

Retrograde filling of the leg nerve cut at the coxal/femoral joint in the metathorax of a normal, unoperated cockroach routinely dyes motoneurons in the ipsilateral anterior ventral quarter of the ganglion, together with median cells which are identified with the common inhibitory neurons described by PEARSON and FOURTNER<sup>10</sup> (Figure 2a). There is no indication in the normal animal that contralateral cells send axons across the ganglion and out of the leg nerve on the opposite side.

Three months after rotation the ganglia appear normal apart from their dorso-ventral inversion, twisted connectives and persistent wound tissues. Peripheral nerve trunks exit to the nearest limb – they do not cross back to their side of origin. If the leg nerve of a rotated ganglion is now filled with dye, the result is the same as in an unoperated ganglion: only neurons ipsilateral to the filled nerve are stained (Figure 2b). However, more cells are filled as other peripheral nerve trunks become closely associated in the wound tissue and take up the dye intended for nerve 5. In a total of 15 assays on rotated metathoracic ganglia there were no signs of contralateral cells being filled by the migrating dye. Clearly left neurons

have sent axons into the right limb and right into left. The appearance of co-ordinated movements in the legs of the operated segment suggests that these ingrowing axons synapse with appropriate muscles previously innervated by equivalent neurons on the opposite side of the ganglion.

Neurons which are displaced often show considerable powers of growth in reaching an appropriate target cell even if the original direction of axon growth is reversed<sup>11</sup>. In these experiments however motoneurons do not grow back to their side of origin when they are disconnected from it and offered the opposite side in which to grow. If the neurons are classified as right or left innervating then the cues by which they distinguish right from left are ineffective at this stage. It is more likely that the leg motoneurons which are the progeny of a bilaterally symmetrical set of mother cells<sup>12</sup> are not distinguished by their side of origin and that they do not discriminate between opposite sides of the mid-line during growth. The duplicate classification of the motoneurons finds its counterpart in the sensory system. The axons of receptor neurons do not respect the mid-line during normal growth<sup>13</sup>. When left and right cerci are exchanged in crickets, the regenerating axons of the sensory neurons enter the ipsilateral half of the terminal abdominal ganglion where they synapse with cells of the opposite side to that which they normally innervate<sup>14</sup>.

The development of coordinated limb movements in the operated animals indicates that the regenerating motoneurons make functional contacts with the muscles of the limb into which they are induced to grow. Further experiments are required to confirm this and to analyse the response of the operated cockroaches to the behavioural problems with which they are confronted.

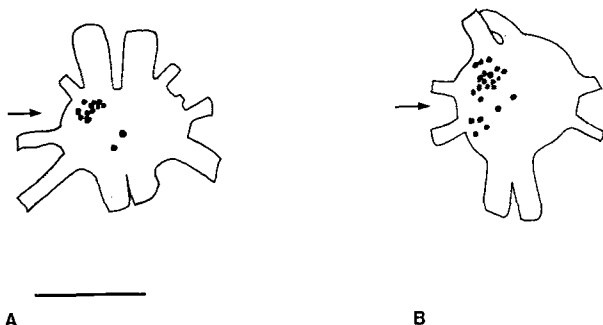


Fig. 2. Neurons stained by retrograde diffusion of cobalt chloride into leg nerve 5 of one side (arrowed).

A) Control ganglion. B) After rotation and regeneration of peripheral nerve trunks. More cells fill in the operated ganglion because nerve trunks associate in wound tissue and take up dye intended for nerve 5. Scale: 1 mm.

<sup>10</sup> K. G. PEARSON and C. R. FOURTNER, *Can. J. Zool.* 51, 859 (1973).

<sup>11</sup> E. HIBBARD, *Expl Neurol.* 13, 289 (1965).

<sup>12</sup> C. M. BATE, *J. Embryol. exp. Morph.* 35, 107 (1976).

<sup>13</sup> P. A. LAWRENCE, in *Cell Patterning* (Ciba Foundation Symposium 1975), vol. 29.

<sup>14</sup> J. PALKA and M. SCHUBIGER, *Proc. natn. Acad. Sci., USA* 72, 966 (1975).

