An Assay to Detect In Vivo Y Chromosome Loss in Drosophila Wing Disc Cells

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ABSTRACT Loss of the Y chromosome in Drosophila has no impact on cell viability and therefore allows us to assay the impact of environmental agents and genetic alterations on chromosomal loss. To detect in vivo chromosome loss in cells of the developing Drosophila wing primordia, we first engineered a Y chromosome with an attP docking site. By making use of the Fc31 integrase system, we site-specifically integrated a genomic transgene encompassing the multiple wing hair (mwh) locus into this attP site, leading to a mwh+Y chromosome. This chromosome fully rescues the mwh mutant phenotype, an excellent recessive wing cell marker mutation. Loss of this mwh+Y chromosome in wing primordial cells then leads to manifestation of the mwh mutant phenotype in mwh-homozygous cells. The forming mwh clones permit us to quantify the effect of agents and genetic alterations by assaying frequency and size of the mwh mosaic spots. To illustrate the use of the mwh+Y loss system, the effects of four known mutagens (X-rays, colchicine, ethyl methanesulfonate, and formaldehyde) and two genetic conditions (loss- and gain-of-function lodestar mutant alleles) are documented. The procedure is simple, sensitive, and inexpensive.

KEYWORDS chromosome loss Y chromosome multiple wing hair wing mosaic spots Drosophila

Elaborated mechanisms ensure the maintenance of genome integrity and stability in cells (Musacchio and Salmon 2007; Vakifahmetoglu et al. 2008). Loss of a chromosome usually disrupts the genetic balance, and the ensuing condition leads mostly to cell death. However, some monosomic cells can occasionally survive and propagate their unusual condition to their descending cells. The abnormal conditions may lead to human disabilities such as mental disabilities, miscarriage, and cancer. In humans, aneuploidy, which includes monosomy, has been regarded as a hallmark of cancer (Pellman 2007; Torres et al. 2010; Williams et al. 2008; Li et al. 2010; Tang et al. 2011).

Changes in the cell’s heritable material can be classified into three major types: (1) point mutations, (2) chromosomal breaks that may alter the amount of DNA in the cells, and (3) changes in chromosome number. There have been quite a number of assays developed to detect the first and the second types of mutations, and several of those have been used on a large scale (Zeiger 2004; Claxton et al. 2010). To detect gain and/or loss of the chromosomes, a number of the so-called aneuploidy test procedures were elaborated mostly in the 1980s and 1990s. They are proficiently overviewed in panel reports such as the Food and Drug Administration’s Redbook or the OECD Test Guidelines for Genotoxicity and Mutagenicity Testing. The aneuploidy test procedures usually make use of yeasts, Drosophila, or cultured mammalian cells. There are two main reasons why they are not routinely used and included in the batteries of mutagenicity test procedures. (1) The aneuploidy-detecting assays are not sensitive enough to observe rare events in a generally limited number of target cells. The high background noise, especially in the karyotyping-based procedures, sets a strong limit on the use of several of the proposed procedures. (2) Most of the aneuploidy test procedures are quite sophisticated and are usually rather expensive.

To overcome these issues, we developed an assay to detect in vivo loss of the Y chromosome in cells of the developing wing imaginal discs of Drosophila melanogaster. We selected to develop this assay based on the following observations and data. (1) Gain and/or loss of the Y chromosome with its nine Y-linked genes (Carvalho et al. 2001) has no impact on viability of the diploid imaginal disc cells. (2) Importantly, several thousand cells can be exposed to physical, chemical, or biological "treatments" in a single developing wing disc. Roughly one-half of the proliferating wing disc cells will give rise to

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the wing blade, a chitinous structure that is easy to mount and analyze. By using an appropriate marker, the individual genotype of about 30,000 wing blade cells can be determined.

