# SOMATIC MUTATION AND RECOMBINATION TEST (SMART) IN *Drosophila melanogaster*

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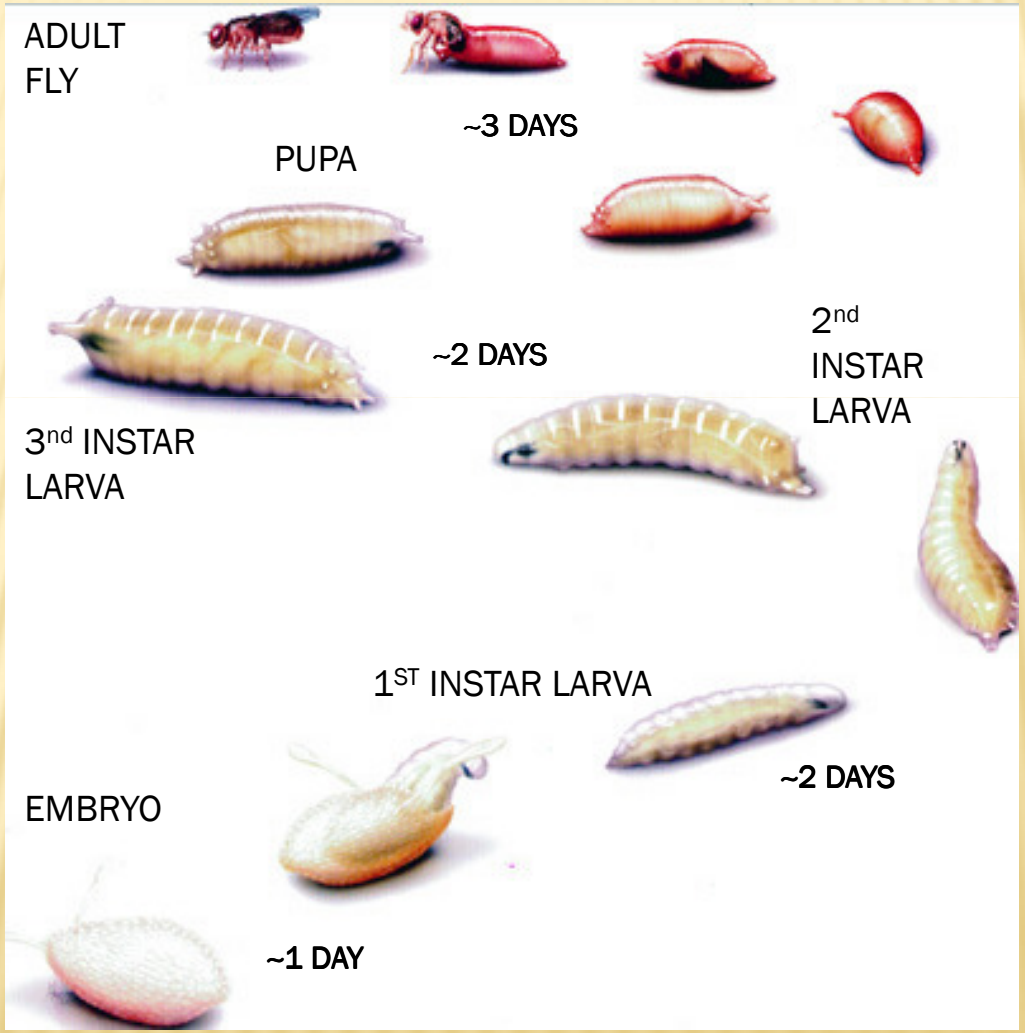
# The fly *Drosophila melanogaster*

- Higher eukaryote dipter with short life cycle and abundant progenie that has been studied in biology labs for over a century.
- Member of the *melanogaster* group of the subgenus *Sophophora*
- Easy and economic to raise.

# LIFE CYCLE OF *Drosophila*



# GENOME

- The genome has been completely sequenced (Adams *et al*., 2000) and encodes approximately 17,000 genes, most of them located in three of its four chromosomes.

Adams M.D., Celniker S.E., Holt R.A., EvansC.A., Gocayne J.D., et al. The genome sequence of *Drosophila melanogaster. Science* ***287:***2185-2195, 2000.
http://www.ncbi.nlm.nih.gov/gene?LinkName=genome_gene&from_uid=47

# *Drosophila* VS HUMAN GENES

- *Drosophila* has 548 unique sequences that complement human genes that codify for hereditary diseases (77%) (Reiter *et al.*, 2001).

- Between *Drosophila* and mammals, the global identity of nucleotides or amino acids is aproximately of 40% homology, nevertheless, for functional domains it can be from 80 to 90% (Bhan, 2011).

Rubin G.M.*et al*. Comparative genomics of the eukaryotes. *Science* **287**: 2204–2215, 2000. Reiter, L. T.,Potocki, L.Chien,S., Gribskov,M. and Bier E. A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. *Genome Res.***11:**1114–1125, 2001. Chien S., Reiter L.T., Bier E, Gribskov M. 2002 Homophila: human disease gene cognates in Drosophila. Nucleic Acids Res. 30:149-151, 2002. Bhan P.U., Nichols C.D. Human disease models in *Drosophila melanogaster*and the role of the fly in therapeutic drug discovery. *Pharmacol. Rev.* **63**:411–436, 2011.

# XENOBIOTIC METABOLISM

Insecticide and plant metabolism genes are highly enriched in the insect Malpighian (renal tubules), the midgut and the larval fat body, implicating them in xenobiotic metabolism with roles analogous to the vertebrate liver.

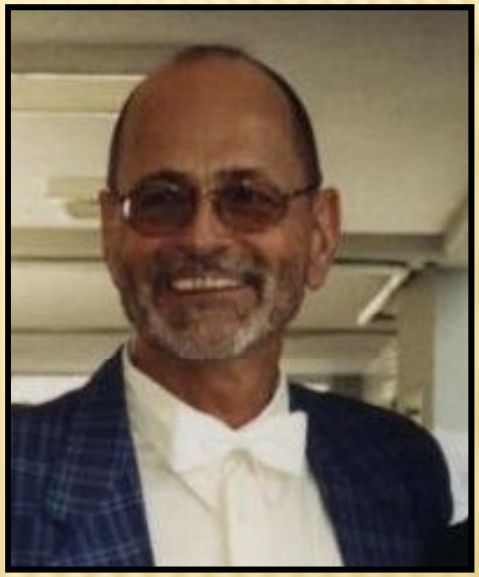
*Pandey UB, Nichols CD. **Human disease models** in ***Drosophila melanogaster*** and the **role** of the **fly** in **therapeutic drug discovery**. Pharmacol Rev. 2011 Jun;63(2):411-36. **Yang J, McCart C, Woods DJ, Terhzaz S, Greenwood KG, ffrench-Constant RH, Dow JA. **A Drosophila systems approach to xenobiotic metabolism.** Physiol Genomics. 2007;30(3):223-31

# *CYP450*$_S$ IN *Drosophila*

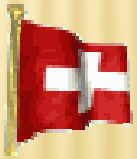
| | | | | |
|---|---|---|---|---|
| 103 GENES<br><br>FB2014_05, released September 9th, 2014 | 23 GENES with experimental known functions (induced by xenobiotics) | 7 GENES related with ecdisone functions | 27 GENES electron transporters deduced by similar sequence percentage. | 42 GENES active in larval phase. |

# SMART or WING SPOT TEST

- More than 400 compounds have been tested.
- Markers expression in the wings
- Just one generation to obtain results
- Induced or high *P450s* expression.
- Somatic recombination can be calculated
- ~48,000 cells/fly



**Dr. Ulrich Graf**

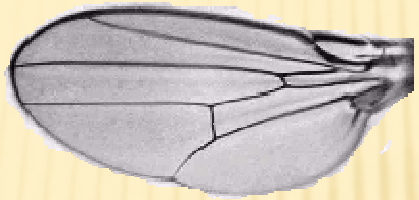


Swiss Federal Institute of Toxicology (ETH)

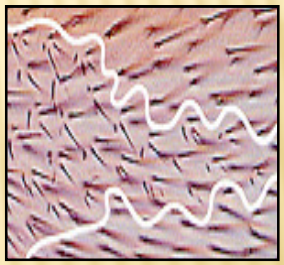
# STRAINS AND WING MARKERS

"*multiple wing hair*"

*mwh/ mwh*



Wild wing



*mwh* 3-0.3

"*flare*"

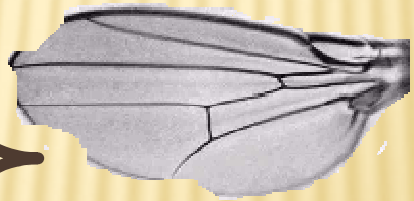
*flr*$^{3}$/ TM3, *Bd*$^{S}$

&

"Oregon-*flare*"

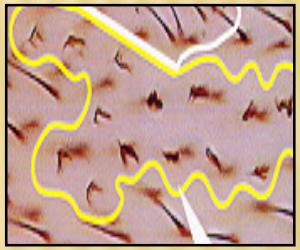
ORR(1);ORR(2); *flr*$^{3}$/ TM3, *Bd*$^{S}$

*Rst(2)DDT* (2-65)



*Bd*$^{(S)}$

3-92.5



*flr*$^{3}$ 3-38.8

*mwh/mwh*

*fh³ / TM3 , Bd⁵*

# flare STRAIN

| | + *flr*$^{3}$ + | + + TM3, *Bd*$^{S}$ |
|---|---|---|
| + *flr*$^{3}$ + | + *flr*$^{3}$ +/+ *flr*$^{3}$ + | + *flr*$^{3}$ +/+ + TM3, *Bd*$^{S}$ |
| + + *TM3, Bd*$^{S}$ | + *flr*$^{3}$ +/+ + TM3, *Bd*$^{S}$ | + + *TM3, Bd*$^{S}$/+ + TM3, *Bd*$^{S}$ |

