Analysis of phenotypes altered by temperature stress and hipermutability in *Drosophila willistoni*

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ABSTRACT. *Drosophila willistoni* (Sturtevant, 1916) is a species of the *willistoni* group of *Drosophila* having wide distribution from the South of USA (Florida) and Mexico to the North of Argentina. It has been subject of many evolutionary studies within the group, due to its considerable ability to successfully occupy a wide range of environments and also because of its great genetic variability expressed by different markers. The *D. willistoni* 17A2 strain was collected in 1991 in the state of Rio Grande do Sul, Brazil (30°05'S, 51°39'W), and has been maintained since then at the *Drosophila* laboratory of UFRGS. Different to the other *D. willistoni* strains maintained in the laboratory, the 17A2 strain spontaneously produced mutant males *white*-like (white eyes) and *sepia*-like (brown eyes) in stocks held at 17°C. In order to discover if this strain is potentially hypermutable, we submitted it to temperature stress tests. Eighteen isofemale strains were used in our tests and, after the first generation, all the individuals produced in each strain were maintained at 29°C. Different phenotype alterations were observed in subsequent generations, similar to mutations already well characterized in *D. melanogaster* (*white*, *sepia*, *blistered* and *curly*). In addition, an uncommon phenotype alteration with an apparent fusion of the antennae was observed, but only in the isofemale line nº 31. This last alteration has not been previously described as a mutation in the *D. melanogaster* species. Our results indicate that the *D. willistoni* 17A2 strain is a candidate for hypermutability, which presents considerable cryptic genetic variability. Different factors may be operating for the formation of this effect, such as the mobilization of transposable elements, effect of inbreeding and alteration of the heat-shock proteins functions.

KEYWORDS. *D. willistoni*, 17A2 strain, temperature stress, genetic variability, hypermutability.

RESUMO. Análises de fenótipos alterados pelo stress de temperatura e hipermutabilidade em *Drosophila willistoni*. *Drosophila willistoni* (Sturtevant, 1916) é uma espécie do grupo *willistoni* de *Drosophila* que apresenta ampla distribuição geográfica desde o sul dos Estados Unidos (Flórida) e México até o norte da Argentina. Esta espécie tem sido alvo de muitos estudos evolutivos dentro do grupo devido à sua considerável capacidade de explorar de forma bem sucedida diversos tipos de ambientes e também por sua grande variabilidade genética expressa através de diferentes marcadores. A linhagem 17A2 de *D. willistoni* foi coletada em 1991 no Estado do Rio Grande do Sul, Brasil (30°05'S, 51°39'W), e vem sendo mantida desde então no laboratório de *Drosophila* da Universidade Federal do Rio Grande do Sul. Diferentemente das demais linhagens de *D. willistoni* mantidas no laboratório, a linhagem 17A2 já produziu espontaneamente machos do tipo *white* (olhos brancos) e do tipo *sepia* (olhos marrons) em estoques mantidos a 17°C. A fim de avaliar se esta linhagem é potencialmente hipermutável, nós submetemo-la a experimentos de estresse por temperatura. Dezoito isolinhagens foram utilizadas e, após a primeira geração, todos os indivíduos de cada isolinhagem foram mantidos a 29°C. Diferentes alterações foram observadas nas gerações seguintes, similares com mutações já bem caracterizadas em *D. melanogaster* (*white*, *sépia*, *blistered* e *curly*). Além destas, uma alteração com aparente fusão nas antenas foi encontrada, mas somente em indivíduos da isolinhagem nº 31. Esta última alteração não foi previamente descrita como uma mutação na espécie *D. melanogaster*. Nossos resultados indicam que a linhagem 17A2 de *D. willistoni* é uma candidata a hipermutabilidade, além de apresentar uma variabilidade genética importante. Diferentes fatores podem estar atuando para a formação deste panorama observado, tais como mobilização de elementos transponíveis, seleção por endocruzamento e alteração na função de proteínas de choque térmico (heat-shock proteins).

PALAVRAS-CHAVE. *D. willistoni*, linhagem 17A2, estresse por temperatura, variabilidade genética, hipermutabilidade.

The 17A2 strain of *Drosophila willistoni* Sturtevant, 1916 was collected in 1991 at an agronomic station in Eldorado do Sul, state of Rio Grande do Sul, Brazil (30°05'S, 51°39'W) and since then has been maintained in the *Drosophila* Laboratory of the Department of Genetics at UFRGS (Universidade Federal do Rio Grande do Sul, Brazil). This strain has been used in cytogenetic studies that detected a high level of polymorphism for paracentric chromosomic inversions and male recombination (Santos-Colares *et al*., 2004).

Differing to the findings in the greater part of material collected in natural environments, the 17A2 strain of *D. willistoni* already presented spontaneous male mutants *white*-like (white eyes) and *sepia*-like (brown eyes) in stocks, which had been maintained at 17°C in laboratory. These mutations were selected by controlled crossings and stable strains were obtained.

