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Gonotrophic Development in *Hypoderma lineatum* and *H. bovis* (Diptera: Oestridae), with Notes on Reproductive Capacity

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ABSTRACT Gonotrophic development in *Hypoderma lineatum* (Villers) and *Hypoderma bovis* (L.) is characterized by initiation and complete growth in larval and pupal stages. Developing oöcytes through six stages to the definitive egg are described. Polyotrophic ovarian development is modified; only primary and secondary complements of eggs survive and develop simultaneously. The female emerges from the puparium with its life-long complement of eggs fully developed. Capability to mate and immediately oviposit on host cattle is well suited to the short life span of the nonfeeding fly. Mean reproductive capacity of *H. lineatum* is 516 ± 54 (SD) eggs, significantly higher than the 478 ± 70 (SD) eggs of *H. bovis*. This system of ovarian development is more efficient than that of other musciform Diptera and contributes to maintenance of cattle grub populations, even when adult fly populations are decimated in areas undergoing organized control.

KEY WORDS Insecta, *Hypoderma*, gonotrophic development, reproduction

CATTLE GRUBS, *Hypoderma lineatum* (Villers) and *Hypoderma bovis* (L.), are serious pests of cattle in the Northern Hemisphere. Even under intense control pressure, *Hypoderma* populations have an unexpected ability to rebound to precontrol levels (Rich 1965, Tarry 1984, Scholl et al. 1986).

After a 7-mo migration within the host, the first instar perforates the skin of the host's back, molts to the second instar and later to the third. Larval development and growth in the "warble" or hypodermal cyst may vary from 3 to 8 wk, depending on the time of arrival to the back (Scharff 1950). The mature larva emerges from the host's skin through the breathing hole, drops to the ground, and moves to a sheltered site to form the puparium within which pupal formation and adult development occur.

Nogge (1967) and Boulard (1968) reported that differentiation and early development of female and male gonads of *Hypoderma* begin in the late second instar. Histological evidence was presented of gonadal elaboration of a mesodermic structure that formed cysts and follicles at the beginning of the third instar.

Adult *Hypoderma* have no functional mouthparts. Females, whose life span is only 3-5 d, must fly to find a mate and oviposit, using only the energy derived from larval resources accumulated during lengthy development in their bovine hosts. Females emerge with a full complement of eggs and must wait only 1 h until they have hardened to flight capability before they are able to mate

and oviposit (Bishopp et al. 1926, Mote 1928). The two sexes meet at specific aggregation sites (Gansser 1956, Catts et al. 1965).

Descriptions of the reproductive organs (Carpenter & Hewitt 1914, Mote 1929, Gooding & Weintraub 1960), mating and oviposition (Weintraub 1961), and hybridization experiments between the two species (Weintraub et al. 1968) have been reported but do not describe gonotrophic development.

The number of eggs per *Hypoderma* female has been estimated to be 400 (Gibson & Twinn 1936), 500 (Bishopp et al. 1929), and 500-800 (Scharff 1950). Many of these estimates were based on few specimens, and some (for example, 800 eggs) may have been repetitions of earlier general estimates in the literature. Evstaf'ev (1982) estimated reproductive capacities in the U.S.S.R. to be 383 and 426 eggs per female of *H. lineatum* and *H. bovis*, respectively.

Increased use of modeling of insect populations, including *Hypoderma*, has created a need for more accurate measurements of reproductive capacity and a description of the gonotrophic cycle leading to oviposition for these two species (Weintraub 1978). Here we address this need, using a technique described for gonotrophic development in other Diptera (Vogt et al. 1974, Scholl 1980).

Materials and Methods

Third instars, age unknown, of both species were manually removed from their warbles, dissected, and assigned to approximate age based on size and development of pigmentation. Larvae of *H. lineatum* that emerged from the backs of cattle were collected and held in an incubator in 50-60% RH

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at 15:9 (L:D) photoperiod and 27 and 15°C, respectively, for pupation and development to adult emergence. At least three specimens were frozen each day at -40°C for later dissection. Because of a shortage of available *H. bovis* puparia, only three female pupae of known developmental age were dissected for comparison with the developmental sequence for *H. lineatum*. Ovaries were removed from about 150 *H. lineatum* and 75 *H. bovis* larvae and stained with neutral red dye to enhance photography as described by Vogt et al. (1974) for *Phaenicia* (= *Lucilia*) *cuprina* (Wiedemann). Representative samples of ovaries were photographed and assigned to age classes by the method described by Scholl (1980) for the stable fly, *Stomoxys calcitrans* (L.). We use the terminology of the descriptions by Fraenkel & Bhaskaran (1973) and Thomas (1985) for pupal descriptions and developmental sequences.

