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Number of chromosomes, Feulgen-DNA content, and nuclear phenotypes in domestic and wild specimens of *Panstrongylus megistus*

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Specimens of *Panstrongylus megistus* with different levels of adaptation to a domestic way of life had some of their nuclear and chromosomal characteristics compared, in an attempt to differentiate them by cytological means. The number of chromosomes in male testes was found not to differ. The nuclear frequency and phenotypes of the Malpighian tubules were also found to be the same. On the other hand, Feulgen–DNA values of metaphase chromosomes in insects from a population with a sylvatic distribution were found to be larger than those in specimens from a domestic population. The differences in Feulgen–DNA values, however, may not represent real differences in DNA content, but may possibly be due to differences in DNA–protein complexes.

Panstrongylus megistus is one of the most important vectors of Chagas' disease in Brazil, because of its wide distribution, high rates of infection with *Trypanosoma cruzi*, and its geographically variable power of invading artificial ecotopes. This species occurs in extra-Amazonian sylvatic regions, and becomes adapted to a domestic way of life in areas where human behaviour has changed natural forests into open land (Forattini, 1980).

Sylvatic populations of *P. megistus* are found in local areas of the São Paulo state covered by perennial rain forest (Serra do Mar system). As well as sylvatic populations, populations showing domesticated behaviour are found in the São Paulo plateau, in the North-Northeast region of the state. In this area there are residual patches of tropical forest, surrounded by large areas of agricultural and cattle-raising land dotted with human habitations, exposed to colonization by *P. megistus* (Forattini *et al.*, 1978).

Significant morphological differences have not been found when comparing these two populations of *P. megistus*. This fact has increased the interest in comparative studies at the cellular level, especially because among the various interpretations of the synanthropy of the species is a suggestion that it is polytypic (Pessoa, 1962).

In this work the number of chromosomes and their Feulgen–DNA content in male testes have been determined in both domestic and sylvatic populations. Since the nuclear

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phenotypes and the number of nuclei per organ vary among species of Triatominae, especially in Malpighian tubules (Mello, 1971; Mello and Lima, 1978; Andrade, 1984), these nuclear characteristics have also been investigated in *P. megistus*.

MATERIALS AND METHODS

Fifth instar nymphae of *Panstrongylus megistus* Burmeister 1835 were used. Insects from populations showing domestic behaviour were obtained from São João da Boa Vista (São Paulo state) and from the south of the Minas Gerais state (Fig. 1). The insects from a sylvatic population were from Juquiá (São Paulo state) (Fig. 1), and were first generation specimens reared in the laboratory.

Fig. 1. Localization of the distribution of *P. megistus* populations, the specimens of which were studied in this work. C, continental (domestic) populations; L, Juquiá (sylvatic populations).

The number of chromosomes was determined in squashes of testes of at least ten nymphae of each population. The preparations were stained with lacto-acetic orcein or subjected to Feulgen reaction. Acid hydrolysis conditions pertinent to Feulgen reaction were: 4N HCl at 24°C for one hour and five minutes.

Feulgen–DNA values in arbitrary units were obtained for meiotic metaphase plates with automatic scanning cytophotometry, using Zeiss equipment and a Microdata computer. Operating conditions were: 100/1·25 objective, optovar 2, measuring diaphragm dia. = 0·1 mm, field diaphragm dia. = 0·2 mm, LD-Epiplan 16/0·30 condenser, 0·5 × 0·5 μm spot size, and $\lambda = 575$ nm.

The nuclear phenotypes of somatic cells were studied in whole-mounted Malpighian tubules. The nuclear frequencies were also determined for these organs.

RESULTS

The number of chromosomes of the wild population specimens of *P. megistus* was found to be the same as that of the insects with domestic behaviour, that is $2n = 18$ autosomes, X_1X_2Y (Figs. 2–6). As previously shown for domestic *P. megistus* specimens and for other Triatominae species bearing X_1 and X_2 chromosomes (Ueshima, 1966; Schreiber *et al.*, 1972), no pairing of the sex chromosomes was detected in metaphase I. During metaphase II the phenomenon of 'touch and go' pairing described by Ueshima (1966) was observed for the sex chromosomes of gently squashed plates of specimens from both insect populations.

The Feulgen–DNA values determined for the metaphase plates of division I and II of meiosis in males were plotted as frequency histograms (Fig. 7). It could be shown that the values for either metaphase I (4C values) or metaphase II (2C values) differ when comparing the insects from domestic and sylvatic populations (Table 1). The Feulgen–DNA values of the chromosomes of the sylvatic population were slightly larger than those of the domestic insects (Fig. 7, Table 1).