Mutations are very rare events in nature and in *Drosophila* the mutation rate has been found to be of the order of 1 in 100,000, with a variation between 1 in 20,000 and 1 in 200,000 gametes (Gardner & Snustad, 1986). When a series of spontaneous mutations occur, we can suspect of the hypermutability phenomenon.

Although spontaneous mutations have been reported in previous studies of the *D. willistoni* species, stable sibling strains from the altered phenotypes have been difficult to establish. Valiati *et al*. (1999), working with individuals captured in urban sites in the state of Rio Grande do Sul noted some alterations such as a male with a small leg instead of the normal antenna and alteration of a portion of the eye tissue (ommatidia). They also observed a female with only one wing and a sterile female, which had a halter in place to the wing in the third thoracic segment. In this case, the efforts to establish mutant isostrains were not successful.

Only recently Goñi *et al.* (2002) obtained stable strains of spontaneous mutations in *D. willistoni* from individuals collected in Montevideo, Uruguay (34°53'S, 56°16'W). Two mutations *yellow*-like and *dull red*-like were segregated from F2 of the EM1.00 strain. In later generations *white* and *pink* mutations appeared. All the mutant strains demonstrated good viability. By controlled crossings with subsequent cytogenetic analysis, it was shown that the *white*, *pink* and *yellow* loci are located in the X chromosome.

An alteration in the eye with a phenotype similar to the mutation already described for *Drosophila melanogaster* Meigen, 1830 known as *eyeless* (*ey*) was also found in *D. willistoni* (Goñi *et al.*, 2002). This type of mutation, in which total or partial eye loss occurs, was analyzed at the molecular level by Ludwig *et al.* (2003), but it was not possible to identify the agent responsible for this alteration. These authors suggested that the effect could be related to spot mutations, mutations in genes neighboring the *ey* region, or even mutations that might have occurred in another gene having a phenotype similar to the *eyeless* (review in Gehring, 2001).

Some environmental factors, such as chemical or physical stress may favor the appearance of morphological alterations due to intrinsic genetic or physiological events, such as: 1) mobilization of the Transposable Elements (TEs) in the host genome, (revision by Capy *et al.*, 1998); 2) selection by inbreeding, where a population is generated from only a few individuals (Loeschcke *et al.*, 1997); 3) and by alterations involving Heat-Shock Proteins (HSPs) during development (Petersen & Young, 1991). This latter possibility does not necessarily involve changes in the DNA sequences, but alterations in the expressivity of the cryptic genetic variability (Rutherford & Lindqüist, 1998).

As all the organisms are strongly affected by their surrounding environments, the environmental factors play an important part in shaping the ecology and evolution of biological systems. Environmental stresses are especially important at many levels of biological organization (Hoffmann & Parsons, 1997; Hoffmann & Hercus, 2000), and one of the ways by which virtually all organisms combat stress is through the heat-shock response (Lindquist & Craig, 1988; Morimoto *et al.*, 1994; Feder & Hoffmann, 1999).

Heat-shock proteins (HSPs) are the main constituents of this response and typically function as molecular chaperones, which assist in maintaining cellular protein structure. In *Drosophila*, during thermal stress, occurs the synthesis of these proteins (Sørensen *et al.*, 2003) and the members of the Hsp70, for example family, are capable of mitigate cellular damage (Lindquist, 1981; Parsell *et al.*, 1993; Krebs & Loeschcke, 1994; Feder *et al.*, 1996; Sørensen *et al.*, 2003; Gong & Golic, 2006). However, a cost of expressing the heat-shock response was identified and the increased survival after acclimation is accompanied by reduced fertility (Loeschcke *et al.*, 1997).

The Hsp90 was proposed to act as a general evolutionary capacitor by releasing the effect of cryptic genetic variation under stressful environment (Rutherford & Lindquist, 1998). However, more recent studies have revealed that the influence of Hsp90 on the variation of particular traits was very limited (Milton *et al.*, 2005). This suggests that the ability of chaperones, and Hsp90 in particular, to buffer phenotypic variation is not so general, but might rely on very specific interactions more tightly involved in particular phenotypes. Recent studies in *D. melanogaster* make a link between chaperones and chromatin regulators. Cold temperature induces a dark pigmentation, in particular in posterior segments, while higher temperature has the opposite effect. The homeotic gene Abdominal-B (Abd-B) has a major role in the plasticity of pigmentation in the abdomen. Temperature acts on melanin production by modulating a chromatin regulator network, interacting genetically with the transcription factor bric-a-brac (bab), a target of Abd-B and Hsp83, encoding the chaperone Hsp90. Genetic disruption of this chromatin regulator network increases the effect of temperature and the instability of the pigmentation pattern in the posterior abdomen (review in Gilbert *et al.*, 2007). Interesting, evidences show that *Drosophila* heat-shock genes are especially susceptible to the insertion of Transposable Elements (TEs) in nature and this can have dramatic fitness consequences, creating variation on which selection can act (Walser *et al.*, 2006; Chen *et al.*, 2007).