# OREGON –flare STRAIN
# ORR(1);ORR(2); *flr*$^{3}$/ TM3, *BD*$^{S}$ STRAIN

- Chromosome 2 contains the gene *Cyp6g1* with the dominant mutation *Rst(2)DDT* that codes for the inducer protein of :
  - *Cyp6a8* and *Cyp6a9* genes located on chromosome 1
  - *Cyp6a2* gene located on chromosome 2

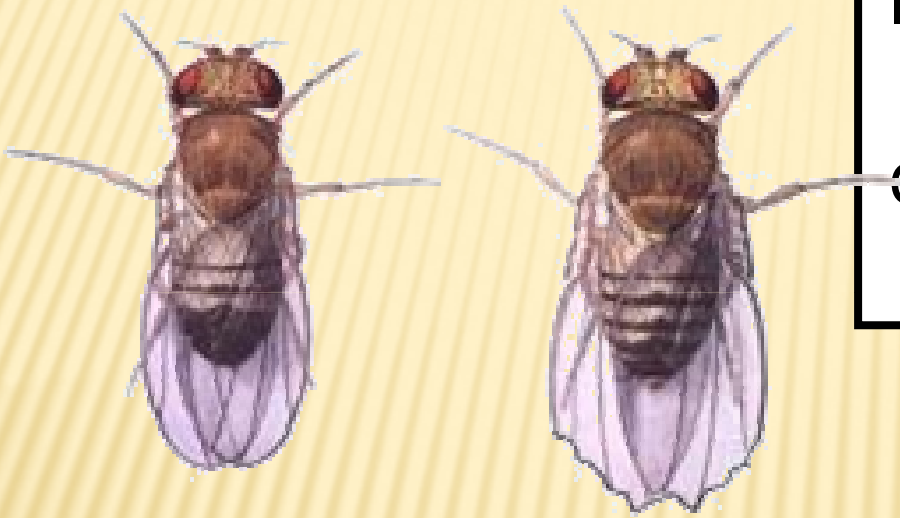
- This strain has a higher expression of *P450*s than the *flr*$^{3}$/ TM3, *Bd*$^{S}$ strain (Frölich and Würgler, 1989).

# SMART

Genotoxicity Bioassay

based in

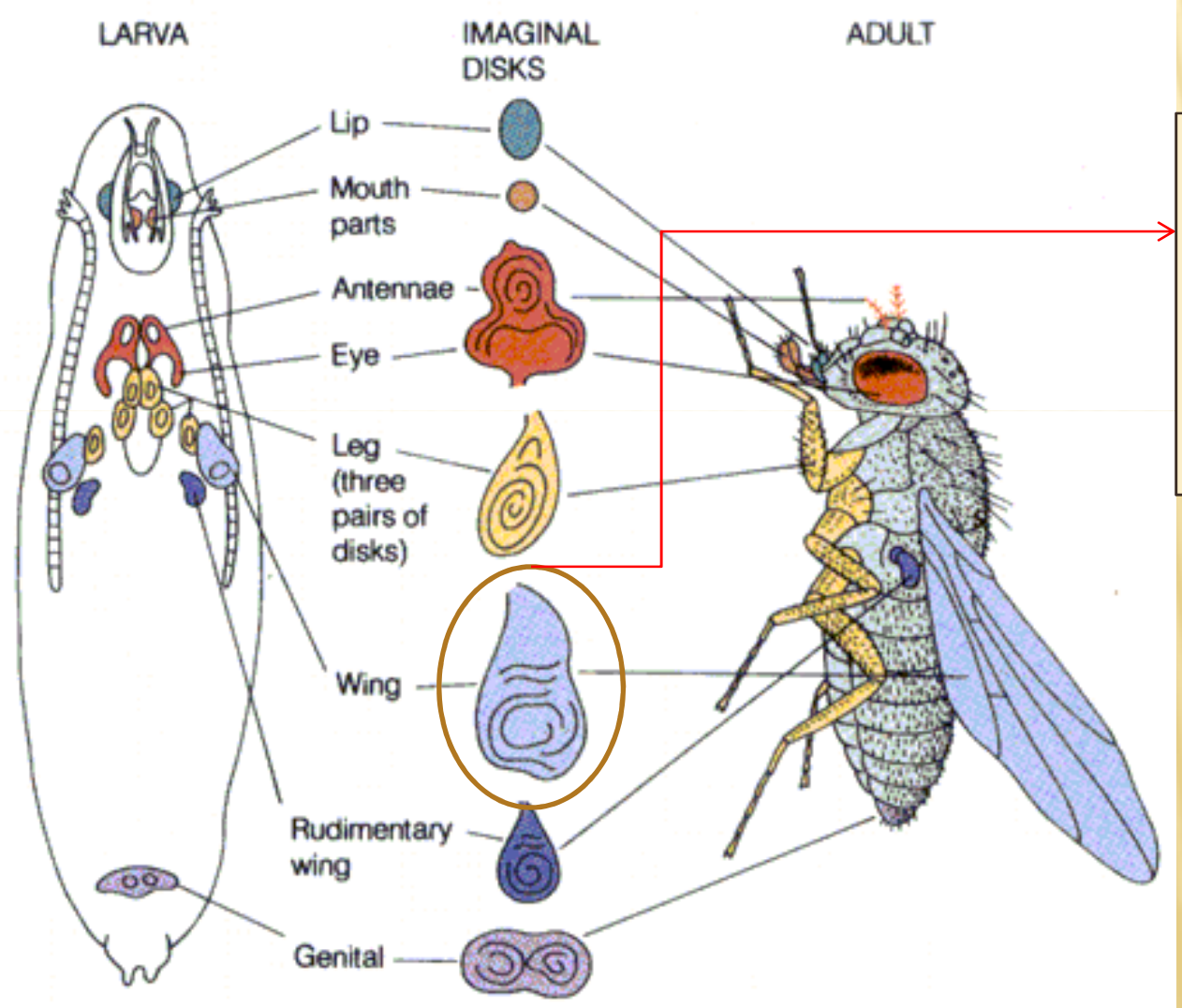
loss of heterozygosity of markers in the imaginal cells of *D. melanogaster* larvae.



**DNA DAMAGE**

**Deletion, point mutation, aneuploidy and somatic recombination.**

# IMAGINAL DISKS

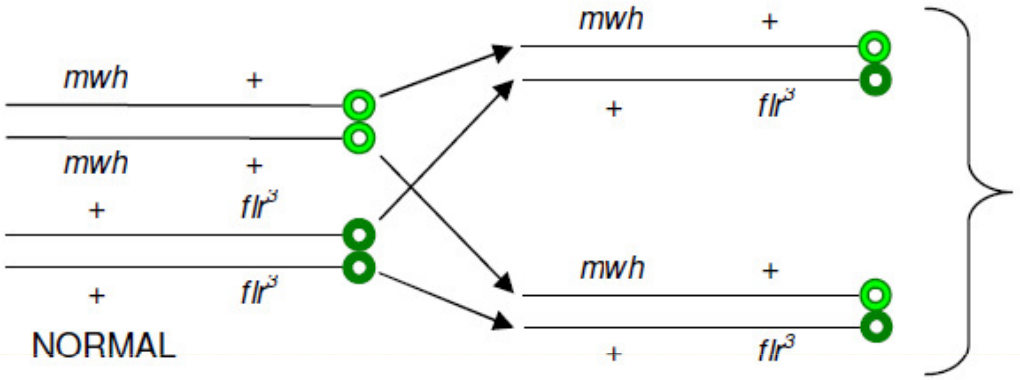


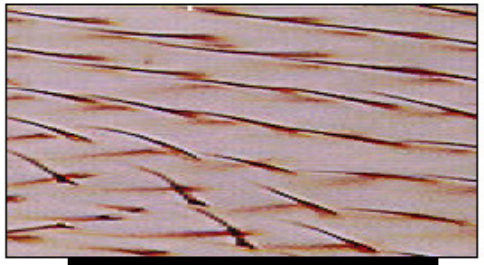

~50 cells at 72h of life (3nd instar larva).

~24,000 cells at the end of metamorphosis (10 days).