In this report we (1) describe the generation of a Y-attP chromosome that permits site-specific integration of transgenes using the $\Phi$C31 integrase, (2) the insertion of a multiple wing hair (mwh$^+$) transgene into the Y chromosome, and (3) document the use of the mwh$^+$/Y chromosome to detect and quantitatively characterize the in vivo loss of the mwh$^+$/Y chromosome by quantifying the effects of X-rays, colchicine, ethyl methanesulfonate (EMS), and formaldehyde as well as the loss- and the gain-of-function lodestar mutant alleles. The assay is simple, sensitive, takes approximately 1 week to complete, and is very inexpensive.

**MATERIALS AND METHODS**

**Construction of the mwh$^+$/Y chromosome**

To generate an attP docking site in the Y chromosome, we made use of the P conversion or replacement method (Sepp and Auld 1999). The donor strain was y $^{w67C6}$, P$^{y+EG7} = CaryP$\phi$attP1 integrated into chromosome 2R (Groth et al. 2004; Markstein et al. 2008). The acceptor strain was y $^{w+}$/Dp(2;Y)G, P$^{y+wmc = hs-hid}$/Y with a segment of the second chromosome integrated into the Y chromosome (Starz-Gaiano et al. 2001). Mobilization of P$^{y+EG7} = CaryP$\phi$attP1 was done using y $^{w+}$/LrCyO; D/TM3, $^{ry^{frk}}$ Sb P(Delta2-3)99B as a transposase source (Robertson et al. 1988). Presumable mobilization events to the Y chromosome were identified as yellow$^+$ marked males that maintained the L or CyO chromosomes, indicative for loss of the original donor chromosome. Subsequently, linkage of the yellow$^+$ marker, associated with the attP site, to the Y chromosome was verified after simple chromosome segregation of the Dp(2;Y)G, P$^{y+EG7} = CaryP$\phi$attP1$ Y chromosome (from now on abbreviated as Y-attP) from males in the parental generation to males of the next generation. Several independent Y-attP chromosomes were generated using this method. To ensure the loss of the hs-hid portion contained within the original P$^{y+wmc = hs-hid}$ element, fertilized females were allowed to lay eggs for 3 days, followed by the removal of the adults, further development of the larvae for 2 more days, and a heat shock of the larvae at 37$^\circ$C for 1 hr. After eclosion of the developing pupae, no males where observed in the P$^{y+wmc = hs-hid}$ element containing stock (0 XY males, 0%; 0 X0 males, 0%; 440 XX females, 100%; 0 XXX females, 0%), whereas males did eclose from the Y-attP stock, indicating full removal of the P$^{y+wmc = hs-hid}$ element (70 XY males, 31.1%; 1 X0 males, 0.4%; 154 XX females, 68.5%; 0 XXX females, 0%).

To test the receptiveness of the Y-attP chromosomes, we tested the integration of a white$^+$ containing attP-Flp[acman]-Ap$^R$ clone by coinjection with the $\Phi$C31 integrase encoding mRNA, as described earlier (Groth et al. 2004; Venken et al. 2006). Stocks were then generated that contain the Y-attP chromosome as well as the $\Phi$C31 integrase present in y $^{M(vas-int.B)}$ZH-2A w$^+$ on the X-chromosome (Bischof et al. 2007). These stocks were then retested for receptiveness using attP-Flp[acman]-Ap$^R$ (Venken et al. 2006).

The entire mwh locus is present in the FlyFos-030330 clone: DNA clones from the original fosmid library are marked with an eye-expressed DsRed fluorescent marker driven by the 3xP3 eye specific promoter, for which transgenic flies can easily be identified in a white mutant background (Ejsmont et al. 2009). Moreover, these clones do not contain insulator sequences shielding the transgene from the surrounding environment; however, that ended up not being a problem, as exemplified in the Results section. The FlyFos-030330 sequence was integrated into the Y-attP chromosome as well as into the VK16 docking site in the 47C cytological region of the second chromosome (Venken et al. 2006, 2009), resulting in Dp(2;Y)G, P$^{y+EG7} = CaryP$ FlyFos-030330|attP Y (from now on abbreviated as mwh$^+$/Y) and PBac[y$^+$-attP-3B FlyFos-030330] VK00016 (from now on abbreviated as VK16 mwh$^+$). Hence, the engineered mwh$^+$/Y chromosome (as well as the VK16 mwh$^+$ line) carries the following markers: yellow$^+$, DsRed, and mwh$^+$. In this stock, X0 males appeared at a very low frequency (3/1366 males, 0.2%, that were nonrescued mwh mutant and sterile).

