cuticle of the dorsal 9^{th} + 10^{th} segment, inserting on the heavy mid-ventral cuticle of each valve. The second group extend from mid-dorsal origins in each valve to mid-ventral points of insertion (Fig. 26). Opposing muscles extend in a transverse manner through the valves, the dorsally situated ones, extending from mid-dorsal origins in segments 9 + 10 to dorso-lateral insertions on each valve, while ventral transverse muscles extend between the anterior origins

of each valve.

Muscles associated with posterior areas of the valves are tergosternal in their attachments (Fig. 17). Muscles extending from mid-dorsal to mid-ventral points of insertion, function in the compression of the valves, while dilator muscles extend from lateral positions.

Lateral muscles attached to the cuticle surrounding the ovipore are quasi-circular in design, inserting on various points of the dorsal, lateral, and ventral areas of the cuticle. These muscles together with the muscles inserting on the oviduct, help in squeezing the eggs through the oviduct and ovipore.

Epidermal Gland Cells:

Epidermal cells of the ovipositor are normally flattened and their lateral cellular borders are indistinct (Fig. 28). Exceptions are found in: I) the ventral intersegmental membrane between segments 8 and 9 + 10 (Fig. 21) the dorsal and lateral surfaces of the dorsal valves, (Fig. 30) isolated cells located on the dorsal and lateral surfaces of the 8^{th} and 9^{th} + 10^{th} segments.

The epidermal cells of the ventral intersegmental membrane, between segments 8 and 9 + 10 (Fig. 21) are large columnar cells, similar to the "class 1" epidermal gland cells described by Noirot and Quennedey (1974). These cells are variable in size, the peripheral cells being smaller. Large central cells have an average depth of 23.5 μ and width of 10 μ. Nuclei are well defined and basal, averaging 8.9 μ in diameter. The cell matrix is granular having basal invaginations of the cell membrane and apical microvilli. Cells of pharate (1-2 days prior to adult emergence) and one-day-old females have few, if any, secretory vacuoles (Fig. 31). Between 2 and 3 days post-emergence vacuoles appear and are concentrated in the basal cell matrix (Fig. 32). From 3 to 5 days after eclosion the areas of vacuole concentration within the cell shift so that, by day 5, the major concentrations are in the apical area of the cell (Fig. 33, 34).

Another group of glandular epidermal cells is present in the epidermis of the dorsal and lateral surfaces of the dorsal valves (Fig. 35). These glands are typically setiform (Snodgrass 1926) and are similar to the modified "Class 1" epidermal gland cells

associated with secretory trichogen cells described by Noirot and Quennedey (1974). Typically 3 cells form each glandular unit: a large trichogen cell communicating with a tubular seta, a tormogen cell closely associated with the trichogen, and a modified epidermal cell (Figs. 36, 37, 38). The large trichogenous cells average 150 μ in depth and 100 μ in diameter have large basal nuclei, a dense granular cytoplasm, and contain in the apical cell area, dense osmiophilic structures resembling lipid droplets.

Isolated gland cells found on the dorsal and lateral surfaces of segments 8 and 9 + 10 are similar to the glandular cells of the dorsal valves, they are of similar size, contain large osmiophilic droplets and are associated with long tubular setae.

Figure 14: Sagital section through the terminal abdominal segments of Euxoa sp. Od-oviduct, Hg- hind gut, T7-7th tergite, Ism- intersegmental membrane, T8 - 8th tergite, T 9 + 10- fused 9th - 10th tergites, DV-dorsal valves, EV- evagination of the 9th + 10th segments, A- anus, Op- ovipore, Pg- pheromone gland, CO-copulatory opening, AA- anterior apophysis, B- bursa copulatrix., CB- cervix bursae, PA- posterior apophysis, DS- ductus seminalis.

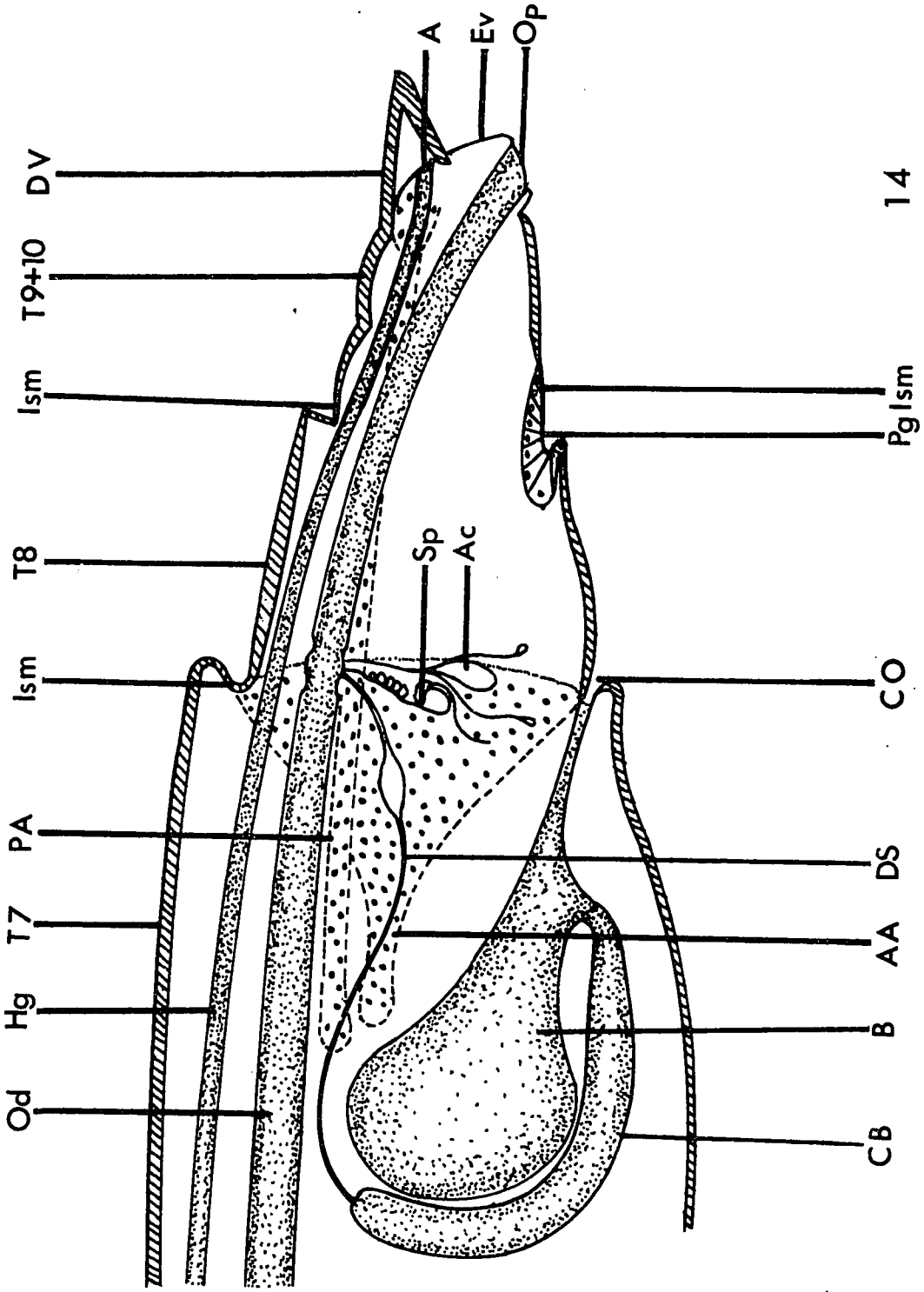

A
Ev
Op
DV
T9+10
Ism
T8
Ism
PA
T7
Hg
Od
Sp
Ac
PgIsm
CO
DS
AA
B
CB
14

Figure 15: Dorsal view of extended segments 8 and 9 + 10 in Euxoa species.

Figure 16: Dorsal aspect of the dorsal valves of Euxoa species. Note the long setae-S.

Figure 17: Lateral aspect of the terminal segments of Euxoa species Note the microtricheae (Mt), of the intersegmental and anterior 9^{th} - 10^{th} segments.

Figure 18: Ventral aspect of the 8^{th} segment of Euxoa species. Note the midventral grouve (Gr).

Other abbreviations as in Fig. 1.