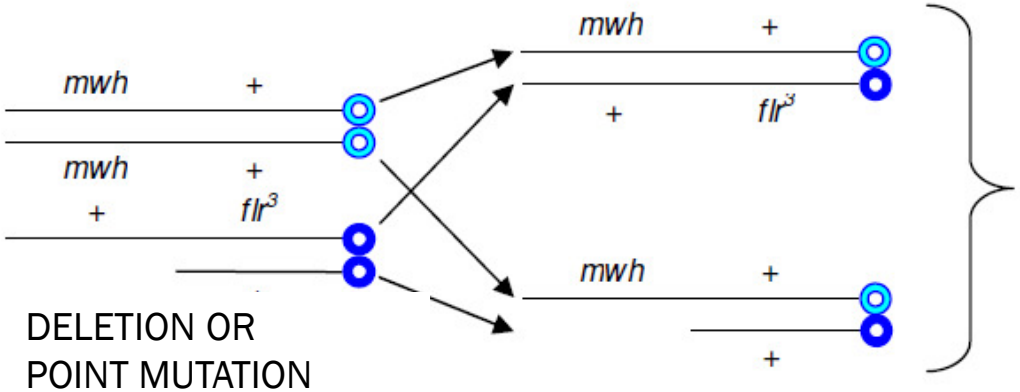
http://www.pearsonhighered.com/mathews/ch28/fi28p45.gif

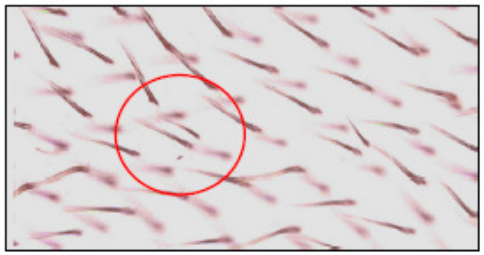
# LOSS OF HETEROZYGOSITY BY DELETION OR POINT MUTATION



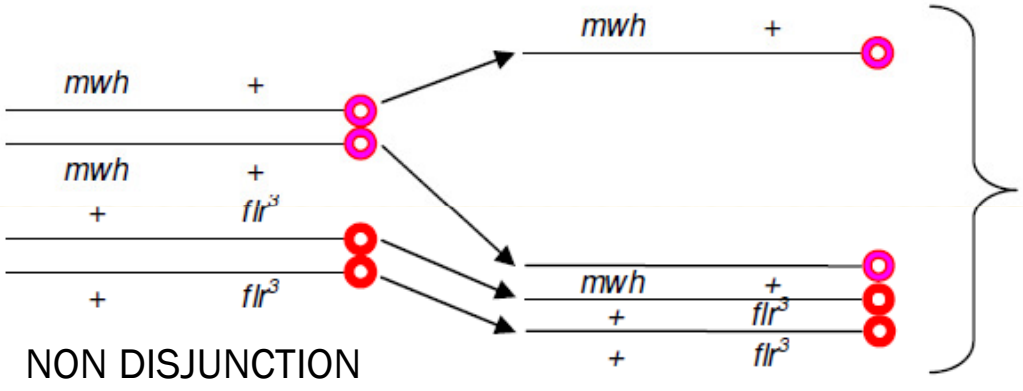
Normal trichomes

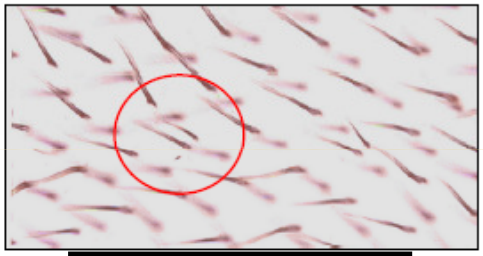


Single *mwh* spot

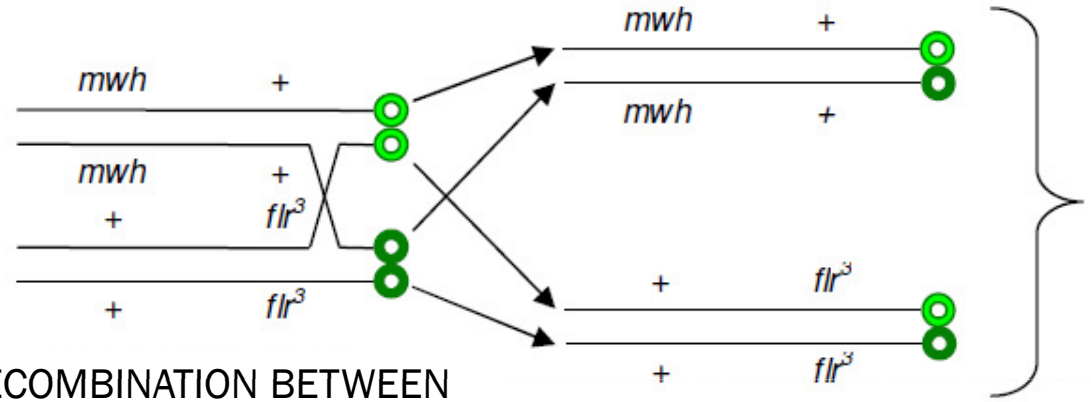
# LOSS OF HETEROZYGOSITY BY ANEUPLOIDY



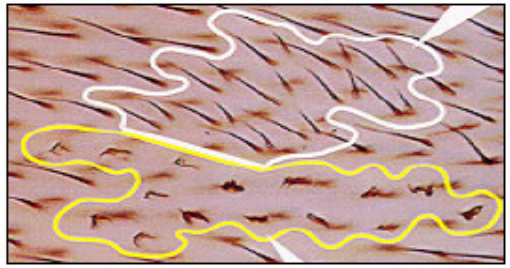



Single *mwh* spot

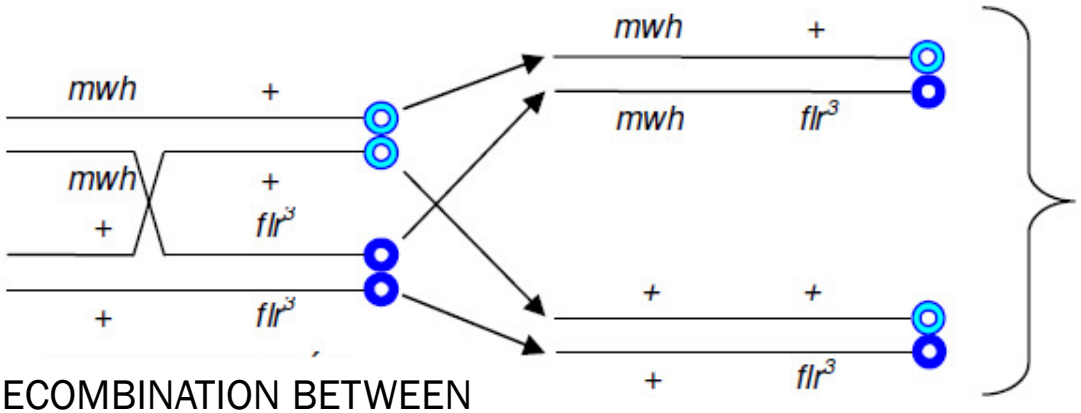
# LOSS OF HETEROZYGOSITY BY SOMATIC RECOMBINATION



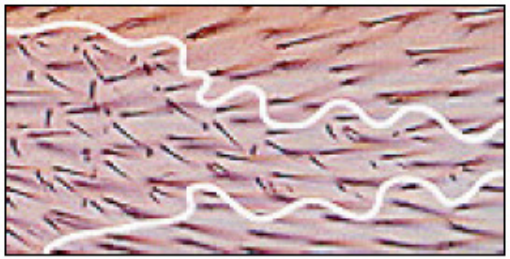

RECOMBINATION BETWEEN CENTROMERES & MARKERS



Twin spot




RECOMBINATION BETWEEN THE MARKERS

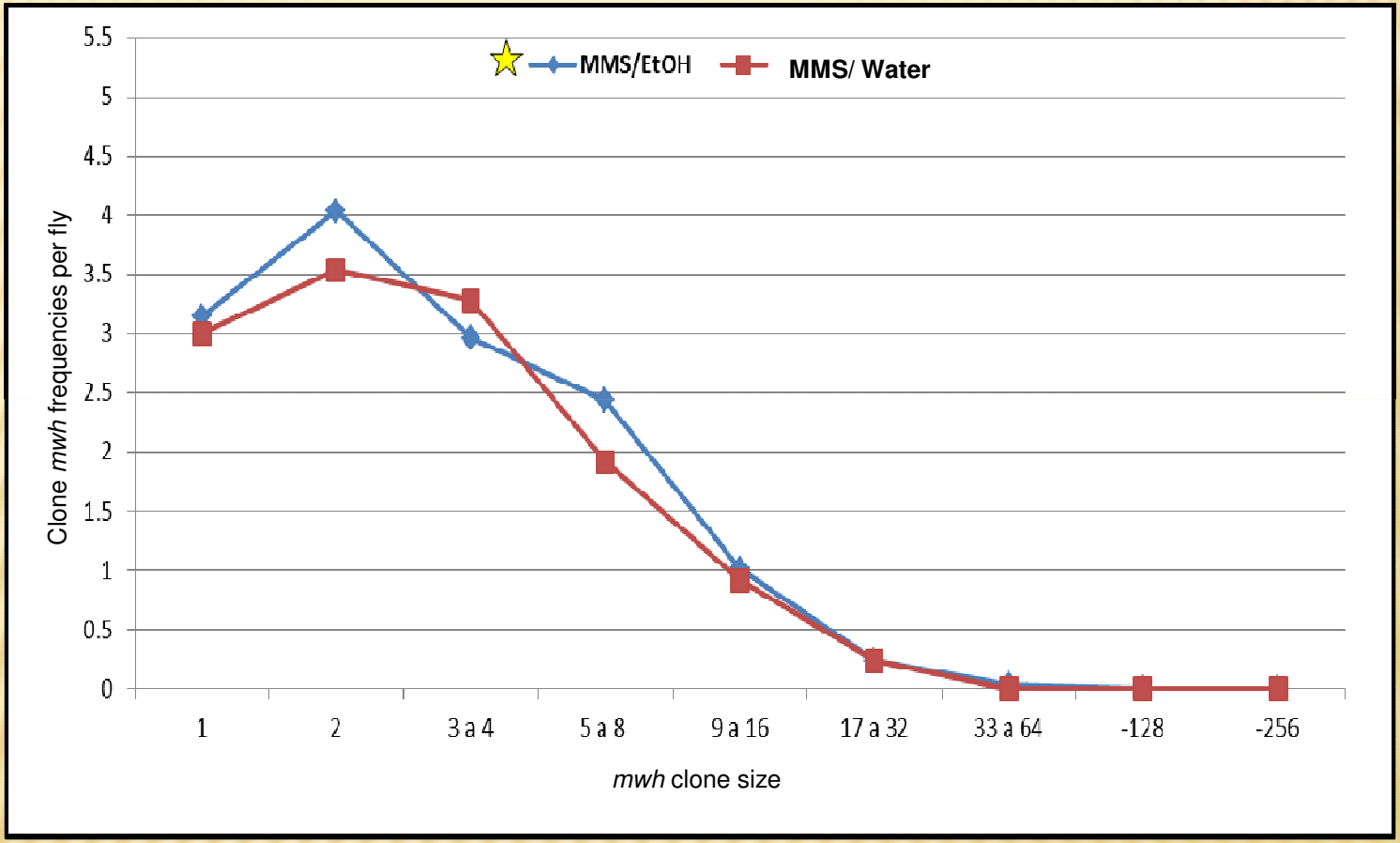


Single *mwh* spot

# TYPES OF SPOTS IN WINGS (CLONES)

| | SINGLE SMALL | SINGLE LARGE | TWINS |
|---|---|---|---|
| CAUSES | Aneuploidy of 3rd chromosome or late damage by secondary metabolites. | Early damage by direct effect. | Somatic recombination between the centromeres and markers. |
| *mwh* or *flr* clones | 1-2 cells expressing either marker | >3 cells expressing either marker | >2 cells expressing both markers |

# CITOTOXICITY




Clones size are produced by a number of cell division cycles after damage. We use Kolmogorov-Smirnov analysis of distribution of the mean accumulated *mwh* clone size classes, $p < 0.05$.