The w/mwh$^+$/Y; mwh strain

The mwh$^+$/Y chromosome was then integrated into a w$^{1118}$/ mwh$^+$ background to create a w$^{1118}$/ mwh$^+$; mwh$^+$ stock. The w$^{1118}$ (shortly w) allele allows the detection of the fluorescent DsRed marker (that marks the presence of the mwh$^+$/Y chromosome) and hence the convenient identification of the occasional loss of the mwh$^+$ Y chromosome in w0; mwh males.

Wing blade cells homozygous for the mwh marker mutation, linked to the third chromosome, produce two to five trichomes per cell instead of the regular single trichome seen in wild-type or mwh/mwh$^+$ heterozygous cells (Figure 1). The trichomes (hairs) are usually short and possess abnormal polarity (Yan et al. 2008). Single mwh homozygous cells can easily be detected in the midst of broad fields of wild-type cells (Szabad et al. 1983).
Treatments

Eggs were collected in 8-hr shifts from the w/mwh+Y; mwh strain on standard Drosophila corn meal food with live yeast and kept at 25°C throughout. We also raised flies on the Formula 4-24 Instant Drosophila Medium (Carolina Biological Supply Company) to assess if the culture conditions can influence the frequency of mwh+Y chromosome loss. The hatching larvae developed on standard Drosophila food and were treated 84–92 hr after egg laying, the event that marks the commencement of embryogenesis. There are approximately 5000 to 6000 cells in a developing wing blade primordium at this mid-third instar stage of development (Bryant and Levinson 1985). The remaining 28–36 hr to puation (at 120 hr after egg laying) allow approximately three rounds of mitoses before the cessation of cell proliferation (Bryant and Levinson 1985). The 84- to 92-hr-old larvae keep on foraging for 4–12 more hours (Rodriguez Moncalvo and Campos 2009). Hence, the tested chemicals can enter their digestive tract and reach the cells of the wing primordia.

For treatments, the 84- to 92-hr-old larvae are floated off the food with a 14% NaCl solution, collected on a nylon mesh, washed with tap water, dried up briefly, and transferred onto standard Drosophila food into which the substance to be tested was mixed. Larvae finished development on this food in case of colchicine and formaldehyde treatments (Table 1). The floated larvae can also be immersed into a solution for exposure to chemicals, as was the case during EMS treatment, or irradiated before transferring back to standard Drosophila food (Table 1).

To assess the "genetic effects" on loss of the mwh+Y chromosome, we analyzed wings of (1) w/mwh+Y; mwh ldsHor-rvP2/mwh Df(3R) ED5218 males that lack the product of lodestar (lds), a member of the Snf2 family of helicase-related genes (Szalontai et al. 2009). The abbreviations are as follows: ldsHor-rvP2 is a complete loss-of-function lds allele (Szalontai et al. 2009), and Df(3R)ED5218 is a small deficiency generated by the deletion of the segment encompassed between two FRT site containing transposons (Ryder et al. 2007) that removes lds and a few adjacent loci. (2) The w/mwh+Y; mwh ldsHor-D/mwh lds+ males carried ldsHor-D, a dominant, chromosome instability causing mutation in Drosophila (Szabad et al. 1995; Szalontai et al. 2009). (3) In the w/mwh+Y; mwh lds-/+ males that developed (i) on standard Drosophila food or (ii) in the FRT site containing transposons (Ryder et al. 2007) that removes lds and a few adjacent loci.

