Nuclear Phenotype Changes after Heat Shock in *Panstrongylus megistus* (Burmeister)

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The nuclear phenotypes of Malpighian tubule epithelial cells of male nymphs of the blood-sucking insect, Panstrongylus megistus, *subjected to short- and long-duration heat shocks at 40ºC were analyzed immediately after the shock and 10 and 30 days later. Normal nuclei with a usual heterochromatic body as well as phenotypes indicative of survival (unravelled heterochromatin, giants) and death (apoptosis, necrosis) responses were observed in control and treated specimens. However, all nuclear phenotypes, except the normal ones, were more frequent in shocked specimens. Similarly altered phenotypes have also been reported in* Triatoma infestans *following heat shock, although at different frequencies. The frequency of the various nuclear phenotypes observed in this study suggests that the forms of cell survival observed were not sufficient or efficient enough to protect all of the Malpighian tubule cells from the deleterious effects of stress. In agreement with studies on* P. megistus *survival following heat shock, only long-duration shock produced strongly deleterious effects.*

Key words: *Pantrongylus megistus* - heat shock - nuclear phenotypes - cell survival - apoptosis - necrosis

The effect of heat shock on survival and molting incidence in *Panstrongylus megistus* varies with the duration of the shock, the developmental stage and sex of the specimens, and in certain cases, the insect's habits and nutritional states (Garcia et al. 1999).

P. megistus is less resistant to heat shock than *Triatoma infestans*, indicating that no generalization can be made about the responses of different reduviid species to temperature shocks (Rodrigues et al. 1991, Garcia et al. 1999).

As with other stress factors, heat shocks induce cytological changes in *T. infestans*, including nuclear fusion, heterochromatin unravelling, and cell necrosis and apoptosis, as part of the mechanisms of cell survival and cell death, respectively (Mello 1989, Dantas & Mello 1992, Mello et al. 1995, Tavares et al. 1997).

The Malpighian tubule cells in late nymphs of *P. megistus* are highly polyploid (Mello 1975). In males, the most usual nuclear phenotype of this organ has a homogeneous distribution of granulous chromatin and a small but conspicuous heterochromatic body formed by several copies of the Y chromosome (Mello et al. 1986).

Since *P. megistus* and *T. infestans* differ in their normal nuclear phenotypic characteristics (Mello 1971, 1975, Mello et al. 1986), these nuclear phenotypes may be affected differently by heat shock treatment.

In the present study, the nuclear phenotypes of *P. megistus* nymphs were determined after heat shock and the changes compared with those for *T. infestans* under similar temperature conditions (Dantas & Mello 1992).

MATERIALS AND METHODS

Fifth instar male nymphs of a domestic population of *P. megistus* (Hemiptera, Reduviidae), descended from insects obtained in Fazendas Pedra Balão and Pedra Branca in São João da Boa Vista (State of São Paulo) and reared in the laboratory at Sucen (Mogi Guaçu, SP), were used. The control nymphs were maintained at 28ºC and 80% relative humidity, conditions which have traditionally been used for rearing this species in the laboratory at Sucen since 1980.

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The treated specimens underwent heat shock at 40°C for 1 h and 12 h. The choice of this temperature was based on a previous study of survival and molting incidence in *P. megistus* after heat shock (Garcia et al. 1999). The specimens were fasted for 15 days before the shock and after treatment were returned to a normal diet of hen blood once a week.

Malpighian tubule preparations were obtained immediately after heat shock and 10 and 30 days later. The organs from at least three specimens were used for each experimental condition and the corresponding control.

Whole Malpighian tubules were mounted on glass slides, immediately fixed in acetic ethanol for 1 min, rinsed in 70% ethanol for 5 min and air dried at room temperature. The material was then subjected to the Feulgen reaction, with hydrolysis in 4 M HCl at 25°C for 1 h and 5 min. The Feulgen-stained material was rinsed in sulfurous and distilled water, air dried, cleared in xylene and mounted in Canada balsam.

A Zeiss light microscope was used to count the total number of Malpighian tubule epithelial cell nuclei per specimen, to identify the different nuclear phenotypes and to evaluate their frequencies in each specimen. Photomicrographs were obtained with a Zeiss Axiophot II microscope.

A linear correlation was used to evaluate the relationship between the stress conditions and the various nuclear phenotypes.

