RESEARCH NOTE

An Improved Technique for the Dissection of Female Genitalia of Phlebotomine Sandflies (Diptera: Psychodidae), with an Improvement in the Handling of Insects

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Since O Theodor (1932 *Bull Ent Res 23*:17-23) pointed out the value of spermathecae and their ducts in the identification of Neotropical female sandflies, these structures have become the most important to identify females. Unfortunately, it is frequently difficult to see the ducts, principally the common one, when present. The dissection of female genitalia makes possible the observation of details, sometimes revealing useful structures.

R Killick-Kendrick et al. (1994 *Ann Trop Med Parasitol 88*: 183-196) demonstrated the importance of observation of the base of spermathecal ducts for the identification of Kenyan *Phlebotomus (Larroussius)* spp. and described a technique for the dissection of female abdomen. With the insects in Berlese's fluid, they separated the terminal part, using entomological pins (size 00) attached to small wooden sticks, and covered it with a coverslip.

With this technique, it is very difficult to see the spermathecae and ducts during the dissection, because they are fragile, transparent and frequently shrink. Excellent results were obtained by passing the insects in phenol (85%- 24 hr), potassium hydroxide (10%- 12 hr), acetic alcohol (10%- 15 min), acid fucsin (8-10 min), 70ºGL, 90ºGL, 95ºGL and absolute alcohol (15 min each), oil of cloves (24 hr), and dissection and mounting in NC medium (N Cerqueira 1943 *Mem Inst Oswaldo Cruz 39*: 37-41).

The preparation method of this medium is the following (EAB Galati pers. commun.): absolute alcohol (20 ml) and camphor (10 g) are mixed in an Erlenmeyer. Colophony (22 g) and pure alcohol-soluble copal (12 g) are added and the mixture is heated in a water bath. After complete dilution of the previous reagents, terebinthine (10 ml) is mixed to the hot mixture, which is filtered. Finally, eucaliptol (26 ml) is added.

Insects are transferred from one liquid to the other using a small basket of metal mesh (1506 divisions/ cm^2), of the type formerly used for Kato-Katz stool examination (N Katz et al. *Rev Inst Med Trop S Paulo 14*: 397-400). After separating the terminal part of the abdomen, the genital fork with ducts and spermathecae is dissected from adjacent tissues and covered by a small coverslip. Less than 5 min are spent to dissect and mount each insect. With this technique, the stained genitalia can be seen during dissection and is always perfect after mounting; transferring the insects is very easy, with a reduced risk of breaking of appendices.

Berlese's fluid is quicker and easier to use than balsam or NC, principally in ecological studies [RP Lane 1993 *Sandflies (Phlebotominae)*, p. 78-119. In RP Lane & RW Crosskey (eds) *Medical Insects and Arachnids*, Chapman & Hall, London], and its use was recommended by DJ Lewis [1973 *Phlebotomidae and Psychodidae (Sandflies and Mothflies)*, p. 155-179. In KGV Smith *Insects and other Arthropods of Medical Importance*, British Museum (Nat. Hist.), London].

However, Berlese's fluid is not recommended by Lane (*loc. cit.*) for long-term preservation. Many of the Berlese-mounted insects I have examined in a study of *Lutzomyia intermedia* (Lutz & Neiva, 1912) and *L. neivai* (Pinto, 1926) (CB Marcondes 1997 *Mem Inst Oswaldo Cruz 91:* 457-462) had bad female genitalia, sometimes two or three years after mounting, whereas, in some material mounted in balsam in 1937 and 1949, respectively by Drs F Fonseca and DT Lucena, spermathecae and ducts are still visible.

I compared the insects mounted with Berlese's fluid and NC and, although I noted some significant differences in the measurements of structures, they were not as extreme as those observed by A Dampf (1947 *Ann Esc Nac Cien Biol 4:* 423-435).

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