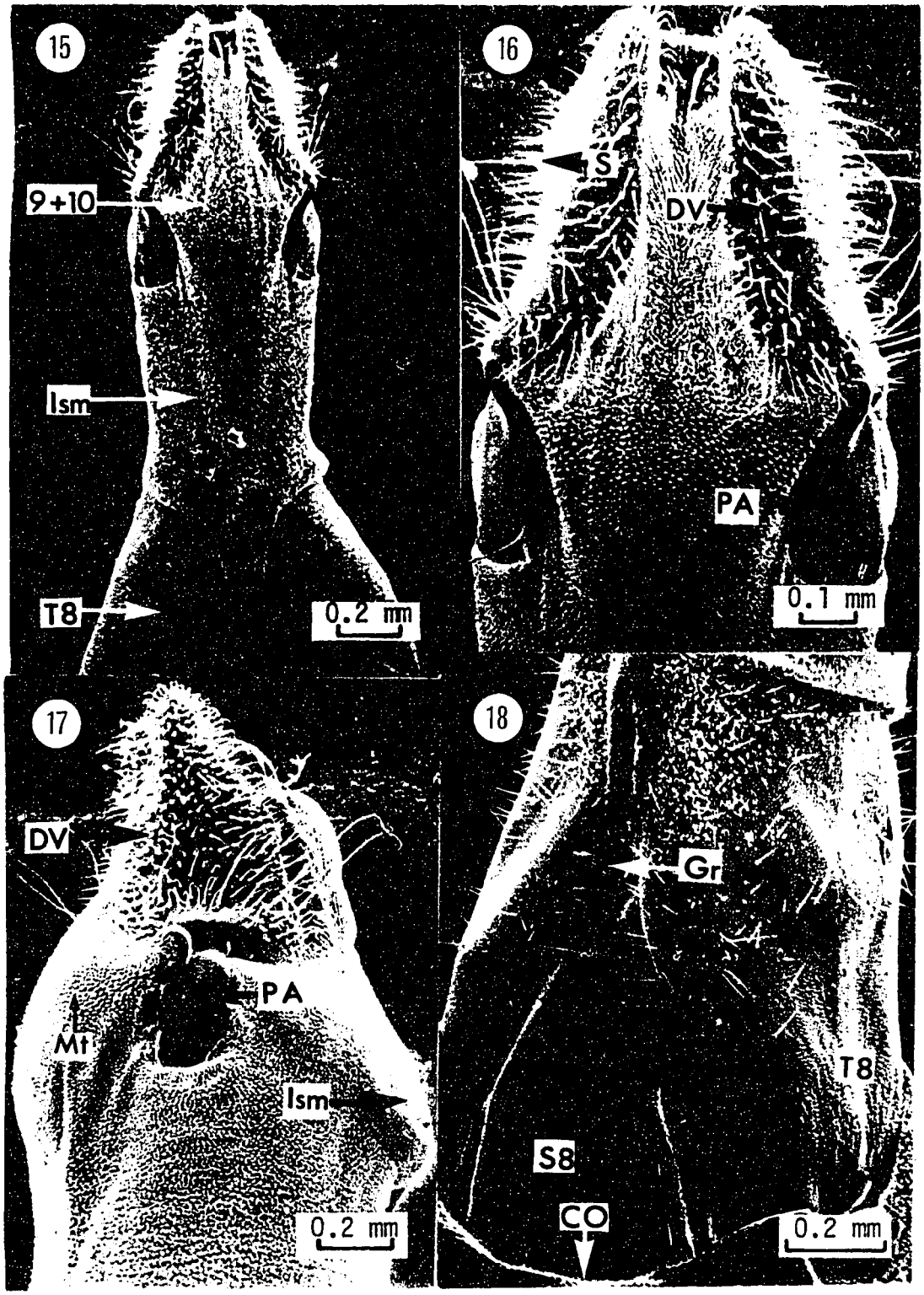
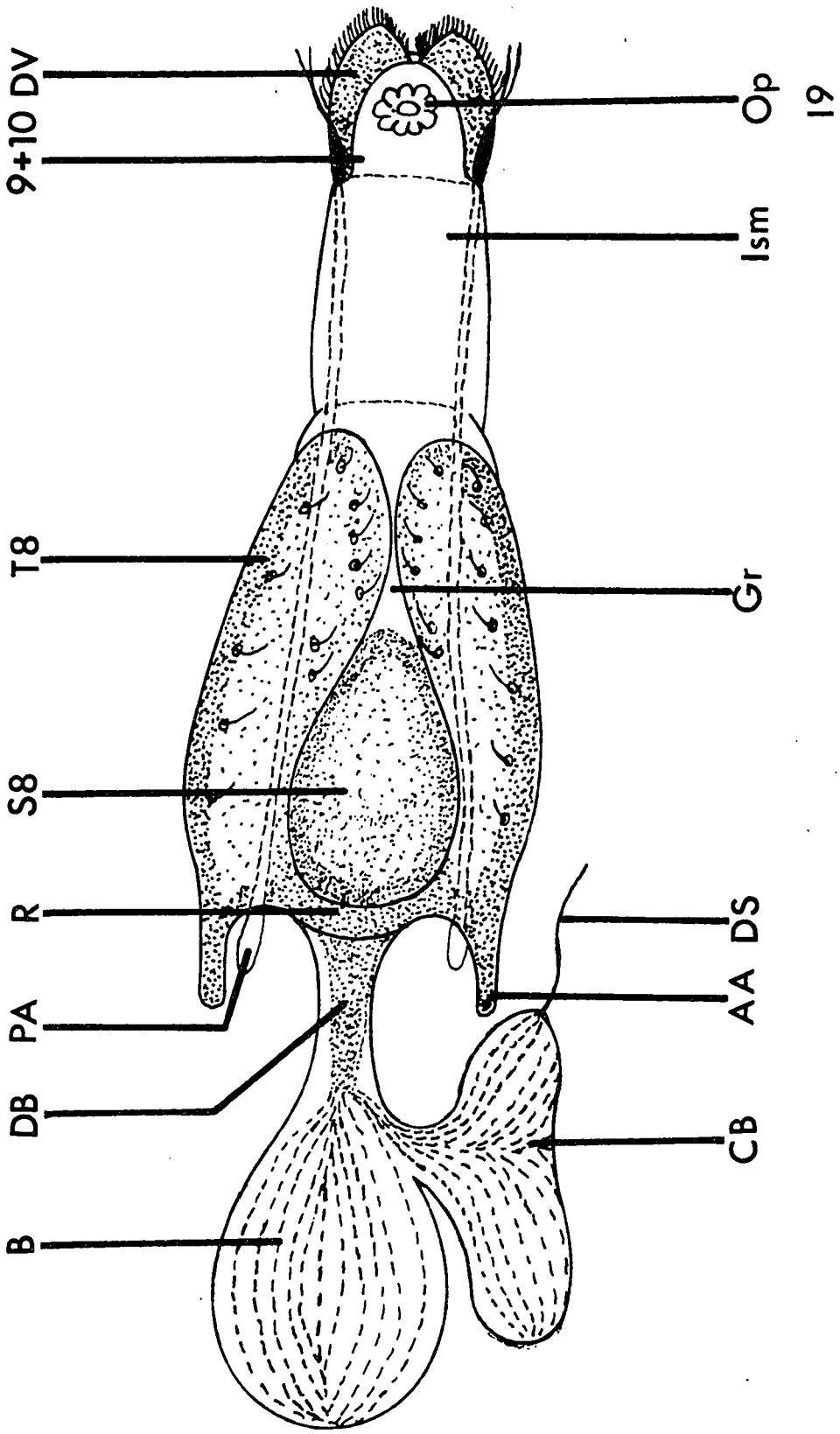

15
9+10
Ism
T8
0.2 mm
16
S
DV
PA
0.1 mm
17
DV
PA
Mt
Ism
0.2 mm
18
Gr
T8
S8
CO
0.2 mm

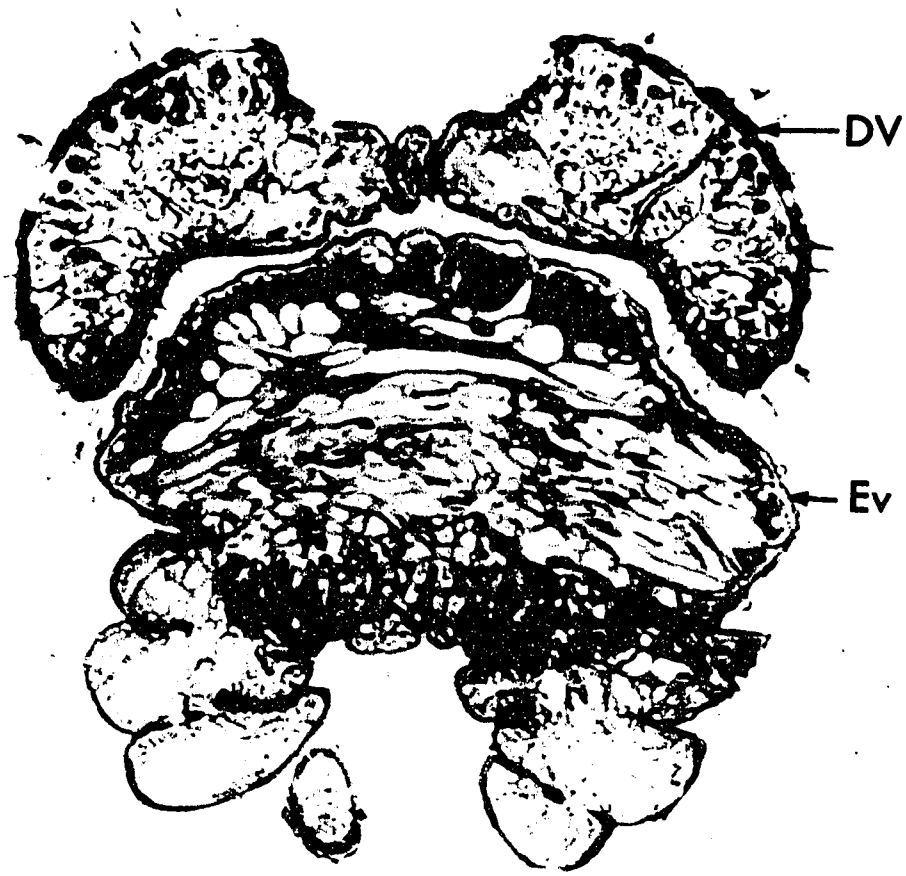
Figure 19: General ventral view of segments 8 and 9 + 10 in Euxoa. Note the extension of the 8th sternite (S8) into the ductus bursae (DB), and the anterior ventral fusion of the 8th tergites to form a ridge (R) over S8. DS-ductus seminalis.