# METHODS. RAISING

- Strains are raised in potatoes flakes added with a fungistatic and bactericide solution.

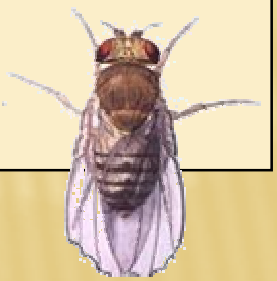
# METHODS: CROSSES

- Isolation under the stereoscopic microscope of virgin females from flare and Oregon-flare strains and mwh males

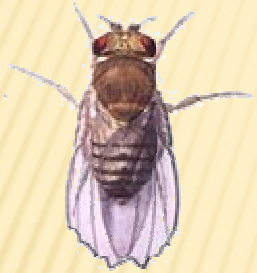
*mwh / mwh*

ORR(1);ORR(2); *flr*$^{3}$/ TM3, *Bd*$^{S}$

*flr*$^{3}$/ TM3, *Bd*$^{S}$



# METHODS: CROSSES



ORR(1);ORR(2);
*flr*$^{3}$/ TM3, *Bd*$^{S}$

X

*mwh* /*mwh*

X

*flr*$^{3}$/ TM3, *Bd*$^{S}$

HB

ST

**F$_1$**

ORR(1);ORR(2);
*mwh flr*$^{3+}$/ *mwh*$^{+}$ *flr*$^{3}$

&

ORR(1);ORR(2);
*mwh flr*$^{3+}$/ TM3, *Bd*$^{S}$

*mwh flr*$^{3+}$/ *mwh*$^{+}$ *flr*$^{3}$

&

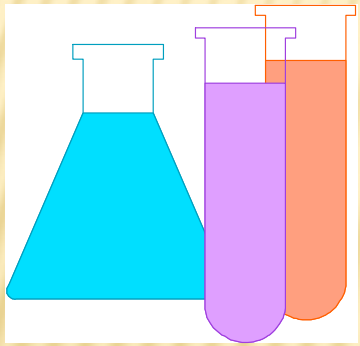
*mwh flr*$^{3+}$/ TM3, *Bd*$^{S}$

# METHODS: F1

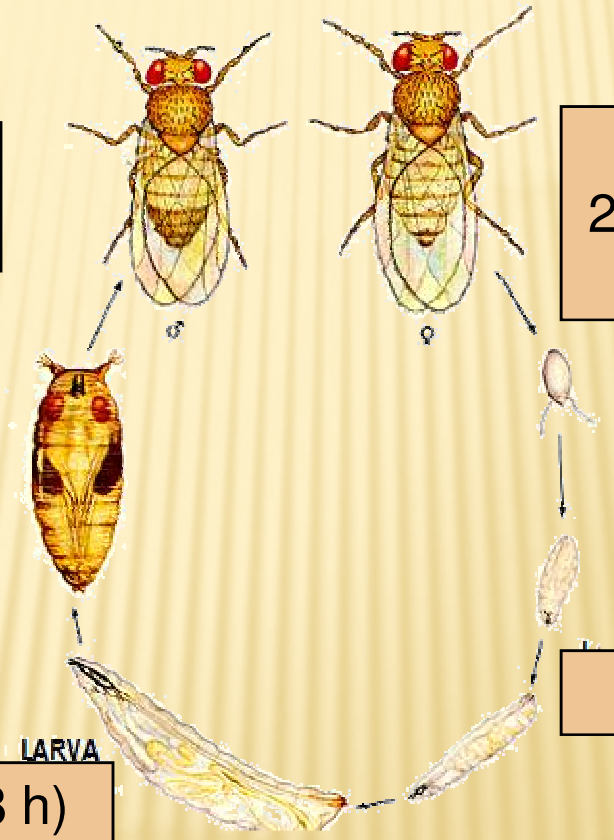
| Males gametes | Virgin females (gametes) | |
|---|---|---|
| | + *flr*$^3$ | TM3, *Bd*$^S$ |
| *mwh* + | *mwh* +/+ *flr*$^3$ | *mwh* +/TM3, *Bd*$^S$ |
| | Wild wings<br><br>Marker-heterozygous flies (MH)<br><br>Single and twin spots can be observed in *mwh/flr*$^3$ heterozygotes | Serrate wings<br><br>Balancer-heterozygous flies (BH)<br><br>Only mwh single spots can be observed as the balancer chromosome TM3 does not carry a flare mutation. |

# METHODS: TREATMENTS

Adults flies collected and stored in 70% EtOH



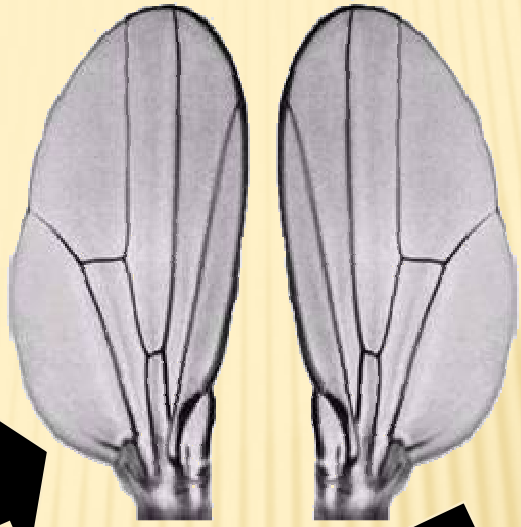
Chronic treatments (~48 h) with Drosophila Instant Medium and solutions