RESULTS

The most frequent phenotype in the control and heat shocked specimens consisted of nuclei with a small heterochromatic body containing copies of the Y chromosome (Mello et al. 1986) in the middle of evenly distribution of lightly stained chromatin (Fig. 1, Table I).

TABLE I

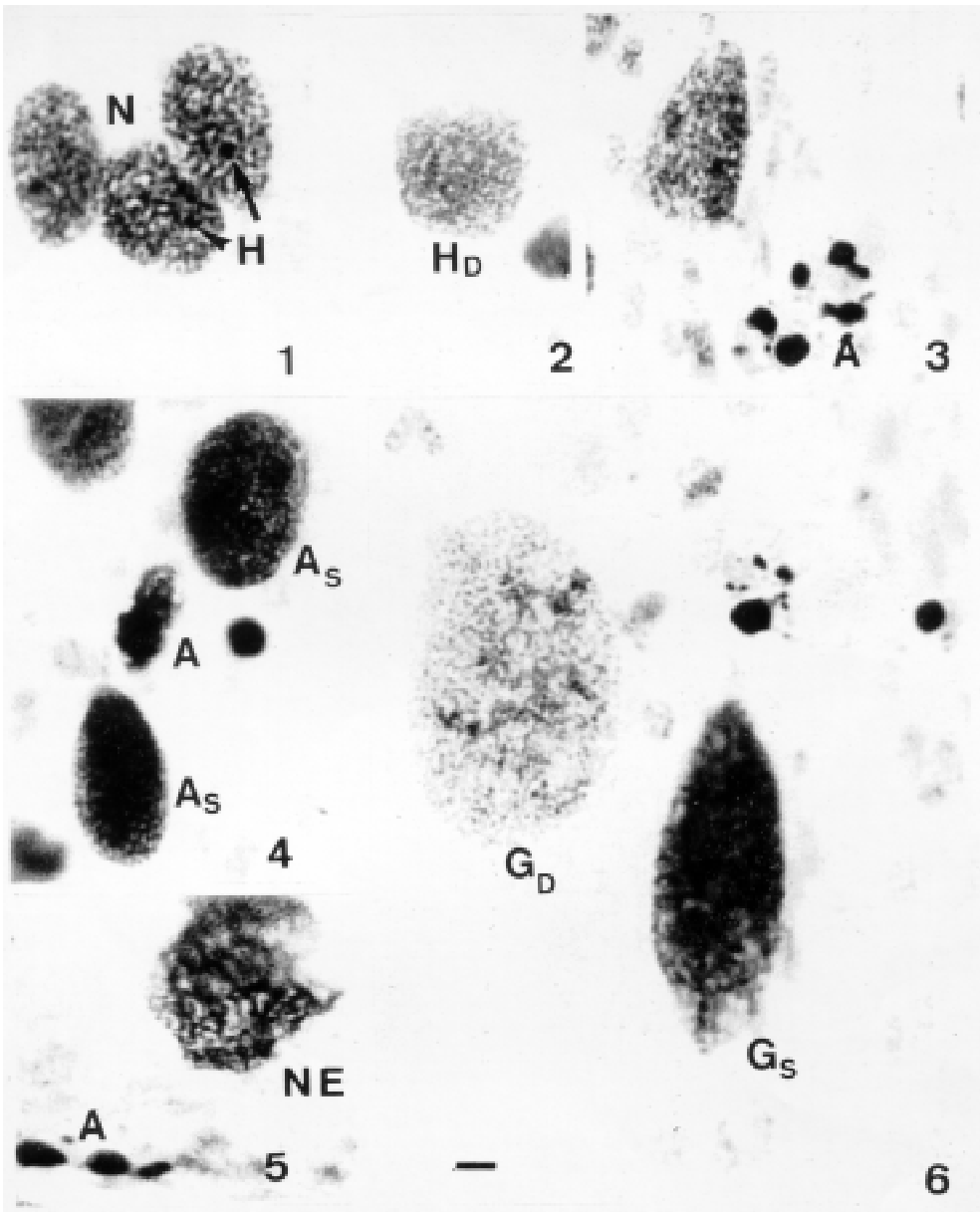
Absolute frequencies of nuclear phenotypes in Malpighian tubule epithelial cells of *Panstrongylus megistus* 5th instar nymphs after heat shock at 40°C

