ACCESSORY GLAND SUBSTANCE AS A STIMULANT FOR OVIPOSITION IN *AEDES AEGYPTI* AND *A. ALBOPICTUS* [1]

SISTER M. GERALD LEAHY [2] AND GEORGE B. CRAIG, JR. [3]

Insemination stimulates egg deposition in *Aedes aegypti* (Macfie, 1915; MacGregor, 1931; Wallis and Lang, 1956). This observation has led to considerable speculation on the nature of the oviposition stimulant. Gillett (1955) showed that the mechanics of mating were not responsible for the stimulus. Males which had mated repeatedly, and hence had their supply of seminal fluid depleted, failed to stimulate females to oviposit, even after frequent copulation. Burcham (1957) suggested that whether the stimulant substance is sperm or seminal fluid, it had to reach the spermathecae to provide the stimulus. He conjectured that either the glandular structure surrounding the perforated base of the spermathecae may produce the oviposition substance directly or produce a humoral material which stimulates some secretory organ of the body (corpus allatum or neurosecretory cells of the brain). He suggested as another possibility that nerve impulses from the spermathecae might be transmitted to some remote secretory organ which in turn secretes the oviposition substance.

The work of Gillett (1955) seemed to narrow the field. He speculated that oviposition could occur when the spermathecae were filled only with spermless seminal fluid. Males which apparently had exhausted their sperm after three or four consecutive matings were rested for very short periods and again mated. The females oviposited in the same period of time as normally mated females but their eggs were infertile. Gillett's data seemed in agreement with the hypothesis that a stimulant substance is produced by the male accessory gland.

MATERIALS AND METHODS. The present experiments were designed to determine the effects of the glandular substance on oviposition. Transplant of the male gland was made into the thorax of virgin females. By this method the oviposition effect of the gland could be objectively evaluated. These conditions eliminated possible nerve or humoral action of stimulated spermathecae.

Implants of the male accessory gland were made both within and between two species of *Aedes, A. aegypti* (Group A) and *A. albopictus* (Group C). The *A. aegypti* strain was from Newala and the *A. albopictus* originated in India. The female mosquitoes were given a blood meal 5 days after emergence and 2 days before transplant. At transplant the reproductive tract of virgin males was extracted into saline and the glands removed. The female was lightly anesthetized with ether and placed on her side under a stereomicroscope at a magnification of 40 diameters. A slit was made in the thorax of the female between the postspiracular sclerite and sternopleuron. The bilobed gland was inserted into the thorax with a needle and the female was transferred to a shell vial.

The controls for the experimental animals included three groups. Because Curtin and Jones (1961) have shown that there are oviposition centers in both the head and thorax of *A. aegypti,* the first group of mosquitoes were injured in the thorax in a manner comparable to that resulting from implant treatment. The second group had transplants of gut into the thorax. The third group was untreated. In normal oviposition, eggs are scattered over a substrate. It is not uncommon for

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[2] Chairman, Biology Department Mount St. Mary's College, Los Angeles, Calif.

[3] Director, Mosquito Genetics Project, University of Notre Dame, Notre Dame, Ind.

a moribund female to oviposit before death. This "death stress" oviposition (DeCoursey and Webster, 1952) results in a single mound of eggs. Since this type of oviposition is unrelated to insemination condition, these mounds of eggs were not included in the present oviposition data.

RESULTS. Implantation of the male accessory gland into females of *A. aegypti* produced increased oviposition (Fig. 1

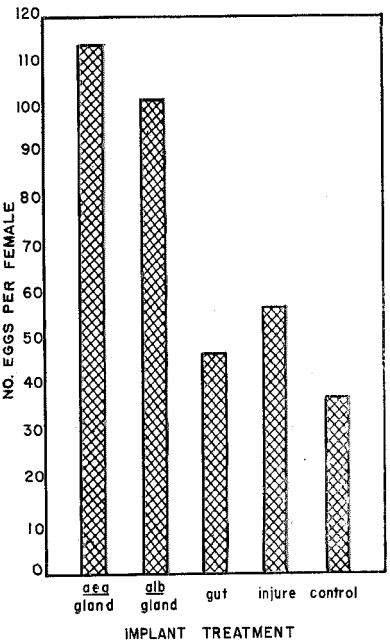

FIG. 1.—Effect of implants of various tissues on oviposition in *A. aegypti*. Eggs were collected four days after treatment. Blood meal was given two days before treatment.

and Table 1). The effect of the stimulus is seen even when the gland implanted comes from another species; i.e., *A. albopictus*. The daily record of the average number of eggs per female is shown in Figure 2. No significant effect of the implant is immediately evident following treatment. However, the stimulant effect of the implant is clearly evident on days three and four.

In mosquitoes of *A. albopictus* a striking increase in egg deposition results from homologous gland implant (Figs. 3, 4 and Table 1). However, heterologous gland transplant has no significant effect.

