Comparative methodologies for estimating *mariner* activity using *white-peach* assay in *Drosophila simulans.*

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Introduction

Transposable elements are DNA segments that can be mobilized within and among genomes. These elements have been pointed out as having great impact on the host genome evolution by their capacity to insert into coding or regulatory regions and induce chromosome rearrangements (Giraud and Capy, 1996; Biémont and Vieira, 2006; Feschotte and Pritham, 2007).

The transposable element *mariner* was originally discovered in the promoter region of the *white* gene of *D. mauritiana* causing an attenuated colorless eye phenotype: this mutant has *white-peach* color eyes (Jacobson and Hartl, 1985; Jacobson *et al.*, 1986). That original *mariner* element, called *wpch* (short term for *white-peach*), is an inactive copy, and it is not able to mobilize itself. Posteriorly, it was experimentally transferred to *mariner*-free strains of *D. simulans* and *D. melanogaster* (Garza *et al*., 1991; Bryan *et al*., 1990). The *D. simulans wpch* strain, containing a *wpch* mutation, does not show evidence of excision in somatic cells or germline, because only this inactive *mariner* copy is present in the genome (Hartl, 2001). In the presence of an autonomous *mariner* copy, which could provide the transposase enzyme, the nonautonomous *wpch* element can be removed from the *white* gene promoter causing reversion of mutation to wild condition in some cells, generating a mosaic pattern of eye pigmentation (Lohe and Hartl, 1996).

Once a transposase source can be provided by crossing these *wpch* mutants with flies carrying potentially active copies of *mariner,* the extend of mosaic formation can be utilized for quantification of *mariner* transposition activity (Capy *et al*., 1990; Bryan *et al*., 1990; Woodruff and Thompson, 2001; Picot *et al*., 2008). This method has been used to describe latitudinal clines of *mariner* transposition activity in *D. simulans* natural populations (Giraud and Capy, 1996; Russell and Woodruff, 1999; Picot *et al*., 2008). In general, these studies use the percentage of mosaic males (PMM) observed in the F1 of crosses between *wpch* female and males of strains to be tested.

Another system for quantification of transposable activity has been proposed. It uses a predefined categorization where the number and size of red-color spots are considered, and flies are classified into a determined category (Figure 1). The larger the red area in mosaic eyes, more active the *mariner* elements from testing strains are considered to be (Giraud and Capy, 1996; Bryan *et al.*, 1990; Medhora *et al*., 1988; Hartl, 1989). However, this classification has been done only by visual inspection and can result in imprecise quantification of areas affected by reversions. Another aspect to be considered is that spot size may reflect the moment of reversion during the eye development more than the degree of activity from certain *mariner* element. When an event of *wpch* excision takes place in early developmental stages within a single cell, many other cells will be produced from that reverting cell, forming a great spot (Haymer and Marsh, 1986). Flies bearing those great spots are classified by the pattern methodology as high activity, but in fact may result from only transposition event.

The objective of this study was to compare methodologies used to estimate the *mariner* activity in *wpch* assays using: i) predefined classes; ii) total red area in the mosaic eyes; iii) number of red spots. Additionally, the effect of temperature was evaluated in generating the mosaic eye pattern.

Materials and Methods

For testing, in this study we used *D. simulans* strains (wild phenotype) collected in Brasília (15º44'47"S; 47º 55' 47"O; Brazil) as transposase source, besides the *D. simulans wpch* lineage. Crosses were performed among 10 males (wild phenotype) and 10 females *D. simulans* (*wpch* phenotype), and three temperatures were analyzed. After crossing, vials were stored at 14°C, 20°C, or 28°C until F1 emergence. 8, 25, and 29 replicas for 14°C, 20ºC, and 28°C were done. F1 generation males carrying *wpch* mutation are expected to show mosaic eyes (Jacobson and Hartl, 1985). The rate of mosaic male formation was evaluated (for PMM analysis), and the mosaic flies were classified into the categories shown in Figure 1. Also, both eyes of each mosaic male were photographed, and the total area occupied by red spots was measured using the software Image Manipulation Program – GIMP 2.6.7. In addition, the number of red spots was also quantified.

