MARKING WITH PIGMENTS FOR IDENTIFICATION OF FLIES IN EXPERIMENTAL POPULATIONS OF *Megaselia scalaris* LOEW

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(With 1 figure)

ABSTRACT

The insect marking technique of Tadei & Mourão (1976) is until now the only experimental method allowing real-age determination of each individual in a population and, consequently, determination of age structure in a given population. We propose an improvement of this technique, used here to determine the population age structure of the geographical strain SR of the *Megaselia scalaris* Loew (Diptera; Phoridae), maintained by serial transfer technique at constant temperatures 25 ± 1.0°C and 20 ± 1.0°C. Determining the age structure allowed the calculation of the real longevity of the flies and the identification of the effect of temperature decisive factors in these are the technique of marking insects, because otherwise we would only have an estimate, and depending on mistakes there in, the effect of the determinant factor (temperature) cannot be detected.

Key words: technique for marking insects, Megaselia, populations, age structure.

RESUMO

Marcação com tinta para identificação das moscas em populações experimentais de *Megaselia scalaris* Loew

A técnica de marcação de insetos de Tadei & Mourão (1976) é, até o momento, o único método experimental que possibilita determinar a idade real de cada indivíduo na população e, conseqüentemente, determinar a estrutura etária da mesma. Para isto propomos um aprimoramento dessa técnica, utilizada aqui para determinar a estrutura etária de populações da linhagem geográfica SR do díptero forídeo *Megaselia scalaris* Loew, mantidas pela técnica da transferência seriada em câmaras com temperatura constante de 25 ± 1,0ºC e 20 ± 1,0ºC. O estabelecimento da estrutura etária permitiu calcular a longevidade real das moscas e detectar o efeito ambiental temperatura, sendo fator determinante neste trabalho a marcação dos insetos, pois se não o fosse, teríamos somente estimativas e, dependendo do erro cometido na estimação, o efeito do fator de interesse (temperatura) poderia não ser detectado.

Palavras-chave: técnica de marcação de insetos, Megaselia, populações, estrutura etária.

INTRODUCTION

The age determination of each individual in populations is important in understanding population dynamics. Specifically, the formulation of a model allowing the establishment of population age structure could make possible the study of birth and death processes so that some characteristics like birth rate, mortality, and longevity, which constitute important parameters for understanding the whole process, could be described. Tadei & Mourão (1976) proposed a technique developed for *Drosophila* which made possible the marking of insects. Age of individuals in the population could be identified after etherized flies were spread out on a sheet of white paper and sprayed using a plastic flask (like those commonly

used for deodorants) which released a fine mist of paint particles. The paint solution had the following composition: 100 ml of distilled water, 50 ml of Coralmur white base, and from 8 to 10 g of Coralcor pigment paste. According the pigment paste was of different colors according to its purpose. For the marking process, the mist had to be of fine droplets, so that the marking process wold have no effect on mortality and, consequently, on individual longevity. In Corradi & Mourão (1980), and Corradi, *et al*. (1980), this technique was used with *Drosophila*.

The current objective was to introduce modification of the technique for marking insects, designed to apply to experimental populations of *Megaselia scalaris* (Diptera; Phoridae), as well as to determine the environmental temperature effect, in an experiment planned to ascertain population age structure and the real longevity of the flies.

MATERIAL AND METHODS

The adaptation of the Tadei and Mourão technique uses a painting device adapted to a low pressure air compressor (Jetmaster II – 2.3 PCM). The device constructed according to the principle of the Venture tube. It consisted of two pieces of copper capillary tube perpendicularly placed, so that two of the tips were in close proximity (Fig. 1). The air compressor was connected to the extremity of one of the tubes through a rubber hose. The extremity of the other was submerged in a recipient containing paint. When air is released by the compressor, suction occurs and, through the two extremities very in close proximity, the paint mist forms marks the etherized flies.

Although use of this equipament facilitated marking, it demanded too much work; besides, as the paint dried, the flask would clog, a problem solved by using thin copper threads for cleaning. This modification required a change in the marking paint composition.The results combining two products Coral, both soluble in water: Coralmur, a white latex base, and Coralcor pigments whose mixture follows a factory code. The proportion, tested in a pilot experiment and accepted as having no effect on the fly mortality, specifically on *Megaselia scalaris* Loew (Diptera, Phoridae), called for two portions of distilled water for one of paint.