Experimental conditions	Nuclear phenotypes									
	A	A_S	NE	G	G_{NE}	G_{HD}	G_S	HD	N	Total
Control, t_0	10	1324	1255	25	4	3	0	147	5302	8070
	0	1345	192	1	1	0	0	54	8786	10378
	6	2347	1037	0	0	0	0	35	10499	13951
1 h shock: t_0	54	2405	2292	12	1	2	15	143	9934	14858
	77	1295	655	11	2	0	0	284	7914	10238
	18	1447	984	3	0	0	0	340	13714	16506
12 h shock: t_0	16	2661	1402	1	0	0	4	119	13354	17557
	3	2244	676	13	7	2	4	224	15389	18562
	5	2954	1655	12	4	3	51	1048	11678	17410
Control, $t_{10\ days}$	7	1729	325	6	0	0	0	98	9039	11204
	9	2482	116	0	0	0	0	76	6479	9162
	16	3252	728	0	0	0	0	268	11428	15692
1 h shock: $t_{10\ days}$	37	2783	1477	14	0	3	17	667	9904	15236
	25	1385	1272	68	20	17	22	275	3978	7062
	35	2703	1696	229	87	33	20	525	10834	16162
Control, $t_{30\ days}$	25	2587	3	0	0	0	0	173	10242	14506
	11	1351	1882	10	0	0	8	295	8169	11726
	23	2704	1962	5	0	0	0	424	10673	15791
	8	1408	1522	5	0	1	0	164	7271	10379
1 h shock: $t_{30\ days}$	11	3960	1164	1	0	0	0	864	11206	17224
	23	3973	1576	0	0	0	0	335	10386	16283
	8	2754	866	3	0	0	0	130	7122	10239
	19	2218	745	5	0	0	0	130	7122	10239
12 h shock: $t_{30\ days}$	93	2329	2995	43	75	13	165	148	11894	17755
	96	4787	840	19	15	0	12	124	12446	18339
	47	3046	2445	14	3	3	17	204	7766	13555

A: apoptosis; A_S: suspected apoptosis; NE: necrosis; G: giant nuclei; G_{NE}: giant nuclei under necrosis; G_{HD}: giant nuclei with heterochromatin decondensation; G_S: giant nuclei suspected of apoptosis; HD: heterochromatin decondensation; N: normal.

Other nuclear types observed in all the experimental conditions were generally more frequent after heat shock (Table I). These included nuclei with heterochromatin unravelling (Fig. 2), apoptotic nuclei (Fig. 3), nuclei suspected of apoptosis (Fig. 4), necrotic nuclei (Fig. 5) and giant nuclei (Fig. 6). In some cases giant nuclei exhibited signs of necrosis or were suspected of apoptosis (Fig. 6). Some giant nuclei were also observed in which heterochromatin decondensation was suspected. Apoptosis and necrosis were defined here in terms of their classic morphological characteristics (Kerr 1971, Kerr et al. 1972).

There was a significant decrease in the relative frequency of normal nuclei with heat shock and its duration (Fig. 7a, Table II). The absolute frequency of nuclei with heterochromatin unravelling increased just after the short and long shocks, and 10 and 30 days after the short shock (Table I). However, in terms of relative frequencies, the in-



Figs 1-6: nuclear phenotypes in Feulgen-stained Malpighian tubules of *Panstrongylus megistus* specimens subjected to heat shock. Bar = 10 µm. A: apoptosis; A_s: suspected apoptosis; G_D: necrotic giant nucleus; G_S: giant nucleus with suspected apoptosis; H: heterochromatin; H_D; unravelled heterochromatin; N: normal; NE: necrosis.

TABLE II

Linear correlation between stress factors and nuclear phenotypes

	D	°C	H	A	A_S	NE	G	G_D	G_{HD}	G_S	HD
°C	.05										
A	-.03	**.29**	-.18								
A_S	**.38**	.08	-.02	.06							
NE	**.30**	.13	.06	.12	-.13						
G	-.18	.12	**-.22**	.06	-.01	**.26**					
G_D	-.02	**.23**	.03	.07	-.07	**.30**	.86				
G_{HD}	**-20**	.07	-.18	.08	.01	**.27**	.93	.79			
G_S	.13	**.31**	**.37**	.18	-.14	**.47**	**.24**	.63	**.33**		
HD	.09	**.38**	**-.34**	**.25**	.05	.15	.20	.06	.18	.08	
N	**-.47**	**-.27**	.07	**-25**	-.56	-70	**-.31**	**-.27**	**-.33**	**-.33**	**-.39**