Survival in the experimental animals was 100 percent (94/94) on the day following transplant and 64 percent on day four. Untreated control survival was 81 percent (34/42) on day four.

DISCUSSION. These effects of gland transplant in *A. aegypti* are comparable to the effect of mating on oviposition in three ways: (1) The transplant induces a striking increase in percent of females ovipositing and number of eggs per ovipositing female; (2) The time pattern of oviposition is similar to that of mated females. Normally, oviposition does not follow immediately after mating but occurs over a period of several days as in these experiments. Gillett (1955) has shown that fed virgin females of Newala

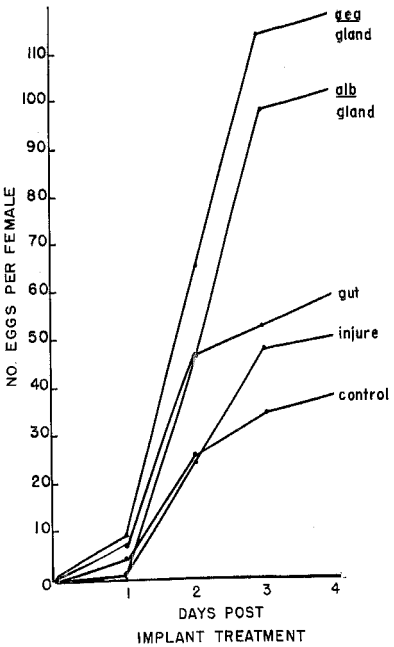

FIG. 2.—Daily record of oviposition resulting from implants of various tissues in *A. aegypti*. Blood meal was given two days before treatment. Treatment was given day 0.

TABLE 1.—Effects of implants of male accessory gland on oviposition in *aeg-N* and *alb-I*. Females were given a blood meal 2 days before treatment and were subsequently kept in oviposition vials.

Ovipositing female	Source of implant	No. of ♀♀	Day post treatment when eggs counted	Oviposition		
				% of ♀♀ ovipositing	No. eggs per ovipositing ♀	No. eggs per ♀
aeg	..	20	4	65	52	38
aeg	*aeg*	27	4	90	126	117
aeg	*alb*	20	4	90	113	102
alb	..	15	4	0	0	0
		15	8	13	8	1
alb	*alb*	20	4	80	49	54
		20	8	90	91	82
alb	*aeg*	17	4	12	60	7
		17	8	29	34	10

will defer oviposition for as long as 3 weeks, but then on mating will oviposit on the second and third day; (3) The heterologous gland transplant into *A. aegypti* produces a marked rise in oviposition. This is comparable to the results of heterologous insemination of *A. aegypti* (Leahy and Craig, in press). Thus, with *A. aegypti,* both homologous and heterologous gland transplants parallel the oviposition effect of both kinds of insemination.

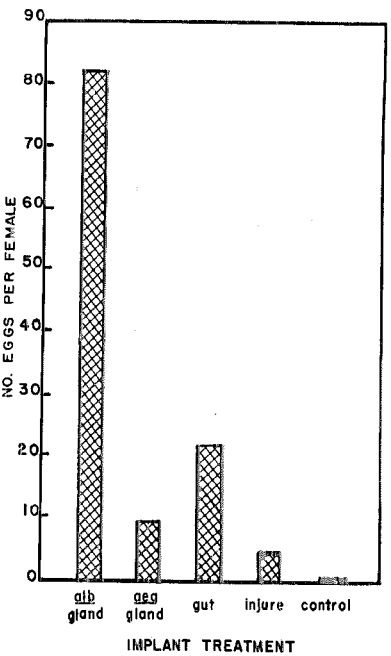

FIG. 3.—Effect of implants of various tissues on oviposition in *A. albopictus.* Blood meal was given two days before treatment. Eggs were collected eight days after treatment.

In *A. albopictus* there are similar parallels between implant-oviposition and insemination-oviposition. As in *A. aegypti* these similarities are found both within and between species. Homologous gland transplant had a stimulant effect on oviposition in *A. albopictus* whereas heterologous gland transplant had no effect. These divergent results of gland implant agree with data on the effect of insemination on egg deposition (Leahy and Craig in press).

Heterologous implants had opposite results for the two species. *A. aegypti* responded with increased oviposition. *A. albopictus* did not respond. This difference is also reflected in Buck's (1942) studies on oviposition resulting from the hybridization of *A. aegypti* and *A. albopictus.* He reported normal size egg clutches from inseminated *A. aegypti* females x *A. albopictus* males, very small ones from inseminated *A. albopictus* females x *A. aegypti* males.