With SR strain flies from Seropédica, the State of Rio de Janeiro, Brazil, kept in the Biology Department of IBILCE-UNESP of São José do Rio Preto, six experimental populations were started and maintained at constant temperature (25 ± 1.0^{o} C and 20 ± 1.0^{o}C), with three replicas at each temperature. The serial transfer technique (Buzzati-Traverso, 1955, cf. Tadei & Mourão, 1981) was used to conserve the populations. This technique consists of introducing the founder flies into a 250 ml bottle with fresh medium (day 1-Monday) to deposit their eggs for two days. They were then transferred (day 3-Wednesday), without etherization, to another bottle with fresh medium for two more days, and after this period they were again transferred (day 5-Friday), without etherization, to another new bottle where they stayed for three days. When they started to emerge from the pupae, the imagoes were collected three times a week, on the same days that the population was transferred to the new bottles (Monday, Wednesday, and Friday). These young flies were etherized, counted, and marked with different-color paints for identification and age structure study, and then added to the adult population. The adult flies were thus maintained in a single bottle with fresh medium, while the other bottles contained eggs, larvae, pupae, and recently-emerged flies. Every 7 days (Mondays), before the young fly addition, the adults were etherized, counted for sex and color, and soon afterwards transferred to a new bottle. The populations kept at constant temperature (25 ± 1.0^{o}C) were maintained in 15 bottles, while the populations at 20 ± 1.0^{o}C were kept in 18 bottles.

RESULTS AND DISCUSSION

The monday censuses lasted 36 weeks for, the bottles with the adults of the population; marking allowed us to identify flies with 3, 5, 7, 10, 12, 14, 17, 19, 21,... days of age. Thus, the population the age structure was able to be described and is contained in Tables 1 and 2, which present only female data.

The data produced at 25^{o}C (Table 1) show that 98% of the flies were concentrated in the 3-14 day age groups while at 20^{o}C (Table 2) the same percentage occurred in the 3-21 day age groups.

Tables 1 and 2 show that at 25^{o}C the concentration of flies of 3, 5, and 7 days was greater than that at 20^{o}C, when the relative frequencies of these classes were reduced and the frequencies of the next classes consequently increased. At 20^{o}C there were survivors until the twenty-eighth day, whereas at

25°C, after the twenty-first day, there were practically no more survivors.

The 95% confidence intervals of the replicas 1, 2, and 3 are described in Table 3, respectively, for temperatures of 25°C and 20°C. For each age, at each temperature, the overlapping (intersection) of confidence intervals of replicas 1, 2, and 3 is observed, showing that the replica effect is not significant.

Table 4 shows 95% confidence intervals for the means of replicas 1, 2, and 3, at temperatures of 25°C and 20°C. At each age, for the two temperatures, the intervals are disjointed (empty intersection), except those of 12, 14, and 17 days. This result shows the significant effect of temperature on the population size and the distribution of this effect on the age of the flies.

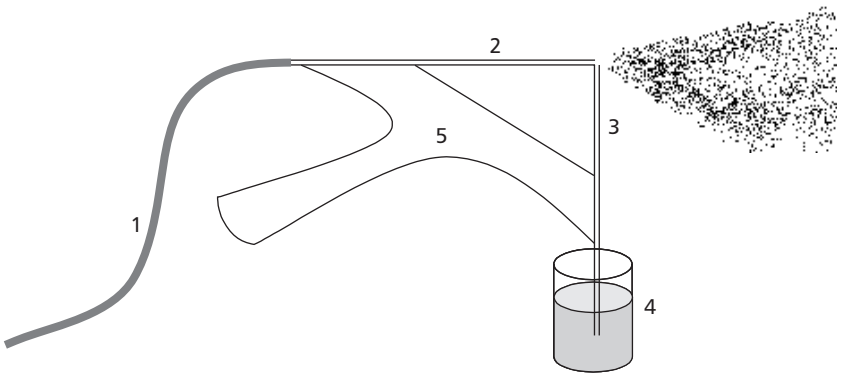

Fig. 1 — Illustration of the painting device: 1. rubber hose; 2. copper capillary tube 2 mm in diameter; 3. copper capillary tube 1 mm in diameter; 4. paint recipient; and 5. support for copper tubes.

$\overline{X} \pm S_{\overline{X}}$

TABLE 1

Mean number of females ( ) by age groups (in days), for the populations SR_1, SR_2, and SR_3 kept at 25°C.