Wing preparation and scoring

The w/mwh+Y; mwh male flies were aged for 1 to 2 days after eclosion. They were dipped first into a 96% ethanol for a few seconds, transferred into water, and their wings were detached. The wings were mounted in Faure’s mounting medium such that wings of every male were positioned in pair-wise fashion. Except for the hinge region, the wings were screened at ×400 magnification (Figure 1). The number and size of the mwh clones were recorded. The screened area of a wing blade contains about 30,000 cells (Garcia-Bellido and Merriam 1971). In determining the number of mwh clones and their size, we followed a published protocol (Graf et al. 1984). Classification of the clones composed of ≥2 mwh homozygous cells is straightforward. Single cells were considered to be mwh homozygous if they carried at least two chromosomes that pointed into different directions. Two mwh cells were classified as a single clone if they were on the same wing surface and were not separated by more than three normal cells. To determine the average clone size, the mwh clones were classified into size classes that represent the minimum number of cell divisions required, following the loss of the mwh+Y chromosome, for the formation of clones composed from 1, 2, 3–4, 5–8, etc. mwh cells (Table 1). We presume that the mwh+Y chromosome is lost from only one of the daughter cells during mitosis.

RESULTS

Constructing the mwh+Y chromosome

To screen for chromosome loss, we generated a mwh+Y chromosome into which an mwh+Y genomic rescue fragment is integrated at an attP docking site (see Materials and Methods). The mwh+Y transgene in this chromosome rescues the mwh mutant phenotype. The resulting w/mwh+Y; mwh stock was used to detect in vivo loss of the mwh+Y chromosome (Figure 2). Note that the presence of an efficient attP docking site on the Y chromosome also can be used to integrate other markers that may allow optimal live labeling of male embryos and young larvae.

Loss of the mwh+Y chromosome during mitosis leads to the formation of a cell without mwh+Y function. This cell is fully viable and propagates its new character onto its descending cells during the oncoming mitoses. The daughter cells will stay together and form a mwh clone (mosaic spot), in an mwh homozygous genetic background, after metamorphosis in the wing blade. In other words, groups of cells that display the mwh mutant phenotype on the wings of w/mwh+Y; mwh males should reflect events involving the loss of the mwh+Y chromosome.

The background mwh clone frequency

Principles of the quantification are as follows. Assuming equal contribution of the wing disc cells to the wing blade, the number of cells in a wing disc primordium at the time of mwh+Y chromosome induction is C/2m, where C is the number of the screened cells in a wing blade [C = 30,000 (Garcia-Bellido and Merriam 1971)] and m is the average clone size. Because generally only one of the daughter cells becomes mwh-labeled after the loss of the mwh+Y chromosome during mitosis, m needs to be multiplied by two. Screening N wings implies the analysis of N C/2m target cells exposed to the treatment and the number of these cells can easily reach 104. Considering that a single mwh-labeled cell will give rise to one clone, n, the number of mwh clones in N wings, equals the number of the target cells that gave rise to daughter cells without the mwh+Y chromosome. Therefore, f, the frequency of mwh+Y chromosome loss, is f = n/2N C.

To determine the spontaneous frequency of mwh+Y clone formation, we analyzed wings of (1) w/mwh+Y; mwh males that developed (i) on standard Drosophila food or (ii) on the 4-24 instant Drosophila medium and (2) wings of w/mwh+Y; mwh ldsHor-D/mwh lds+ males. Because the frequency of the mwh clones were not significantly different in the aforementioned types of wings (86 clones/58 wings, 22/16 and 56/34, respectively, P > 0.05, χ² test), we pooled the data and used the 164 mwh clones in 108 wings as the control frequency throughout the present study (Table 1).