The normal oviposition pattern for *A. albopictus* varies in some respects from

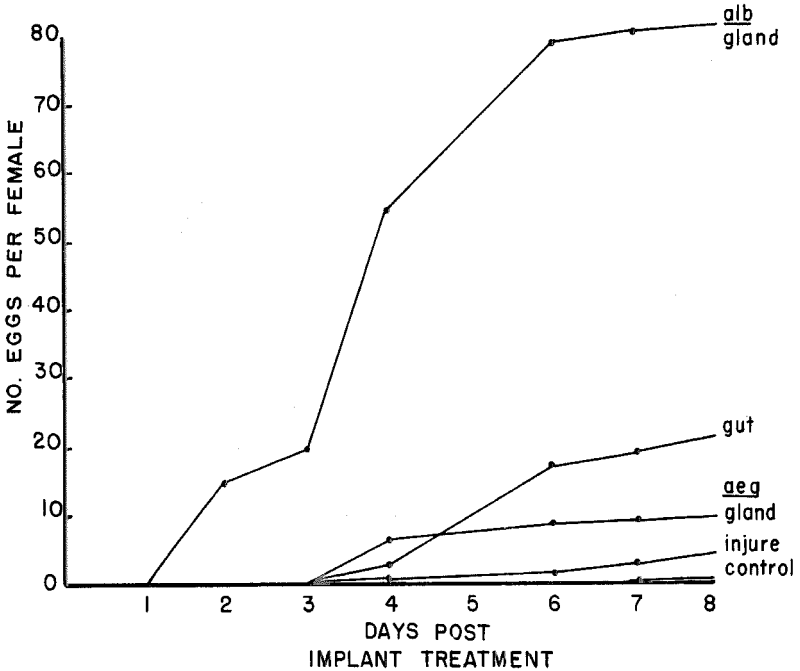

FIG. 4.—Daily record of oviposition resulting from implants of various tissues on *A. albopictus*. Blood meal was given two days before treatment. Treatment was given day 0.

that of *A. aegypti*. The oviposition of *A. albopictus* consequent upon mating is characterized by a lower rate and greater length of time. Indications of these same variations appeared in the present experiments (Table 1). When the implant of *A. aegypti* is made into *A. aegypti* the average number of eggs per female is 117 in 4 days. However, when the homologous implant is made in *A. albopictus* only 54 eggs per female are produced in 4 days, and 82 by 8 days.

The gland transplant of *A. albopictus* into *A. aegypti* has particular interest for two reasons. First, a trans-specific action is evident. Woodhill (1949) observed a similar result on the sub-specific level in his hybridization experiments with *Aedes scutellaris*. There, mating provided an obvious stimulus to egg deposition even when eggs were sterile. Secondly, we may infer from the results of this implant that the species difference in oviposition is due primarily to the response of the female and not to a deficiency in the stimulant substance provided by the male gland. Implants of the male gland of *A. albopictus* produced higher oviposition in females of *A. aegypti* than in females of its own species. For example, in homologous transplant experiments 80 percent of *A. albopictus* females oviposited and there was an average of 49 eggs deposited per female. However, when the male gland from *A. albopictus* was implanted into female *A. aegypti*, 90 percent oviposited at a rate of 113 eggs per ovipositing female.

CONCLUSIONS. The present experiments indicate that a stimulant substance is produced by the male accessory glands of *A. aegypti* and *A. albopictus*. Contrary to above opinions it is not necessary for the stimulant to reach the spermathecae to produce its effect. Even when it enters

through the thorax of virgin females it provides a marked oviposition stimulus.

Action of the accessory gland in other Diptera is under current investigation. A chemical analysis of the glandular substance is also proposed.

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TECHNIQUES FOR OBTAINING VIABLE EGGS OF *LEPTOCONOPS BEQUAERTI* KIEFFER, *CULICOIDES FURENS* POEY, AND *CULICOIDES BARBOSAI* WIRTH AND BLANTON, (DIPTERA: CERATOPOGONIDAE)

JOHN R. LINLEY

Sandfly Research Unit, Ministry of Health, Jamaica

Jamaica is an extremely beautiful island with a thriving tourist industry. However, the welfare of tourism is constantly threatened by the activities of various biting insects, notably certain mosquitos and some species of biting Ceratopogonidae. The latter play such an important part in these considerations that a research unit was established in 1959 with the specific task of investigating the biology of these insects.

It soon became apparent that the problems were being caused almost entirely by the three species named in the title of this paper. *Leptoconops bequaerti* Kieffer bites throughout the day in strong sunlight and quite high winds (Kettle and Linley, 1960a), and is in fact a particular nuisance as it breeds in white sand of the type found on the most attractive beaches. In fact, one well respected hotel in the Montego Bay area was breeding its own problem beneath the beach shelters provided for visitors.

Culicoides furens Poey and *C. barbosai* Wirth and Blanton are active mainly at dawn and dusk, and are associated with the coastal mangrove swamps. There are large areas of such swamp in Jamaica, often adjacent to development projects already completed or to localities with po-