Transposable Elements (TEs) constitute a substantial component of the *Drosophila* genome (Lindsley & Zimm, 1992). According to experimental estimates (Harada *et al.*, 1990; Nuzhdin & Mackay, 1995), the rates of spontaneous transpositions are approximately 10^{-5} - 10^{-6} events per TE copy per sperm per generation. Direct and indirect evidence has accumulated in favors of the phenomenon of TE transposition being induced by external stress factors, of which temperature treatment and γ-irradiation are the most important. It was demonstrated that heat shock induces transcription (necessary for retrotransposition) of the *copia* retrotransposon (Strand & McDonald, 1985). However, the control of TE activity is dependent of the host genome and its interaction with environmental factors. In *Drosophila*, some retrotransposons were mobilized after heat-shocks in certain laboratory lines (Ratner *et al.*, 1992; Zabanov *et al.* 1994; Vasilyeva *et al.*, 1999), while in other experiments that effect was not found (Arnault *et al.*, 1997; Vázquez *et al.*, 2007).

In view of the appearance of spontaneous mutations in the 17A2 strain and the possibility that this might be a clue to the identification of agents responsible for the variability of this species, we designed this study to identify different alterations in the phenotypes which occur when individuals of this strain are submitted to physical stress (by temperature increase), as well as to describe in detail the type of alteration found in the antenna morphology, herein demonstrated for the first time for the *Drosophila* genus.

Because the species *D. willistoni* presents extensive genetic variability expressed by various markers such as polymorphism chromosomal for paracentric inversions and enzymatic polymorphism (Ayala & Powell, 1972), a descriptive investigation such as we present in this study can provide a base for future research, which could lead to a better understanding of the possible causative agents of this *D. willistoni* genetic variability.

MATERIAL AND METHODS

The stocks of *D. willistoni* populations were maintained by general massal crosses in a standard culture medium (MARQUES *et al.*, 1966) inside a chamber kept at a constant temperature of $17°C \pm 1°C$ with approximately 60% relative humidity. Two strains were used in this work: WIP-4 (a strain maintained in laboratory since 1966 and that never presented spontaneous mutation) as negative control and 17A2 (a strain colleted in 1991), target of this work. Initially, for obtaining the isostrains of the each strain, forty natural-born virgin females and males were crossed. Only eighteen strains of each population initially selected (the number is indicating in table I), produced offspring for the continuities of the experiment. After the first generation, descendents of each of these strains were submitted to temperature stress being maintained in culture chambers at 29°C. In each new generation, after brief anesthesia in ethylic ether and visualization on stereomicroscope, the individuals were analyzed and counted. All the mutants were removed and submitted to controlled crossings (a mutant phenotype male crossed with a wild type virgin female) in an effort to obtain stable mutant strains. The strains from the population WIP-4 did not show individuals with mutant phenotypes (data not shown). Thus, the number of controlled crossings is the number of mutant individuals obtained in each generation, for each isofemale line in 17A2 strain, as described in table I.

Individuals of wild phenotype were placed in a new culture medium to breed and deposit their eggs again. The strains were followed-up for eleven generations. The phenotypic alterations detected in each generation were photographed with a stereomicroscope. Ten male individuals of the 17A2 strain and ten of the mutant *blistered* strain had been dissected in EPHRUSSI & BEADLE (1936) serum for the withdrawal of the testes in order to evaluate the structure, appearance and presence/absence of spermatozoa. The results of these comparative analyses were also photographed with a stereomicroscope.

Antennae that showed alterations were analyzed separately on permanent slides mounted with Entellan® (Merck). Slides visualization and image capture was achieved by using a stereomicroscope. Samples of these individuals demonstrating antennae alterations were also dehydrated and fixed for subsequent analyses of the antennae in a Scanning Electron Microscopy (SEM). Normal antennae were processed as controls. The procedures for the preparation for these samples and the image capture were made at the Electronic Microscope

Table I. Total frequency of individuals obtained in each generation in the 17A2 *D. willistoni* isostrains submitted to temperature stress. (T, total number of individuals; M, number of individuals with morphological alterations).

Isostrain	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
1	T:216	T:145	T:158	T:84	T:123	T:88	T:157	T:110	T:77	T:41
	M:1	M:2	M:1	M:0	M:2	M:1	M:0	M:13	M:0	M:1
2	T:112	T:238	T:32							
	M:0	M:1	M:0							
4	T:56	T:566	T:40	T:693						
	M:0	M:2	M:0	M:28						
6	T:47	T:472	T:199	T:122	T:114					
	M:0	M:2	M:0	M:1	M:2					
7	T:84	T:664	T:75	T:166						
	M:0	M:21	M:1	M:3						
8	T:124	T:31								
	M:0	M:0								
9	T:72	T:1026	T:462	T:113	T:40					
	M:0	M:2	M:1	M:2	M:5					
10	T:67	T:605	T:146	T:133	T:88					
	M:0	M:3	M:10	M:4	M:0					
13	T:42	T:1145	T:250	T:154	T:50					
	M:0	M:12	M:9	M:2	M:8					
20	T:86									
	M:0									
23	T:155	T:23	T:11							
	M:3	M:0	M:0							
26	T:8									
	M:0									
27	T:256	T:113	T:62							
	M:2	M:4	M:0							
28	T:26									
	M:0									
29	T:320	T:137	T:73							
	M:13	M:0	M:2							
30	T:16									
	M:0									
31	T:311	T:92	T:540	T:68	T:363	T:174	T:263			
	M:4	M:13	M:2	M:10	M:6	M:4	M:37			
33	T:42									
	M:0									