The mwh clones were grouped into size classes. A size class defines the minimum number of cell divisions required between the induction of a clone and its formation to the observed size. The distribution of the mwh clones among the size classes seem to follow the "half-by-half" rule, i.e., there are twice as many clones in class I as in class II, twice as many in class II as in class III, and so on (Table 1). If we assume a (1) constant frequency of loss of the mwh+Y chromosome throughout the subsequent rounds of mitoses and (2) equal contribution of the wing disc cells to the final wing blade cell population, the 164 mwh clones are expected to be distributed as follows
### Table 1 Features of mwh mosaicism

| Treatment and/or Genotype<sup>a</sup> | Wing, N | mwh Clone, n | mwh Clone Frequency, n/N | Size Class (I-VIII)<sup>b</sup> and the Number of mwh Cells per Clone | Average Clone Size<sup>c</sup> (mwh Cell per Clone), m | Frequency of Clone Induction, f |
|-------------------------------------|---------|--------------|--------------------------|---------------------------------------------------------------|-------------------------------------------------|---------------------------------|
| Control                            | 108     | 164          | 1.5                      | 96 39 22 5 2 0 0 0                                             | 1.7 ± 1.0                                      | 1.7 × 10<sup>-4</sup> |
| Su-var(2)103                       | 40      | 56           | 1.4                      | 27 17 7 4 1 0 0 0                                             | 1.9 ± 1.0                                      | 1.8 × 10<sup>-4</sup> |
| Su(var)3-9<sup>pm</sup>            | 32      | 52           | 1.6                      | 28 13 9 2 0 0 0 0                                             | 1.8 ± 0.9                                      | 1.9 × 10<sup>-4</sup> |
| X-rays; 1000 Rad (150 kV, 0.5 mm Al; 500 Rad/min) | 12      | 119          | 9.9<sup>**</sup>         | 37 31 19 15 9 4 2 2                                          | 2.6 ± 1.5                                      | 17.2 × 10<sup>-4</sup> |
| X-rays; mwh<sup>+</sup> VK16; 1000 Rad (150 kV, 0.5 mm Al; 500 Rad/min) | 40      | 7            | 0.2<sup>**</sup>         | 5 1 1 0 0 0 0 0                                             | 1.4 ± 0.9                                      | 0.2 × 10<sup>-4</sup> |
| Colchicine (1 μg/mL in the food. From 84–92 hAEL on) | 4       | 63           | 15.8<sup>**</sup>        | 8 15 16 11 6 7 0 0                                           | 2.8 ± 1.5                                      | 29.4 × 10<sup>-4</sup> |
| EMS (25 mM for 4 hr at 84–92 hAEL) | 18      | 57           | 3.2<sup>**</sup>         | 24 16 9 2 4 2 0 0                                           | 2.2 ± 1.4                                      | 4.6 × 10<sup>-4</sup> |
| Formaldehyde<sup>d</sup> (0.05M in the food. from 84–92 hAEL on) | 46      | 136          | 2.9<sup>**</sup>         | 92 31 12 1 0 0 0 0                                           | 1.4 ± 0.7                                      | 2.8 × 10<sup>-4</sup> |
| Ids<sup>hor-D</sup>/Ids<sup>*</sup> | 14      | 73           | 5.2<sup>**</sup>         | 38 26 7 2 0 0 0 0                                           | 1.7 ± 1.0                                      | 5.9 × 10<sup>-4</sup> |
| nub-Gal4; UAS-Ids<sup>hor-D</sup>; Ids<sup>*</sup>/Ids<sup>*</sup> | 24      | 247          | 10.3<sup>**</sup>        | 99 64 45 31 6 2 0 0                                          | 2.2 ± 1.2                                      | 15.1 × 10<sup>-4</sup> |
| Ids<sup>hor-D</sup>/Df(3R)ED5218<sup>e</sup> | 24      | 164          | 6.8<sup>**</sup>         | 84 43 23 11 3 0 0 0                                          | 1.9 ± 1.0                                      | 8.7 × 10<sup>-4</sup> |

<sup>a</sup> and ** indicate significantly different from the control at P < 0.05 and P < 0.01, respectively. hAEL, hours after egg laying.