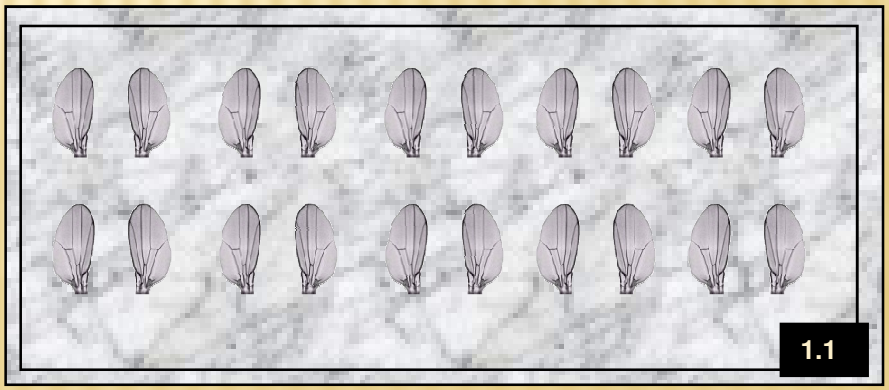



Eggs laying for 8h, 25°C & 65 % relative humidity

Larvae 72 ± 4h

# METHODS: WING ANALYSIS

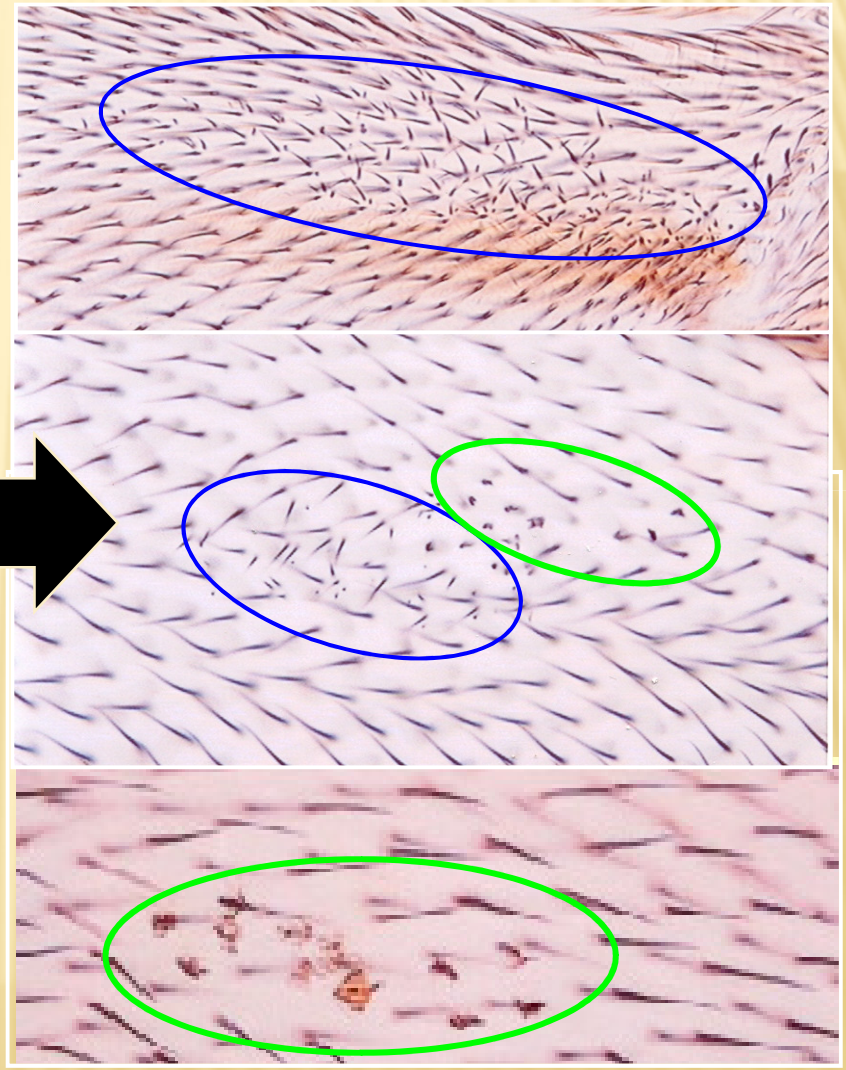






Trans-heterozygotes
(*mwh flr*$^{3+}$ / *mwh*$^{+}$ *flr*$^{3}$)

Slide

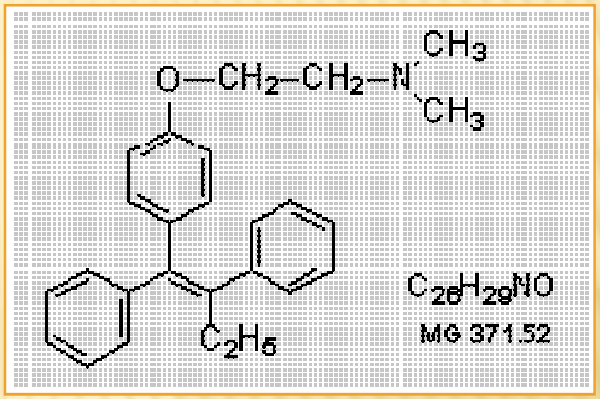
# METHODS: SCORING (40x)



# METHODS: STATISTICS


- ✓ Software SMART PC-version 2.1 based in the conditional binomial test according to Kastenbaum–Bowman significance levels (Frei & Würgler, unpublished $p<0.05$)
- ✓ Mann-Whitney-Wilcoxon U test.

# TAMOXIFEN




The two most common side effects of tamoxifen therapy are risk for a secondary endometrial cancer and an increased risk of a thromboembolic event. It also includes the increased risk for a pulmonary embolus.

Smith (2014) reported that the incidence for endometrial cancer was 3.1% in the tamoxifen group vs. 1.6% in the placebo group. Endometrial cancer–related mortality was 0.4% in the tamoxifen group vs. 0.2% in the placebo group.

The expected benefits of tamoxifen therapy in terms of reduction of breast cancer recurrence and breast cancer–related mortality outweighed the expected toxicities and potential negative outcomes.

Smith GL. The long and short of Tamoxifen therapy: A review of the ATLAS Trial. J Adv Pract Oncol. 5(1):57-60, 2014.

# TAMOXIFEN ADDUCTS

- Tamoxifen is metabolically activated via alpha-hydroxylation (CYP3A4) and sulfate conjugation (sulfotransferase) to give a reactive species that binds to DNA predominantly at the N(2)-position of guanine, producing pro-mutagenic lesions.*
- Xenobiotic metabolism of oxide-4-nitroquinoline (4-NQO) produces acetoxy-aminoquinoline, which leads to purine adducts.**

*Brown K. Is tamoxifen a genotoxic carcinogen in women? Mutagenesis 24(5):391-404,2009. **Mirzayans,R., Bashir,S., Murray,D. and Paterson,M.C. (1999) Inverse correlation between p53 protein levels and DNA repair eficiency in human fibroblast strains treated with 4-nitroquinoline 1-oxide: evidence that lesions other than DNA strand breaks trigger the p53 response. Carcinogenesis, 20, 941±946.

# TAMOXIFEN AND 4-NQO CONTROL

| TAMOXIFEN | 0.66 mM | 1.66 mM | 3.33 mM |
|---|---|---|---|
| ST CROSS | | | |
| MH flies | ↓ (Small spots) | ↑ | ↑ |
| BH flies | - | - | ↑ |
| HB CROSS | | | |
| MH flies | - | ↓ (Large spots) | ↓ (Large spots) |
| BH flies | - | - | - |
| 4-NQO | 2.5 mM | 5.00 mM | |
| ST CROSS | | | |
| MH flies | ↑ | ↑ | |
| BH flies | ↑ | ↑ | |
| HB CROSS (~1.7 fold higher than in ST cross; ~73% recombination) | | | |
| MH flies | ↑↑ | ↑↑ | |
| BH flies | ↑↑ | ↑↑ | |

# WEAK GENOTOXICITY

- The results showed genotoxic effects of TAM at 1.66 and 3.33 mM in the ST cross only and without a clear dose-response effect. This suggests a weak genotoxicity of this anti-oestrogen.
- The negative results obtained with TAM in the HB cross may indicate efficient detoxification of the compound by the increased xenobiotic metabolism present in this cross.
- 4-NQO showed genotoxic effects in the ST & HB crosses with a clear dose response effect.

**Mutagenesis** vol. 19 no. 3 pp. 187–193, 2004 DOI: 10.1093/mutage/geh020

# Genotoxicity of tamoxifen citrate and 4-nitroquinoline-1-oxide in the wing spot test of *Drosophila melanogaster*

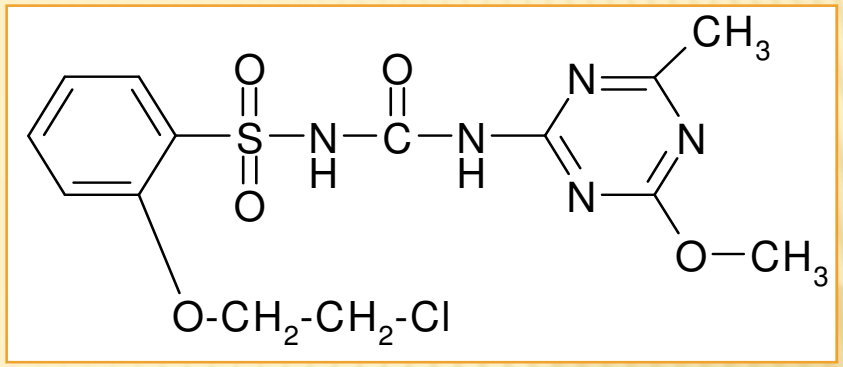
**M.Eugenia Heres-Pulido[1], Irma Dueñas-García[1], Laura Castañeda-Partida[1], Antonio Sánchez-García[1], Martha Contreras-Sousa[1], Ángel Durán-Díaz[1] and Ulrich Graf[2,3]**

[1]Laboratorio de Genética Toxicológica, FES Iztacala, Universidad Nacional Autónoma de México, 54090 Tlalnepantla, Estado de México, México and [2]Institute of Animal Sciences, Section Physiology and Animal Husbandry, Swiss Federal Institute of Technology, Schorenstrasse 16, CH-8603 Schwerzenbach, Switzerland

**Tamoxifen (TAM) is an anti-oestrogen used for treatment and prevention of human breast cancer, but it is also related to human endometrial and uterine cancer. The wing spot test in *Drosophila melanogaster* was employed to determine the genotoxic effects of TAM and 4-nitroquinoline-1-oxide (4-NQO), a carcinogen that produces adducts similar to TAM–DNA adducts detected in rodent liver and human liver microsomes. As *Drosophila* spp. have no oestrogen receptor, no effects can result in binding of TAM to a receptor. Chronic treatments with TAM citrate were performed with 3-day-old larvae of the standard (ST) and high bioactivation (HB) crosses of the wing spot test at concentrations of 0.66, 1.66 and 3.33 mM. In addition, the carcinogen 4-NQO was administered at 2.5 and 5.0 mM. Somatic spots on normal wings from marker-heterozygous flies and on serrate wings from balancer-**

uterine cancer in breast cancer patients receiving TAM therapy (IARC, 1996). The mechanism for this increase is unclear, although two plausible hypotheses have been proposed: (i) TAM has agonistic effects on oestrogen receptors (ERs) which induces the development of endometrium or uterine tumour cells; (ii) cancer is caused by TAM–DNA adducts that lead to mutations in those cells (see Poirier and Schild, 2003). The two principal routes of TAM metabolism are α-C-hydroxylation of the ethyl group that produces α-hydroxytamoxifen (α-OHT), defined as group II adducts (Randerath *et al.*, 1994), and xenobiotic metabolism by cytochrome P450 2D6 (CYP2D6), which yields detectable levels of 4-OHT in human liver microsomes (Dehal and Kupfer, 1997; White, 1999), described as group I adducts (Randerath *et al.*, 1994). The concentrations of TAM correlate with the *in vitro* production of 4-OHT (Crewe *et al.*, 2002). Low TAM and 4-OHT levels significantly increased CYP3A4 expression (Desai *et al.*, 2002). It has been demonstrated that CYP3A4 is the only cytochrome responsible for yielding α-OHT (Boocock *et al.*, 2002) in correlation with protein and DNA adduct formation (Notley *et al.*, 2002). CYP2B prevents the production of TAM–DNA adducts (Stiborová *et al.*, 2002). In a human lymphoblastoid cell line (MCL-5) with elevated CYP1A1 and transfected human cDNAs (CYP1A2, CYP2A6, CYP2E1 and CYP3A4), exposure to TAM increased micronuclei and caused aneuploidy and structural

# TRIASULFURON HERBICIDE




Triasulfuron (TS) is a widely used sulfonylurea herbicide which inhibits the acetolactate synthase in broad-leaf weeds and in some wheat crop grasses (*Triticum aestivum* L.). Residues can be found in soil and superficial water with high toxicity to primary producers. In cereals, TS metabolism depends on P450s, the age of seedlings and the interaction with compounds

# DIRECT TREATMENTS

- We used Triasulfuron (TS) and Amber®75WG at the 10-fold crop field application concentration (0.5 mg/mL).
- As Kaya *et al*. (2004) we demonstrated that the positive control Basagran®480 (0.24mg/mL) was genotoxic only in the HB cross.
- TS and Amber®75WG produced similar genotoxic effects in the ST & HB crosses.