19

Figure 20: Cross section through the dorsal valves and evagination of segments 9 + 10 in Euxoa, DV-dorsal valves; Ev-evagination.

20

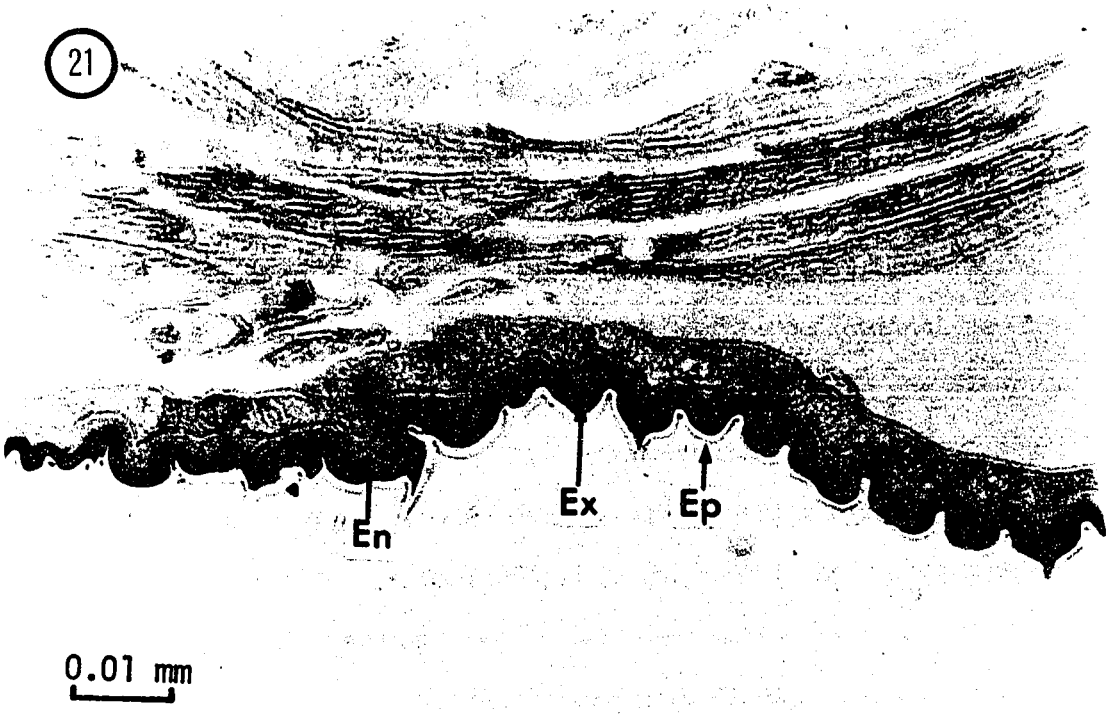


0.3 mm

Figure 21: Polychrome stained cross section through the posterior ventral area of segment 8 showing the endocuticle (En), exocuticle (Ex), and epicuticle (Ep).

Figure 22: Sagittal section of anterior part of the 9^{th} tergite. Notice the thick exocuticle.

Figure 23: Cross section of intersegmental membrane between segments 8 and 9 + 10. Notice the thick endocuticle.


21
En
Ex
Ep
0.01 mm

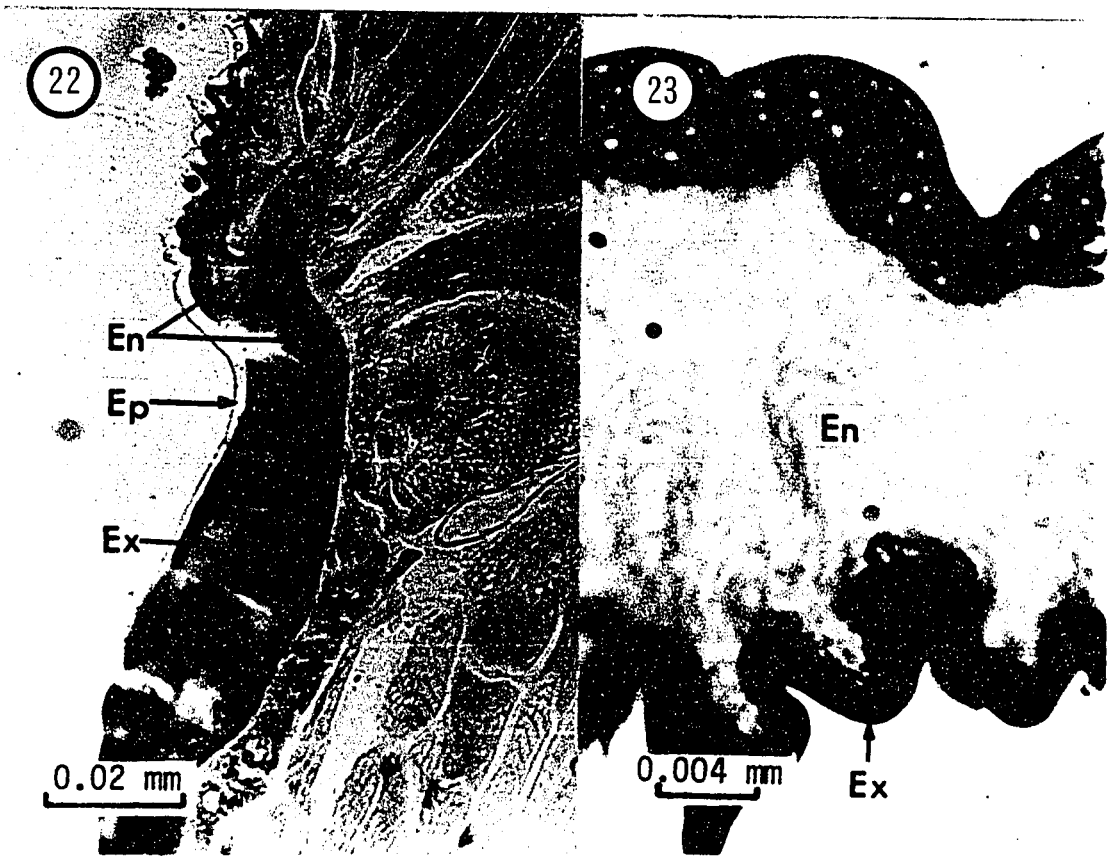

22
En
Ep
Ex
0.02 mm
23
En
0.004 mm
Ex

Figure 24: Longitudinal musculature in the ovipositor of Euxoa species (Lepidoptera:Noctuidae). Abdominal segmentation denoted by Roman Numerals-VII, VIII, and IX + X; Dorsal valves-DV; Intersegmental membrane-Ism; Anterior apophysis-AA; Posterior apophysis-PA. Numerical description of the musculation is as follows: I-Longitudinal retractor of segments IX + X, originates from the anterior margin of tergite VII; 2-Longitudinal retractor of segment VIII, originates from the anterior margin of tergite VII; 3-Longitudinal retractor of the anterior apophysis, arises from a lateral origin on the anterior margin of tergite VII; 4-Longitudinal retractor of tergite VIII; 5-Longitudinal protractor muscle of segment VIII; 6-Longitudinal protractor muscles of the Ism and segments IX+X; 7-Ventro-lateral longitudinal retractor muscles of the Ism and segments IX+X; 8-Dorsal longitudinal retractor of segments IX+X; 9-Medio-lateral longitudinal retractor muscles of the Ism and segments IX+X; 10-Retractor of the sex pheromone gland, arises from muscle group 9; II- Interapophyseal muscle, arises from muscle group 8 and inserts on the posterior apophysis; 12-Retractor of the ovipore evagination, arises from muscle group 9. Wherever possible muscle descriptions are those of Arnold and Fischer (1977).

