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DETERMINATION OF PARITY IN PINNED SPECIMENS OF THE BITING MIDGE, *CULICOIDES VARIIPENNIS*

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Parity determinations on females of the biting midge *Culicoides variipennis* (Coquillett), the principal vector of bluetongue and epizootic hemorrhagic disease viruses in the US (Jones et al. 1981), have been done with specimens that were fresh or preserved in alcohol (Akey and Potter 1979). The method of parity determinations based on changes in tergite patterns (Potter and Akey 1978) has worked especially well for specimens preserved in alcohol that were collected from northeastern Colorado by personnel or associates of this laboratory. I became interested in the possibility that the parity of pinned specimens previously collected from this area could also be determined by tergite patterns. This study was conducted to develop a technique to determine parity in pinned specimens by either the Dyce (1969) or the Potter and Akey (1978) method for parity determination.

MATERIALS AND METHODS

A single group of female flies of known parity status were obtained for controls from a colony of *C. variipennis* (Sonora strain, 000 line; Jones et al. 1969) as follows: 20 nulliparous females were killed at 24–48 hr of age; 20 females of the same age were blood fed for 90 min and killed immediately; and 20 more females were blood fed, allowed to oviposit, and then killed. These 60 females were dried at 70 C° for 24 hr and treated as pinned specimens.

Field specimens had been collected from sheep-baited traps in 1964 near Hudson, Colorado and at that time had been mounted on blocks with minuten pins. These specimens were somewhat shriveled and had darkened considerably with age.

Each pinned specimen was dipped in relaxing fluid[1] (Boesel 1977), gently removed from the minuten pin, and submerged in a 2 dram vial (ca 9 ml) of relaxing fluid for 24 hr. The relaxing fluid was replaced with a 50% solution of ethyl alcohol for 24 hr. Next, a 15% solution of potassium hydroxide was used to replace the alcohol and clear opaque coloration that was often present in body parts of these dry specimens. In this crucial step, the time required was dependent on the condition of the specimen. The specimens used in this study required at least 15 but never more than 24 hr (ambient room temperature ca. 25°C). However, care was taken not to decolorize the tergite patterns nor the ventral abdominal pigmentation. The potassium hydroxide solution was replaced with a neutralizing solution of 15% acetic acid. After 4 hr, the acid was replaced by 75% ethyl alcohol and the specimens were kept in it until examined for parity by tergite pattern and/or ventral abdominal pigmentation.

Chloral hydrate (Nesbitt's solution, see Borror et al. 1976) and phenol (Hetherington's solution, see Barbosa 1974) were also evaluated as clearing agents with some of the pinned specimens from the 1964 collection.

RESULTS AND DISCUSSION

Of the 60 control flies of known parity, 98% (59) were determined correctly and the single error was a nulliparous female judged to be parous. Examination of previously pinned specimens (262 females collected near Hudson, Colorado from sheep-baited traps) showed 107 females to be nulliparous and 155 to be parous. Assuming an accuracy rate of 98%, these 2 groups would be correct to within 2 or 3 specimens.

Specimens required a cursory examination for evidence of blood or eggs before treatment with potassium hydroxide since the clearing

[1] Boesel relaxing fluid: 450 ml of 95% ethyl alcohol, 350 ml distilled water, 150 ml ethyl acetate, and 50 ml benzene.

treatment removed evidence of either a blood meal or a gravid condition.

Chloral hydrate required ca.2 wk to clear the abdomens sufficiently and phenol did not clear them at all. Since chloral hydrate is much slower acting than potassium hydroxide, it would be of use to an investigator who could not closely monitor the more rapid clearing action of potassium hydroxide.

Specimens cleared for parity determinations by either potassium hydroxide or chloral hydrate were not suitable for repinning but could easily be mounted on microscope slides. If it were essential to preserve wing patterns (colorations) then the wings would have to be removed and held separate prior to clearing.

This is the first technique known to this author for determining parity of pinned specimens. It permits parity investigations to be made on pinned or dried collections that have potential epidemiological significance in addition to the taxonomic value for which they may have been first prepared. This technique will probably remain limited in usefulness to parity determinations in *Culicoides* unless external pigmentation changes related to oogenesis are also found to occur in other ceratopogonids or mosquitoes.

Parity data from the pinned specimens used in this technique study will be reported elsewhere as part of a study on the parity of *C. variipennis* captured in bait traps and from collections of biting midges attracted to animals.

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SOME FACTORS AFFECTING THE USE OF *NEOAPLECTANA* SP. FOR MOSQUITO CONTROL

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There are several strains and species of *Neoaplectana*, some of which are the most easily and economically mass producible nematodes pathogenic to insects. As the infective stage of the nematode is resistant to a wide variety of environmental conditions, it is likely that the nematodes could survive in a range of mosquito habitats. The infective stage can be dispersed by all common spray methods used for insecticides. The drawback to its general use (against forest and agricultural pests) has been its susceptibility to desiccation. This would not pose a problem in the aquatic habitat of mosquitoes.

A range of culicids has been found to be susceptible to invasion by the DD-136 strain of *Neoaplectana carpocapsae* (Table 1). These laboratory results indicated the possibility of utilizing this parasite for controlling certain mosquito species. However, irrespective of the natural larval habitat(s) of these species all infectivity tests have been carried out in smooth-bottomed containers. Such results make no provision for the length of time the nematode would normally be available to the