D: days after shock; °C: temperature; H: shock duration; A: apoptosis; A_s: suspected apoptosis; NE: necrosis; G: giant nuclei; G_D: necrotic giant nuclei; G_{HD}: giant nuclei with heterochromatin unravelling; G_s: giant nuclei with suspected apoptosis; HD: heterochromatin unravelling; N: normal with usual heterochromatin body.

value Moderate correlation (between 20% and 50%)
value High correlation (> 50%)

crease in nuclei with heterochromatin unravelling was significant only immediately after short and long shocks (Fig. 7b, Table II); the frequency of this phenotype decreased after long shocks compared to short shocks (Fig. 7b). There was no significant correlation with the time after the shock (Table II). The appearance of heterochromatin decondensation was moderately correlated with that of apoptotic nuclei (Table II).

A few giant nuclei were detected (Table I). The relative frequency of this nuclear phenotype decreased immediately after long shocks when compared to short shocks (Fig. 7c), i.e. there was a slight negative correlation between shock duration and phenotype frequency (Table II). There was no correlation between the relative frequency of morphologically normal giant nuclei and the time after the shock or the shock temperature, but a moderate correlation was observed between giant nuclei with heterochromatin decondensation and the time after the shock (negative), between the occurrence of necrotic giants and shock temperature (positive) and between giants suspected of apoptosis and the shock temperature and duration (positive) (Table II).

There was a high correlation between giant nuclei with normal morphological characteristics and necrotic giant nuclei as well as between necrotic giants and giants suspected of apoptosis (Table II).

The absolute and relative frequencies of apoptotic nuclei increased immediately after heat shock (Fig. 8a, Tables I, II), but there was no significant correlation between these frequencies and the time after shock or the duration of the shock (Table II).

The relative frequency of nuclei suspected of apoptosis did not increase with the heat shock temperature or duration, but there was a moderately significant correlation between the increase in this frequency and the time after shock (Fig. 8b, Table II). The absolute and relative frequencies of necrotic nuclei increased significantly 10 and 30 days after the heat shock (Tables I, II, Fig. 8c).

DISCUSSION

The number of nuclei in the Malpighian tubules of fully-nourished laboratory-reared *P. megistus* is about 18,000 (Mello et al. 1986). In the present study, control specimens had a much smaller nuclear frequency, indicating that nuclear fusion and cell death induced by other stressors may have occurred under laboratory conditions. Indeed, the specimens used in this investigation had been moderately fasted prior to the heat shock. Fasting is a stress agent in blood-sucking insects (Andrade & Mello 1987, Mello 1989). The choice of a slight fasting condition for the present study was based on the finding that only after it some 5th instar specimens are capable of surviving a long heat