Center (CME) of UFRGS (Porto Alegre, Brazil) in accordance with the standard procedures adapted at that Center. Some individuals of the isostrains presenting reduction in the number of births were dissected to find out if the reproductive structures showed any kind of alteration.

RESULTS

Of the 18 isostrains initially placed in the chamber at 29°C, five were lost in F2, one in F3, four in F4, two in F5, four in F6 and one in F8 - only the 1B isostrain survived to the end of the experiment (Tab. I). In general this loss was mainly due to the gradual reduction in the number of births. The individuals of the isostrains that presented reduction in the number of births had the female gonads and the male testes morphology normal, but no spermatozoa were produced within this last (Fig. 1).

A great number of individuals with different phenotypic alterations appeared in the different isostrains analyzed (indicated by M in table I). The alterations found resembled some *D. melanogaster* mutants: *white* (white eyes), *sepia* (sepia eyes), *blistered* (bubbles with hemolymph in the wings) and *Curly* (curved wings). During the experiment two individuals with a double-mutation phenotype were observed: one *white*/*Curly*-like and the other *white*/*blistered*-like. Another alteration with the antennae apparently fused was observed. This latter alteration was found in various individuals but only in those of the isostrain number 31 (Fig. 2).

Although the morphologically altered individuals were removed from the isostrains after the count for the establishment of stable strains, the altered phenotypes continued to show up in subsequent generations (Fig. 3) suggesting that there is some factor intrinsic to the
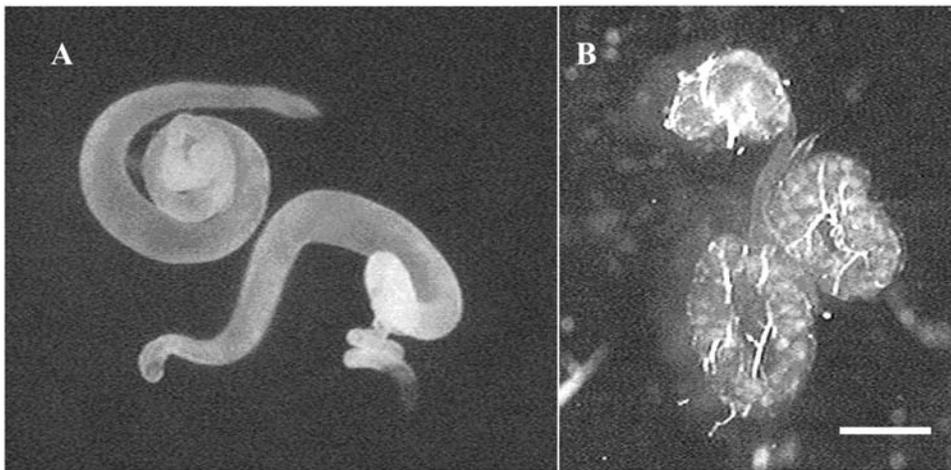

Fig. 1. Stereo microscope illustrations of the *Drosophila willistoni* testes. A. normal testes of a wild male of the 17A2 strain. B. Testes of a male of the *blistered* phenotype obtained from the isostrain 1B. The structure has a transparent aspect because no spermatozoids are present. Bar, 0.5mm
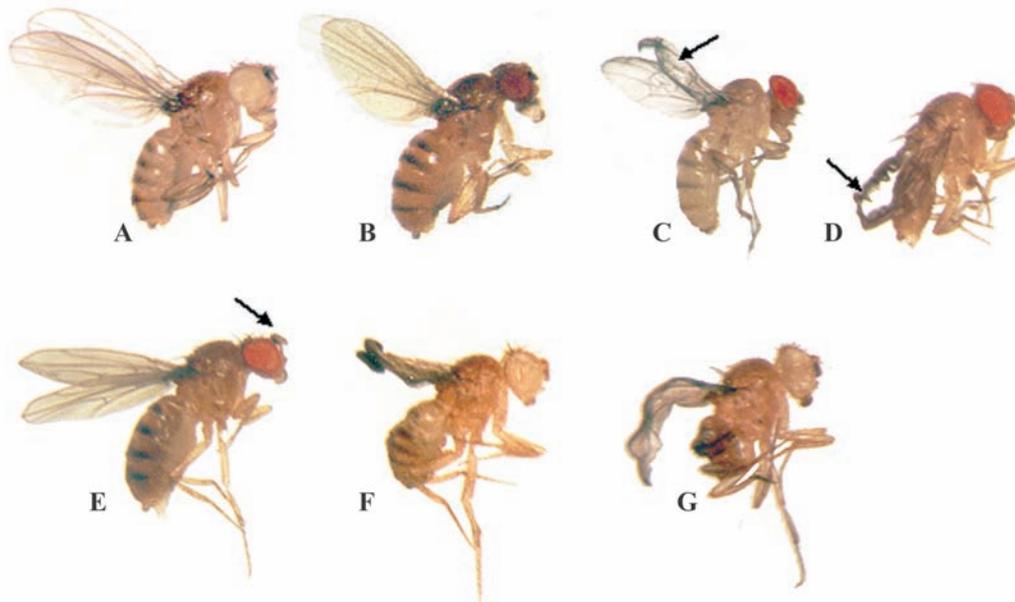

