# A COMPARISON OF TWO TECHNIQUES FOR DISTINGUISHING PAROUS FROM NULLIPAROUS *CULEX TARSALIS* COQUILLETT [1]

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Two techniques recently have been used to distinguish parous from nulliparous *Culex tarsalis* Coquillett. The first is based on the observation that the tracheoles of a mosquito's ovaries become uncoiled when the mosquito develops and deposits eggs. Kardos and Bellamy (1961) and later other workers (Blackmore and Dow, 1962; Burdick and Kardos, 1963) successfully applied this technique to *C. tarsalis,* but all noted that some specimens could not be satisfactorily classified. Kardos and Bellamy considered as intermediate those females with fewer coiled ovarian tracheoles than found in nulliparous specimens, but with more than found in parous specimens that had formed "normal" egg rafts. They believed that intermediate females had "*probably* deposited a smaller than normal egg raft, perhaps autogenously." The second technique is based on the fact that dilatations are formed in a mosquito's follicular tubes when the mosquito develops and deposits eggs. This technique, when applied to *C. tarsalis* (Nelson, 1964), could not be used to determine the number of ovipositions by individual females, as it is used with some mosquito species (Detinova, 1962). However, it was useful for distinguishing parous from nulliparous specimens.

To compare and further evaluate the two techniques for use with *C. tarsalis,* both were applied to colony females of known oviposition histories and to more than 2,000 females collected (during the winter of 1961–1962) in Kern County, California. This note reports the results of these investigations.

Since both techniques were used to classify each mosquito, some changes were made in the usual procedures (Burdick and Kardos, 1963; Nelson, 1964). After dissection, the follicular tubes from one ovary were examined for dilatations, and the other ovary was mounted on a slide for later tracheole examination. Because ovaries were dissected in saline for dilatation examination, the ovary for tracheole examination was washed before mounting to prevent damage to tracheation by salt crystal formation. This was done by gently removing the ovary from the saline with forceps and briefly rinsing it in distilled water.

Only females that were blood-engorged (with blood visible in the abdomen) or empty, i.e. without blood or eggs developed beyond stage II of Christophers (1911) were classified. Females with any well-developed eggs (presumably retained from previous oviposition) in their ovaries were not classified.

With the dilatation technique, a specimen was considered parous when a dilatation was found in two or more follicular tubes of an ovary; or nulliparous when dilatations were not found despite thorough examination of the ovary. With the tracheation technique, a specimen was classified as (1) parous, when nearly all ovarian tracheoles in the mounted ovary were uncoiled (with less than five coiled); (2) nulliparous, when nearly all tracheoles were coiled; and (3) intermediate, when both coiled and uncoiled tracheoles were present,—in a range between the preceding two conditions. Although the scheme for tracheation classifications was followed as closely as practicable, these classifications were by nature more subjective than those of the dilatation technique.

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Initially, a co-worker prepared 100 colony *C. tarsalis* with known oviposition histories in a test series and then submitted them to the author as "unknowns" for classification by both techniques. The series consisted of 70 empty and 30 blood-engorged females, the latter having fed on blood the night before they were dissected. When making classifications, the author knew only that parous and nulliparous specimens were randomly represented in both the engorged and empty groups, and that the parous females had oviposited once.

Upon dissection, three specimens had some well-developed eggs in their ovaries and therefore were omitted from the test series. All but one of the remaining 97 mosquitoes were correctly classified with the dilatation technique. With the tracheation technique, 80 of the 97 females were classified as parous or nulliparous; 77 correctly. Seven specimens were considered intermediate. The ovarian tracheation of 10 engorged females was obscured by accumulation of yolk in the egg follicles, and the females could not be classified.

From October through April, 1961–1962, 2,363 "wild" *C. tarsalis* were classified by both techniques (Table 1) and

TABLE 1.—Classification of 2,363 female *Culex tarsalis* with the dilatation and tracheation techniques.

| Dilatation classifications | Tracheation classifications | | | |
|---|---|---|---|---|
| | Nulliparous | Parous | Intermediate | Totals |
| Nulliparous | 1,727 | 32 | 26 | 1,785 |
| Parous | 26 | 495 | 57 | 578 |
| Totals | 1,753 | 527 | 83 | 2,363 |

2,280 could be classified as either nulliparous or parous by both techniques. There was agreement of classification in more than 97 percent of these specimens. Of those 83 specimens considered intermediate by tracheation examination, nearly 70 percent were classified as parous with the dilatation technique, and this indicates they probably were parous.

An additional 676 wild females were classified with the dilatation technique that could not be classified by their ovarian tracheation. Included were 547 engorged females in which egg yolk obscured tracheation, and 129 (including both empty and engorged specimens) whose ovarian mounts were otherwise unsatisfactory.

These results demonstrate the value of both techniques for distinguishing parous from nulliparous *C. tarsalis,* but also indicate significant advantages of the dilatation technique. The most obvious advantage is that the need to classify some specimens as intermediate, which posed a major problem to Burdick and Kardos (1963) in using the tracheation technique, is eliminated.

Egg development was at or beyond late Christophers' stage II in the 547 blood-engorged females that could not be classified with the tracheation technique. Since engorged females with eggs in stage III or late stage II are common, the ability to classify such specimens is a further important advantage of the dilatation technique.

Finally, some specimens could not be classified because of unsatisfactory slide mounts,—a problem limited to the tracheation technique. Burdick and Kardos (1963) also found that a significant proportion (about 8 per cent) of their ovarian mounts were unsatisfactory for classification. When the tracheation technique was used to classify *C. tarsalis* in Colorado (Blackmore and Dow, 1962), up to one-fourth of the deplete females in some collections could not be classified.

To summarize, the present study compared two techniques for classifying female *C. tarsalis* into nulliparous and parous categories. Classifications based on the presence or absence of follicular dilatations were more accurate than those based on tracheation characteristics and could be applied to a wider variety of specimens.

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