Pupal duration was expressed as a percentage of development rather than number of hours or days, to accommodate a larger variety of rearing conditions. Data from reared puparia showed that the time span from larval exit from the host until adult emergence could range from 13 to more than 72 d, or daily rates of 7.69 and 1.39%, respectively, for the extremes of environmental conditions. The comparison of pupal characteristics and gonotrophic development was made in the context of percentage of development that had elapsed.

Reproductive capacity of each species was determined by dissection of 105 *H. lineatum* and 122 *H. bovis* females that had been reared and had oviposited in the laboratory (Weintraub 1961). After maximal oviposition in the laboratory, the flies were dissected, and counts were made of eggs remaining in the genital tract. This number was added to the total number of eggs laid to determine reproductive capacity. A Student's *t* test was performed to test differences in egg production between the two species.

Voucher specimens have been sent to the Department of Entomology, University of Guelph, Guelph, Ontario, Canada.

Results

Gonotrophic Development. Late third instars almost fully grown but still without pigmentation (Fig. 1) had ovarioles filled with granules of no obvious structure or organization (Fig. 2). When the larval cuticle began to change from white-yellow to tan-gray, two Stage 0 follicles formed in each ovariole (Fig. 3). The more proximal of the two was at first slightly larger and more differentiated, but by the time the larval cuticle began to melanize (Fig. 4), both follicles in Stage 1 were entirely enclosed, with a cell wall and nurse cells visible (Fig. 5).

When the fully melanized larva (Fig. 6) emerged from the host's back for pupariation, both follicles

in each ovariole in early Stage 2 were equal in size and stage of development (Fig. 7). Yolk deposition, which became visible at this point, was restricted to the distal 10% of each follicle.

It is very difficult to age *Hypoderma* larvae accurately; however, emergence from the host is a distinct developmental landmark, consistent for both species, with which assignment to a particular age can be based (Table 1). Shortly after pupariation, the oocytes began to enlarge and a greater proportion (20–35%) of each follicle was occupied by yolk (Fig. 8) for the remainder of Stage 2. At this stage the nurse cell chamber still was the dominant feature of the egg. The shape of the egg changed from nearly round at the beginning of Stage 2 to ovoid.

Stage 3 represented the period of greatest growth in size of the egg. By the end of this stage the expanding yolk and oocyte had reduced the nurse cell chamber to <25% of the total length of the egg (Fig. 9). During Stage 3, the nucleus of the *Hypoderma* egg moved to the middle of the follicle as the disintegrating nurse cells were pushed to the distal end. The ovoid follicle found at the beginning of this stage became distinctly elongated. At the end of Stage 3, >50% of pupal development had occurred (Table 1).

At the time the pupa entered the red-eyed pharate adult stage (Fig. 10), the oocyte entered Stage 4 and the disintegrating nurse cells still occupied <25% of the total egg length (Fig. 11). As the pupa approached 60% of its development, a feature characteristic of the *Hypoderma* egg began to form. With most of the egg volume occupied by yolk, and the nucleus often found at the margin of the follicle (Fig. 12), the proximal end of the developing chorion began to "bud" within each follicle and the attachment organ (Cogley et al. 1981) took shape (Fig. 13 and 14). This organ and the egg proper continued development through the rest of Stage 4 to the 80th percentile of pupal development.

The final 20% of pupal development was concurrent with Stage 5 egg development; here the chorion hardened, the disintegrated nurse cells were reduced to a small cluster of cellular debris, and the fully developed attachment organ reached its definitive form (Fig. 15). Even at this stage of development, eggs were still paired within each ovariole, and dissections normally revealed two ova enclosed within a thin, common follicular membrane (Fig. 16).

