In one of the vials, six males showing brownish eye color were observed. The brownish eye color in these males shows resemblance with *garnet* eye color, a sex linked mutation of *D. ananassae* reported by Hinton (1980). These males were crossed with wild type females and the resulting progeny from this cross were normal. When these flies were pair mated, some of the males obtained from this cross showed *garnet* eye color. These males were pair mated with females from the same cross, which resulted in the production of *garnet* eye color females. A separate homozygous line was established by using females and males showing *garnet* eye color. In order to test the inheritance pattern, virgin *garnet* eye color females were collected from the stock and mated with wild type males. All the F_1 males showed *garnet* eye color demonstrating sex linked inheritance. Figure 1 shows a mutant male with *garnet* (*g*) eye color. Thus it is concluded that *garnet* eye color mutation in *D. malerkotliana* is a sex linked recessive mutation that was induced by X-rays. It is a new mutation being reported for the first time in this species.

Figure 1. Garnet eye color phenotype in *Drosophila malerkotliana.*

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New spontaneous wing mutant *curly* in *Drosophila willistoni* strain GdH4-1.

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Introduction

In the 20th century the family Drosophilidae has become the focus of increased interest in the areas of genetics, development, and evolution, their most known species *Drosophila melanogaster* being used as a model organism in several experimental studies, including of human diseases (Leopold and Perrimon, 2007; Vosshall, 2007). However, the genus *Drosophila* comprises 15 subgenera and about 1,400 species, with high diversity and wide geographic distribution. The subgenus *Sophophora* (Sturtevant, 1939) comprises 332 species divided into eight groups, among which is the *willistoni* group (Bächli, 2008).

In the beginning of the 21st century the complete genome sequencing of 12 *Drosophila* species shows the interest in the genus as eukaryote models (Clark *et al.*, 2007; Schaeffer *et al.*, 2008). Other species can also become important, as their biology and genetics reach an information level to allow its use as experimental models. The *D. willistoni* is regarded as a model organism for evolutionary studies, and aside from *D. melanogaster*, has the largest number of structural genes mapped, which render it the only Neotropical species included amongst the complete genome sequenced 12 *Drosophila* species (Clark *et al.*, 2007; Schaeffer *et al.*, 2008). The strain of *D. willistoni*, selected by the "Drosophila 12 Genomes Consortium" group to be sequenced, was collected on Guadalupe Island and named GdH4-1 (Guadaloupe, France, Tucson Center 14030-0811.33) and does part of the *Drosophila* Species Inventory held at the Laboratório de Experimentação em *Drosophila* (LE*D*-UFPE). The aim of this work is to describe a new mutant phenotype showing rolled up wing tips in the strain GdH4-1 of *D. willistoni*, noticed during its maintenance.

Material and Methods

The *Drosophila willistoni* GdH4-1 strain is cultivated in a rich culture medium containing cereal flours, bananas, yeast, sugar, honey, antifungal methylparaben and phosphoric and propionic acids, in 60 ml glass bottles capped with foam stoppers. For the preparation of the culture medium all nutrients are weighed, then mixed and cooked in a microwave for 15 minutes, stopping every 2 minutes to mix. The room temperature is kept at 22 ± 1°C with constant humidity. The cultivation flasks are maintained by adding water and yeast regularly. During this procedure, in a single culture glass of generation F14, were found 6 specimens with the upward curved wing tips phenotype. These flies were separated from the others by successive changes of glass (without ether anesthesia) and a mass culture was initiated, without counting how many flies were females or males, since certainly the females were already inseminated. This new mutant strain was successively transferred to new vials and selected by the removal of wild-type flies, in order to improve the phenotypic trait (curled wings) until generation F9, when no more wild-type flies hatched.

To determine the inheritance pattern for the new trait, some crosses were performed between the curled wing mutant and the wild-type parental strain GdH4-1 of *Drosophila willistoni*.

Results and Discussion

The first wing mutant phenotype was found in *D. melanogaster* by Morgan (1915) and named *curled* (*cu*) (*appud* Bridges and Morgan, 1923). Since 1915, this same mutant phenotype was described as a series of mutations, sometimes with dominant inheritance pattern and other times as recessive. These mutants includes *Curly* (Ward, 1923), *Upturned* (Ball, 1935), *Curlyoid* (Curry,

1939), *curly* (Goldshmidt, 1944), *Curled 3* (Meyer, 1952), *curlex* (Lindsley and Grell, 1968), and *curled* in X (Krivshenko, 1958). The rolled up wing tips phenotype found spontaneously in the strain GdH4-1 of *D. willistoni* is similar to the *curly* (*Cy*) phenotype described by Ward (1923) in *D. melanogaster*. Classically, the *curly* mutant generally presents curved shape wings with a clearer color membrane, also finer in texture. The degree of the wings curvature varies from a curly bend upward to a rotation of 360°, the more the wing is rolled-over the more wrinkles are present (Ward, 1923). In *D. melanogaster* the *curly* phenotype, which is the better studied of this mutant series, is a dominant trait associated with two large inversions in the second chromosome (Ward, 1923).