## The Potency of Male Accessory Gland Material in the Mosquito (*Aedes aegypti*)<sup>1</sup>

J. C. JONES and B. V. MADHUKAR

Department of Entomology, University of Maryland, College Park (Maryland 20742, USA), 30 October 1975.

**Summary.** Injections of male accessory gland material from *Aedes aegypti* into the hemocoels of virgin female mosquitoes indicate that the potency of the secretion is equivalent to the amount of semen which a male normally places within the female. This estimation is far less than had been previously calculated. It is suggested that the term *matrone* for male accessory gland material is inappropriate since it does not convert a maid into a matron but prevents reinsemination of an impregnated female.

Male *Aedes aegypti* mosquitoes normally inseminate 5 females<sup>2</sup>. Shortly after females are inseminated, virgin males will not force-copulate with them<sup>3</sup>. The male normally ejaculates semen consisting of one-fifth or less of the secretion within a pair of large accessory glands and a relatively small amount of spermatozoa and fluid from the seminal vesicles directly into the seminal bursa of a virgin<sup>2</sup>. Within less than 5 min after being inseminated, the female transfers many spermatozoa into 2 spermathecae<sup>2</sup>, and over the next few days all of the semen within the bursa is resorbed into her hemolymph, leaving

this sac clear and flat like that of a virgin<sup>4</sup>. As soon as the spermatozoa have reached the spermathecae, the female is said to be impregnated<sup>5</sup>, to distinguish this event from the presence of spermatozoa elsewhere within her body.

CRAIG<sup>6</sup> thought that the reason inseminated mosquitoes do not become reinseminated is either because the female avoids copulation or because the male cannot insert his aedeagus into the vagina. SPIELMAN et al.<sup>7</sup> found, however, that inseminated females copulate with additional males. They proposed that these females were reinseminated but quickly ejected the second ejaculate. JONES<sup>4</sup>

observed that initially inseminated females copulate again and do so repeatedly under free-flying conditions, but that following even long subsequent copulations females were not reinseminated and ejected no material from their bursae. The bursa has no muscles with which to eject semen<sup>8</sup>. Long after the bursa is empty, males copulate with but do not reinseminate impregnated females. We still do not know how inseminated or impregnated females prevent ejaculation by potent males.

CRAIG<sup>6</sup> reported that 24 h after implanting a single male accessory gland into each of a series of 86 virgins of the Rockefeller strain of *A. aegypti* none of the females were inseminated by many males. He calculated<sup>6,9</sup> that 1 male has enough material in his accessory glands to prevent insemination of 64 to 80 virgins. He reported that injection of 1  $\mu$ l of a dilution equivalent to 5 to 62.5 individual glands in 1 ml of saline completely prevented insemination of virgin mosquitoes.

We implanted 1 male accessory gland into the abdomen of each of 10 virgin females of the Bangkok strain of *A. aegypti* and 3 days later placed the females with 20 virgin males in a 1 cu ft cage and allowed them to cohabit 24 h. One of the 9 survivors was inseminated. We separately homogenized 2, 8, 25 and 50 male accessory glands in 1 ml of mosquito saline<sup>10</sup> and injected 1  $\mu$ l from each concentration into 10 to 20 virgin females, after which they were allowed to co-habit for 24 h with an equal number of virgin males. In almost every case, the females were found to have been impregnated. 17 out of 18 injected with 1  $\mu$ l from 50 glands were impregnated. Even after injection with 1  $\mu$ l from 100 glands/1 ml saline, 2 out of 7 females were impregnated in one test and 4 out of 10 in a second. We thought that our failure to prevent impregnation might be due to the fact that we had not added seminal vesicle material, so we injected 11 virgins with 1  $\mu$ l of an homogenate of 12 vesicles and 24 male accessory glands in 1 ml saline and found that all 9 surviving females were impregnated after co-habitation with males. Since CRAIG<sup>9,10</sup> did not actually use homogenates of isolated male accessory glands but sonified the terminalia or ground-up the whole mosquitoes, we sonified 100 male

terminalia (equivalent to 200 accessory glands) in 1 ml saline and injected 1  $\mu$ l of the supernatant into 17 virgins. 24 h after co-habitation with 32 males, 14 females were impregnated and 3 were not inseminated.