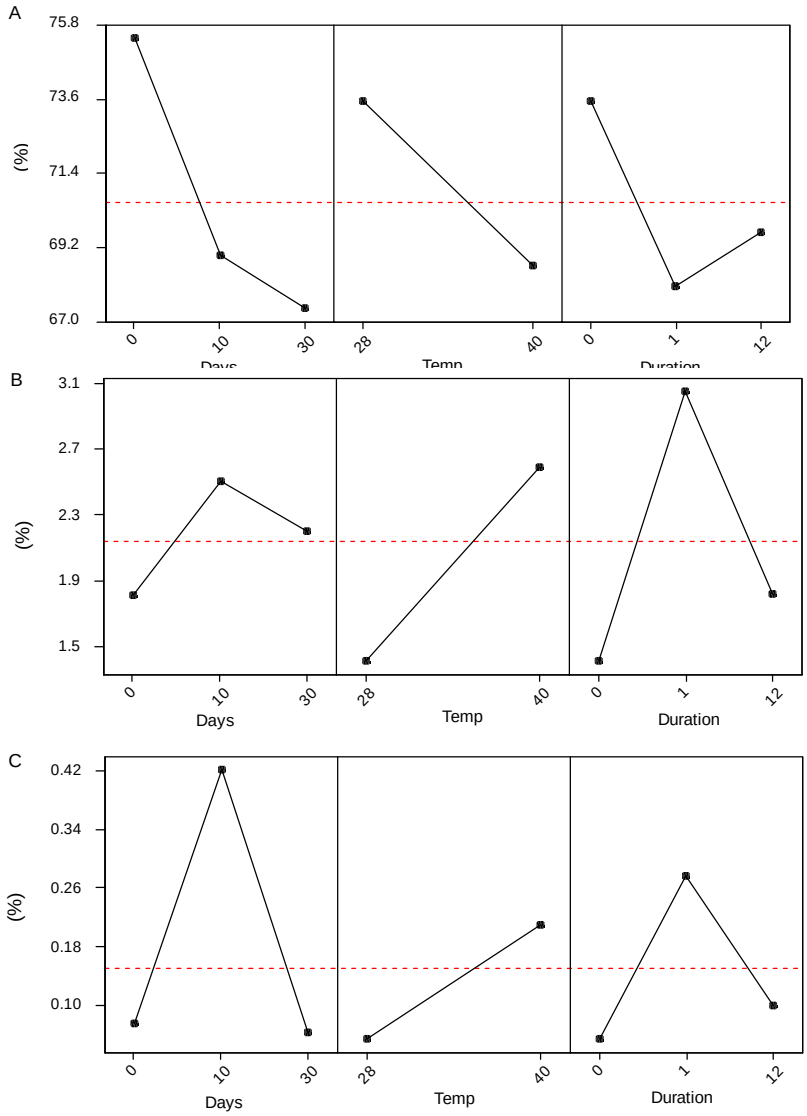


Fig. 7: influence of time after shock, shock temperature and shock duration (h) on the relative frequency of normal nuclei (A), normally-sized nuclei showing heterochromatin unravelling (B) and giant nuclei (C) of *Panstrongylus megistus*.

shock (Garcia et al. 1999). The observation that some altered nuclear phenotypes also occurred in the control specimens supports the idea that some stressing effect other than heat shock was involved. Maybe an occasional and unexplained refusal of some specimens to feed a blood meal added some fasting period to that intentionally provoked.

Nuclear phenotypes differing from the normal phenotype seen in *P. megistus* have also been reported for *T. infestans* subjected to heat shock or other stressing agents (Álvares-Garcia 1988, Mello 1989, Dantas & Mello 1992, Mello et al. 1995). Such phenotypes may reflect mechanisms of cell survival (heterochromatin unravelling, nuclear fusion) or cell death (apoptosis, necrosis) in response to stress (Dantas & Mello 1992, Tavares et al. 1997).

Heterochromatin unravelling occurred even long after heat shock in *P. megistus.* This situation differs from that for *T. infestans* in which such unravelling is most frequent 10-120 min after the shock (Dantas & Mello 1992). If heterochromatin unravelling leads to the activation of silent genes during stress (Simões et al. 1975), its effects may be longer-lasting in *P. megistus* than in *T. infestans.* It is not known whether heterochromatic zones contain genes for heat shock proteins (hsp).

In various reduviid species, giant nuclei are produced by nuclear and/or cell fusion (Wigglesworth 1967, Mello & Raymundo 1980,