24

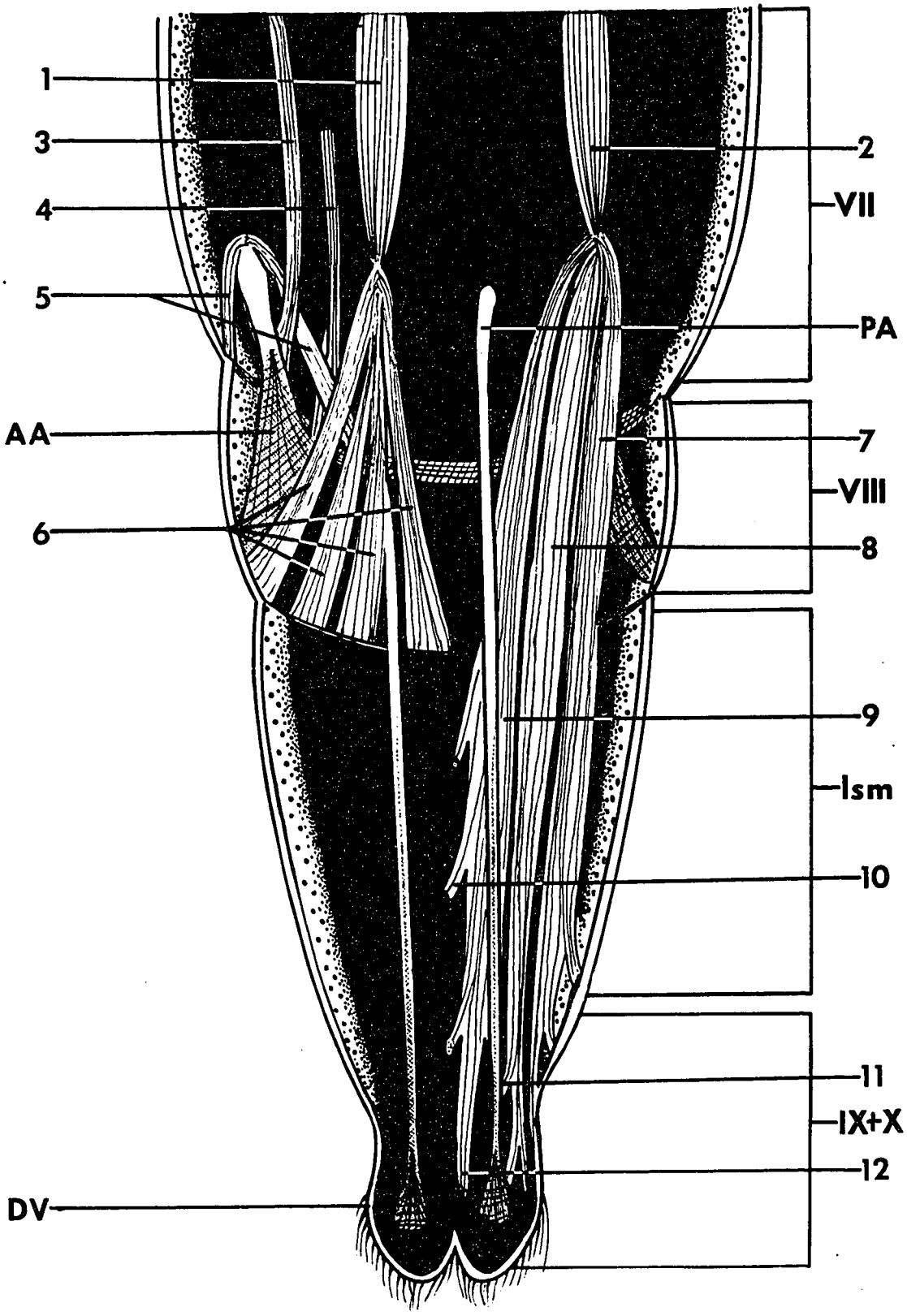
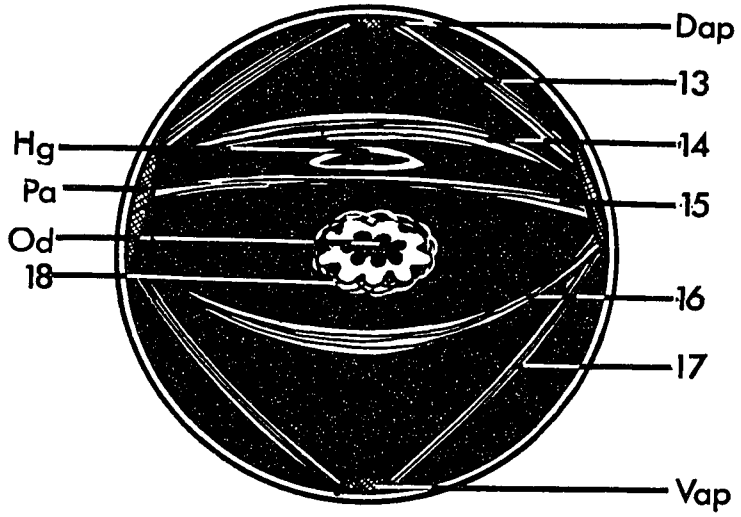


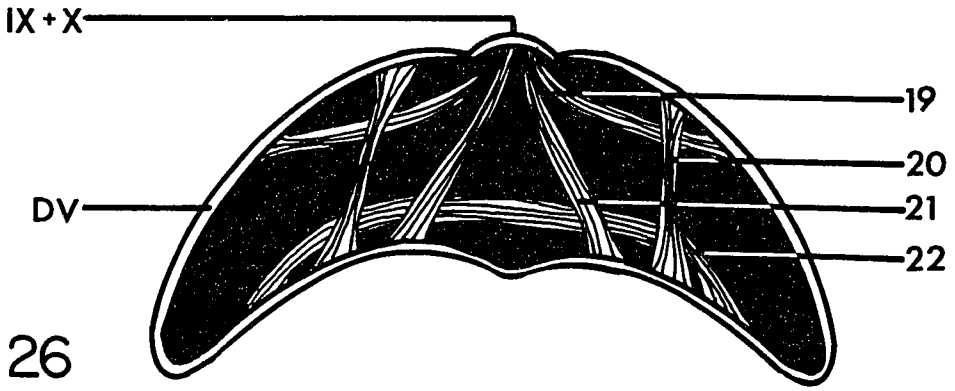
Figure 25: Cross section through segments 9 + 10 of E. declarata. Dap-dorsal apodime, H.g - hind gut, Pa - origin of the posterior apophysis. Od-oviduct, Vap-ventral apodime. Numerical description of muscles: (13) Dorso-lateral compressor muscle; (14), (15), (16) Lateral compressor muscles; (1&) Ventro-lateral compressor muscle; (18) Quasi-circular muscles inserted on the oviduct.

Figure 26: Cross section through the point of origin of the dorsal valves (Dv) on segments IX + X. Numerical list of muscles: (19) Dorso-lateral oblique muscles; function in opposition to muscles (20) and (21) which are terg-sternal compressor muscles; (22) Ventro-lateral oblique muscles.

Figure 27: Cross section through the mid dorsal valves muscles (23) and (24) are tergosternal in their insertion .



25



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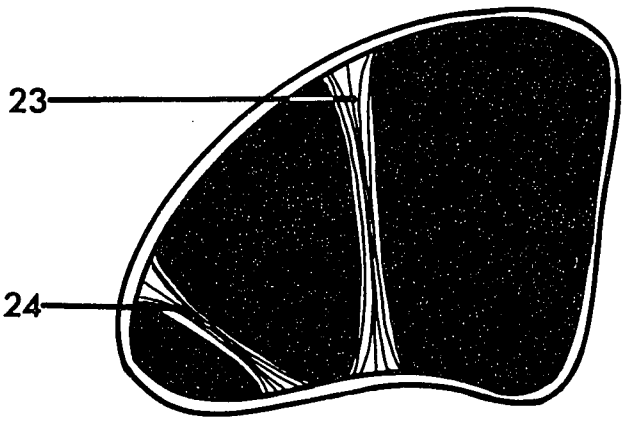
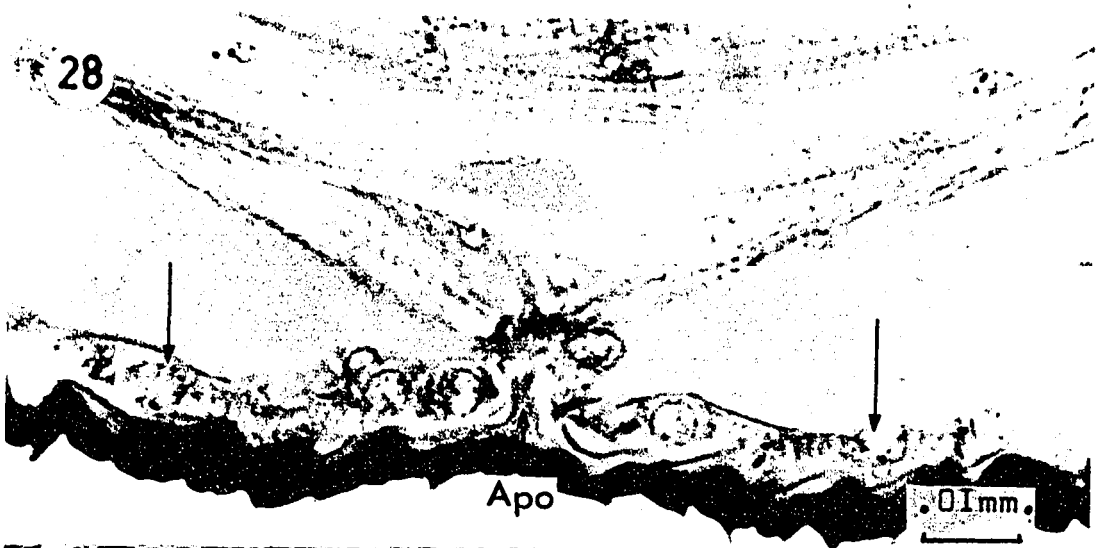
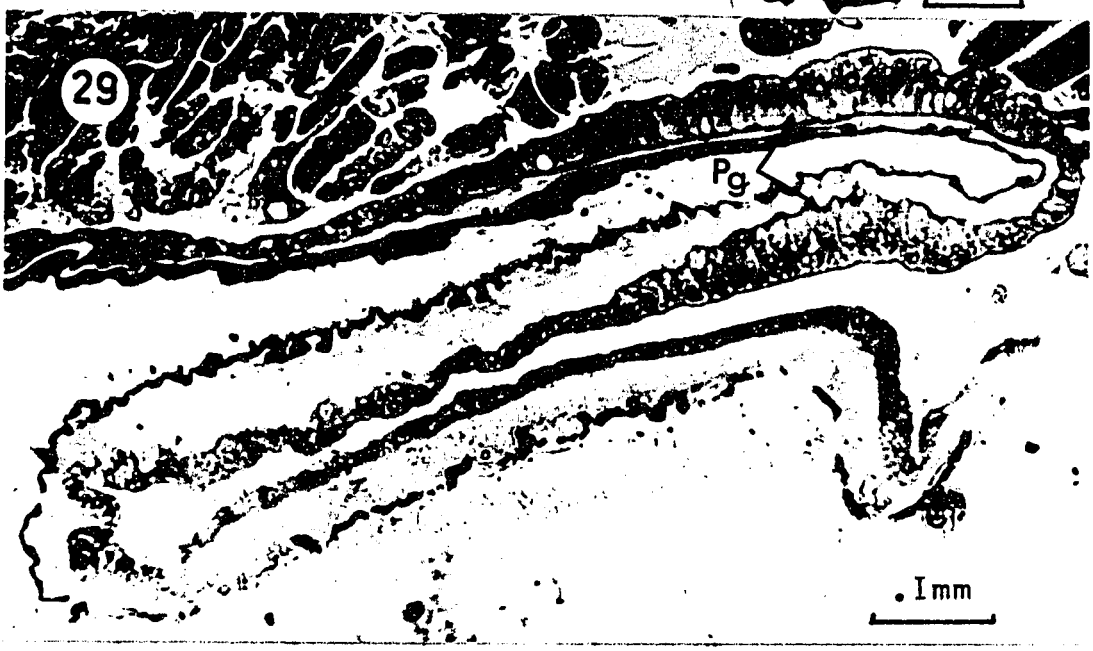


