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Karyotypic characterization of Muscina stabulans (Fallen) (Diptera: Muscidae) using conventional staining, silver staining and C-banding

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Karyotypic characterization of *Muscina stabulans* (Fallen) (Diptera: Muscidae) using conventional staining, silver staining and C-banding

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SUMMARY — Chromosome analysis of *Muscina stabulans* with Giemsa and silver nitrate staining and C-banding revealed an example of an exception among Muscidae. *M. stabulans* also presents the diploid chromosomal set consisting of 10 biarmed chromosomes, being 4 pairs metacentric and one pair submetacentric. None of the pairs was found heteromorphic. Positive but small C-band regions were always located on the centromeric regions of all chromosomes; an intersticial C + band appears only on the short arm of chromosome 2. In meiotic plates only chromosome 2 was observed bearing one Ag-NOR site on the short arm too.

Key words: chromosomes, C-banding, Ag-NOR, *Muscina stabulans*, false stable-fly.

INTRODUCTION

Muscina stabulans is widespread throughout the world and is usually associated with waste products of livestock and agricultural production (WILLIAMS *et al.* 1985). In southeastern Brazilian egg-laying poultry facilities *M. stabulans* is recognized as being a numerically important member of the arthropod fauna from July to November (AVANCINI and UETA 1990). Its potential as carrier of pathogens to humans and domestic animals is comparable to that of *Musca domestica* (LECLERCQ 1969; WILLIAMS *et al.* 1985).

Despite the false stable fly's importance in health and sanitation, very little is known about its chromosomes and its genetics. Questions remain concerning the karyotype of this fly. METZ (1916) apparently thought that *M. stabulans* had six pairs of chromosomes. BOYES *et al.* (1964) observed the presence of only five pairs, describing the probable absence of the sexual chromosomes. These authors, however, did not publish the karyotype or mention which classification they used to name the chromosomes of *M. stabulans*.

Among muscoid Diptera, $2n = 12$ is the most constant diploid complement, with five pairs of autosomes and one sexual pair, heteromorphic.

Therefore, *M. stabulans* (subfamily Muscinae) along with *Haematobia irritans* (subfamily Azeliinae) (AVANCINI and WEINZIERL 1994; PARISE 1994) and very few other species are exceptions within this group.

In our laboratory, studies of the Muscidae have been undertaken to improve the characterization of the karyotypes of several species, especially those that have a different number of chromosomes than the mode, to contribute to an understanding of evolutionary mechanism. *Haematobia irritans* (AVANCINI and WEINZIERL, 1994; PARISE, 1994) has already been examined, and some other species belonging to the seven different subfamilies present in the New World (CARVALHO *et al.* 1993) will be soon.

In the present contribution, we report the karyotype of *M. stabulans* using conventional staining. The amount and position of constitutive heterochromatin in *M. stabulans* were also examined through C-banding. The location of nucleolar organizer regions were investigated using silver impregnation.

MATERIALS AND METHODS

Fly Rearing. — *Muscina stabulans* from the 2nd generation kept in laboratory culture were used in this study. The rearing conditions for all life stages were 24° ± 2° C, 40-50% relative humidity, light regime of LD 12:12. The colony was started from flies collected in a private poultry facility located 40 km southwest of the State University of Campinas campus. Larvae were reared in a mixture of animal ration plus ground beef in a proportion of 4:1. Adults were fed on sugar cane 24 hours/day and on ground beef only few hours/day. Water was always available.

Chromosome preparations. — Third instar larvae were dissected in 0.85% saline to remove the brain. Adult males were dissected to remove the testes. The material was squashed in a drop of 45% acetic acid after fixation in 3:1 ethanol:acetic acid for 4 minutes. The coverslip was removed after imersion in liquid nitrogen. Embryonic tissues, prepared in the same way, were used to better locate the secondary constriction due to the less condensed state of the chromosomes of those tissues.

Chromosome morphology. — For morphological studies the slides analysed were mainly those stained with 2% Giemsa in 0.1M phosphate buffer at pH 7.0 for 20 minutes.

Mean descriptive values of the karyotype were calculated from information obtained from a minimum of one well-spread mitotic metaphase plate measured from each of the 10 individuals.

The nomenclature used for the description of chromosome morphology is that proposed by LEVAN *et al.* (1964).

C-banding. — C-banding using Giemsa staining was performed according to the technique of SUMNER (1972) with slight modifications.

Nucleolar silver staining. — The colloidal silver method described by HOWELL and BLACK (1980) and some modifications of it were used to tentatively identify the nucleolar organizer regions (NORs).

Pictures were taken with a Zeiss photomicroscope, using Agfa Copex X-Pan film.

RESULTS

Chromosome morphology.

The diploid chromosome number of *M. stabulans* is $2n = 10$ (Fig. 1). All the elements of the karyotype are biarmed, with four of the pairs metacentric (1, 2, 3 and 5) and only one submetacentric (4) but not typical as documented in Table 1. The size of the chromosomes varies from 7.42 ± 0.13 to 4.9 ± 0.10 µm. Pairs 1 and 2 are not only the largest but also those which present the lowest arm ratio which makes them the most typical metacentric of the set (see Table 1). The most commonly observed secondary constrictions are located only on these two chromosomes: two on the long arm of pair 1 and one on the short arm of pair 2 (Figs. 2 and 3). These constrictions are not conspicuous enough, however, to be observed in metaphasic plates other than those from embryonic tissues (Fig. 2). We did not find any obvious heteromorphic pair in any of the plates we analysed.