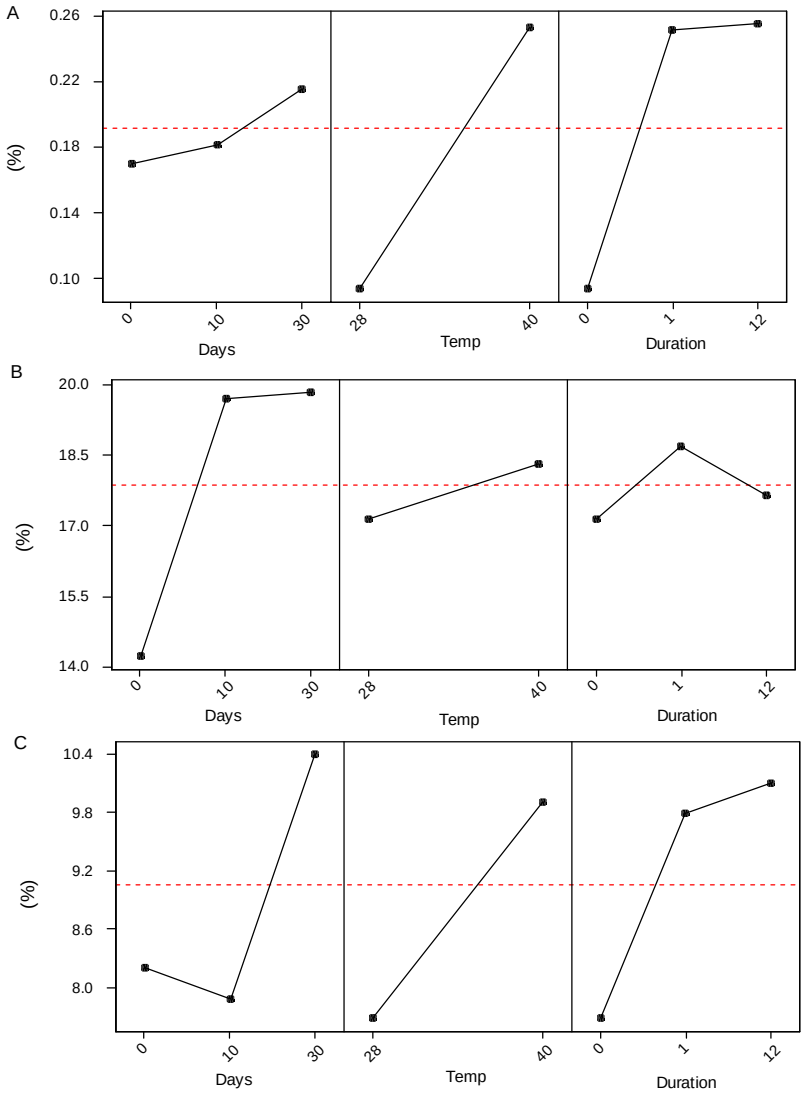


Fig. 8: influence of time after shock, shock temperature and shock duration (h) on the relative frequency of apoptotic (A), suspected apoptotic (B) and necrotic (C) nuclei of *Panstrongylus megistus.*

Mello 1989, Dantas & Mello 1992). These nuclei have been suggested to be involved in cell or organ survival mechanisms under unfavorable conditions. In this regard, the high mortality rates seen in *P. megistus* subjected to long heat shock (Garcia et al. 1999) suggest that giant nuclei were not present in sufficient numbers and/or were not efficient enough to protect the insects from heat shock-induced damage. Indeed, the relative frequency of giant nuclei decreased significantly with the shock duration whereas the number of giants suspected of apoptosis was weakly correlated with this factor.

P. megistus survivors of heat shock showed a significant decrease in the frequency of giant nuclei 10 to 30 days after the shock. In *T. infestans*, the highest frequency of giants occurs 30 days after the shock (Dantas & Mello 1992). In addition to a decrease in new fusions, the fact that some giant nuclei in *P. megistus* exhibited necrosis or were suspected of apoptosis probably also contributed to the reduction in their frequency. It is possible that in *P. megistus* cells and nuclei resulting from fusion may be more susceptible to metabolic failure than in *T. infestans*.

When the stress is enhanced beyond a certain level, the presence of hsp is incapable of protecting the cells and apoptosis program is initiated (Lindquist & Craig 1988, Maihos et al. 1993,

Samali & Cotter 1996). If the stress exceeds this level, cell death by necrosis predominates (Samali & Cotter 1996). Thus, murine mastocytoma cells subjected to a temperature of 43-44ºC show an increase in apoptotic index. At a temperature of 45ºC, both apoptosis and necrosis are observed in these cells while at 46-47ºC only necrosis is found (Harmon et al. 1990). Similar responses have been described in other cell types (Sakaguchi et al. 1995).

In the Malpighian tubules of *P. megistus*, apoptosis and necrosis occurred simultaneously, especially after hyperthermia. Only the apoptosis program intensified immediately after the heat shock. The frequency of the apoptotic nuclei then remained unchanged whereas necrosis, which was not significantly affected immediately after heat shock, intensified with the shock duration and time after shock.

Hsp expression, apoptosis, heterochromatin unravelling and nuclear fusion were thus not apparently sufficient to protect all *P. megistus* Malpighian tubule cells from the deleterious effects of heat shock. In terms of insect survival, only long shocks proved to be strongly deleterious (Garcia et al. 1999). In the few survivors of long shocks, there was probably no additional degeneration since no significant difference in the frequency of necrotic nuclei was observed compared to insects subjected to short shocks.

The individual variations in response to hyperthermia were similar to those seen in *T. infestans* after other stressing agents and suggest that specimens of *P. megistus* may also vary in their resistance to different stressors, including heat shock.

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