27


28
Apo
.01mm

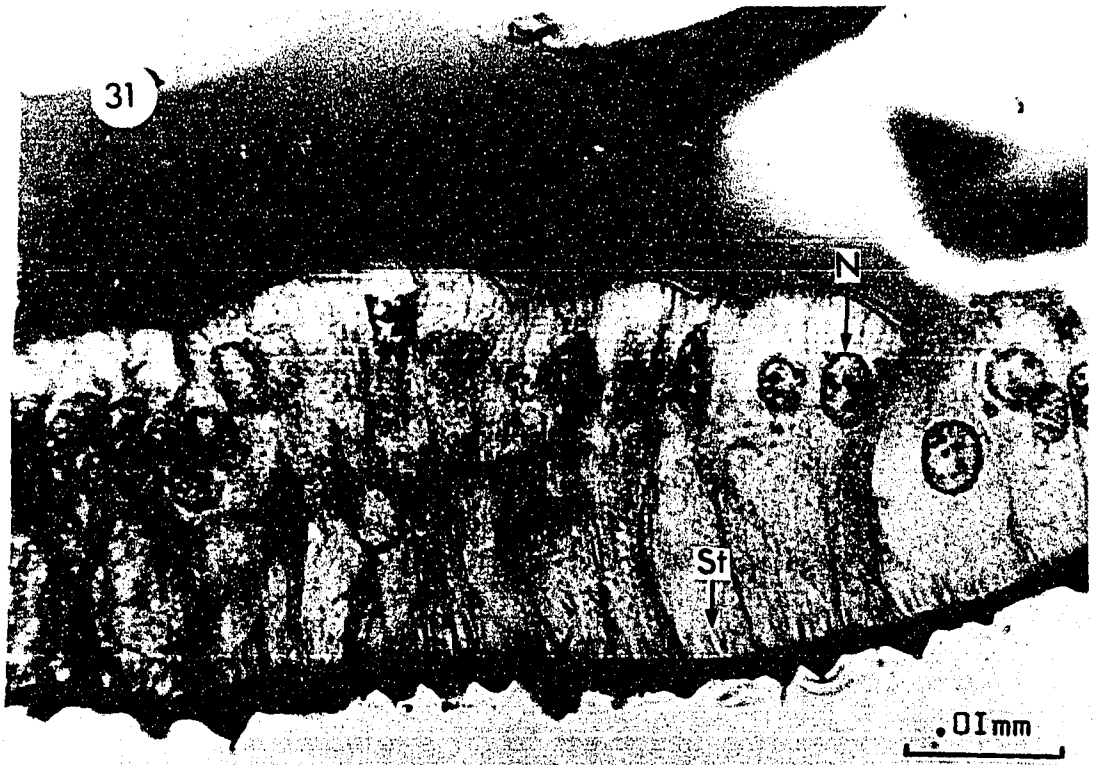

29
Pg
.1mm

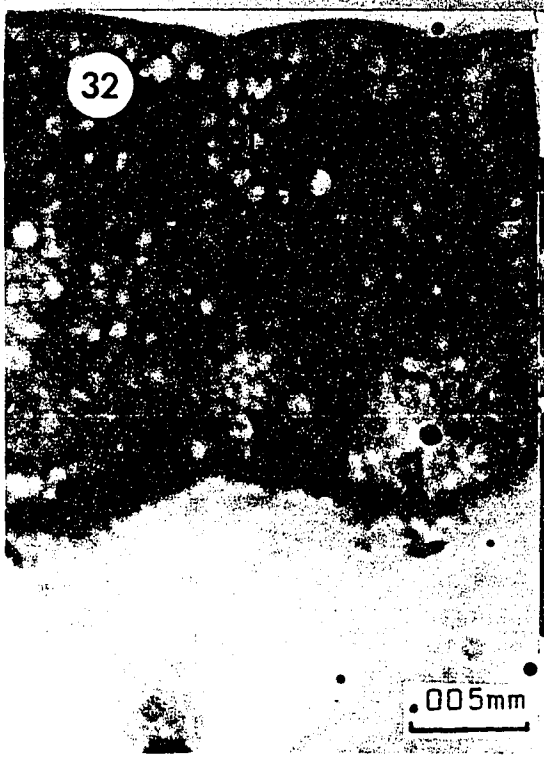

30
.1mm

Figure 31: Sagittal section through the pheromone gland of a 1 day old E. campestris: N.-nucleus, St-structures extending from the apical cell membrane.

Figure 32: Cross section through the pheromone gland of E. declarata, 3 days post emergence. Note concentration on vacuoles in basal cell Area-Arrow.

Figure 33: Cross section of a day 5 pheromone gland. Note concentration of vacuoles has shifted to apical cell area-Arrow.


31
N
St
.01mm


32
.005mm


33
.005mm

Figure 34a: Electron micrograph of the apical cell area of a 5 day old pheromone gland cell. Note the concentration of membrane bound vesicles (MV) in the apical area, and infoldings (I) of the apical cell membrane. Inset (B) shows cuticular surface structure over the pheromone gland note the microtrichea (Mt) and infoldings (arrow). These infoldings may represent the limits of individual cells (See Wakeo & Summinmoto 1969).

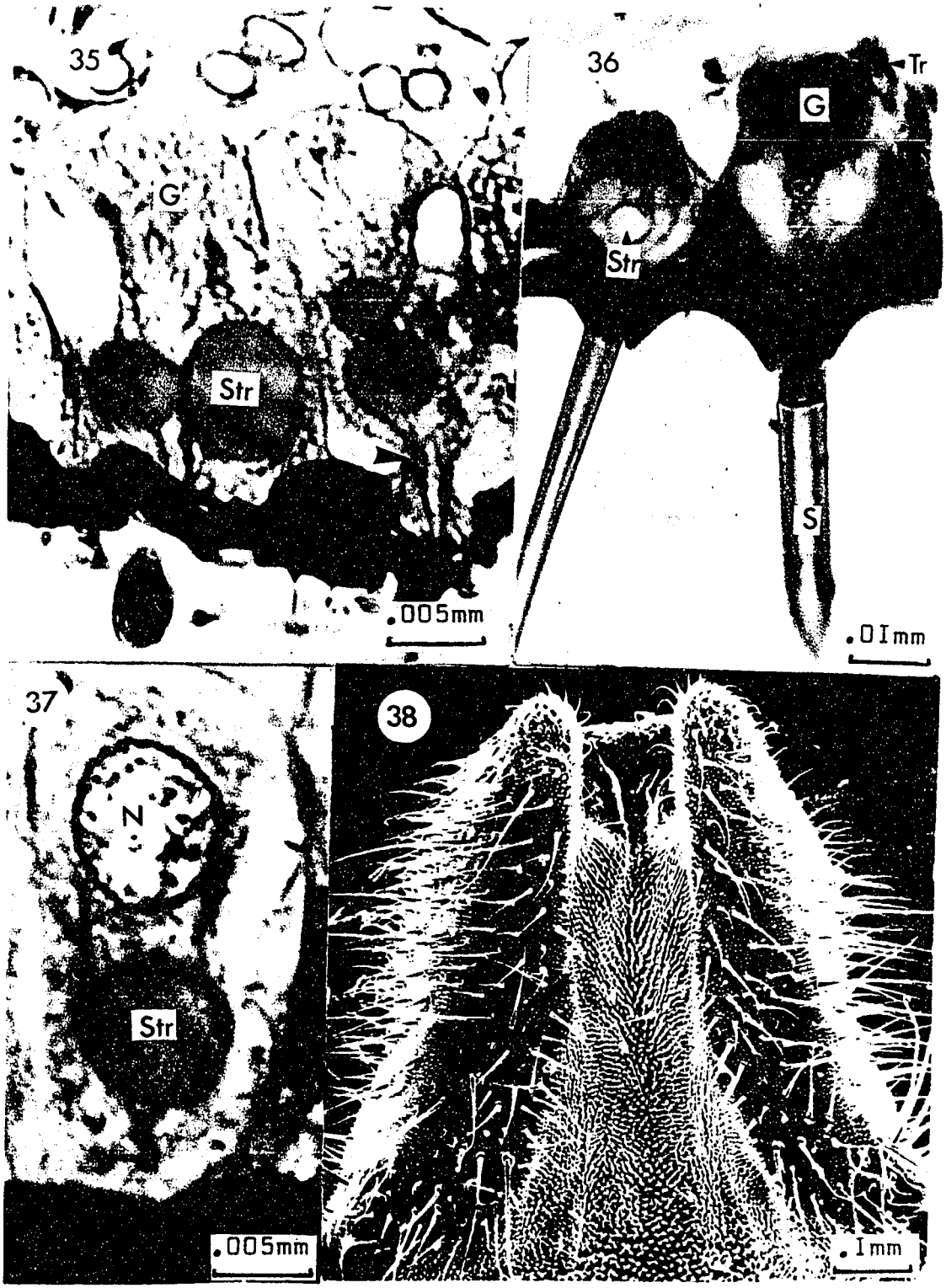

b
Mt
.005mm
34
MV
MV
MV
I
Iμ

Figure 35: Section through the dorsal valve of Euxoa declarata showing glanduar cells (G), osmiophylic structures resembling droplets (Str.). Also note the duct associated with the osmiophylic structures (Arrow).