<sup>b</sup> All the males carried the w<sup>1118</sup>-labeled X, the mwh<sup>+</sup>Y chromosome, and were homozygous for mwh. Su-var(2)<sup>103</sup> is a dominant suppressor mutation of position-effect-variegation and Su(var)3-9<sup>pm</sup> is an exceptionally strong position-effect-variegation enhancer mutation (Schotta et al. 2003; Ebert et al. 2004).

<sup>c</sup> The minimum number of cell divisions (I–VIII) required—after the loss of the mwh<sup>+</sup>Y chromosome—until the formation of clones composed from 1, 2, 3–4, etc., mwh cells. It was assumed that only one of the daughter cells becomes mwh-labeled after the loss of the mwh<sup>+</sup>Y chromosome during mitosis.

<sup>d</sup> Calculated from the average size class by making use of the linear relationship between size classes (I–VIII) and the log average clone size within the different size classes.

<sup>e</sup> 0.05M formaldehyde mixed into the food allows 50% of the larvae develop to adult (Szabad et al. 1983).
among size classes I-V: 85, 42, 21, 11, and 5. Because the observed
(96, 39, 22, 5, and 2) and the expected distributions are not signifi-
cantly different ($P > 0.05, \chi^2$ test), the incidence of $mwh^+Y$ chro-
mosome loss appears to be constant throughout the subsequent
rounds of cell divisions in cells of the wing discs (Table 1). The
spontaneous frequency of $mwh^+Y$ chromosome loss is $1.7 \times 10^{-4}$/
cell division (Table 1). The spontaneous loss of the $mwh^+Y$ chro-
mosome appears to happen randomly because the distribution of
the 164 $mwh$ clones among the 108 wings follows the Poisson dis-
tribution: the observed and the expected values are not signi-

Table 2 Distribution of the spontaneous $mwh$ clones in the control wings

| Number of wings with $N, mwh$ Clones |
|--------------------------------------|
| 0       | 1       | 2       | 3       | 4       | 5       | 6       |
|---------|---------|---------|---------|---------|---------|---------|
| Observed| 30      | 28      | 29      | 10      | 7       | 4       |
| Calculated*| 23.7   | 35.9    | 27.3    | 13.8    | 5.2     | 1.6     |

*Based on the Poisson distribution $P(i) = e^{-i}/i!$, where $n=N/N$ and $n=164$, $N=108$. division, in which the $mwh$-labeled cells seem to participate, replace
the lost cells and hence larger than normal clones develop. The
average clone size was 2.6 $mwh$ cells and thus the frequency of
$mwh^+Y$ chromosome loss is $17.2 \times 10^{-4}$, about 10-fold the control
level (Table 1).

Some of the X-ray–induced $mwh$ clones on the wings of the
$w/mwh^+Y; mwh$ males might have originated through the loss of
function of the $mwh^+$ gene in the $mwh^+Y$ chromosome. To estimate
the contribution of the lost $mwh^+$ gene function in the frequency of
the $mwh$ mosaic spots, we inserted the $mwh^+$ gene contained within
the FlyFos-030330 clone (Ejsmont et al. 2009) into the $attP$ docking
site at 47C (VK16) on the right arm of the second chromosome
(Venken et al. 2006, 2009). After X-irradiation at a 1000 Rad of
$w/Y; VK16 mwh^+/[In(2LR)Gla]$ male larvae, seven $mwh$ clones de-
veloped on 40 wings (Table 1). A comparison of the 119/12 and the
7/40 frequencies clearly shows that the vast majority of the $mwh$
clones on wings of the $w/mwh^+Y; mwh$ males originated due to chro-
mosome loss and that the contribution of point mutations in the
$mwh^+$ gene is very low. It is also highly unlikely that the
seven $mwh$ clones (of 40 wings) originated through X-ray–induced
mitotic recombination since the $In(2LR)Gla$ chromosome effectively
suppresses recombination in the 47C area where the VK16 landing
site is (Venken et al. 2006).