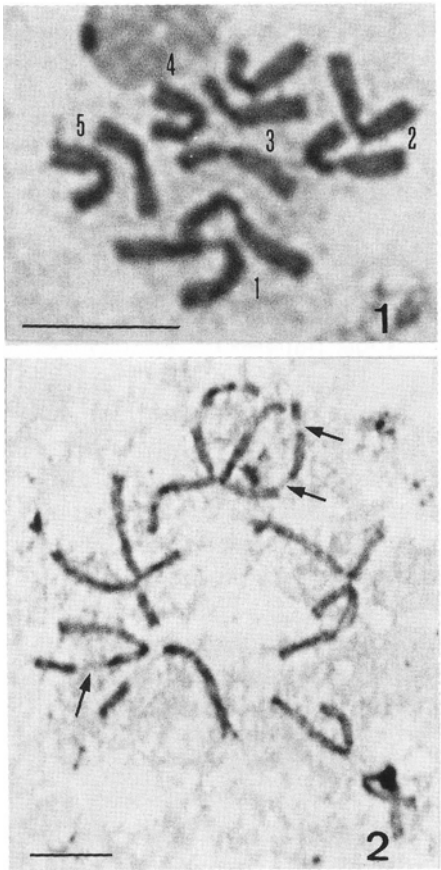
Ag-NOR.

Silver impregnation did not allow us to identify NORs in mitotic chromosomes. In the great majority of mitotic preparations, all the centromeric regions were stained, indicating the lack of specificity of this method. However, using meiotic plates, we found pair 2 consistently stained at the interstitial position,

TABLE 1 - Morphometric data of mitotic chromosomes of *Muscina stabulans*. Nomenclature after LEVAN *et al.* (1964).

Chromosome	1	2	3	4	5
Length (µm) + SD	7.42 ± 0.13	6.46 ± 0.22	5.46 ± 0.15	5.29 ± 0.14	4.9 ± 0.10
Arm ratio	1.16	1.16	1.52	1.76	1.31
Centromeric index	45.85%	46.17%	39.62%	36.44%	43%
Relative lenght	24.60	21.20	18.50	17.30	16.20
Designation	m	m	m	sm	m

Haploid genome lenght (*n*) 29,71 µm



Figs. 1 and 2. — Silver impregnation in mitotic metaphases. Fig. 1. Karyotype of *Muscina stabulans* (larval brain). Fig. 2. Secondary constrictions in chromosomes 1 and 2 (arrows) (embryonic tissues). Bars 5 μm.

on the short arm (Fig. 4), around the region where the secondary constriction was observed.

The results above are reinforced by the fact that the silver staining technique revealed that the majority (93%) of the cells had only one nucleolus and the remaining portion (7%) only two. This indicates the existence of only one pair of nucleolar-organizing regions in the karyotype (Fig. 5).

C-band.

No large segments of constitutive heterochromatin were detected in the complements of *M. stabulans* studied. All the chromosomes showed a common

C-banding pattern basically characterized by the presence of clearly visible heterochromatin blocks in the centromeric region (Fig. 6). In one of them, pair 2, the constitutive heterochromatin is also placed interstitially along the short arm, in a region related to the secondary constriction (Figs. 6 and 7). We did not observe any telomeric band.

DISCUSSION

Our results on chromosome number of *Muscina stabulans* confirm the description of BOYES *et al.* (1964) of five pairs of chromosomes and the absence of an obvious heteromorphic pair.

According to our results, *M. stabulans* does not possess a single wholly C-banded chromosome nor an arm with an extensive C-banded region, characteristics that are common in the Dipteran sexual chromosomes (ULLERICH 1976; BEDO 1980; EL AGOSE *et al.* 1992). EL AGOSE *et al.* (1992) described the Y chromosome of *Musca domestica* as uniformly C-banding positive and the X

