DIAPAUSE AND FAT BODY FORMATION BY CULEX RESTUANS THEOBALD

(DIPTERA, CULICIDAE)

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The problem of the diapause and fat body formation by *Culex restuans* became of special interest when observations of the ecology of this species led to the conclusion that the majority of the overwintering adult population consisted of young females which had not taken a blood meal. This was contrary to the popular theory that, with the exception of the species reported by Frohne in 1953 (1), most mosquitoes which hibernate as adults utilize nutritional reserves in their fat body derived from a late fall season blood meal. Consequently, special study of the ecology of *C. restuans* was initiated during 1956.

PROCEDURE

Year-around sampling of the mosquito population was conducted during 1956, 1957 and 1958. Collections were taken from winter hibernation sites in two study areas each month from November through February. Beginning in March, weekly collections were taken from known diurnal resting places and weekly sampling of established aquatic sites for larval activity was continued from then until the water was frozen solid in the following winter. Adult females were collected from diurnal resting places by hand aspirator and transported to the laboratory in cardboard cartons. All blood-engorged *Culex* were then sorted from the collection and maintained in cages until oviposition occurred. Since adult female *C. restuans* were difficult to distinguish from *Culex pipiens* and *Culex salinarius*, egg rafts were isolated and allowed to hatch so that identification of specimens was confirmed by examination of the larvae. The number of engorged females in each collection was noted throughout the mosquito breeding season.

Larvae were obtained for laboratory study from egg rafts oviposited by adults collected in the field. In August 1956, 300 laboratory-reared adult females were divided into six groups and each group placed in a lantern chimney cage. These were provided with water, wet filter paper around the sides and cotton pads soaked in 5 per cent sucrose solution for feeding. In a similar cage, a group was maintained consisting of 50 females which had previously taken a blood meal and oviposited an egg raft. All seven cages were placed in cool storage hibernation conditions (at 40° F.) early in September. They were inspected each week thereafter during the next four months and the incidence of fat body formation and mortality noted.

In September 1957, a similar series of cages were set up. However, in these, each of three cages contained 100 females obtained from field collections in hibernating sites. These females all appeared to possess fat bodies or to have engorged with clear fluid. Each of three other cages contained 100 laboratory-reared females which had fed from one to five weeks on 5 per cent sucrose solution.

Results

Adult female *C. restuans* left hibernation places late in March and April, depending upon the onset of warm weather. Shortly thereafter, during April, May and June, increasing numbers of blood-engorged specimens appeared in diurnal resting places. This continued until mid-July when up to 75 per cent of the females in collections contained blood. From August through October the incidence of blooded females declined and there was an increase in the number of young adults in collections. Very large numbers of these were encountered in diurnal and hiberation places during the fall season and by October, the majority of the females were engorged with clear fluid or contained fat bodies.

The development of larvae in aquatic sites was not detected until late in the spring. However, by June 1, all stages of larvae were collected. Larval populations continued to increase throughout the summer and into the fall, when large populations of fourth instar larvae were observed.

During summer months laboratory populations of *C. restuans* were reared from egg rafts oviposited by wild females late in June. From these, adults were obtained and maintained in one-cubic-foot cages for study. They took blood meals readily during the early summer period and fed from a variety of hosts (man, rat, mouse, chicken and egg embryo). However, late in the summer and during the fall season increasing difficulty was encountered in inducing blood feeding. There appeared a decided preference for engorging with sucrose solution, and fat bodies developed in females which had not previously taken a blood feeding.

During the fall of 1956, in six groups of laboratory-reared females which were fed only on sucrose solution and then placed in experimental hibernation conditions, the incidence of fat body formation was again noted. All of these females developed fat bodies and survived the first few weeks of hibernation. However, the group of blood-fed females sustained high mortality. This continued, until January 1, only one of the 50 survived. At this time 68 per cent of the 300 sucrose-fed females still survived, and the experiment was terminated at this time.

During the fall and winter of 1957-1958, in a similar series of tests to compare survival and fat body formation among wild and laboratory reared females, little difference was observed in survival rates. The majority of the females in both groups developed fat bodies and survived well during the first four months. However, late in January 1958, heavy mortality occurred in both groups when accidental desiccation occurred in the cages.