Colchicine binds tubulin and inhibits microtubule polymerization.
Hence, colchicine effectively functions as a “mitotic poison” or spindle
poison. It is therefore expected to induce a high frequency of
$mwh$ clones in wings of the $w/mwh^+Y; mwh$ males, as indeed shown
in Table 1. The average size of the $mwh$ clones was 2.8 cells after col-
chicine treatment, and the frequency of clone induction was $29.4 \times$
$10^{-4}$. Although approximately 80% of the clones (50/63) appeared in
the expected I-IV size classes, several grew unusually large and were

Figure 2 Overview of the strategy. Tool-generation phase: $P$ element conversion was used to replace a white* marked $P$ element with an $attP$ site
and yellow* marker containing $P$ element. A FlyFos clone containing the entire $mwh$ marked was integrated into the $attP$ site using the $PhiC31$
integrate. This transgene was combined into an $mwh$ mutant background and rescued the homzygous $mwh$ phenotype. Mutagen assay phase:
The resulting flies were allowed to develop until a late larval stage and treated with mutagens (see Materials and Methods). Adult wings were
removed, analyzed for the presence of $mwh$ clones, and the frequency of such clones calculated.
assigned to classes V and VI (Table 1). This indicates that colchicine induces cell death in the wing primordia followed by intercalary regeneration, in agreement with previous data documenting that cells die most likely through the induction of aneuploidy (Isaenko et al. 2002).

EMS is routinely used as a mutagen in Drosophila (Lewis and Bacher 1968). It induces mostly point mutations and some chromosomal breaks in wing imaginal disc cells but does not appear to induce detectable levels of aneuploidy in germline cells (Szabad 1986). The present assay clearly shows that EMS induces the formation of mwh clones, although with low but significantly greater frequency as in the control (Table 1). We surmise that most of these clones are probably due to EMS-induced mutations in the mwh + gene present on the mwh Y chromosome and to chromosomal loss. Indeed, a 4-hr 8 mM EMS treatment induced mutations at a rate of 8.8 x 10⁻⁴ in the wing disc cells, a value similar with the 4.6 x 10⁻⁴ value reported here (Table 1) (Szabad and Bennetttova 1986).

Formaldehyde induced a subtle but significant elevation in the frequency of the mwh clones (Table 1). However, in line with the mosaic spots that originated through formaldehyde-induced chromosomal breaks (Szabad et al. 1983), the mwh clones remained very small, and consequently the frequency of mwh clone formation was rather low: 2.8 x 10⁻⁴ (Table 1). The generally small size of the 136 mwh clones is most likely the consequence of a delay in action of the formaldehyde between its uptake in the digestive system and its ability to reach the wing disc cells. Formaldehyde has been known to induce mutations through small-scale chromosomal rearrangements without compelling evidence of induced chromosome loss in yeast and cultured mammalian cells (Zimmermann and Mohr 1992; Speit and Merk 2002; Speit et al. 2011). To elaborate on the origin of the mwh clones in wings of the w/mwh; Y/mwh males after formaldehyde treatment, we analyzed 20 wings of w; Y, VK16 mwh +/Im(2LR)Gal; mwh males in which the mwh clones cannot be caused by chromosome loss or recombination. Because only two mwh clones formed (each with one mwh homozygous cell) on 20 such wings, this result is suggestive that at least some of the 136 mwh clones emerged due to formaldehyde-induced loss of the mwh Y chromosome.

**DISCUSSION**

Spindle assembly checkpoint and mitotic catastrophe are cellular machineries that guard over chromosome/genome stability in the course of the subsequent cell divisions (Musacchio 2011; Vitale et al. 2011). Failed or disturbed functions of these surveillance mechanisms lead usually to cell death. However, some of the cells may escape the attention of the aforementioned mechanisms and survive. Many of these cells are aneuploid and may become the source of mental retardation, miscarriage, and cancer (Pellman 2007; Gordon et al. 2012; Holland and Cleveland 2012; Pfaus and Amon 2012). The aforementioned well-established findings necessitate the elaboration of robust, reliable, and cheap aneuploidy test procedures.