Fig. 2. Altered phenotypes obtained in individuals of *Drosophila willistoni* 17A2 isostrain submitted to temperature stress. Picture shows female individuals with altered morphologies: A. *white* (white eyes); B. *sepia* (brown eyes); C. *blistered* (arrow indicates the presence of blisters on the wings); D. *Curly* (curved wings indicated by the arrow); E. with apparently fused antennae (indicated by the arrow) - see figure 5 for details of the mutant structure; F. Female *white* and *Curly*; G. *white* and *blistered*.

genomes of the individuals, that causes the reappearance of the alteration.

Of the controlled crossings carried out with the different altered phenotypes, only one strain with origin in a male *blistered*-like was established. This individual had been crossed with a natural-born female. The descendents of this crossing were selected and maintained in stock in the laboratory at 25ºC for approximately eight months. However, after this period,
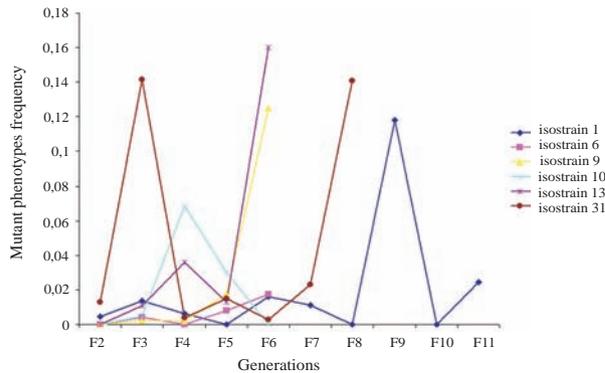

Fig. 3. Frequencies of mutant phenotypes which appeared during 11 generations in the different 17A2 isostrains (represented by different colors). The interruption in the frequency plot indicates that the isostrain was lost. Only those isostrains which remained longer in the experiment were used.

the number of individuals produced gradually declined until finally the strain was entirely lost.

No previous descriptions of *D. melanogaster* individuals with the antenna alteration phenotype described here were found in the available literature. The pair of antennae is located in the frontal portion of the fly's head and is divided into four segments. In these, bristles and aristae are found and the entire structure performs hearing (GÖPFERT & ROBERT, 2001; TODI *et al.*, 2004) and olfactory (FISHILEVICH & VOSSHALL, 2005) functions. A sketch of the *D. melanogaster* antenna structure is provided in figure 4.

On mutants of *D. willistoni* isostrain 31 it can be seen that the altered antennae are very close to each other giving the impression when observed at glance that they form a single structure (Fig. 5). We checked that these altered antennae were not different from those observed in wild type flies as regards to the number of segments and bristles of the aristae, but these were always smaller (Fig. 6). Analyses carried out using SEM confirmed the difference in the structures size and further revealed that the antennae became approximated, giving the impression that they constituted only one structure, because they were closely implanted in the head of the flies (Fig. 7).

When crossings were made (at 25°C) between the individuals carrying the antenna alterations and wild type
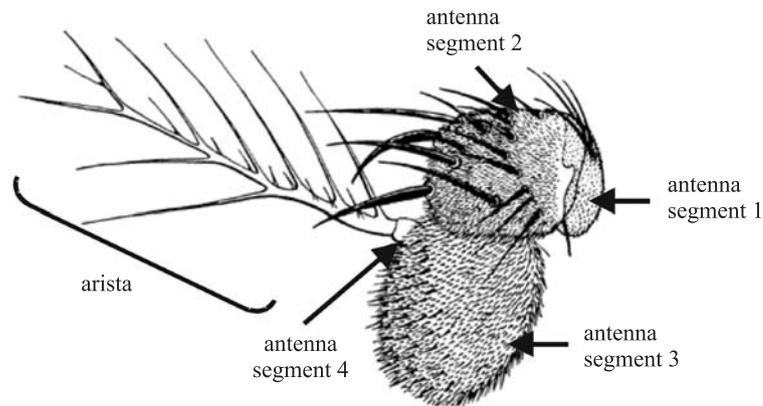