Discussion

The problem of diapause and fat body formation by *C. restuans* became of interest during study of potential mosquito vectors of eastern equine encephalitis in Connecticut. Of the four species of *Culex* commonly found hibernating in Connecticut, *C. restuans* was

selected for study for a number of reasons. It was the only one of the four species known to be a good host and laboratory vector of the eastern equine encephalitis virus (Chamberlain *et al.* (2)). It enters houses and feeds upon man and takes blood from a wide variety of other hosts. In addition, it was one of the few species which was found abundantly in all areas where encephalitis virus activity occurred in Connecticut (Wallis, *et al.* (3)). However, following study of hibernating mosquitoes in areas of known virus activity, it was suggested (Wallis, *et al.* (4)) that since as far as it is known, it is necessary that a blood meal from an infected host be taken by the mosquito in order for it to become infected, it was highly improbable that the eastern equine encephalitis virus was harbored by hibernating *Culex* if they entered diapause without blood feeding. Therefore, it was considered important to determine experimentally if blood feeding was necessary for fat body formation and hibernation. Numerous observations of the ecology of *C. restuans* indicated that blood feeding was not necessary, and did not generally occur in nature. Huge larval populations built up late in the fall and adults from this late season breeding had little time in which to find a host and obtain a blood feeding before hard frosts drove the populations into hibernation sites for the winter. Furthermore, the incidence of blooded females steadily declined in late summer and adults were no longer taken in fall biting collections. This was similar to the situation in the laboratory where the females could no longer be induced to take blood late in the summer, and instead, exhibited a decided preference for feeding upon sucrose solutions. When under experimental conditions the sucrose-fed females repeatedly formed fat bodies and successfully went into diapause in hibernation conditions, whereas blood-fed females did not survive, it was concluded that blood was not necessary for fat body formation and winter survival. Thus, the observations and experiments here support the hypothesis that species of hibernating *Culex* which enter diapause without blood feeding were unlikely to serve as overwintering hosts of the eastern equine encephalitis virus.

Since this study was initiated, results have been reported of work on the hibernation of another species, *Culex tarsalis*, which are very similar to results in this study, and which led the authors to the conclusion that *C. tarsalis* was not a likely host for harboring the western equine encephalitis virus (Rush *et al.* (5), Bennington *et al.* (6)). This, and the report of Tate and Vincent (7) that *Culex pipiens* females did not take blood before entering hibernation, and Frohne's (1) observations on *Culex territans*, leads to the speculation that perhaps more *Culex* mosquitoes exhibit similarities to Frohne's Type V life cycle. At least those species with more northern distribution, even though they do not specifically fit his definition in that more than one generation a season occurs, may, during the late fall generation, undergo diapause before blood feeding and egg deposition, as he describes in the *Culiseta impatiens* life cycle.

SUMMARY

The diapause and fat body formation by *Culex restuans* was studied from 1956 to 1959. In the field, the incidence of blooded females was high in the spring and summer, but declined rapidly in the fall, when hibernation places were filled with large populations of young adults. These females, instead of containing blood, were filled with a clear fluid and developed fat bodies. Laboratory colonies reared during the summer became reluctant to take blood in the fall and developed a preference for engorgement on sucrose solution. Sucrose-fed females, when placed in experimental hibernation conditions, developed fat bodies without previously having taken blood. The good survival of these females was contrasted with poor survival and unsuccessful hibernation of blood-fed females.

REFERENCES

1. Frohne, W. C. 1953. Natural history of *Culiseta impatiens* (Wlk.), (Diptera, Culicidae), in Alaska. Trans. Amer. Microscop. Soc. 72:103-118.
2. Chamberlain, R. W., Sikes, R. K. Nelson, D. B., and Sudia, W. D. 1954. Studies on the North American arthropod-borne encephalitides. VI. Quantitative determinations of virus-vector relationships. Amer. Jour. Hyg. 60:278-285.
3. Wallis, R. C., Jungherr, E. L. Luginbuhl, R. E., Helmboldt, C. E., Satriano, S. F., Williaman, L. A., and Lamson, A. L. 1958. Investigation of eastern equine encephalomyelitis. V. Entomologic and ecologic field studies. Amer. Jour. Hyg. 67 (1):35-45.
4. Wallis, R. C., Taylor, R. M., McCollum, R. W., and Riordan, J. T. 1958. Study of hibernating mosquitoes in eastern equine encephalomyelitis and epidemic areas in Connecticut. Mosq. News 18(1):1-4.
5. Rush, W. A., Brennan, J. B., and Eklund, C. M. 1958. A natural hibernation site of the mosquito *Culex tarsalis* Coquillett in the Columbia River Basin, Washington. Mosq. News 18(4): 288-293.
6. Bennington, E. E., Sooter, C. A., and Baer, H. 1958. The diapause in adult female *Culex tarsalis* Coquillett (Diptera: Culicidae). Mosq. News 18 (4): 299-304.
7. Tate, P., and Vincent, M. 1936. The biology of autogenous and anatogenous races of *Culex pipiens*. Parasitology 28:115-145.

BOOK NOTICES

LABORATORY MANUAL FOR INTRODUCTORY ENTOMOLOGY, by Clifford J. Dennis (East Central State College, Ada, Okla.). 65 pp., illus. Wm. C. Brown Co., Dubuque, Iowa. Price, $2.00.

MITES, OR THE ACARI, by T. E. Hughes. 225 pp., illus. Essential Books, 16-00 Pollitt Drive, Fair Lawn, N. J. Price, $6.75.