these cells are probably less affected by crowding and do not reduce the synthesis of type I collagen at confluency[12] as may occur in ALL fibroblasts. Density-dependent changes in the types of collagens synthesized by fibroblasts from different origins have been reported[12,13].

The ratio of collagen types here reported for normal human bone marrow stromal cells differs to that previously informed for murine stromal cells[14]. The discrepancy could be attributable to the different origin of the stromal cells or to the fact that in our studies cells were labeled for collagen synthesis at a low serum concentration[15].

These studies give additional support to previous observations on the existence of a population of damaged stromal cells in the bone marrow of ALL patients at diagnosis or during the early stages of therapy[5,16]. One may speculate that defects in the establishment of the extracellular matrix, such as those described in the murine bone marrow system[3], may contribute to an altered hemopoiesis.

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Puff activity after heat shock in two species of the *Drosophila obscura* group

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Summary. When individuals of *Drosophila guanche* are submitted to heat shock, five new puffs are induced. These puffs usually do not appear during normal development. Comparing these results with those obtained in *Drosophila subobscura*, also belonging to the *obscura* group, differences between the induced puffing pattern of both species have been found.
Key words. Drosophila guanche; Drosophila subobscura; puffs; heat shock.

It is well established that living organisms respond to heat shock by important changes in their gene expression[1-3]. Drastic alterations in the puffing pattern level after heat shock treatment have been observed in several *Drosophila* species: *D. busckii*[4], *D. melanogaster*[5,6], *D. hydei*[7]. These reports show that two effects clearly characterize the heat shock response at the puffing level: first, a new set of specific puffs is induced and second, the majority of puffs active during normal development (developmental puffs) decrease their activity. However, this model appears to be not so simple. *D. subobscura* displays some variability in the puffing response depending on the heat shock conditions[8]. Different responses were found depending on treatment temperature and an important number of developmental puffs do not decrease in their activity. Some of them maintain and some others increase their expression. This behavior contrasts with the remarkable conservation of the response described in a very wide variety of organisms, from *E. coli* to man[9].

In this work we compare data about the heat shock puffing response in *D. guanche* with those obtained by Pascual and de Frutos[8] in *D. subobscura*.

D. guanche, like *D. subobscura*, is considered to be a member of the *obscura* group of *Drosophila*[10-13]. Because of their cytogenetic characteristics both species are closely related and belong to the same cluster. A high degree of homology between the banding patterns of polytene chromosomes in the two species is evident, except for the sexual chromosome[14]. While *D. subobscura* exhibits a rich inversion polymorphism, *D. guanche* seems to be a monomorphic species. We used the *D. guanche* TF2 strain, descending from individuals captured in the Canary Islands. The TF2 strain differs from *D. subobscura* in at least six inversions[15].

Individuals synchronized at the beginning of prepupa formation (O-h prepupa), and incubated at 19 ± 1 °C, were exposed at 31 °C or 37 °C, during a period of 30 min. After temperature treatment all prepupae were dissected in Drosophila Ringer solution. For experimental treatments the method described by Pascual and de Frutos[8] and for cytological procedures the method described by de Frutos and Latorre[16] were used. Controls were carried out with synchronized prepupae which remained at 19 ± 1 °C, during the incubation period.

Five nuclei were sampled from each of the 60 individuals analyzed (20 individuals for each temperature, 31 °C or 37 °C, and control). Only two levels of activity were taken into account for each locus: (+) puff of maximum or medium size and (O) small size or no puff. For each experimental treatment, the number of type (+) observations related to the total observations, gives the frequency of appearance of each puff. Consequently, a high frequency of puffing would indicate a high gene activity.