Fig 4. Sketch representing one of *Drosophila melanogaster* antenna with the respective structures adapted from BRYANT (1978).
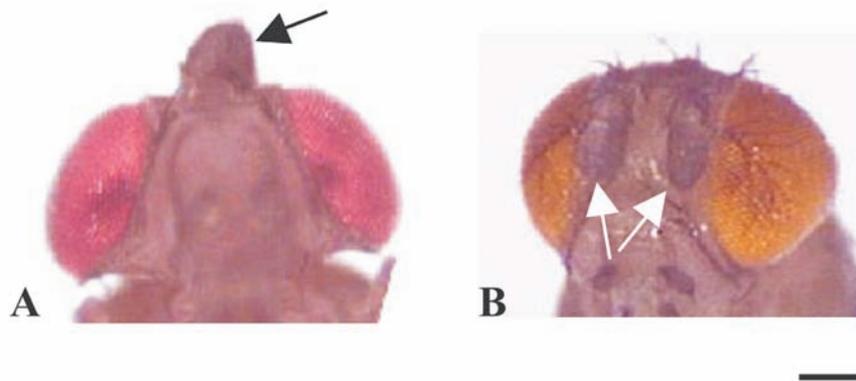

Fig. 5. Stereo microscope view of D*rosophila willistoni* antennae. A. An individual with altered antennae (arrow) which appeared in the isostrain 31. B. View of the antennae of a wild individual (arrows). Note that the altered antennae appear to be a single structure. Bar, 0.5mm

Fig. 6. Optical microscope view of de antennae of *Drosophila willistoni*. A. Antenna with the wild phenotype of an individual from the 17A2 strain. B. Antenna with altered phenotype of an individual for isostrain 31. Bar, 0.5mm.
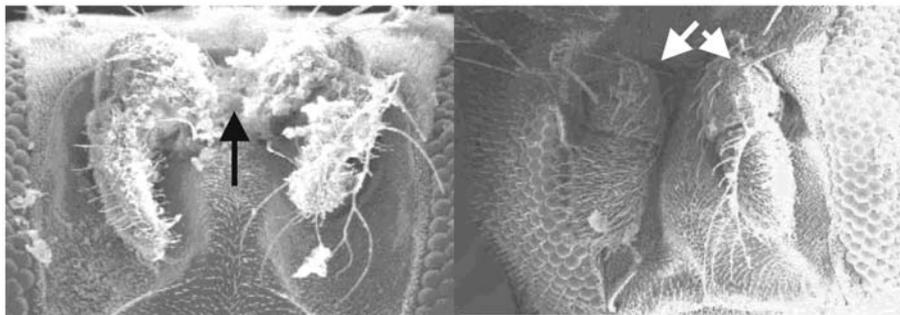

Fig. 7. Frontal view analysis of *Drosophila willistoni* antennae (SEM). To the left are the badly-formed antennae which occur in the individuals of the isostrain 31. Note the close implantation of the structures in the head (arrow) which is quite different from that of individuals with the wild 17A2 isostrain phenotype (to the right), where the antennae are implanted farther apart and the structures are well defined (arrows). 400X magnification.

flies, the offspring did not show the alterations presented by their parents. Despite of this, generations of the 31° strain maintained at 29°C continued to produce these malformations. In view of this, it was not possible to derive stable strains from these individuals.

DISCUSSION

Our descriptions of the *D. willistoni* altered phenotypes produced through temperature stress indicate that an intrinsic factor in the genome of the individuals causes the alterations to appear. This idea is bourn out by the fact that the altered phenotypes continued to show up in subsequent generations, although in each generation the affected individuals were removed from isostrains after the count.

So it was not possible to relate the inviability of strain with the increased number of mutants as observed in the strains 4, 9, 13 and 31 (Tab. I). Besides this, some strains, although presented low number of mutants, were also lost after few generations. However, we cannot completely discard the possibility that the former strains could be lost due to a higher intrinsic genomic instability.

Organisms have developed a series of mechanisms to cope with environmental stress, including increased expression of stress proteins and changes in metabolism and hormone concentrations (PLETCHER *et al.*, 2002; SØRENSEN *et al.*, 2005; LANDIS *et al.*, 2004). It has been shown that some of the same mechanisms are also affected by intrinsic stresses, such as inbreeding and aging (KRISTENSEN *et al.*, 2005; PLETCHER *et al.*, 2002; LANDIS *et al.*, 2004; PEDERSEN *et al.*, 2005). The deleterious consequences of inbreeding, environmental stress and the interactions between them are of serious concern in diverse fields of biological sciences, such as evolutionary genetics, conservation biology, and animal breeding. Recently, KRISTENSEN *et al.* (2006) found that the effect of temperature stress on gene expression is accentuated in inbred compared to out bred lines of *D. melanogaster*. On the basis of these results the authors conclude that inbreeding and environmental stress, both independently and synergistically affect gene expression pattern. It also appears to have occurred in the present study. The majority of the alterations observed in the *D. willistoni* 17A2 strain are mutations well characterized in *D. melanogaster*. Those which affect the eye pigmentation, for example, show alterations in gene sequences involved in ommatidia pigmentation metabolic pathways, and may cause the brown (*sepia* mutant) and white eyes (*white* mutant) to appear, contrary to what happens with wild type individuals who have red eyes. The *white* gene is involved in the production and distribution of the omochrome pigments (browns) and pteridines (reds) found in the adult flies compounding the eyes and ocelli, as well as in the adult males testes and in the larvae Malpighi tubules. The specific function of the transport

protein, associated to the membrane, is to connect ATP-precursor pigments in the omochrome and pteridines pathways (SULLIVAN & SULLIVAN, 1975; DRESSEN *et al.*, 1988; TEARLE *et al.*, 1989).