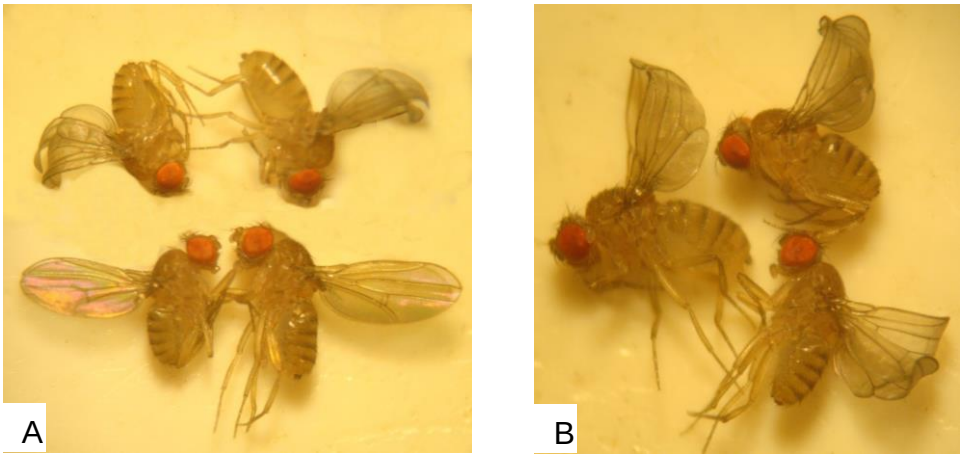

Figure 1: Comparison (A) between individuals of *curly* (*Cy*) mutants (above) and wildtype (below) of *Drosophila willistoni*. Males are positioned to the left, and females at right. The variation in expression of *Cy* phenotype (B), from a subtle to a more severe phenotype.

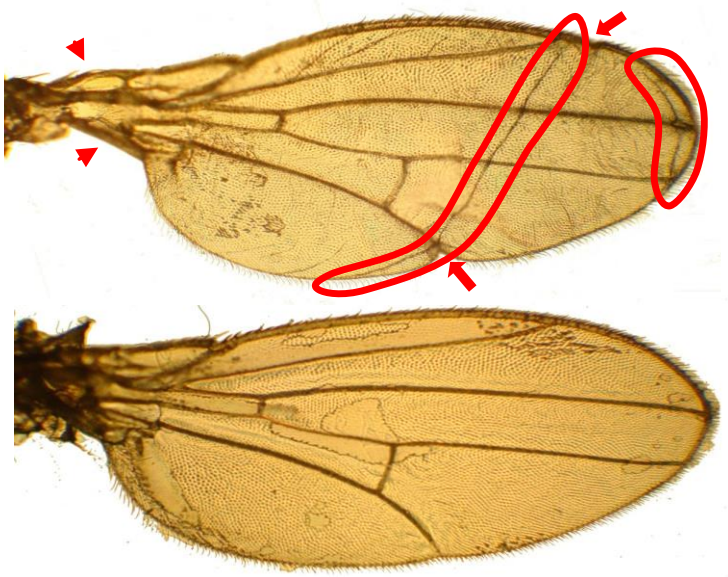

Figure 2: Structure of the wings of *Cy* mutant and wildtype of *Drosophila willistoni*, pointing out the details of the wing of the fly mutant (top), compared to wild (bottom). Modificafion of basal areas (arrowheads), recesses in the perimeter (arrows) and new lines wrinkles (circled) can be seen in the mutant, possibly caused by the curved wing. Both wings are in the same magnification (100×).

The *curly* (*Cy*) mutant phenotype found in *D. willistoni* GdH4-1 is shown in Figure 1, compared to the wildtype wing of the original strain (A), and a series of *curly* mutants (B), varying from a subtle to the most severe phenotype. This series is fully similar to that presented in Pavelka *et al*. (1996) for *D. melanogaster*, so the new mutant of *D. willistoni* is named here as "*curly*". A clearer comparison between mutant and wild type wings is shown in Figure 2. The mutant wings show recesses near vein ends and wrinkles which can be confused with new crossveins. Both recesses in the wing perimeter and the wrinkled lines were probably caused by curvature of the

wings. The effect of full phenotype expression seems also to modify significantly the axillary and jugal areas of the wing base.

The initial impression was that the new *curly* (*Cy*) phenotype of *D. willistoni* extended the life cycle of the strain to approximately 60 days. However, when the experiments to establish the generation time were performed, by generation F11 of the mutant strain, the developmental time was about 21 days, very similar to the GdH4-1 strain.

There was no significant difference between crosses in both directions (*i. e.*, female *Cy* × male *cy* or female *cy* × male *Cy*), which leads to the conclusion that the *curly* mutation of *D. willistoni* is not linked to the X chromosome. The F1 showed a frequency of 96.02% of *Cy* mutants (241:10), very near to 100% expected for a Mendelian dominant allele inheritance. Since the phenotype is highly variable, the individuals marked as wild types could be in fact mutants with the more subtle phenotype, which could made the F1 mutant frequency raise to 100%. This seems to be a situation very similar to that described by Ward (1923), who described that some phenotypically wildtype flies were in fact mutants in a genetic background that promoted the suppression of the *curly* phenotype. The F2 generation has shown the frequency of 2.34:1, which can be fairly accepted as similar to the expected 3:1 Mendelian proportion. The deviation can be due to partial lethality, since in some crosses a very few individuals were recovered in F2. This lethality is presumably dependent on the individual genetic background. The availability of mutants, as the *curly* described here and the series of eye mutants described by Soler and Goñi (2012) and the chromosome gene arrangement of these mutants (Goñi and Valente, 2012), are very important to make *D. willistoni* a candidate to become an experimental model. Its genomic characteristics distinctive from *D. melanogaster* (Clark *et al.*, 2007; Schaeffer *et al.*, 2008) can increase even more such interest.

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Erupt-like mutants from a natural population of *Drosophila melanogaster*.

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Mutations affecting head structures (eyes and antenna) were isolated from a natural population of *D. melanogaster* from Nalchik (North Caucasus, Russian Federation). The screen for