Days	Replicates			
	SR_1	SR_2	SR_3	Mean
	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$	$\overline{X} \pm S_{\overline{X}}$
3	124.1 ± 9.9	121.3 ± 9.9	120.1 ± 9.9	121.8 ± 5.7
5	88.3 ± 9.9	94.3 ± 6.5	85.7 ± 6.4	89.4 ± 4.0
7	114.9 ± 9.9	108.1 ± 9.9	116.9 ± 9.9	113.3 ± 6.0
10	41.5 ± 5.9	38.3 ± 5.8	37.1 ± 6.0	38.9 ± 3.4
12	24.1 ± 4.1	23.2 ± 3.4	18.9 ± 2.6	22.0 ± 2.0
14	20.9 ± 3.8	18.5 ± 4.6	19.5 ± 4.5	19.6 ± 2.5
17	9.2 ± 2.1	3.6 ± 1.0	6.0 ± 1.9	6.2 ± 1.0
19	3.7 ± 1.4	1.7 ± 0.7	0.9 ± 0.4	2.1 ± 0.5
21	1.2 ± 0.4	0.7 ± 0.4	0.9 ± 0.4	0.9 ± 0.2
24	0.4 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.1
26	0.3 ± 0.2	----- ± ----	----- ± ----	----- ± ----
28	0.1 ± 0.1	----- ± ----	----- ± ----	----- ± ----

TABLE 2

Mean number of females ($\overline{X} \pm S_{\overline{X}}$) by age groups (in days), for the populations SR_1, SR_2, and SR_3 kept at 20°C.

TABLE 3

The 95% confidence intervals of the populations SR_1, SR_2, and SR_3, at 25°C at 20°C.

Temperatur	Days
	3
	5
25°C	7
	10
	12
	14
	17
	19
	21
	24
	26
	28
20°C	

For longevity calculation, the Tadei & Mourão (1981) formulation was used, as it takes into account the age structure of each population, obtaining this weighted average: $\overline{X} \pm S_{\overline{X}}^{k}$, x_i representing the fly age, and the relative frequency of observed flies in each age group.

The occurrence pattern of the age groups at 25ºC and 20ºC, verified respectively in the three replicas, shows which age groups can determine the population size as a result of the temperature variation.

Table 5 presents the mean longevity values of the females at 25ºC and 20ºC. The statistical test for replica effect, as expected, was not significant; but the t-Student unilateral test for verification of the temperature effect was significant (t = 1.76 and p = 0.04 < 0.05), showing that the mean longevity of the flies at 20ºC was superior to that at 25ºC.

The determination of the real mean longevity of the flies can only be done when the population age structure is experimentally established through the insect marking technique.

When the age structure of a population is unknown, the mean longevity of the flies can be estimated by Levene's method (cf. Dobzhansky and Pavlovsky, 1961).

$$\overline{X} = \sum_{i=1}^{k} x_i \, fr_i$$

TABLE 4

The 95% confidence intervals for the mean of the populations SR_1, SR_2, and SR_3, at 25°C and 20°C.

Days	Temperature	
	25°C	20°C
3	[110.6; 133.0]	[62.3; 70.3]
5	[81.6; 97.2]	[43.6; 58.0]
7	[101.5; 125.1]	[57.0; 77.0]
10	[32.2; 45.6]	[22.3; 32.1]
12	[18.1; 25.9]	[15.5; 22.9]
14	[14.7; 24.5]	[22.1; 34.7]
17	[4.2; 8.2]	[7.1; 11.7]
19	[1.1; 3.1]	[4.2; 8.8]
21	[0.5; 1.3]	[4.9; 10.7]
24	[0.1; 0.5]	[1.7; 4.5]
26	–	[0.5; 3.3]
28	–	[0.1; 1.7]

TABLE 5

Mean longevity of females ( ) for experimental populations SR_1, SR_2, and SR_3 kept at constant temperatures (25 ± 1.0ºC and 20 ± 1.0ºC).

Replicates	25ºC	Replicates	20ºC
	$\overline{X} \pm S_{\overline{X}}$		$\overline{X} \pm S_{\overline{X}}$
SR_1	7.2 ± 0.6	SR_4	9.1 ± 0.8
SR_2	6.8 ± 0.5	SR_5	9.1 ± 0.8
SR_3	6.8 ± 0.5	SR_6	9.0 ± 0.8
Mean	6.9 ± 1.0	Mean	9.1 ± 0.5

This is a mathematical model and, like all models, is a simplification of natural processes; as such, it is based on theoretical premises which describe the phenomenon, whether natural or experimental. This methodis, however, valid only as a model, when all the premises are satisfied. Thus, estimate reliability depends on that.

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