For the location of the puffs, the chromosome map of

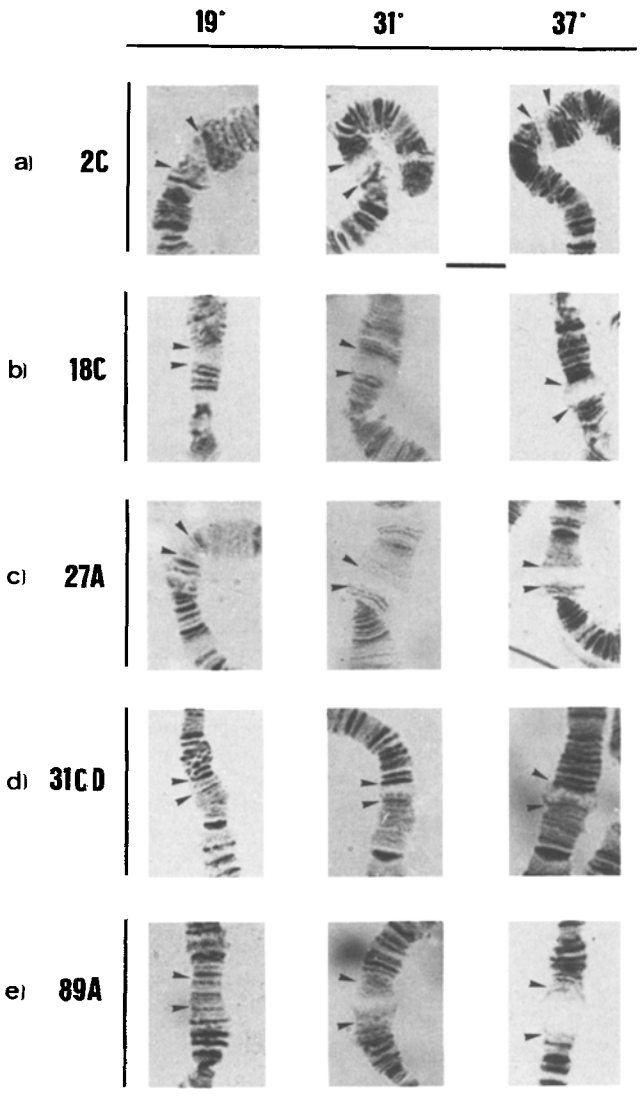

Figure 1. Puffs induced after heat shock treatments in *Drosophila guanche*. Bar = 5 μm.

D. guanche[14] was used. Because of strong homologies between the banding patterns of polytene chromosomes of *D. guanche* and *D. subobscura*, the nomenclature used on the *D. guanche* map is the same as that proposed by Kunze-Mühl and Müller[17] for *D. subobscura*.

In *D. guanche*, the heat shock induces a set of puffs that usually do not appear during normal development: 2C (A chromosome), 27A and 31CD (J chromosome) and 89A (O chromosome) (fig. 1, a, c, d, e). It seems important to point out puff 18C, which became highly active after temperature treatment (fig. 1,b). This puff appears active in some developmental stages, such as 18-h prepupa (unpublished data), but was never seen to be active at O-h prepupa. The table shows the frequency of puffing activity after heat shock (at 31 °C and 37 °C) in *D. guanche* and *D. subobscura*. Puffing frequency (%) is indicated as $5 \leq * < 30$, $30 \leq ** < 80$, and $80 \leq *** \leq 100$. Figure 2 shows the location of heat shock puffs of both species with respect to the standard gene arrangement of *D. subobscura*. From the table and figure 2, several features can be noted:

1) None of the puffs induced by temperature shock (at 31 °C or 37 °C) appear in the control group. It follows that these puffs can be considered as heat-inducible puffs in the strict sense.

2) The response to heat shock is not dependent on temperature in *D. guanche*. The same loci are puffed at both tested temperatures (31 °C and 37 °C). In contrast, *D. subobscura* behaves in different ways at 31 °C than at 37 °C. There is induction of differential gene activity at specific loci: 2C, 27A (after heat shock at 31 °C), 14AB and 31C/D (after shock at 37 °C).

3) *D. guanche* shows a stronger response after heat shock at 37 °C than at 31 °C, while in *D. subobscura* the response to heat shock is higher at 31 °C than at 37 °C. It could be assumed that 31 °C is the optimum heat shock for *D. subobscura* and 37 °C for *D. guanche*. It is possible that *D. guanche* tolerates higher temperatures than *D. subobscura*, because the first species is endemic to warm habitats, while *D. subobscura* is adapted to cold ambients.

4) Remarkable differences were found in the heat shock puffing patterns between the two species, especially in the A chromosome. Only puff 2C shows activity in *D. guanche*. The bands 14AB and 15DE never puff in this species. The heat shock puff 2C was used as a cytological landmark to compare the banding pattern of the A chromosome in the two species[14]. The most important differences between the banding pattern of *D. guanche* and that of *D. subobscura* are located in the A chromosome[14]. The pattern of induced puffs on the O and J chromosomes is more conservative. It is worth mentioning the behavior of 89A and 94A puffs on the O chromosome. In *D. subobscura* both puffs reach the largest size after treatment at 31 °C; whereas at 37 °C they are only slightly active. It has been demonstrated by 'in situ' hybridization, that both puffs encode for the hsp70 (de Frutos, in preparation). In *D. subobscura*, as well as in other *Drosophila* species, the most important hsp is hsp70[8]. However, in *D. guanche* only 89A is active. Locus 94A never shows puff activity in the last species.

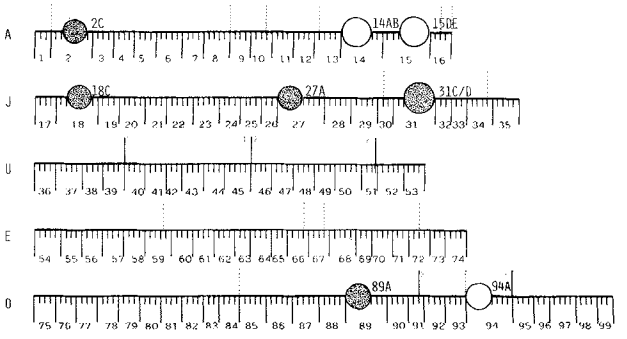

Figure 2. Location of heat shock puffs in *D. guanche* and *D. subobscura* with respect to the standard gene arrangement of *D. subobscura*. ◎ Heat shock puffs present in both species. ○ Heat shock puffs only present in *D. subobscura*. ····· Indicates the boundaries of specific inversions of *D. guanche*. —— Indicates inversions described in both species.