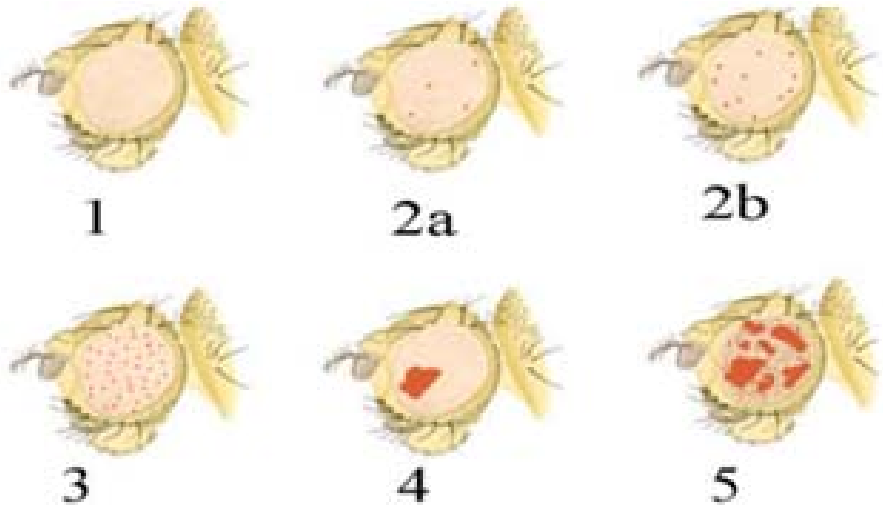

Figure 1. Classification of mosaic males according to standard methodology in six categories.

Results and Discussion

The PMM observed at the analyzed temperatures was: 20.88% at 14°C, 61.47% at 20°C, and 66.96% at 28°C. These results agree with other previous studies that showed the influence of temperature in the rate of *mariner* transposable element transposition (Giraud and Capy, 1996; Russell and Woodruff, 1999; Picot *et al*., 2008). Once high transposition rate occurs in higher temperature, as confirmed by our temperature test assay, all other experiments were performed at 28°C. Every male fly emerged from crossing experiments was classified into one of six categories using the pattern system showed in the Figure 1. Alternatively, the entire red pigmented area was measured for both eyes. As shown in the Figure 2, the standard deviation observed for the pigmented area among classes encompasses the average observed in all categories. These findings show that the use of conventional mosaic categories is not totally effective for quantifying the *mariner* activity in *wpch* crossing assay, once the classes form a continuous array. Besides which, the major critics to both methodologies either the visual classification using the mosaic pattern or the measurement of pigmented area is that the size of spots is seriously affected by the moment of *mariner* excision during fly development.

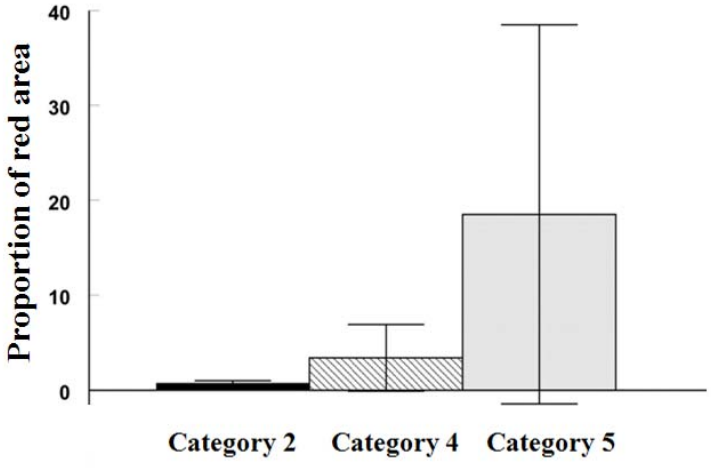

Figure 2. Proportion of red area in mosaic eyes classified in three categories based on pattern methodology, at 28°C.