It is roughly estimated that the average female mosquito has a hemolymph volume of 1–4  $\mu$ l. Since the male ejaculates about one-fifth of his accessory gland secretion into a female, and since it is all absorbed into the hemolymph, it can be calculated that he places an amount equivalent to 25 to 100 glands in 1 ml. Our injection experiments indicate that females must contain the equivalent of at least 100 glands if they are not to be inseminated.

CRAIG et al.<sup>6,11</sup> thought that the male accessory glands were responsible for preventing copulation and insemination, and were responsible for increasing the number of eggs developed and laid by the mosquito. CRAIG<sup>12</sup> coined the term *matrone* because he believed that the secretion acted on the female to convert her from 'a maid to a matron'. It seems to us that this term is inappropriate for several reasons. It is not used by females for fertilization of the eggs and it specifically acts to prevent the male from reinseminating an already inseminated or impregnated female. The secretion does not prevent copulation. The failure to be inseminated more than once is not a characteristic of either a mother or a matron.

<sup>1</sup> Scientific Article No. A2098, Contribution No. 5054 of the Maryland Agricultural Experiment Station.

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<sup>3</sup> J. C. JONES and R. E. WHEELER, J. Morph. 117, 401 (1965).

<sup>4</sup> J. C. JONES, Am. Zool. 10, 311 (1970).

<sup>5</sup> J. C. JONES and H. G. SHEFFIELD, Mosq. News 30, 650 (1970).

<sup>6</sup> G. B. CRAIG, JR., Science 156, 1499 (1967).

<sup>7</sup> A. SPIELMAN, M. G. LEAHY and V. SKAFF, Biol. Bull. 132, 404 (1967).

<sup>8</sup> J. C. JONES and H. G. SHEFFIELD, Mosq. News 30, 270 (1970).

<sup>9</sup> G. B. CRAIG, JR., Miscell. Publ. ent. Soc. Am. 7, 130 (1970).

<sup>10</sup> G. C. ODLAND and J. C. JONES, Annis ent. Soc. Am. 68, 613 (1975).

<sup>11</sup> M. S. FUCHS, G. B. CRAIG, JR., and E. A. HISS, Life Sci. 7, 835 (1968).

<sup>12</sup> G. B. CRAIG, JR., personal communication.

## Isolation of a Soldado-Like Virus (Hughes Group) from *Ornithodoros maritimus* Ticks in Ireland<sup>1</sup>

J. E. KEIRANS, C. E. YUNKER, C. M. CLIFFORD, L. A. THOMAS, G. A. WALTON<sup>2</sup> and T. C. KELLY<sup>2</sup>

U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Institutes of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton (Montana 59840, USA); and Department of Zoology, University College, Cork (Ireland), 4 November 1975.

**Summary.** Three isolations of a virus of the Hughes group were obtained from seabird ectoparasites, *Ornithodoros (Alectorobius) maritimus*, on Great Saltee Island, Ireland. The agent is closely related to Soldado virus, originally obtained from related ticks near Trinidad, West Indies, and represents the second recorded tickborne arbovirus in Ireland.

The virus reported here (RML 59972) was isolated in 1972 from ticks collected by one of us (T.C.K.) from seabird nesting areas in southern Ireland. A sample of *Ornithodoros (Alectorobius) maritimus* Vermeil and Marguet was collected beneath a stone on an east facing ledge, 2 m below a 30 m high Lewisian granite-gneiss cliff summit of Great Saltee Island (52.07 N, 6.36 W) 7 km S of County Wexford in southeast Ireland. *Uria aalge* (guillemot or common murre) is the most numerous seabird species nesting here, but kittiwakes (*Rissa tridactyla*), razorbills (*Alca torda*), and shags (*Phalacrocorax aristo-*

*telis*) also occupy ledges, crevices, and holes in the surrounding area.

Adult (64 male, 50 female) ticks were sent to the Rocky Mountain Laboratory (RML) where they were identified as *O. (A.) maritimus*, segregated by sex (5 male or 5 female per pool each, except one pool of 7 female), pooled and processed for virus isolation (55 male, 37 female) or

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<sup>2</sup> Department of Zoology, University College, Cork, Ireland.