When the female fly emerged, the eggs began to fill the lateral oviducts and common oviduct; the attachment organ preceded the egg proper (Fig. 17). When the flies hardened to flight condition, females of both species were capable of mating and ovipositing (Fig. 18). During oviposition, the attachment organ grasped each hair, much as a sausage is placed inside a bun. *H. lineatum* normally attaches its eggs in long strings (Fig. 19), the attachment organs touching each other (Fig. 20),

Fig. 1-8. F1, follicle 1; F2, follicle 2; N, nucleus; NC, nurse cells; Y, yolk. Bar lines in Fig. 1, 4, and 6, 5 mm; all others 100 μ m. (1) Middle to late *Hypoderma lineatum* third instar, dorsal view. (2) Ovarioles from middle to late third-instar *H. lineatum*. (3) Ovarioles from late third-instar *H. lineatum* with two distinct follicles forming within each ovariole; F1 formed slightly ahead of F2; both in Stage 0. (4) Late third-instar *H. lineatum* with cuticle partly melanized. (5) Stage 1 follicles from late third-instar *H. lineatum*; both follicles of equal size and development. (6) Fully melanized *H. bovis* larva which had emerged from animal. (7) Stage 2 follicles from emergent *H. lineatum*; nurse cells and nucleus readily discernible. (8) Late Stage 2 follicles from *H. lineatum* pupa; nurse cells still evident, yolk deposition in progress.

Table 1. Development, larval and pupal descriptions, and gonotrophic development of *Hypoderma*

% development ^a		Description	Gonotrophic development
$\bar{x}$	Range		
—	—	Third instar	Stages 0–1
5	0–10	Pupariation	Stage 2
10	5–15	Prepupa	
15	10–20	Cryptocephalic pupa	
20	10–30	Phanerocephalic pupa; white-yellow eyes	Stage 3
35	25–45	Pharate pupa; yellow-orange eyes	
45	35–55	Pupal-adult apolysis; brownish red eyes; first appearance of ocelli as light orange markings to developed ocelli	Stage 4
65	50–80	Melanization of thoracic sclerites; brown eyes; setae on head becoming darker; general head color from light to dark gray	
80	75–90	Deeper head and thoracic melanization almost black on frons; hairs appear on thorax and abdomen	Stage 5
90	85–95	Silvery appearance of head and thorax as pupal cuticle separates from adult in preparation for emergence	
100	90–100	Emergence	

^a Percent development was measured from the time that grubs were collected daily in catching devices on host cattle. Variable development was observed, ranging from dark tan grubs (which do not pupariate or develop further), through completely melanized mature larvae, to formed puparia. As a result, we have accommodated ranges of development around an estimated mean.

while *H. bovis* attaches its eggs in the same manner, but singly on each hair.

Puparia, whose opercula can be easily removed, can be aged to a relatively accurate percentile of physiological development by comparison with information in Table 1. Removal of the operculum also exposes the head for ready determination of sex. Generally, a space >2 mm between the compound eyes indicates a female pupa. Puparia handled in this manner can be returned to an incubator for further maturation with no additional mortality for the remainder of their development to emergence (Weintraub, unpublished).

Reproductive Capacity. Adult female *H. lineatum* and *H. bovis* from the Montana-Alberta area had an average reproductive capacity of 515.9 and 478.2 eggs per female (SD = 53.5 and 70.4; range 377–665 and 243–634), respectively, the reverse of relative reproductive capacities of the two species given by Evstaf'ev (1982). Reproductive capacities in the two species were statistically different ($t = 4.48$; $df = 225$) at the $P < 0.001$ level. This was consistently true, although *H. bovis* females are larger and stouter than *H. lineatum* females. The increased bulk seems to be mainly the flight muscles required for *H. bovis*'s very active oviposition attack.

Discussion

Nogge (1967) and Boulard (1968) described the development of *Hypoderma* spp. gonads from sexually undifferentiated tissue in first instars through early ovarian differentiation in the second instar, to the appearance of gonocytes with precursors of follicular cells in a germarium in the early third instar. In the late third instar, with both its follicles formed, we could not discern a functioning germarium of the type normally associated with dipteran ovarian development. The sequence suggests that the germarium formed early and was absorbed

as the paired follicles developed. This unusual pattern for Diptera may have evolved with the short life span of the adult *Hypoderma* female. The developmental pattern and the absence of a germarium do not permit *Hypoderma* to form subsequent batches of eggs, as do other Diptera. *Hypoderma* also differ from other dipterans in the simultaneous development of the two follicles within each ovariole during all five stages of egg development.