# METABOLISM OF WINTER WHEAT

- TS metabolism in winter wheat implies its hydroxylation (Cyp71C6v1).
- Winter wheat seedlings were immersed for 4 h in both herbicides, and aqueous extracts (AEs) of the roots were prepared to expose the larvae.
- The AEs from TS treatments yielded statistically significant lower spot frequencies in the HB cross than in the ST cross.
- Differences between the two crosses must be related to their different P450s levels.
- Wheat metabolism did not modulate the genotoxicity of Basagran®480 in the HB cross.

Mutation Research 653 (2008) 70–75

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

# Genotoxicity of triasulfuron in the wing spot test of *Drosophila melanogaster* is modulated by winter wheat seedlings

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ARTICLE INFO

*Article history:*
Received 24 December 2007
Received in revised form 15 March 2008
Accepted 19 March 2008
Available online 27 March 2008

*Keywords:*
SMART
Herbicides
Amber® 75WG
Bentazon
Basagran® 480

ABSTRACT

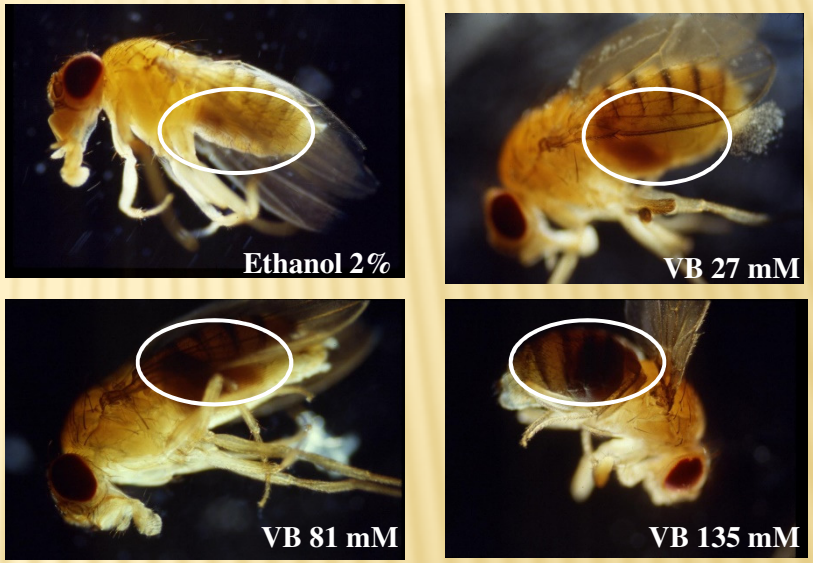
Triasulfuron (TS) is a widely used sulfonylurea herbicide which inhibits the acetolactate synthase in broad-leaf weeds and in some wheat crop grasses (*Triticum aestivum* L.). Residues can be found in soil and superficial water with high toxicity to primary producers. In cereals, TS metabolism depends on cytochromes P450 (CYPs), the age of seedlings and the interaction with compounds. The genotoxicity of TS was demonstrated in the wing spot test of *Drosophila melanogaster*, an *in vivo* assay based on the loss of heterozygosity of the *mwh* and *flr* markers in the wing imaginal disk cells of larvae fed with chemical agents. Chronic treatments with analytical grade TS, commercial formulation TS (Amber® 75WG) (0.5 mg/mL) and commercial formulation bentazon (Basagran® 480) (0.24 mg/mL) were performed with three-day-old larvae of the standard (ST) and the high bioactivation (HB) crosses with regulated and high constitutive levels of CYPs, respectively. To demonstrate the effect of winter wheat metabolism on TS genotoxicity, *T. aestivum* L. seedlings were immersed for 4 h in these herbicides, and aqueous extracts (AEs) of the roots were prepared to expose the larvae. TS and Amber® 75WG produced similar genotoxic

# VERBASCOSIDE AND CAFFEIC ACID

Verbascoside (VB) is a phenylpropanoid glycoside isolated from *Buddleja* species. They are known as ‘‘tepozan’’ and described in the Aztec manuscript the ‘‘Códice Badiano’’ as a plant used to treat the skin squamous disease ‘‘mentagra”.

VB purified from *B. cordata* and its constituent caffeic acid (CA) (0, 27, 57, 81, 135, 173 mM) were screened to determine their possible genotoxicity.

VB was not genotoxic at any of the concentrations tested in both crosses. CA decreased the spontaneous frequencies of small and total spots and showed some toxicity in the ST cross because the low imago survival.




The amount of VB residue as determined by HPLC in the adult flies that were fed on it, indicated a low metabolism of this compound, which explains the absence of genotoxicity.

Food and Chemical Toxicology 50 (2012) 1082–1090

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

# Verbascoside is not genotoxic in the ST and HB crosses of the *Drosophila* wing spot test, and its constituent, caffeic acid, decreases the spontaneous mutation rate in the ST cross

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ARTICLE INFO

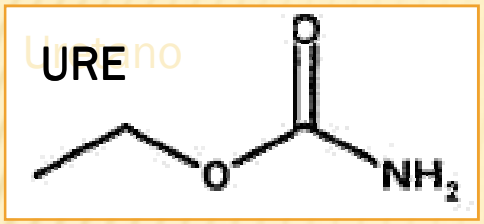
*Article history:*
Received 21 July 2011
Accepted 5 December 2011
Available online 11 December 2011

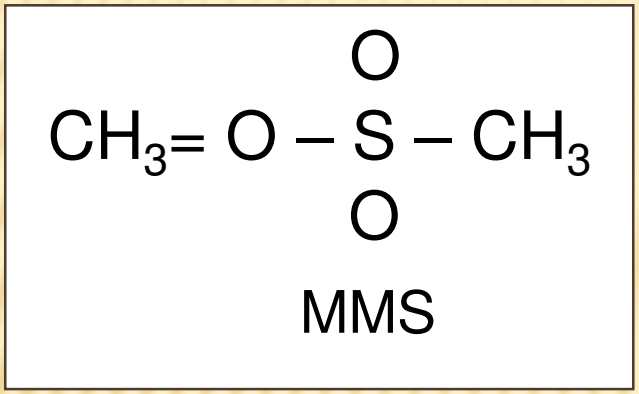
*Keywords:*
Verbascoside
Phenylpropanoid
*Drosophila melanogaster*
SMART
Buddlejaceae
Cyp450s

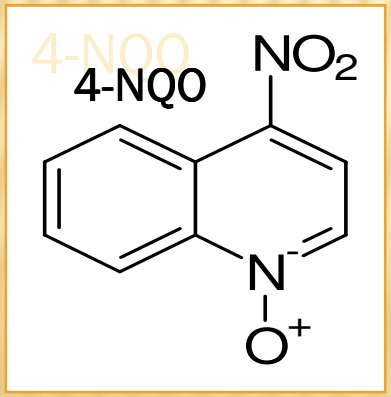
ABSTRACT

Verbascoside (VB) is a phenylpropanoid isolated from *Buddleja* species, some of which originate in Mexico, and was first described in the sixteenth century in the codices of Mexican traditional medicine. VB is present in alcohol extracts and is widely used in the north of Mexico as a sunscreen. VB absorbs UV-A and UV-B radiation and has high antioxidant and anti-inflammatory capacities. VB and its constituent caffeic acid (CA) were screened to determine their genotoxic activity using the *Drosophila* wing spot test. Third instar larvae (72 ± 4 h) of the standard (ST) and high bioactivation (HB) crosses, with regulated and high levels of cytochrome P450s (Cyp450s), respectively, were exposed to VB or CA (0, 27, 57, 81, 135, and 173 mM). VB was not genotoxic at any of the concentrations tested in both crosses. The amount of VB residue as determined by HPLC in the adult flies that were fed with VB indicated a low metabolism of this compound, which explains the absence of genotoxicity. CA decreased the spontaneous frequencies of small and total spots and showed putative toxicity in the ST cross.

# BROCCOLI




Broccoli (*Brassica oleracea* var. italica) has been defined as a cancer preventive food. Nevertheless, broccoli contains potentially genotoxic compounds as some isothyocyanates.

We performed treatments with organically grown broccoli (OGB) and co-treatments with the promutagen urethane (URE), the direct alkylating agent methyl methanesulfonate (MMS) and the carcinogen oxide-4-nitroquinoline (4-NQO) in the ST and HB crosses.

We used fresh market broccoli (FMB) as a non-organically grown control.

# ORGANICALLY GROWN BROCCOLI (OGB)

- In both crosses, the OGB (100%) added with Tween–EtOH (5%) yielded the expected reduction in the spontaneous genotoxicity rate.
- OGB co-treatments modulated the URE effect in the HB cross.
- MMS showed synergy and 4-NQO modulation in both crosses.