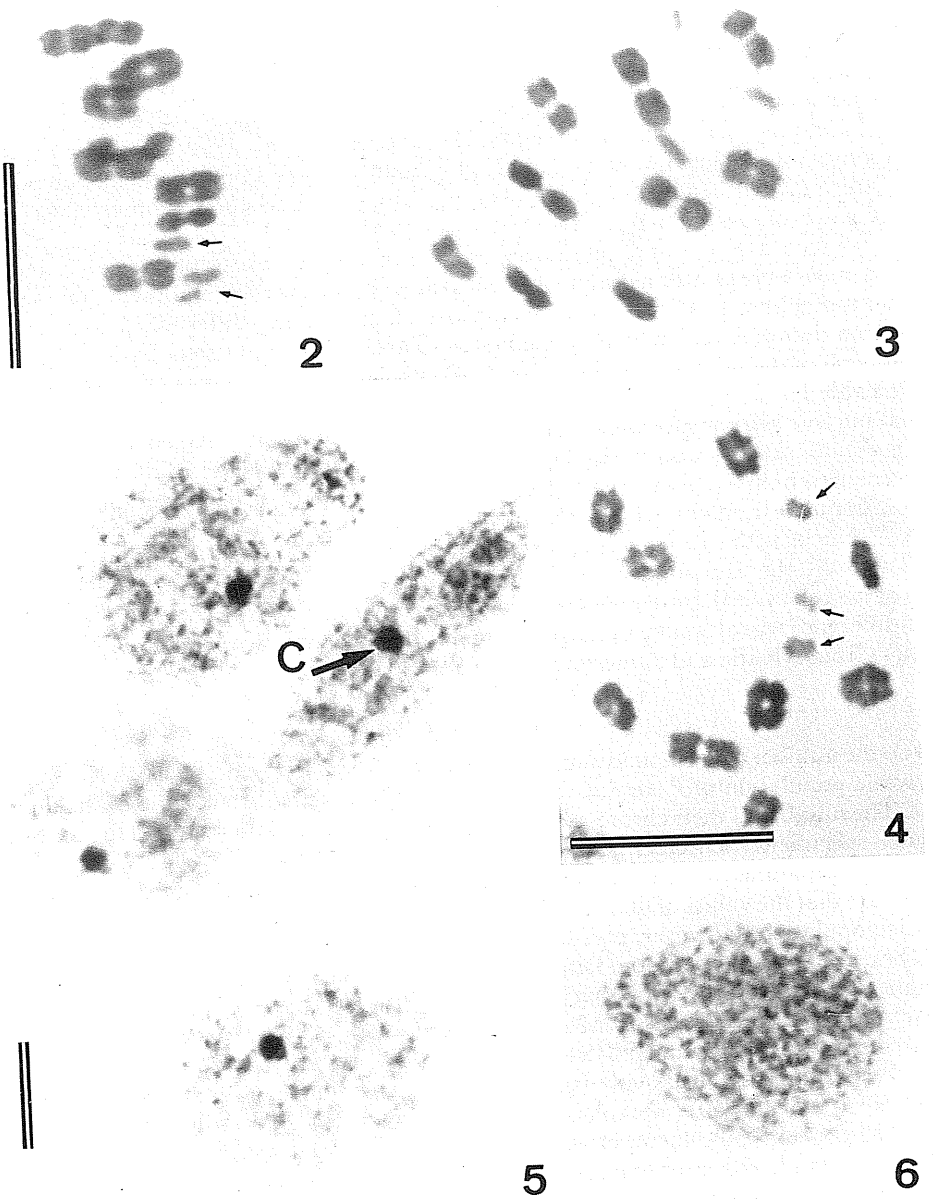
Two nuclear phenotypes were found in the Malpighian tubule nuclei of *P. megistus*: one exhibits a small chromocentre (Fig. 5), whereas the other is devoid of it (Fig. 6). Both phenotypes were present in the organs of insects from the two populations. However, the former were the most frequent in males, while only nuclei without chromocentres were found in females (Table 2). Nuclei with a degenerating appearance were also observed in some insects. Eventually (Table 2), the non-condensed chromatin appeared filamentous, looking like certain phases of the cell cycle detected in polyploid cells of other hemipteran species (Geitler, 1939).

No difference was observed in the nuclear and chromocentre frequencies when comparing specimens from sylvatic and domestic populations (Table 3).

DISCUSSION

Based on the number of autosomes and sex chromosomes of male meiotic cells, the specimens of the sylvatic population of *P. megistus* could not be differentiated from those of the domestic insects. The number of their chromosomes agrees with previous data recorded for continental *P. megistus* specimens (Schreiber *et al.*, 1972).