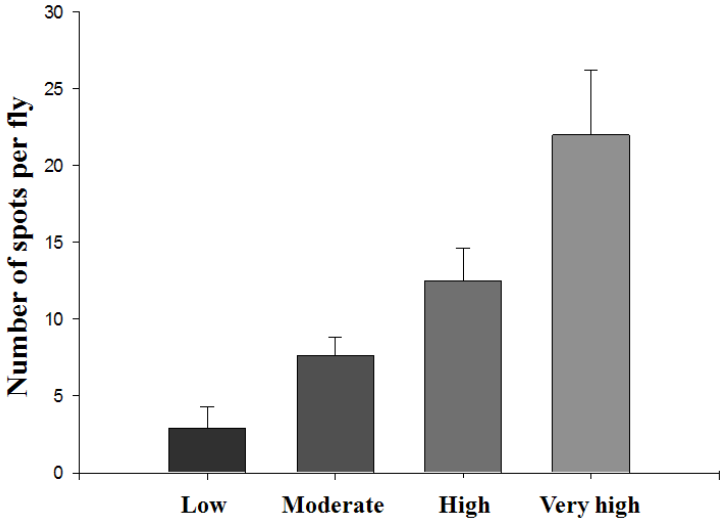

Figure 3. Average number of spots according to the four classes proposed by the new methodology.

An alternative method for quantification of transposition rate was done by counting the number of red spots and comparing it with the standard categorization analysis. The data show that the spot number is more accurate for quantification of transposition activity than area analyzes, because the variation in spot number within categories is relatively smaller than red size area (Figure 3).

We propose that *mariner* transposable activity can be classified into four categories using the number of spots in both eyes. Flies with 1 to 5 spots are classified as low activity. Flies with 6 to 10 spots are classified as moderate activity. Flies with 10 to 15 spots are classified as high activity, and finally flies with more the 15 spots are classified as very high activity. A similar classification was proposed by Capy *et al*. (1990).

When the reversion of *wpch* mutation occurs in developmental early stages, few spots appear, but they form a large pigmented area. When the reversion occurs in later stages, few cells revert back to wild condition per reversion event, giving the small spots (Giraud and Capy, 1996; Bryan and Hartl, 1988; Hartl, 1989). Thus, the number of spots reflects more accurately the transposable activity level of the element, allowing a more detailed understanding of their behavior.

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An instant fly medium and a convenient method to dispense it.

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Introduction

Most small research and teaching laboratories use instant media to grow fruit flies, with Carolina Formula 4-24 being a popular choice, although simpler and less expensive media such as mashed bananas (Bennett, 1961) or instant mashed potatoes (Duenas *et al*., 2002) can also be used. Other than the higher price relative to cooked versions and other types of media (Formula 4-24 is currently $5.13 per lb from Carolina Biologicals in a 50 lb bag), the principal disadvantage of instant medium comes from how it is commonly mixed in the laboratory: the dry food powder and water are added separately to individual vials or bottles, a procedure that will result in variable ratios of dry media to water across the bottles or vials (unless each bottle or vial is individually weighed after the sequential additions of dry medium and water). This is not a trivial concern: dietary depletion – produced by varying the amounts of macronutrients relative to agar – is well known to increase the lifespan of fruit flies, as well as to affect the expression of behaviors, including locomotion. Although the mechanism underlying the lifespan extension produced by dietary depletion is controversial, the end result – longer lived and differently acting flies – is not.

On the practical side, filling even a relatively small number of vials (50-75) in this manner is tedious. To address this problem, Laverty (1986) fabricated a device that will concurrently fill multiple vials with approximately the same amount of dry media, to which water is then added with a repeater pipette, reducing the labor associated with filling vials. Since the amount of dry medium added to each vial will still vary with this apparatus, however, the food concentrations will vary across individual vials. Rather than adding the dry ingredients and water separately into individual vials, mixing the dry ingredients and water prior to dispensing ensures that all vials and bottles will contain media of the same concentration. Described here is a recipe for an inexpensive medium similar to that described by Duenas *et al*. (2002) with physical properties similar to Formula 4-24, as well as a simple and efficient method to dispense rapidly this or any other instant medium by using a vertical screw-type sausage stuffer.