Figure 36: Epidermal gland cells of the dorsal valve. Note the tubular setae (s) associated with glandular cells (G) Tormagen cell (Tr), and osmophylic structures (Str). This section was not post-fixed in OsO_4, the droplet-like structures remaining transparent.

Figure 37: Epidermal gland cell of the dorsal valve showing the nucleus (N) and dense osmiophilic structures (Str).

Figure 38: SEM of the dorsal valves and associated sensory and glandular setae of E. declarata.


35
G
Str
.005mm
36
Tr
G
Str
S
.01mm
37
N
Str
.005mm
38
.1mm

DISCUSSION

Pheromone Release

Of the several mechanisms operating in premating isolation of insect species, ethological isolation, which often involves different mechanisms of chemical communication, is considered the most important (Mayr 1970, Tamaki and Honma 1976). Although most closely related sympatric species are attracted to different, species-specific chemicals, pheromonal blends, or racemic mixtures (Roelofs and Carde 1974a, Shorey 1976b, Carde et al 1977), there are some co-occurring species whose attractants or pheromones are sufficiently similar to be cross attractive (Sanders 1971 a and b, Kaae et al 1973a, Roelofs and Carde 1974a, Tamaki and Honma 1976). Field release-recapture experiments have shown that cross attractancy occurs between campestris and rockburnei under natural photoperiod conditions and between campestris and declarata when the calling periods of females of the 2 species were synchronized by manipulation of the photoperiod (Byers unpublished). Also preliminary results on male responses to synthetic chemicals, using the electroantennogram technique, indicate that all 3 species respond to the same chemicals (Underhill unpublished).

When closely related cross attractive species co-occur, temporal differences in pheromone release and associated mating behaviors have been suggested as being effective in the prevention of gene exchange (Roelofs and Carde 1974a, Shorey 1974, 1976b). Most commonly, mating rhythms are not completely exclusive and function in conjunction with other mechanisms to fulfill repro-

ductive isolation (Kaae et al 1973b, Roelofs and Carde 1974a, Liebherr and Roelofs 1975, Tamaki and Honma 1976, Carde et al 1977, Grant 1977). Cases do exist, however, in which temporal isolation is proposed (Callosamia sp. Brown 1972, Ferguson 1972), or documented (laboratory and wild strains of tobacco budworm, Raulston et al 1976) as the sole component of reproductive isolation (also see Roelofs and Carde 1974a).

The calling periods of the sympatric pairs (declarata - campestris and declarata - rockbunei) are temporally exclusive at 20°C with separations of 5.4 and 8.0 hrs. occurring between the times of maximum calling. It is, therefore, not unreasonable that the temporal isolation of pheromone release should play a major role in the reproductive isolation of declarata from the other 2 species, and may, in fact, account for the mating discrimination noted by Byers and Hinks (1978). This is supported by preliminary field release-recapture, and olfactometer experiments (Byers unpublished) which indicate that the period of male responsiveness closely approximates the calling period of the conspecific females. Further support comes from the data on temperature depression which show that, at lower temperatures, the calling periods of all 3 species are advanced - an expected result which occurs in most species studied to date (Carde et al 1975a, Comeau et al 1976, Dreisig 1976, Marks 1976). But when at low temperature the calling period of campestris and rockburnei remain essentially exclusive from declarata, there being at least a 4.5 hr. difference in the mean maximum times of calling at each temperature.

The circadian periods of pheromone release by the parapatric

pair, campestris and rockburnei, are, to a large extent separate at 20°C with a difference of 2.5 hrs. occurring between the mean maximum times of calling. There is, however, an overlap between the decline of calling by campestris and the initiation of calling by rockburnei. Under field conditions the number of sexually active males in a population is considerably greater than the number of sexually receptive females because: (1) males eclose several days earlier than females and become sexually mature earlier (Byers pers. comm.) and (2) females, but not males, are less sexually active for several days after mating than virgin females (Byers 1978, Raulston et al 1975, Marks 1976 and Sanders 1975). Thus a male bias in the operational sex ratio (defined, by Emlen 1977, as the ratio of sexually active males to sexually receptive females in a population at any time) exists resulting in male competition for available receptive females, and except at very low population densities, females are expected to attract a mate soon after the calling period begins. Therefore under natural conditions, with males present, the observed intersection of calling periods is of little significance and consequently the periods of pheromone release must be considered as major of reproductive isolation. Support for this hypothesis comes from the work of Raulston et al (1976) who found that a 2 hr. difference in mating activity periods of laboratory and wild strains of tobacco budworm, Heliothis virescens, resulted in a very low degree of interstock mating when laboratory-reared moths were released into the native population. Also the rockburnei stocks available for this study originated from an area which does not support campestris and as such these

moths may have adopted an earlier calling period than rockburnei originating from areas supporting both campestris and rockburnei. Character displacement is considered by Dodson and Dodson (1976) as a major mechanism of species isolation and has been regarded as the probable cause for differences in the calling periods of European and North American strains of the gypsy moth (Roelofs and Carde 1974a).

Mechanisms of isolation act to inhibit gene flow between potentially interbreeding species, and therefore, among broadly sympatric species, ethological barriers must not only be variable between species, but also constant among different populations of the same species. All intra-specific stocks used in studies at 20°C were similar in their circadian periods of pheromone release. The best evidence for intra-specific constancy comes from data obtained from 2 stocks of declarata, one from Lethbridge Alta. and the other from Lake Louise Alta. These 2 sites have extremely different ecological conditions. Lethbridge is 920 m. above sea level, has a mean summer (May-August) air temperature of 15°C, receives 216 mm. of precipitation during the summer months, and is considered semi-arid grassland. The Lake Louise site is a montane meadow, at an elevation of 1.534 m., having a mean summer air temperature of 9.8°C, and receiving 231 mm. of precipitation during the summer (Environment Canada 1973). In the latter site the nightly temperature often falls below 10°C during the adult flight period. Obviously these 2 populations, inhabiting vastly differing environments, have undergone differential selection in order to survive in their respective niches. Characters expected to undergo niche related selection include: differences in larval

growth rates, prepupal aestivation periods, and pupation periods (Hinks and Byers 1976). However these environmental selective pressures have not affected the reproductive activity periods of these populations. Similarly stocks collected at sites 650 km. apart (declarata stocks 1 & 3) show no differences in the periodicity of pheromone release. These sites were however of similar ecological conditions. Eastern stocks of both declarata and campestris were not available for study but will provide the ultimate test for intra-specific constancy when tested.

Effects of temperature on calling:

The effects of temperature depression on both calling and male response periods have been well studied in many nocturnal Lepidoptera (Saario et al 1970, Sower et al 1971, Carde and Roelofs 1973, Roelofs and Carde 1974a, Shorey 1974, Carde et al 1975a, Gorsuch et al 1975, Comeau et al 1976, Marks 1976, Bollinger et al 1977). In all cases both seasonal and daily temperature depressions cause shifts in the reproductive periods to warmer periods of the afternoon or night. This shifting of circadian activity rhythms is by no means limited to reproductive rhythms (see Dreisig 1976) and is an adaptive response to the increased demand for metabolic energy during activity at lower temperatures (Carde et al 1975a, Marks 1976). Carde et al (1975a) suggest that although such phase shifts are advantageous for energy conservation, there might be increased difficulty in the maintenance of temporal partitioning among interfertile, co-occurring, cross-attractive species.

It is expected that if the circadian periods of pheromone release and male response are major components of reproductive isolation, then temperature-induced shifts in mating periods must still maintain sufficient separation to ensure exclusive reproductive periods. Under all temperature regimes the calling period of declarata remained distinct from those of both campestris and rockburnei, a difference of 4.5 hrs. occurring between "T" values of declarata and campestris at 5°C. Interestingly, although the difference in "T" values (ΔT) was diminished by 1.5 hrs. or more between declarata - campestris, and declarata - rockburnei as temperatures decreased, Δ T remained almost constant between campestris and rockburnei. This suggests that perhaps a minimum difference of about 2 hrs. is necessary to ensure reproductive isolation. A similar value has been reported by Raulston et al (1975) as effectively isolating native and laboratory stocks of the tobacco budworm.