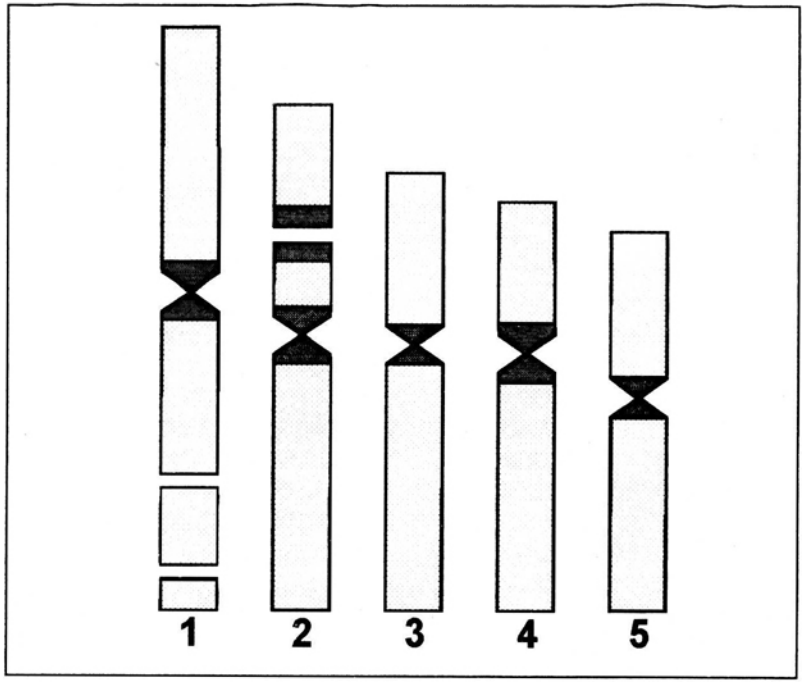
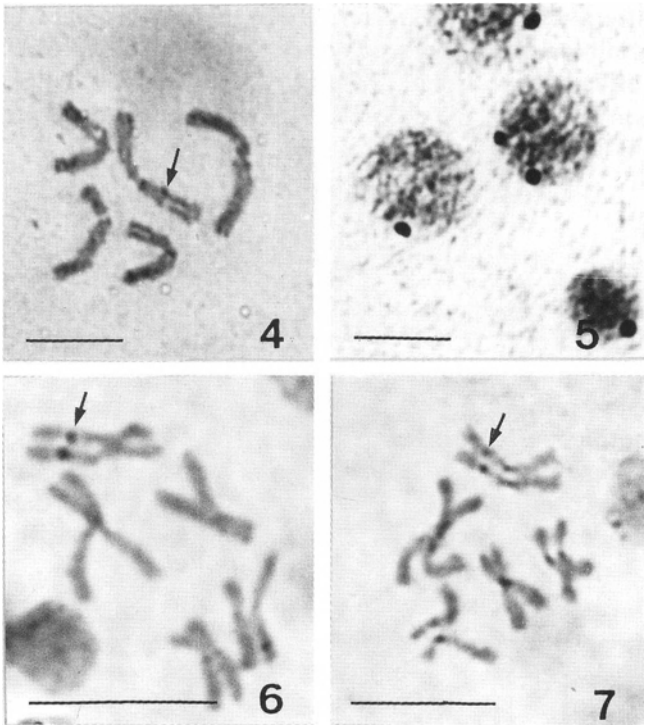


Fig. 3. — Idiogram of *Muscina stabulans* based on mean values shown in Table I. Dark regions are C-band positive.



Figs. 4 and 5. — Silver impregnation. Fig. 4. Meiotic metaphase: chromosome 2 bearing NOR (arrow) (testis). Fig. 5. Nucleoli of interphase nuclei revealed by silver impregnation (larval brain).

Figs. 6 and 7. — C-banded metaphases of *Muscina stabulans* (larval brain). Arrows show the interstitial band on chromosome 2. Bars = 5 μm.

with one dark region on each side of the non-staining centromere, followed by a short, light region and a distally darker region on each arm. If we take the C-banding pattern of *M. domestica* (EL AGOSE *et al.* 1992) as the standard for Muscidae, our data on *M. stabulans* C-banding reinforces the opinion of loss at least of part of sexual chromosomes' material, that is the constitutive heterochromatin which can not be identified in the chromosomal set anymore. Therefore, independently of the mechanisms involved in the diminishing of one chromosome pair, it seems apropriate to conclude that it is accompanied by loss of most of C-banded material present in the sexual chromosomes.

With results similar to those reported by AVANCINI and WEINZIERL (1994) and PARISE (1994) for *Haematobia irritans* and by HAGELE and RANGANATH (1983) for *Drosophila nasuta,* we were not able to obtain stained NORs in

mitotic chromosomes of *M. stabulans* by means of silver impregnation. This method is successfully employed to identify the sites that were transcriptionally active during the preceding interphase in most vertebrate (SCHWARZACHER and WACHTLER 1993, review) and in some groups on insects (SUJA and RUFAS 1987). These two Muscid flies present one similarity in the Ag-NOR position: in both, only one site was found by this method, and in both, the NOR is not terminally located. Therefore, at least one of the secondary constrictions observed in *M. stabulans,* that located on chromosome 2, probably represents a NOR. Moreover, it seems that segments adjacent to the NOR are constitutively heterochromatic, as has been described for some vertebrates (SCHMID 1982; KING *et al.* 1990), as well as for some invertebrates (CABRERO *et al.* 1986).

Very little is known about NOR positions and patterns of C-banding in Muscidae species for comparison with our results.

Because the diploid complement of $2n = 12$ is the most common in Cyclorraphan Diptera it might be generalized that this karyotype structure is more primitive among these flies. However, the presence of flies carrying $2n = 10$ distributed through different subfamilies of Muscidae shows that this generalization is not a simple matter. Accepting the current classification of the family Muscidae would require an explanation that many independent losses of chromosomes occurred in the evolutionary history of this group. In our future papers on the cytogenetics of the Muscidae we hope to add more data to this controversial issue.

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