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# Anesthetic effects of two chemicals on the fertility of *Drosophila willistoni*.

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The use of chemicals as anesthetic substances to handle large number of flies both in the laboratory as well as in the field work have a practical interest to Drosophilists. The most common chemical substances used to anesthetize flies are ether and carbon dioxide (Ashburner and Thompson, 1978), but they present some disadvantages: high cost and short anesthetic effect (rarely lasts longer than 10 minutes). As an alternative, Fuyama (1977) proposed the use of triethylamine as an anesthetic for *Drosophila* with prolonged effects. A drop (less than 0.1 ml) of triethylamine is enough for one dose, and since it is less volatile than ether, retains its potency for one hour or more.

According to Fuyama (1977), the effects caused in the mortality and fertility for the use of triethylamine of *D. melanogaster* are similar to those caused with ether. We have been using triethylamine as the routine chemical to anesthetize flies in our research work (Goñi *et al.*, 1997, 1998) as well as in the teaching classroom. One of our research projects is the study of genetic variation on natural populations of *D. willistoni* from Uruguay (Valente *et al.*, this issue). Our experience indicates that *D. willistoni* is more sensitive under laboratory conditions than *D. melanogaster*, so would like to test the effect of two anesthetic substances, ether and triethylamine (= T.E.), in the fertility of *D. willistoni*.

Three wild type strains were used to test the influence of T.E. and ether in the fertility of *D. willistoni*: SULS $96b^2$, SUMU $98e^5$, both derived from isofemale lines collected in Piriapolis City, Maldonado, February 1996 (the former), and Facultad de Agronomía, Montevideo City, May 1998 (the later), and WIP4, a strain maintained for 30 years in laboratory conditions from the State of Bahía, Northwest of Brazil. Flies were anesthetized in a chamber made of a 50 ml polypropylene conical tube (30 mm diameter of clear, good quality tube) adapted as follows: the tube is cut to its conical base, now its tip and plugged by sponge cork with a hole in the middle. Flies are transferred to the polypropylene tube through its tip and plugged with the sponge. An ear cotton ("cottonete") previously wet with ether at one end is introduced to the tube and let hang half way down the tube until the flies are immobilized. Vapors of T.E. are dispensed through a 500 ml polyethylene wide mouth wash bottle previously filled with cotton where 1 to 2 ml of T.E. was previously dropped. Stocks and experimental matings were cultured at 25ºC constant temperature in 25 × 95 mm shell vials containing 10 ml of a medium composed of corn meal, brewer's yeast, glucose, agar and propionic acid. Experimental crosses involved 5 females and one male, 3-day-old virgin adults of each sex per mating vial, after 4 days, the parents were transferred to a vial containing fresh medium for a second week's progeny sample. A total of five matings were performed for each treatment (T.E., ether and control). In the control matings, virgin adults were selected with an aspirator. The progenies of each mating were counted and classified by sex (data not shown).

Table 1 shows the total progeny recorded in each cross and under each treatment. A nonparametric test was then applied. For each case (corresponding to the intracrosses of WIP-4, SULS $96b^2$ and SUMU $98e^5$ strains) a Kruskal-wallis test was performed in order to compare the three samples

Table 1. Total progeny in *D. willistoni* using different anesthetic treatments.

| Cross | T.E.* | | | | | | Ether | | | | | | Control | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total progeny/ cross | | | | | | Total progeny/ cross | | | | | | Total progeny/ cross | | | | | |
| | 1 | 2 | 3 | 4 | 5 | Total | 1 | 2 | 3 | 4 | 5 | Total | 1 | 2 | 3 | 4 | 5 | Total |
| WIP-4 x WIP-4 | 6 | 19 | 4 | 11 | 9 | 49 | 235 | 184 | 170 | - | 181 | 770 | 82 | 19 | 354 | 485 | 289 | 1229 |
| SULS 96b[2] x SULS 96b[2] | 34 | 44 | 3 | - | - | 81 | 10 | 289 | 85 | 22 | 20 | 429 | 179 | 279 | 307 | 248 | 45 | 962 |
| SUMU 98e[5] x SUMU 98e[5] | 132 | 106 | 103 | 51 | 66 | 458 | 134 | - | 69 | 133 | 413 | 749 | 315 | 55 | 232 | 269 | 111 | 1073 |

*Triethylamine

(corresponding to the T.E., ether and control treatment). In each case we have three samples of size 5, which we regard as independent and identically distributed. In order to avoid ties, and fulfill the hypothesis that each observation comes from a continuous distribution function, we added to each sample, a sample of 5 independent identically distributed uniform random variables in the interval [0.0.1]. The statistic to be considered is:

$$S = \frac{12}{5N(N+1)} \sum_{i=1}^{3} \left( R_i - \frac{5(N+1)}{2} \right)^2$$

Where $R_i$ is the sum of rank of the elements $i$ sample, in the joint sample of size N = 15 (Gibbons 1985). The nonparametric statistic is based on the deviations of the sum of ranks of each sample, with respect to its expected value under the null hypothesis (that each sample comes from the same population). The null hypothesis is rejected for large values of S. In the first case, WIP-4, a value of 6.98 was obtained for *S*, which gives a p-value $\leq 0.05$. In the second case, 96b2, a value of 6.72 was obtained for *S*, which gives a p-value $\leq 0.02$. Finally, in the case of 98e5, a value of 2.48 was obtained for *S*, which gives a p-value $\geq 0.1$. So, in this case, there is not enough evidence to reject the null hypothesis. Based on these results we can conclude that in general, the use of triethylamine or ether decreases the fertility of *D. willistoni* though we have not enough evidence to conclude the same in the case of 98e5. Our data may be insufficient to generalize that these two anesthetic chemicals decrease the fertility of *D. willistoni,* since in one case, 98e5, we do not have enough evidence to reach the same conclusion.

It is known that sensitivity of ether in *D. melanogaster* is influenced by a number of factors, *i.e.*, temperature during the etherization, the ether dosage or the length of exposure, also the sex and age of the flies, and also cytoplasmic and polygenic components (see Ashburner and Thompson, 1978). More data are needed to know the factors that may influence the observed sensitivity of T.E: and ether on fertility in *D. willistoni.*

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