The *sepia* (*se*) mutant has eyes of a brown color in the eclosion, which darkens to sepia, and becomes still darker with advancing age. It has normal ocellar pigmentation. Chromographically, the *se* eyes are characterized by the absence of red pigments (drosopterin and isodrosopterin, for example), and by an accumulation of yellow pigments. About ten *sepia* alleles have been fully described for *D. melanogaster* (LINDSLEY & ZIMM, 1992).

Other mutations alter the structure of the upper and lower epithelia of the wings causing curvatures (in the *curly* mutant) or the formation of haemolymph blisters between the epithelia (in the *blistered* mutant) (LINDSLEY & ZIMM, 1992).

The *Curly* (*Cy*) is a dominant mutation in *D. melanogaster* that affects the genes necessary to form the wing structure in the adult fly. The mutation *Curly*, one of the most important tools in genetics analysis in *Drosophila*, results in curled wings at 25°C but in normally straight wings at 19°C (GRIFFITHS *et al.*, 1996). It is used, for example, as a preferential marker of the second chromosome in genetic laboratories. The curvature of the wings in the *Curly* fly is caused by an unequal concentration of the upper and lower epithelia during the dry period after the emergence from the pupa (LINDSLEY & ZIMM, 1992).

The *D. melanogaster blistered* mutant, on the other hand, presents formations of haemolymph blisters between the wing surfaces. Some of these mutations are caused by defects in the *Drosophila* integrin proteins transmembrane heterodimers, which are part of basal junctions, and maintain the two wing surfaces united (FRISTROM *et al.*, 1993). Mutation in the integrin genes, such as *inflated* (*if*) and *myosferoid* (*mys*), cause defects in the dorsal-ventral adhesion of the wing intervenal cells also resulting in blisters on the wings (BROWER & JAFFE, 1989; WILCOX *et al.*, 1989; ZUSMAN *et al.*, 1990).

We cannot definitely state that the phenotypes observed in *D. willistoni* have their origin in the same types of mutations previously described for *D. melanogaster*, because it would be necessary to make a study at the molecular level of each type of alteration to confirm the similarity unequivocally. Moreover, the alteration resulting in jointed antennae and small aristae, which we found in the isostrain 31 of 17A2 strain, has no similar description in *D. melanogaster*. This result leads to the premise that multiple other factors may be involved in the production of the *D. willistoni* altered phenotypes which result from exposure to temperature stress. Some hypotheses for the possible agents responsible for this phenomenon are developed below.

The first hypothesis is related to mobilization of the Transposable Elements. High temperatures associate with permissive genomes (VÁZQUEZ *et al.*, 2007) may favors mobilization of these elements by an increase in the production of transposase (ENGELS, 1989) and, consequently, the phenomenon of gonadal disgenesys (PETERSEN & YOUNG, 1991). In fact, some mutations in *D.*

melanogaster were characterized as being the result of the increased mobility of the Transposable Elements. The loco *white* of *D. melanogaster* is a hotspot for insertions presenting, for example, several alleles originated through insertions of Transposable Elements such as *Doc, roo* and *copia* (LINDSLEY & ZIMM, 1992).

LORETO *et al.* (1998) described a hypermutable strain of *D. simulans* Sturtevant, 1919 whose causal agent was subsequently characterized as being related to the mobilization of a transposable element similar to *hobo* (TORRES *et al.*, 2006). Considering that the majority of *Drosophila* mutations are attributed to transposable element mobilizations (FINNEGAN, 1990), this would seem to be an explication pertinent to the phenomenon observed in the *D. willistoni* 17A2 strain.

Furthermore, some transposable elements are in fact present in the genome of the *D. willistoni* 17A2 strain. REGNER *et al.* (1996) analyzed two populations of *D. willistoni* (17A2 and WIP-4) for the presence of the *P* transposable element, by *Southern Blot* and *in situ* hybridization, and detected the presence of apparently active *P* element in the 17A2 strain. LORETO *et al.* (1998) and SASSI *et al.* (2005), using the *Southern blot* technique, also detected the presence of sequences similar to *hobo* in the genome of the 17A2 strain, although with low homology to those of *D. melanogaster.*

REGNER *et al.* (1999) obtained phenotypic alterations in individuals of the WIP-4 and 17A2 strains in experiments, which associated controlled crossings (between and within strains) and temperature stress. The phenotypes obtained were wing alterations of the *Beadex*-like, *held-up*-like and *Notch*-like, as well as an eye alteration similar to *extra-eye*, all having been previously described for *D. melanogaster.* Some individuals with gonadal disgenesys were also obtained, very similar to what happens with *D. melanogaster* when there is mobilization of the *P* element in its genome. This led the authors to postulate that *P* element mobilization might be the agent causing the mutations.