The cytophotometric results established for Feulgen-stained chromosomes, however, demonstrate that the values of the sylvatic population were statistically larger than those of the domestic population. However, possible differences in DNA content, based on these results, should be considered with caution. Different responses to Feulgen reaction may occur in nuclei under the same conditions of the hydrolytic procedure because of differences in chromatin compactness (Mello, 1979, 1983; Mello and Vidal, 1978, 1980). Furthermore, other data deserve consideration. Attempts to detect chromosome areas in *P. megistus* gave contradictory results. Preparations stained with lactoacetic orcein exhibited a total chromosome area of 16·62–50·39 μm^2 ($n = 50$) (metaphases I and II) for *P. megistus* of the sylvatic population and 30·31–60·62 μm^2 ($n = 50$) (metaphases I and II) for the insects of the domestic population. On the other hand, apparent enlargement of the chromosome areas with Feulgen reaction occurred in both populations. The absorbing area of the Feulgen-stained chromosomes for which the Feulgen-DNA values were evaluated in this work, and also determined with the scanning cytophotometer, revealed values in the range of 27·25–92·00 μm^2 ($n = 56$) (metaphase I) and 21·00–50·25 μm^2 ($n = 47$) (metaphase II) for sylvatic *P. megistus* and 23·00–96·25 μm^2 ($n = 69$) (metaphase I) and 15·50–46·50 μm^2 ($n = 41$) (metaphase II) for domestic insects. As chromosomes of the studied populations swell differently with Feulgen reaction, this could lead them to respond differently to DNA and/or apurinic acid breakdown with acid hydrolysis (Mello and Vidal, 1978). Possibly DNA–protein complexes responsible for chromatin compactness could differ, but the DNA content could be the same. In addition,



Figs. 2–4. Metaphase meiotic chromosomes of males of *P. megistus*. The arrows indicate the sex chromosomes. Figs. 2 and 3. Sylvatic specimens. Fig. 4. Domestic specimens. Bars, 10 μm.

Figs. 5 and 6. Nuclear phenotypes of polyploid nuclei of Malpighian tubules of males (Fig. 5) and females (Fig. 6). The arrow indicates the small chromocentre (C) present only in male cells. Bars, 10 μm.

TABLE I

Welch's t-*test (Bickel and Docksum, 1977) and analysis of variance for comparison of the Feulgen-DNA values of metaphase chromosomes of domestic and sylvatic specimens of* P. megistus

Meiotic phase	*Feulgen-DNA values*	
	Sylvatic population ($\bar{X} \pm S$)	*Domestic population* ($\bar{X} \pm S$)
Metaphase I	30·460 ± 1·620	28·895 ± 2·010
Metaphase II	63·218 ± 3·780	59·071 ± 3·959

t-*test*

Meiotic phase	~df	t	P	*Decision*
Metaphase I	68	3·861	0·0003	Highly significant ($P_{0·01}$)
Metaphase II	118	5·927	0·0000	Highly significant ($P_{0·01}$)

Analysis of variance

Metaphase I

Due to	df	SS	SS/df	F *ratio*
Factor	1	50·75	50·75	15·69
Error	82	265·24	3·23	
Total	83	315·99		

Decision, highly significant ($P_{0·01}$).

Metaphase II

Due to	df	SS	SS/df	F *ratio*
Factor	1	524·6	524·6	34·83
Error	121	1822·6	15·1	
Total	122	2347·3		

Decision, highly significant ($P_{0·01}$).

it is worth mentioning that specimens of the domestic and sylvatic populations used in this work can be crossed, giving rise to fertile offspring (Ferraz-Filho, unpublished data). In conclusion, only u.v. cytophotometry can solve the question of difference or similarity in the DNA content of chromosomes of these insect populations. The examination of spermatids, instead of metaphase plates, for comparing Feulgen–DNA values was discarded because nuclear histones are replaced by other basic protein(s) during spermatogenesis of Triatominae, giving rise to different levels of DNA stability towards Feulgen acid hydrolysis in spermatids and spermatozoa (Silva and Mello, 1986). The exact phase of spermatogenesis at which the substitution of the basic proteins occurs is not precisely known in *P. megistus*. The lack

TABLE 2

Frequencies and phenotypes of Malpighian tubule nuclei of P. megistus

Populations	*Sex*	*Total number of nuclei per whole organ*	*Number of nuclei, per whole organ, with*			
			Chromocentre present	*Chromocentre absent*	*Nuclear degeneration*	*Filamentous euchromatin*
Domestic	Male	19 380	18 708	672	—	—
	Male	18 586	18 176	308	102	—
	Male	18 189	17 842	347	—	—
	Male	18 262	17 928	334	—	—
	Male	18 392	18 108	284	—	—
	Female	18 508	—	18 508	—	612
Sylvatic	Male	18 976	18 484	218	274	—
	Male	19 020	18 644	376	—	—
	Male	18 652	18 244	308	—	—
	Female	18 310	—	18 024	286	2 800
	Female	18 500	—	18 500	—	—
	Female	18 500	—	18 500	—	—