# FRESH MARKET BROCCOLI

- FMB controls (100%) added with water or Tw-EtOH (5%) produced genotoxicity.
- Co-treatments modulated URE genotoxicity, diminished MMS damage, and did not change the 4-NQO effect.

Food and Chemical Toxicology 48 (2010) 120–128

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

# Genotoxicity studies of organically grown broccoli (*Brassica oleracea* var. italica) and its interactions with urethane, methyl methanesulfonate and 4-nitroquinoline-1-oxide genotoxicity in the wing spot test of *Drosophila melanogaster*

María Eugenia Heres-Pulido *, Irma Dueñas-García, Laura Castañeda-Partida, Luis Felipe Santos-Cruz, Viridiana Vega-Contreras, Rosa Rebollar-Vega, Juan Carlos Gómez-Luna, Ángel Durán-Díaz

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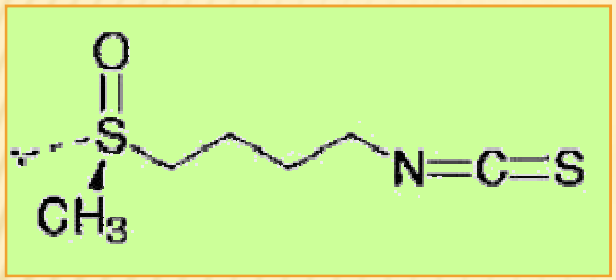
ARTICLE INFO

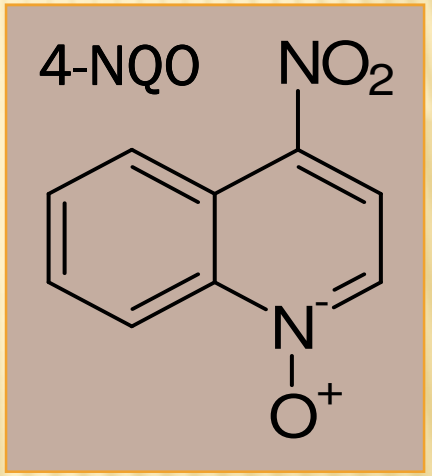
*Article history:*
Received 25 March 2009
Accepted 22 September 2009

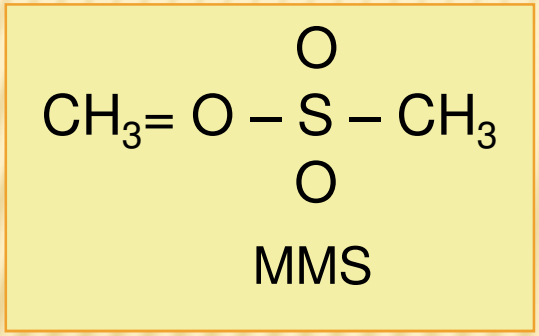
ABSTRACT

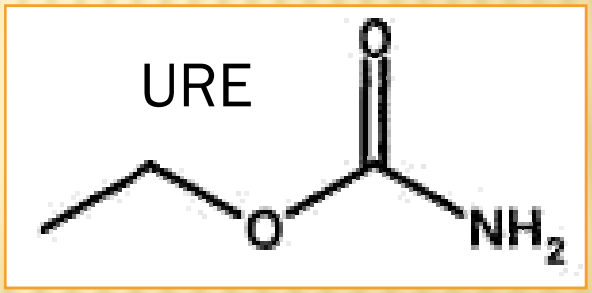
Broccoli (*Brassica oleracea* var. italica) has been defined as a cancer preventive food. Nevertheless, broccoli contains potentially genotoxic compounds as well. We performed the wing spot test of *Drosophila melanogaster* in treatments with organically grown broccoli (OGB) and co-treatments with the promutagen urethane (URE), the direct alkylating agent methyl methanesulfonate (MMS) and the carcinogen 4-nitroquinoline-1-oxide (4-NQO) in the standard (ST) and high bioactivation (HB) crosses with inducible and

# SULFORAPHANE



# SULFORAPHANE (SF)

- SF is an isothiocyanate present in Brassicaceae vegetables, as broccoli, that induce the detoxification of electrophiles and reactive oxygen species (ROS).

- SF has been correlated with chemoprevention mechanisms against degenerative diseases and the Nrf2/ARE system.

# SF

- We tested SF (0.14, 0.28, 0.56 mM) diluted in a DMSO/Tw80/EtOH solution (DTE). This corresponded to 25, 50, 100% of lyophilized broccoli, respectively.

- Co-treatments were of SF added with MMS (0.5 mM) or URE (20 mM), 4-NQO (2 mM), and $H_2O_2$ (20 mM).

# SF GENOTOXICITY & MUTAGENS

| | SF 0.14 mM | | SF 0.28 mM | | SF 0.56 mM | |
|---|---|---|---|---|---|---|
| | CROSSES | | | | | |
| | ST | HB | ST | HB | ST | HB |
| DTE solution | ↑ | --- | --- | ↑ | --- | --- |
| DTE/MMS 0.5 mM | ↓ | --- | ↓ | --- | ↓ | ↓ |
| DTE/URE 20 mM | ↑ | ↓ | --- | --- | --- | --- |
| DTE/4-NQO 2 mM | ↓ | --- | ↓ | --- | ↓ | --- |
| $H_2O_2$ 20 mM | --- | --- | ↑ | --- | --- | ↑ |

# SF & MUTAGENS

- The DTE/SF treatment (0.14 mM) was genotoxic in the ST cross.
- In the HB cross, DTE/SF (0.28 mM) was genotoxic.

- In the ST cross the DTE/SF/MMS treatments showed a tendency to reduce the genotoxic damage caused by MMS, which could be explained by the radical scavenging action of the DTE mixture.

- In both crosses, the results for the URE, 4-NQO and $H_2O_2$, were different and must be related to differential modulation of P450s expression and the SF and DTE scavenger properties.

# DTE (DMSO/TW80/ETOH)

| | Total spots/flies | |
|---|---|---|
| **CROSSES** | **ST** | **HB** |
| **MilliQ water/MMS** | **39.85** | **16.88** |
| **DTE/MMS** | **22.22 ↓** | **13.07 -** |
| **MilliQ water/URE** | **09.15** | **13.32** |
| **DTE/URE** | **04.95 ↓** | **08.20 ↓** |
| **TE/4-NQO** | **02.29** | **01.91** |
| **DTE/4-NQO** | **02.68 -** | **02.54 -** |
| **MilliQ water/$H_2O_2$** | **00.50** | **00.50** |
| **DTE/$H_2O_2$** | **00.31 -** | **00.59 -** |

Food and Chemical Toxicology 50 (2012) 4479–4486

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

# Interactions of sulforaphane and dimethyl sulfoxide with methyl methanesulfonate, urethane, 4-nitroquinoline-1-oxide and hydrogen peroxide in the *Drosophila melanogaster* wing spot test

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ARTICLE INFO

*Article history:*
Received 7 August 2012
Accepted 4 September 2012
Available online 28 September 2012

*Keywords:*
Genotoxicity
Cytochromes P450
Scavenger
Oxidative stress
*in vivo*
Cell division

ABSTRACT

Sulforaphane (SF) is an isothiocyanate present in Brassicaceae, vegetables that induce the detoxification of electrophiles and reactive oxygen species. SF has been correlated with chemoprevention mechanisms against degenerative diseases. We tested if the SF had an effect against methyl methanesulfonate (MMS), urethane (URE), 4-NQO and $H_2O_2$. SF (>95% purity, 0.14, 0.28, 0.56 mM) was diluted in a DMSO/Tw80/EtOH mixture (DTE) corresponding to 25, 50, 100% of lyophilized broccoli. The SF treatment (0.14 mM) was positive for small spots in the ST cross and negative in the HB cross. In the HB cross, SF (0.28 mM) was genotoxic. In the ST cross, the SF treatments showed a tendency to reduce the genotoxic damage caused by MMS, which could be explained by the radical scavenging action of the DTE mixture. In the ST cross, the frequency of small spots in the SF 0.14 mM/URE treatment was similar to that of Water/URE, which can be explained by a DTE and SF scavenger action. In both crosses, the results for the direct oxidants, 4-NQO and $H_2O_2$, were different and must be related to differential modulation of CYPs expres-