The growth of eggs in the ovary, which represents mostly the accumulation of yolk, involves the utilization of much nutrient material. This material is obtained either from the daily food of the insect or from food reserves stored in the body, principally in the fat tissue. Insects that do not feed in the imaginal stage (such as *Hypoderma*) draw all of the egg material from the latter source (Snodgrass 1935).

Hypoderma, and possibly related Oestridae that do not feed as adults, can accumulate nutrients only during the feeding larval stage. Diptera that feed regularly as longer-lived females can produce a series of egg batches over time, whether autogenously or not; the later eggs are developed in the adult. These flies normally oviposit fewer primary eggs than *Hypoderma* but oviposit variable numbers of eggs in subsequently developed batches. However, vulnerable to mortality and physiological constraints, they may die before their full ovipositional potential is realized.

In contrast, the gonotrophic development of *Hypoderma* is initiated in the nutrient-rich environment of the larva and completed by the relatively protected pupa, leaving the adults free to begin quickly the oviposition sequence. Furthermore, an increased number of available eggs is made possible by the simultaneous development of two batches of eggs, even though subsequent batches are forfeited. *Hypoderma* has adapted to a brief adult life span, with active pursuit of an oviposition site on cattle hairs, an apparatus for fastening the eggs

Fig. 9-16. AO, attachment organ; DAO, developing attachment organ; DNC, disintegrating nurse cells; FM, follicular membrane; N, nucleus. Bar line in Fig. 10, 5 mm; all others 100 μ m. (9) Stage 3 follicles from *H. lineatum* with nucleus in center of egg displacing disintegrating nurse cells. (10) Red-eyed pharate adult stage of *H. lineatum*.

Fig. 17-20. (17) Whole ovary from *H. lineatum* adult; common and lateral oviducts filled with eggs, each with attachment organ presented first. (18) Ovipositing *H. lineatum* female. (19) Eggs of *H. lineatum* deposited on hair in stringlike fashion. (20) *H. lineatum* eggs on hair; eggs cleared in xylol show developed larvae.

securely (Cogley et al. 1981), and situated for direct invasion of the host by the hatching larvae (Nelson & Weintraub 1972). Gonotrophic activities that normally become a drain on adult bodily resources coincide with larval access to nutrients, the residues of which are carried forward to fuel the activities of the nonfeeding adult. Female *Hypoderma* imagoes emerge ready for immediate fertilization and oviposition and are able to infest hosts with a large number of eggs in a short time (Weintraub 1961).

All adults of Gasterophilidae and Oestridae have reduced mouthparts and cannot feed (Zumpt 1965), like the two species investigated here. Adult Cuterebridae have similar ecological behavior and host-parasitic life cycles with more developed but nonfeeding mouthparts (Catts 1982), and may provide an intermediate stage relating back to muscoid and calliphoroid roots of this specialized myiasis group (Zumpt 1965). Further study of these forms

has been initiated for possible evolutionary relationships or for determining the ecological role of oogenesis in maintenance and regulation of their populations. It would be interesting to determine if other members of these families follow similar patterns of ovarian development that featured completion within the immature insect stages and simultaneous maturation of more than a primary follicle within each ovariole.

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(11) Stage 4 follicles from *H. lineatum* pupa; disintegrating nurse cells reduced to 25% of total length, both follicles still enclosed within common follicular membrane. (12) Stage 4 *Hypoderma lineatum* follicles with nucleus at margin of cell and developing attachment organ beginning. (13) Developing attachment organs from late Stage 4 *H. lineatum* follicles. (14) Stage 4 *H. lineatum* follicle with disintegrated nurse cells reduced to <10% of egg length at distal end of each follicle. (15) Late Stage 4 follicle from *H. bovis* with disintegrated nurse cells reduced to small cluster of debris and attachment organ nearly developed. (16) Stage 5, fully developed follicles from emergent female *H. lineatum* ovary; both follicles still enclosed within follicular membrane.

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