Among nocturnal Lepidoptera the circadian periodicity of reproductive behavior is regulated either by the dawn phase of the preceeding light period (examples include: T. ni - Sower et al 1971, Choristoneura fumiferana (F) - Sanders and Lucuik 1972, and Argyrotaenia velutinana (Walker) - Carde et al 1975a, Comeau et al 1976), or by the initiation of the scotophase as occurs in Holomelina immaculata (Reakirt) (Carde and Roelofs 1973). In species using "lights on" as the cue for the initiation of reproductive behavior, temperature related phase shifts may advance mating periods into the photophase (Sanders and Lucuik 1972, Carde et al 1975a, Comeau et al 1976). However, among species using the dusk phase as the cue for the initiation of reproductive behavior, temperature-related

shifts never supersede the effect of the dusk phase, and reproductive cycles must occur during the scotophase (see Carde and Roelofs 1973, Carde et al 1975a). Temperature-induced phase shifts occurring in the late calling species, campestris and rockburnei, are of equal magnitude and considerably greater than the phase shifts of declarata. Shifts in the reproductive periods of the former 2 species are not limited by the initiation of the dark phase, the critical temperature below which reproductive activity ceases being reached before temperature-related shifts approach the dusk phase. Among declarata, however, phase shifts are limited by the initiation of the scotophase, and the cue for initiation of calling is the dusk phase. These species appear to be wholly nocturnal in their activity rhythms and are not expected to be seen in flight during daylight hours, unless disturbed. This nocturnal life style is undoubtedly an adaptive response developed, perhaps, as a method of predator evasion.

The critical temperature below which reproductive behaviors cease is below 5°C among the member species of the declarata group. This appears to be very low in considering critical temperatures for other species (T. ni - 11°C, Sower et al 1971; C. fumiferana - 7°C, Sanders and Lucuik 1972; H. immaculata - 16°C Carde and Roelofs 1973; D. castanea - 8°C, Marks 1976). However, unlike other species studied, members of the declarata group are autumn fliers and have distributions which extend into montane regions, where such low temperatures are often encountered, particularly in the latter part of the flight period. It is therefore imperative that these species maintain their mating activity rhythms in sufficiently high numbers to ensure mating and resultant species continuance at

low temperatures.

Calling bouts

The length of individual calling bouts and the number of bouts per calling period are species-characteristic and undoubtedly an adaptive response ensuring the greatest possible chance of conspecific mating (see Sower 1970, Sanders and Lucuik 1972, Swier et al 1977). Female declarata and campestris call for one extended bout during each scotophase for periods which encompass between 1/3 and 1/2 of the species pheromone release period. These calling bouts are, therefore, of considerable length with respect to the calling period of each species. The adaptive significance of these relatively long calling bouts is probably twofold: firstly it ensures that, in dispersed populations (probably a general condition), males have adequate time to seek out a receptive female (Marks 1976), and secondly it reinforces the species specificity of the release period by ensuring that a female will call continuously throughout a major portion of the species-specific calling and male response periods and there is a high likelihood that she will have lured a conspecific mate by the time calling ceases.

The observed differences in calling postures between declarata and the other 2 species at 20°C are not thought to be of importance in the reproductive isolation of the members of the declarata group because in these and in H. immaculata (Carde and Roelofs 1973) temperature decreases cause variation in the calling postures.

Hybrid Calling Periods

The effects of hybridization on insect reproductive behaviors have not been well studied, however, studies have been made on hybrid Ips species (Coleopera: Scolytidae) by Lanier (1970), tussock moths (Hemerocampa) by Grant et al (1975) and budworm moths (Choristoneura) by Sanders et al (1977). Results of these studies show that no simple autosomal or sex-linked inheritance system is ubiquitous throughout the insect orders and that inheritance patterns vary greatly among members of the same order.

The calling periods of the 2 hybrid groups studied show 2 distinct patterns of pheromone release. Although extended and intersecting the calling periods of both parent species, female rockburnei x declarata (F_1) hybrids are definitely more closely allied with declarata than rockburnei, the major activity peak occurring early in the scotophase. Females of the reciprocal cross, declarata x rockburnei (F_1), have a distinctly different calling period which corresponds almost exactly with that of the maternal declarata stock and with apparently no influence of the paternal species, rockburnei, on this hybrid's calling periodicity. Although parental stocks of both hybrid groups were the progeny of only 2 field-collected females and the results at best of observational significance (no chromosome mapping studies or studies on F_2 stocks and backcrosses have been made) two interesting hypotheses have arisen. The first is that calling early in the scotophase may be a dominant characteristic but the maternal female may exert some control over calling periodicity. Therefore when declarata constitute the maternal parent, calling would be expected to occur in the early part of

the scotophase because both dominance and female control would favour such a system. If, on the other hand, rockburnei is the maternal parent stock one would expect an extension of the calling period so as to incorporate the rockburnei period, while still having the largest percentage of synchronous calling early in the scotophase. The second hypothesis is that in the event of naturally occurring hybridization, the progeny might be preferentially absorbed into the declarata stock due to the early peak periods of both hybrid groups. Introgressive hybridization is well documented among plant species and among certain animal species, including some butterflies and moths (Dodson and Dodson 1976). It remains to be seen experimentally how valid these two hypotheses are.

Evolutionary and Phylogenetic Considerations:

That the 3 species of the declarata group are closely related is an unquestionable certainty. However the actual phylogenetic relationships existing within the group are still rather vague.

The data from this study on reproductive periodicities show that the sympatric pairs (declarata-campestris and declarata-rockburnei) have distinctly separate mating periods, while the parapatric pair (campestris-rockburnei) are separated to a lesser degree. This tends to support the view expressed by Byers and Hinks (1978), that declarata and campestris evolved before rockburnei and have completed speciation to the point where co-existance occurs, with little if any interspecific gene flow, rockburnei, however, probably evolved from a founding stock of campestris perhaps during the Wis-

consin Glaciation and has not developed mechanisms of sufficient magnitude to enable it to exist in sympatricity with campestris. If this is correct then the development of a late calling period by rockburnei is an adaptive response to maintain isolation with it's broadly sympatric sibling, declarata, and little chance of interspecific mating between campestris and rockburnei in their zone of contact. It is probable that before rockburnei could extend it's range to encompass that of campestris further barriers to gene flow would have to develop. This could be accomplished by several methods of premating isolation including: 1) development of broader differences in calling periods; 2) development of different sex pheromones; and 3) the development of species-specific pre-copulatory behaviors.

The ovipositor

The success of any natural group of organisms is dependent upon the development of attributes which ensure survival in the organisms' niche. The mode of oviposition in Lepidoptera is geared to provide adequate food sources and protection for immature stages. This has been achieved through the development of a vast number of morphological adaptations of the female terminalia which enable them to oviposit in areas most suitable for larval growth. (Stekol'nikov 1965, 67a, 67b, Mutuura 1972, Matsuda 1976, Arnold and Fischer 1977).

Mutuura (1972), as well as Matsuda (1976), suggest that anatomical modifications of Lepidopteran female terminalia can be classified into 4 types with respect to different modes of oviposition:

(1) the hepialid type where eggs are deposited randomly near the food; (2) the typical lepidopterous type which lay eggs on the surface of the food source; (3) the eriocraniid type which oviposit in the tissues of the host plant, and (4) the tineid type where eggs are laid in crevices of food. Among Euxoa the development of anterior apophyses of moderate length extending from lateral origins on the 8th tergite, an elongated intersegmental membrane between segments 8 and 9, the elongated posterior apophyses, and anterior ventral position of the copulatory opening on the 8th segment was concurrent with the behavioral development of oviposition beneath the soil surface. Although these moths do not lay their eggs on the larval food, the larvae having evolved along polyphagous lines (Hinks and Byers 1976), the afore-mentioned developments of the ovipositor are representative of higher Lepidoptera which have evolved the "Typical Lepidopteran" terminalia of Matuura (1972).

Concurrent with the development of specialized anatomical modifications was the development of areas having a specialized cuticle, and serving specific functions during mating and oviposition. The heavily sclerotized tergites of segments 8 and 9 + 10 provide shape and rigidity to the terminal segments when extended during pheromone release and oviposition, and, when retracted, the 8th tergite acts as a sheath in which the 9th + 10th segments dorsal valves are held. The tergites of segments 9 + 10 protect the unsclerotized cuticle surrounding the ovipore during preoviposition digging. The heavily sclerotized cuticle of the 8th sternite, which has become reduced and modified to form the subgenital plate of the copulatory opening, provides the structure necessary for guiding the male intermittent

organ into the ductus bursae. The anterior position of the copulatory opening is of importance during oviposition because the ovipositor is bent downwards and the copulatory opening tightly shut, thereby preventing the ductus bursae from becoming clogged with dirt. The highly flexible cuticle of the intersegmental membrane, and cuticle of the ventral areas of segments 8 and 9 + 10 are of importance in providing flexibility necessary for extension, and retraction of the abdomen tip, in bending during pre-oviposition burrowing, and in dilation of the terminalia as eggs pass through the oviduct. Finally the dorsal valves must be rigid and heavily sclerotized as they are the primary implements of digging and the origins of the posterior apophyses, necessary for extension and retraction of the terminal segments.

Although the developments of an elongated ovipositor and specialized areas of cuticle were of importance as female Euxoa assumed the habit of oviposition in the soil, the major factor governing the development of subterranean oviposition was the evolution of skeletal musculation which had sufficient strength and structure to facilitate the downward displacement of the ovipositor into the soil.

Extension of the ovipositor from the 7^{th} segment is the result of contraction of the protractor muscles associated with both pair of apophyses and the posterior margins of the 7^{th} and 8^{th} tergites. This utilization of protractor muscles in ovipositor extension during both pheromone release and oviposition appears to be common among noctuids; similar protractor muscles have been described in several subfamilies by Jefferson et al (1966, 1968) and Jefferson and Rubin (1970).