The evolutionary conserved nature of the mechanisms involved in the aforementioned phenomena and processes (Lince-Faria et al. 2009) call for the use of model species to detect chromosome gain and/or loss. Drosophila melanogaster is an appropriate model species for the analysis of numerous basic biologic processes, including mutagenesis (Bellen et al. 2010). There have been a number of Drosophila-based aneuploidy test procedures developed to detect gain and/or loss of chromosomes both in the germline and in the soma (Szabad 1986; Szabad and Wurgler 1987; Rodriguez-Arnaiz et al. 1992; Szabad et al. 1995). However, most of these techniques detect aneuploidy in the female and/or in the male germ line, and there are two major difficulties associated with the germ-line based procedures: (1) a limited number of the germline cells and (2) the long time course between the induction and the detection of the aneuploidy (Szabad and Bennetttova 1986; Szabad et al. 1995). Imaginal discs with ongoing rounds of mitoses and a large number of target cells are ideal “tools” to detect aneuploidy. A previous method based on loss of a white-Y chromosome in photoreceptors (Szabad and Wurgler 1987) was shown to work but has not been included into the battery of the so-called genetic toxicity testing procedures (Zeiger 2004) because (1) small eye clones go undetected and hence the sensitivity of the procedure is rather low, and (2) detection and characterization of the eye clones is relatively complicated and time consuming. These caveats are clearly not an issue in this assay.

Indeed, the Drosophila wing blades appear to be an ideal organ to analyze cellular events. The wings develop as a sack of diploid epithelial cells (discs) in which the successive rounds of cell cycles occur at about 10-hr intervals, the cell number grows exponentially, and mitoses cease soon after pupariation (Bryant and Levinson 1985; Dubatolova and Omylanychuk 2004; Baker 2007; Neto-Silva et al. 2009). Of the approximately 50,000 wing disc cells, about 30,000 compose the wing blade, a chitosinous structure that is flat, highly convenient to mount and analyze, and in which practically every cell forms a trichome (Figure 1). The large collection of trichome marker mutations (Garcia-Bellido and Dapena 1974) set the wing discs apart from the many other cell types, making them highly suitable for testing for aneuploidy.
as appropriate “tools” to study cellular events, including mutagenesis. Szabad et al. (1983) proposed the so-called somatic mutation and recombination test to detect chromosome breaks—through the use of the mwh and the flare marker mutations—and point mutations induced in the mwh gene (Szabad et al. 1983; Surjan et al. 1985; de Andrade et al. 2004). Regrettably, loss of the X (first), the second, or the third chromosomes bring about cell death, and the absence of one of the fourth chromosomes significantly reduces viability of the wing disc cells, but loss of the Y chromosome has no impact on cell viability. After the loss of the mwh Y chromosome, the wing disc cells survive and propagate their new genetic composition to their descending cells that remain together and form an mwh clone in the wing blade (Figure 1 and 2). As described in the present work, formation of the mwh clones in wings of the w1118/mwh Y; mwh males is thus a reliable indicator of chromosome loss. We also show that variegation of the mwh* transgene or point mutations in the transgene play little if any contribution to the formation of the mwh clones.

The number and size of the mwh clones allow a quantitative evaluation of the effectiveness of the environmental or genetic “treatments” to induce the loss of the mwh Y chromosome (Szabad et al. 1983). Our data show that chromosomal loss can be induced by X-rays, colchicine, and formaldehyde, whereas EMS does not cause chromosomal loss. Finally, gain- and loss-of-function mutations in l(2)estarr, previously shown to induce chromosomal instability, also cause chromosomal loss in our assay. In summary, the proposed assay is simple, sensitive and inexpensive.

Based on the present data, we propose the w1118/mwh Y; mwh system is an adequate tool to detect in vivo the effects of environmentally and genetically induced chromosome loss in a higher eukaryotic organisms.

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