Our results are similar to those obtained by REGNER *et al.* (1999), to the extent that we also obtained alterations in 17A2 intra-strain crossings under temperature stress. However, in our experiment the alterations are different to those previously described, which suggests that the time period during which the strain was maintained in the laboratory and new putative transpositions may have influenced the obtained results.

The existence of a relationship between environmental conditions and mobilization of TEs, however, still is an open question. In *D. simulans* natural populations, the *412* element follows a gradient in copy number between the South and North hemispheres (VIEIRA & BIÉMONT, 1996), its copy number being negatively correlated with minimum temperature (VIEIRA *et al.*, 1998). Another example of variation in TE number related with natural environmental variation was given for BARE-1 in barley (KALENDAR *et al.*, 2000). Less severe thermal stresses might have some effect on TE activity, for example high temperature enhances transposition of the *P* element in dysgenic crosses (ENGELS & PRESTON, 1980). Conversely, no effect of culture at the stress temperature of 28°C could be shown regarding the transposition and

the structural mutation rate of 11 TE families (Alonso-Gonzalez *et al.*, 2006).

A second hypothesis that could be advanced to explain our results, especially those related to the gradual loss of the isostrains, could be the effect of depression by inbreeding, given that all isostrains were generated from one single couple. The phenomenon of depression by inbreeding is well known, being characterized by a rapid fecundity and viability decline owing to the increase of homozygotes for unfavorable alleles as the population becomes more and more inbred in the laboratory (Futuyma, 1992). The evidence that some of the males in the isostrains produced no spermatozoa supports this hypothesis.

Inbreeding can lead to a homozygozity of development genes mutant alleles which, being sensitive to temperature effects, result in phenotypes altered by stress conditions. This can have been the reason because the strains were lost after the increase of mutant individuals. Inbred populations may hardly suffer the effect of changes in the environment that non-inbred populations perceive as non-stressful, indicating that intrinsic and extrinsic stresses may interact. There is evidence that such interaction can lead to synergism between genetic and environmental stresses causing normally benign environmental conditions to become harmful (review in Kristensen *et al.*, 2006). In *D. melanogaster*, alleles sensitive to temperature of the e*cdysoneless* (*ecd*) gene lead to third stage mutant larvae with reduced synthesis or release of the ecdysone hormone in the salivary glands when exposed to temperature stress (Henrich *et al.*, 1987). A reduction in ecdysone affects the expression of a proneural gene called atonal (*ato*), which is responsible for initiating the process of the development of numberless segment-specific sensorial organs including the eye, the hearing organ (Johnston's organ located in the antennae) and tension receptors (cordotonal organ located in the paws) (Niwa *et al.*, 2004). The results obtained from the isostrain 31, where the phenotypes with united antennae occurred only at 29°C, may be related to this hypothesis, because when the control crossings were carried out without temperature stress in order to obtain stable strains, altered descendents were not produced.

The final hypothesis, which may explain the appearance of morphological alterations in *D. willistoni* 17A2 strain under temperature stress, may also be connected to proteins involved in the development processes and to the negative effects that higher temperatures may have on them. The higher temperature may interfere in the regulation pathways of embryonic development genes, in a way that mutations might not necessarily occur. Temperature shock during development induces morphological defects in vertebrates and invertebrates (Petersen & Young, 1991).

The Heat Shock Proteins 90 (Hsp90) chaperones the maturation of many regulatory proteins and, in *D. melanogaster*, buffers genetic variation in morphogenetic pathways (Queitsch *et al.*, 2002). When Hsp90 buffering activity is compromised, by temperature for example, cryptic variants are expressed and selection can lead to the continued expression of these traits, even when Hsp90

function is restored (Rutherford & Lindquist, 1998). The loss of isostrains in this work may also be alternatively explained by impaired expression of HSPs. In *D. melanogaster*, alteration in Hsp70 induction is implicated in reduced fecundity (Krebs & Loeschcke, 1994), and overexpression of Hsp70 is associated with increased larval mortality (Krebs & Feder, 1997), retarded growth (Feder *et al.*, 1992), and reduced egg hatching (Silbermann & Tatar, 2000).

Although the principal objective of the present study is the description of the phenotypes resulting from temperature stress in the 17A2 strain, it is possible to conclude that an intrinsic source of genetic variability exists in this strain, although it still remains to be found. None of the three hypothesis discussed above excludes the others and they may indeed be operating simultaneously. More focused experiments are still necessary in the future to identify with greater accuracy which agents are causing the alterations. However, it can be said that this present work has characterized the *D. willistoni* 17A2 strain as a hypermutable strain.

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