Frequency of puffing activity after heat shock in *Drosophila guanche* and *Drosophila subobscura*. Puffing frequency (%) is roughly indicated as: $5 \leq * < 30$, $30 \leq ** < 80$, $80 \leq *** \leq 100$

Loci	*Drosophila guanche*			*Drosophila subobscura*		
	19 °C Control	31 °C	37 °C	19 °C Control	31 °C	37 °C
2C		*	**		**	
14AB						*
15DE					*	**
18C		**	***		**	**
27A		**	***		***	
31C/D		*	*			**
89A		**	***		***	*
94A					***	*

In conclusion, a certain intra and interspecific variability in the heat shock response has been found at the puffing level. This does not agree with the conservative character of the response to temperature. Future molecular analysis will probably explain the unexpected heat shock puff differences found between both species.

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Circulating *Trypanosoma cruzi* from the same cloned population show differences in the ability to infect cells and to cause lethal infection in mice[1]

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Summary. Two subpopulations of circulating parasites displaying different abilities to infect mammalian cells and to cause lethal infection when inoculated into normal mice were demonstrated in the blood of mice acutely infected with *T. cruzi*. Parasites of one subpopulation rapidly penetrated mouse fibroblasts and were readily phagocytized by normal mouse peritoneal macrophages whereas parasites of the other subpopulation showed little ability to invade non-phagocytic cells and resisted phagocytosis. Inoculation of organisms of this latter population into mice resulted in infections with lower parasitemias and longer time to death as compared to controls inoculated with organisms from a population containing both types of parasites. When a population of parasites containing both types of trypanosomes was cultured in acellular medium at 28 °C a decrease in the number of parasites was noted to occur in the initial days of culture. This decrease was not noted when parasites of the subpopulation of trypanosomes resistant to phagocytosis were cultured similarly.
Key words. Trypanosoma cruzi; Chagas' disease; macrophages; mouse fibroblasts.

Heterogeneity in *Trypanosoma cruzi*, the causative agent of Chagas' disease or American trypanosomiasis, was first reported in the original description of the parasite as morphologically distinct 'broad' and 'slender' bloodstream forms[4]. Since then, heterogenous populations of *T. cruzi* have been demonstrated in regard to biological, pathological, and antigenic make-up[5-7]. Because of the suggestion that differences in infectivity might be associated with morphological variations[6], a number of studies have been conducted using parasites from isolates with predominance of either 'broad' or 'slender' forms[8-11]. These studies have been conducted with organisms from cell cultures submitted to different conditions of temperature, culture medium, and incubation periods[8,9] or with trypanosomes obtained by centrifugation on density gradients after passage of the organisms through an ion exchange column[10], a technique which has been shown to selectively deplete subpopulations of parasites[11]. In addition, organisms from cell cultures have been shown to be only partially equivalent to bloodstream trypanosomes[12].

In this work we used a biological system to separate subpopulations of blood trypanosomes from the same cloned population and examined them for their capacity to invade cultured cells, to cause lethal infection in mice and to multiply in acellular cultures.

Methods and results. The Y strain of *Trypanosoma cruzi*, isolated in 1953 from a human case of Chagas' disease[13], was obtained from Dr Z. Brener (Centro de Pesquisas Rene Rachou, Belo Horizonte, State of Minas Gerais, Brasil) and cloned in our laboratory according to the technique described by Goldberg and Chiari[14]. Parasites derived from a single clone were obtained from the blood of adult, male, outbred, Swiss-Webster mice (Simonsen Laboratories, Gilroy, CA) at the peak of parasitemia (7 days after i.p. infection of the mice with 10^5 blood-form trypanosomes). The organisms were purified by centrifugation in 3% dextran as previously described[15], pelleted and resuspended at a concentration of 2×10^6/ml in RPMI 1640 tissue culture medium (GIBCO Laboratories, Grand Island, NY) supplemented with 5% heat-inactivated (60 °C) fetal bovine serum. One half of this trypanosome suspension was kept at 37 °C for 18 h under tissue culture conditions (original population). The other half was distributed over monolayers of murine peritoneal macrophages or monolayers of mouse fibroblasts (L929 cells, ATCC CCLI) on tissue culture slides (Lab-Tek Products, Napperville, Ill.) and cultured as previously described[15]. The organisms present in the supernatant of these cultures after 18 h of incubation (non entering subpopulation) were pelleted and resuspended in tissue culture medium as above. At least 80% of the organisms dispensed were recovered. The cell monolayers were washed, fixed with absolute methanol, stained with Giemsa stain and examined microscopically for intracellular parasites[15].

The in vitro and in vivo infectivity of the parasites of the 'non entering subpopulation' was compared with that of the same number of organisms of the 'original population' which had been kept at 37 °C for 18 h. For the in vitro assay, monolayers of peritoneal macrophages or of L929 cells were infected with trypanosomes of the 'original population' or of the 'non