The downward bending of the terminalia during oviposition is accomplished by contraction of the ventral longitudinal muscles, including the pheromone gland retractor muscle, which arise from the anterior apophyses. The pheromone retractor does not relax during oviposition and the gland and associated cuticle remain involuted and protected. Downward displacement into the soil is made possible by the continuous contraction of the protractor muscles extending from the posterior margin of the 8^{th} tergite to the posterior apophyses, providing the force for descent, and by repeated contraction of the dorsal longitudinal and medial longitudinal muscles which cause rapid lateral and dorso-ventral movements of the intersegmental membrane, segments 9 + 10, and the dorsal valves. Digging movements are further increased by the contraction of: (1) the oblique muscles in the 9^{th} + 10^{th} segments, causing dorso-ventral movements of these segments and the valves without movement of the intersegmental membrane, and (2) the lateral and transverse muscles of the dorsal valves which cause the valves to act alone. The final stage in oviposition begins after a space large enough to accommodate the everted ovipositor is made by the valves. Medial lateral muscles inserted on the cuticle surrounding the ovipore relax and the ovipore is evaginated via increased hydrostatic pressure. Eggs pass down the common oviduct by contraction of the quasi-circular muscles of the oviduct and cuticle surrounding the ovipore.

The pheromone gland

It is evident that the sex pheromone gland of female Euxoa species lies in the ventral intersegmental membrane between the 8th and 9th abdominal segments.

The gross morphology of this area suggests that it is the site of the female sex pheromone gland because: 1) the glandular cells fit the descriptions of sex pheromone gland cells found in other Lepidoptera (Jefferson et al 1966, 1968, Jacobson 1972, Noirot and Quennedey 1974, Baer et al 1976, Smithwick and Brady 1977), 2) the glandular cells are situated in the intersegmental membrane between segments 8 and 9, described as being the site of the sex pheromone gland in all Lepidoptera (Percy and Weatherston 1974, Weatherston & Percy 1977), with the exception of the salt marsh caterpillar, Estigmene acrea (Drury) (MacFarlane and Earle 1970), 3) the area is of the eversible sac type and similar to the sex pheromone gland of Feltia subterranea (F) described by Jefferson et al (1968), and 4) the area is fully exposed only when the female is sexually receptive.

The development of the pheromone gland has been an adaptive process, it's anatomical position evolving so as to provide a maximum area for release, thereby ensuring adequate diffusion of pheromone for male attraction, while being held in a protected position during times when the female is not calling. In species which lay their eggs on the surface of the larval host plant, such as the Tortricidae (Bronskill 1970, Percy and Weatherston 1974, Baer et al 1976), there is little need for protection of the gland as the area is never in contact with the oviposition medium, therefore the phero-

mone gland has developed on the dorsal surface of the intersegment, which provides a large surface area for pheromone diffusion. Among species which lay their eggs on, or in the soil, such as many Noctuidae (Jefferson et al 1968) and notably the Euxoa species studied, the development of a ventral pheromone gland and insertion of a retractor muscle on the cuticle overlying the gland was necessitated to insure that the pheromone gland remains involuted and protected during oviposition.

Mechanics of Extrusion:

During sex pheromone release the terminal abdominal segments are extended in a horizontal fashion from the 7^{th} segment as a result of contraction of the protractor muscles associated with the apophyses and posterior margins of the 7^{th} and 8^{th} segments. The utilization of protractor muscles in extension of the ovipositor during pheromone release appears to be common among noctuid species. Similar muscles have been described in several subfamilies by Jefferson et al (1966, 68) and Jefferson and Rubin (1970). An increase in hydrostatic pressure within the fully extended ovipositor causes the outpocketing of the mid-ventral pheromone gland. During calling longitudinal muscles inserted on the cuticle surrounding the ovipore (muscle 12) remain contracted, the ovipore being involuted. At the cessation of calling hydrostatic pressure is decreased and the pheromone gland retractor muscle (muscle 10) contracts causing the involution of the pheromone gland, after which the ovipositor is withdrawn by contraction of the abdominal

retractor muscles; the 9^{th} + 10^{th} segments normally being pulled in before the 8^{th} segment.

Gland Cell Development

The progressive vacuolation of the pheromone gland cells during adult development appears to follow a general pattern found in pheromone gland development among many species (see Jefferson et al 1966, Waku and Sumatimoto 1969, Percy 1974, Percy and Weatherston 1974, Feng and Roelofs 1977, Smithwick and Brady 1977). In other Lepidoptera this is closely associated with increasing amounts of agranular endoplasmic reticulum, related to lipid production, and increased concentrations of pheromone (Feng and Roelofs 1977, Miller and Roelofs 1977, Smithwick and Brady 1977). Pheromone production among females of the declarata group probably reaches it's peak between 2 and 3 days after adult eclosion, the vacuole concentration reaching it's peak at this stage, and, because these moths are rather long lived (adult life ca. 15-25 days), often mating several times, pheromone production must continue at high levels for several days. The presence of pore canals or other structures connecting the endocuticle with the atmosphere was not discernible with the light microscope and therefore the method by which pheromone reaches the surface is unknown. However it has been postulated that the pheromone travels through the cuticle in epicuticular filaments and to the surface either by simple diffusion across the outer epicuticle layer or through pore canals (Percy 1974, 75, Percy and Weatherston 1974, Lalanne-Cassou et al 1977). Once on the sur-

face of the cuticle evaporation into the atmosphere is undoubtedly aided by the large surface area resulting from the many folds and convolutions in the cuticle overlying the gland.

Trichogenous cells

The trichogenous cells found on the dorsal valves of female Euxoa are similar to secretory glands found in many insect species (see Noirot and Quenedey 1974, Percy and Weatherston 1974, Lawrence and Staddon 1975). Among Lepidoptera such glandular units are associated with the emission of aphrodisiac pheromones by males (Birch 1970, Percy and Weatherston 1974). The apparatus of dispersion in these cases is a brush-like group of modified hairs or scales called hair pencils. Each pencil has a large surface area and many "portholes" (Corbet and Lai-Fook 1977) through which the pheromone is released (Birch 1970, Grant 1971, Grant and Eaton 1973, Corbet and Lai-Fook 1977). In female Euxoa these gland cells secrete their contents through unmodified setae. This would seem to preclude these glands from functioning in the production of an air-borne pheromone due to their small surface area. Although the function of these secretions is unknown at present, the habit of oviposition in the soil, and the position of the glands, on the surface of the primary implements of digging, suggest that the secretion assumes some role in oviposition, perhaps acting as a lubricant, or in cleaning the ovipositor, or as some form of courtship pheromone.

Observations using the light microscope indicate the presence of a large round structure resembling a lipid droplet in the apical

area of the cell, and an apparent duct communicating with the secretory setae. Without electron microscopic observations the constitution of these organelles can only be speculative. However 2 hypotheses seem possible: (1) the organelles are membrane-bound vacuoles which move through the intracellular ducts to the apical cell membrane, fuse with it, and the contents secreted through the associated setae. This hypothesis is not supported by microscopic evidence as no organelles were seen in close association with the apical cell membrane (observations of ca. 1500 sections), (2) each organelle is the microvillar lumen of the trichogen cell which contains densely staining material (Corbet and Lai-Fook 1977). In this case the trichogen, after secreting the associated setae, withdraws the setae forming process and forms a microvillous lumen before the cell assumes it's secretory function. In both cases the setae is not continuous with the surrounding cuticle but articulates with a socket formed by the tormogen cell (Snodgrass 1926, Birch 1970, Corbet and Lai-Fook 1977).

Summary and Conclusions:

The results of this study have shown that:

1) Calling periods of the 3 species of the declarata group are essentially exclusive at temperatures of between 5°C and 20°C. Differences in the mating periods of the 3 species are broad enough to pose a barrier to gene flow between the 3 species.

2) Little variance in the species-specific calling periods were observed among populations originating from different habitats and ranges separated by several hundred Kms.

3) The member species of the declarata group are adapted for life at cool temperatures and as such activity rhythms are maintained at low temperatures.

4) The cue for the initiation of calling is the beginning of the dusk phase.

5) Females exhibit only one major calling bout per night accounting for up to 1/2 of the species-specific calling period. This is a characteristic posessing particular adaptive value: it maintains species specificity and ensures a high probability of mating.

6) Hybrid declarata♀ x rockburnei♂ females have a pheromone release period which closely approximates that of the maternal declarata stock. Female rockburnei♀ x declarata♂ hybrids exhibit an extended calling period which intersects that of both parental stocks, although the major portion of females call early in the scotophase.

7) The sex pheromone gland of Euxoa species is situated in the ventral intersegmental membrane between abdominal segments 8 and 9, and is exposed only during sex pheromone release.

8) Columnar epidermal gland cells composing the pheromone gland undergo progressive vacuolation during the first few days of adult life. Membrane-bound vesicles, most likely containing sex pheromone appear to move from the basal cell area to the apical cell border after production.

9) The presents of the pheromone gland in the ventral intersegmental membrane ensures protection of the gland during oviposition.

10) The extensive musculature of the ovipositor serves in preoviposition burrowing and also functions in extension of the ovipositor during pheromone release. The ultimate extrusion of the pheromone gland

is the result of increased hydrostatic pressure in the abdomen.

11) Trichogenous gland cells are associated with tubular setae on the dorsal and lateral surfaces of the dorsal valves. The function of large droplets contained within these cells is unknown at present.

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