TABLE 3

Welch's t-*test (Bickel and Doksum, 1977) for comparison of the nuclear and chromocentral frequencies of Malpighian tubules of domestic and sylvatic specimens of* P. megistus

Frequencies	~df	t	P	*Decision*
Nuclei	8	−0·507	0·6260	Non-significant ($P_{0·05}$)
Chromocentres	5	−1·598	0·1709	Non-significant ($P_{0·05}$)

of this information, and the possibility of existing differences in the DNA–protein complexes for the insect populations compared, could prejudice the correct evaluation and comparison of spermatid Feulgen–DNA values.

The nuclear phenotypes and frequencies in Malpighian tubules do not differ when comparing insects of the different populations. The finding of a small chromocentre in the Malpighian tubule polyploid nuclei in males only is suggestive that it could represent the Y chromosome. On the other hand, the fact that some nuclei in the organs of males are devoid of a conspicuous chromocentre may indicate unpacking of this heterochromatin, resembling findings in *Triatoma infestans* specimens subjected to physiological stress (Mello, 1983). In support of this hypothesis, work in progress reveals that there is no difference in the Feulgen–DNA content of *P. megistus* Malpighian tubule nuclei with and without a conspicuous chromocentre. Possibly some genes not usually expressed in the Y chromosome of *P. megistus* could be triggered under special physiological conditions (Simões and Cestari, 1982; DiBerardino *et al.*, 1984). Alternatively, the cells containing these nuclei may be mutants whose wild-type product is necessary for condensation of the heterochromatin of the Y chromosome (Gatti *et al.*, 1983).

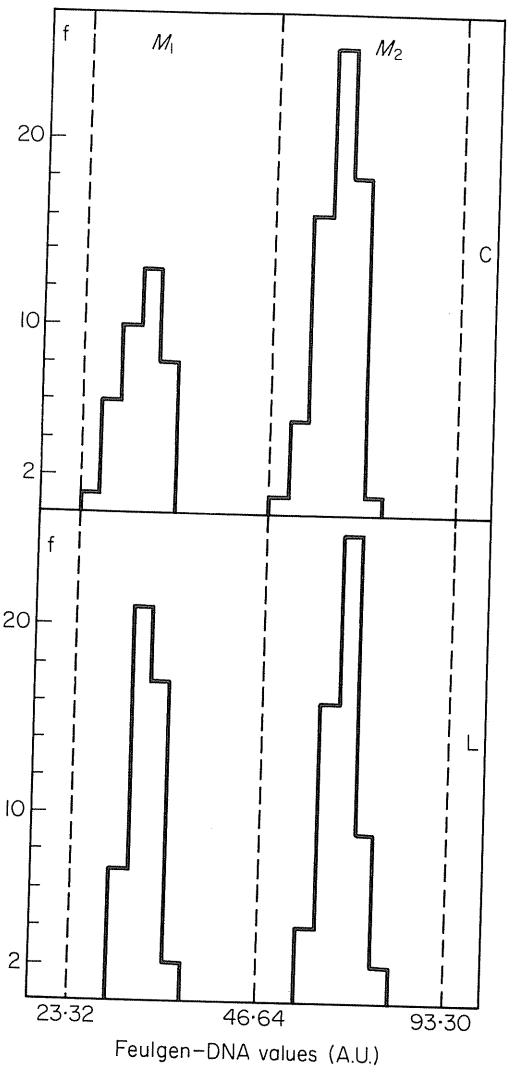


Fig. 7. Frequency histograms plotted for the Feulgen–DNA values of metaphase chromosomes (M_1 and M_2) of *P. megistus* specimens of sylvatic (L) and domestic (C) origins.

Nuclear degeneration has been found in a few nuclei of the Malpighian tubules of *P. megistus*, a finding which has also been reported in specimens of *Triatoma infestans* subjected to starvation or to copper pollution (Mello, 1983; Kubrusly, 1984), but which may also result from mutagenesis for heterochromatin condensation (Gatti *et al.*, 1983).

The cytological data discussed in this work could not discriminate the domestic and sylvatic populations of *P. megistus* from each other, except by a suggestion of differences in DNA–protein complexes. Possibly nuclear protein(s) could be involved in the activation of certain genes, up to the point of promoting changes in species behaviour.

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