# *EXPRESSION OF CYP6G1 & CYP6A2 IN SMART CROSSES*

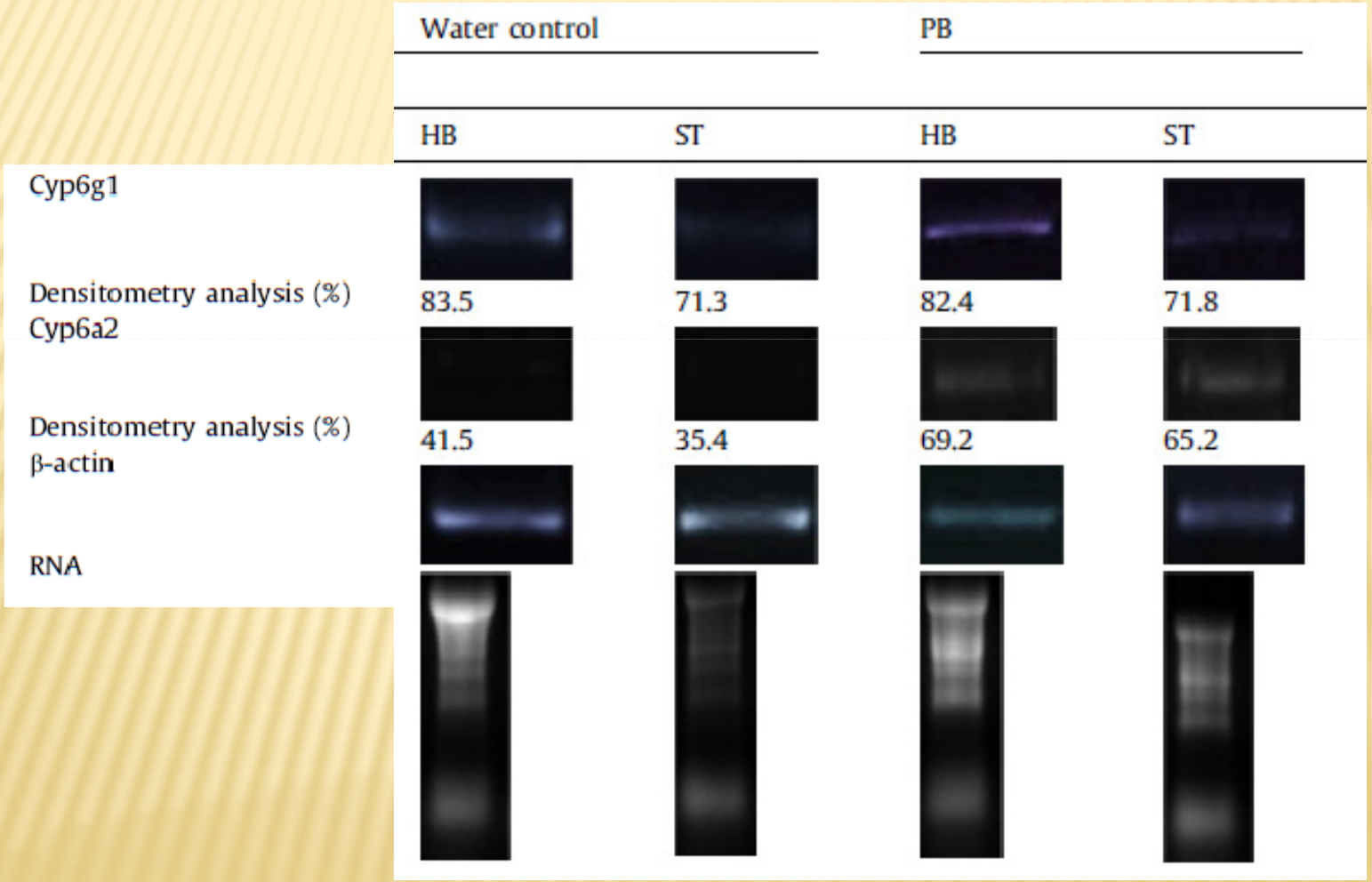
- Constitutive overexpression of *Cyp6g1* and *Cyp6a2* genes in DDT-resistant line Oregon-flare of the *Drosophila melanogaster* wing spot test (SMART) had been reported.

- We compared *Cyp6g1* and *Cyp6a2* expression levels against the $\beta$-actin gene in the standard (ST) and high bioactivation (HB) crosses of SMART treated with sulforaphane (140 μM) or phenobarbital (12 mM) as the control inductor.

- We determined P450s activity by total NADPH consumption.

# *ACTIVITY & EXPRESSION OF CYP6G1 & CYP6A2 IN SMART CROSSES*

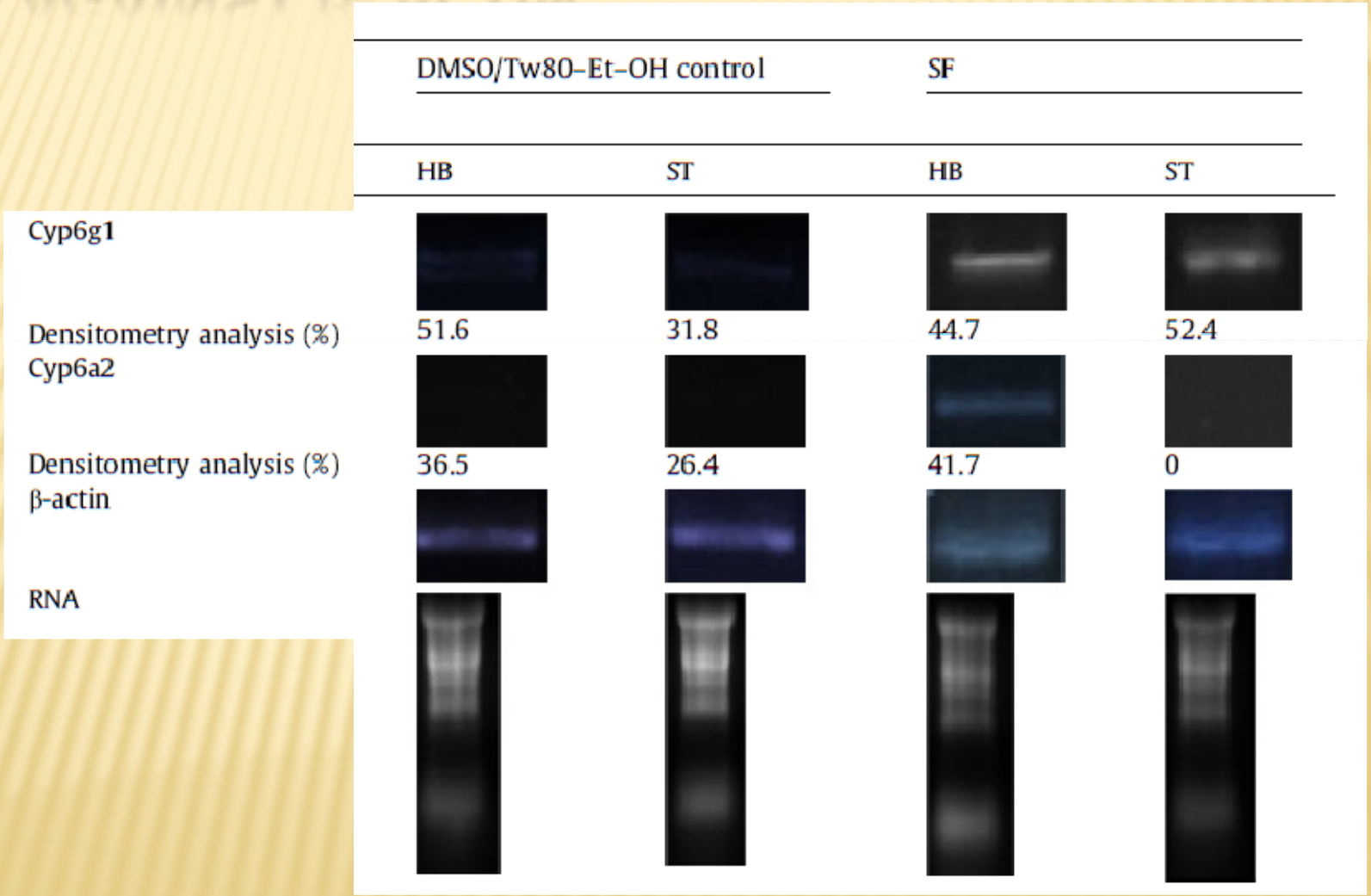
- We confirmed that P450s activity (NADPH consumption) and the expression levels of both genes were higher in the HB cross.
- The *Cyp6g1* levels were higher than those of *Cyp6a2* in both crosses, but lower than the expression of *β-actin*.
- Phenobarbital:
  - Increased the P450s basal activity (NADPH consumption)
  - Did not modify the *Cyp6g1* levels
  - Increased the *Cyp6a2* levels


Water control
PB
HB
ST
HB
ST
Cyp6g1
Densitometry analysis (%)
83.5
71.3
82.4
71.8
Cyp6a2
Densitometry analysis (%)
41.5
35.4
69.2
65.2
β-actin
RNA

# *CYP6G1*, *CYP6A2*, SF & PB

- In the HB cross the DTE/SF:
  - Decreased expression of *Cyp6g1*
  - Increased expression of *Cyp6a2.*
- In the ST cross the DTE/SF:
  - Increased expression of *Cyp6g1*.
  - Inhibited expression of *Cyp6a2.*

# TRANSCRIPTS & DENSITOMETRY ANALYSIS (%) AGAINST β- ACTIN



# SF & *CYP6A2*, *CYP6G1*

- Although the transcript levels were always higher in the HB cross than in the ST, the expression of *Cyp6a2* and *Cyp6g1* was not constitutive, was independent one from the other, and was modulated by SF treatment.

- SF (140 μM) and PB (12 mM) were genotoxic in the ST cross.

Food and Chemical Toxicology 48 (2010) 3333–3339

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

# Sulforaphane modulates the expression of *Cyp6a2* and *Cyp6g1* in larvae of the ST and HB crosses of the *Drosophila* wing spot test and is genotoxic in the ST cross

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ARTICLE INFO

*Article history:*
Received 14 June 2010
Accepted 29 August 2010

*Keywords:*
CYP450s

ABSTRACT

Constitutive overexpression of *Cyp6g1* and *Cyp6a2* genes in DDT-resistant line Oregon-*flare* of the *Drosophila melanogaster* wing spot test (SMART) has been reported. *Cyp6g1* and *Cyp6a2* expression levels were compared against the *β-actin* gene in the standard (ST) and high bioactivation (HB) crosses of the Somatic Mutation and Recombination test (SMART) treated with sulforaphane or phenobarbital as the control inductor. The CYP450s' enzymatic activity was determined by overall NADH consumption. The expression levels of both genes and the CYP450s activity was higher in the HB cross. The *Cyp6g1* levels were higher than those of *Cyp6a2* in both crosses, but lower than the expression of *β-actin*. Sulforaphane

# SOME GRADUATED STUDENTS


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¡Thank you!