Teaching Notes

Ultraviolet (UV) light induced mutations in *Drosophila melanogaster*.

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Ultraviolet (UV) light is a potential threat to the integrity of our genetic material. UV is known to cause mutations in prokaryotic and eukaryotic organisms and is a potent inducer of skin cancer in humans (Friedberg *et al*., 1995; Greaves, 2000). Yet, UV does not penetrate tissues very far, and this makes it difficult to determine if UV can induce mutations in germ cells of higher organisms.

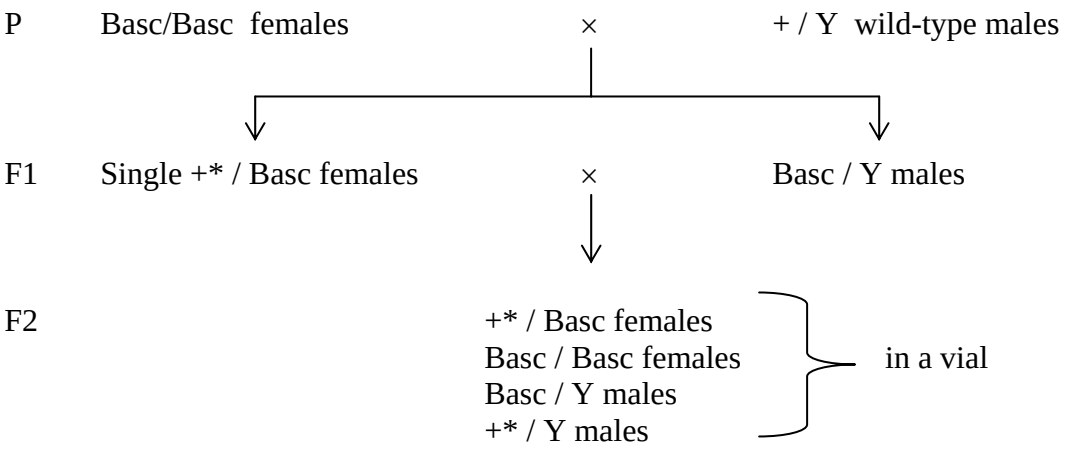
The early observations of the ability of UV to induce germ-line mutations in *Drosophila* were only successful when very young males, before cuticle darkening, were squashed flat to expose their gonads (Mackenzie and Muller, 1940). Even then, only small increases in gene mutations were observed with UV treatments.

The objective of this exercise is to test the ability of UV to induce recessive sex-linked lethal mutations by placing *Drosophila melanogaster* males on a UV transilluminator that is in common use in molecular biology laboratories to view DNA. The rate of mutation in these UV-treated males will be compared to the rate in sibling males that are untreated with UV.

One- to two-day-old *D. melanogaster* wild-type males were etherized and placed directly, abdomens down, on a UV transilluminator. Each male was held down by one or more glass microscope cover slips and was treated with UV for 10 to 60 minutes to determine if UV is toxic to *Drosophila*. In some runs, only the gonad area of the abdomen was exposed to UV by placing a glass cover slip under the flies except for the abdomen. This was done to reduce exposure of the brain and other vital organs to the possible toxic effects of UV. After estimating toxic levels of UV exposure,

males were treated for 11 minutes (whole body) or 45 minutes (gonads only) with UV and were screened for new recessive sex-linked lethal mutations. Males that were not exposed to UV were used as controls.

The UV-treated and control males were mated individually, as shown in the Basc mating scheme below. In this mating scheme, single wild-type males (Canton-S) that do not contain pre-existing X-linked lethals were mated with two or three virgin Basc/Basc females, single F1 heterozygous females were mated with one or two sibling Basc/Y males, and F2 flies were screened for new recessive sex-linked lethal mutations. The absence of +* /Y males (round, red eyes) in the F2 vials means that a new recessive sex-linked lethal mutation has been recovered. Presumptive lethals can be confirmed by mating F2 +* / Basc females with F2 Basc / Y sibling males. A lethal was declared when at least 20 Basc/Y F2 or F3 males and no +* /Y males are recovered. In this mating scheme, B = Bar eye dominant mutation, a = white-apricot eye recessive mutation, sc = multiple X-chromosome inversions, + = wild-type X chromosome from a Canton-S stock, * = UV-treated X chromosome, and Y = Y chromosome in males. The multiple X-chromosome inversions cause the elimination of recombination gametes in the heterozygous F1 females due to duplications and deficiencies of chromosomal material and to acentric and dicentric chromosomes (see Klug *et al*., 2007, for a discussion of this topic). This loss of gametes with recombinant X chromosomes means that a new lethal mutation on the wild-type X chromosome will not be recombined onto the Basc chromosome and be missed as a new mutation. Single F1 +* / Basc females were used in this mating scheme so that a single X-chromosome is tested in each F2 vial. For more details on the Basc mating scheme for recessive sex-linked lethal mutations with *D. melanogaste*r (see Mason *et al*., 1985, 1987).



Toxicity caused by UV was determined by the percentage of males that died after treatments in one day. The toxicity results of UV treatments are shown in Table 1.

From these results, it is clear that long-time whole-body or gonad UV treatment of *D. melanogaster* males is toxic. This, however, does not mean that the UV is reaching the germ cells of these males. UV could be toxic in these *Drosophila* males because it is causing damage to DNA, proteins, and other components of somatic cells, without reaching the gonads. Hence, the males also need to be tested for the ability of UV to induce genetic damage in germ cells.

Hence, we treated *D. melanogaster* male gonads with 11 or 45 minutes of UV and tested for recessive sex-linked lethal mutations using the Basc mating scheme shown above. One lethal mutation was also identified in sibling wild-type males that were not treated with UV (the controls). The results are shown in Table 2.

Table 1. Toxicity Tests.

Minutes of UV Treatment	Number of Flies Treated	Number of Flies Dead After One Day	% of Flies Dead After One Day
Whole Body Treatments with UV:			
0	31	1	3
10	6	2	33
11	53	5	9
12	21	7	33
13	25	9	36
14	44	5	11
15	5	4	80
20	5	5	100
25	6	6	100
Gonad Treatments with UV			
20	31	16	52
25	37	19	51
30	33	14	42
45	28	23	82
60	25	24	96

Table 2. Recessive Sex-Linked Lethal Mutations in *Drosophila melanogaster*.

	Lethals	Total Chromosomes Scored	% Lethals
Controls (no UV)	1	627	0.2
UV Treatments			
11 min whole body	2	340	0.6[a]
45 min gonads only	2	79	2.5[b]
Total	4	419	1.0[c]

[a]Fisher exact P = 0.28.
[b]Fisher exact P = 0.04
[c]Fisher exact P = 0.16

The spontaneous mutation rate is similar to the rate reported in the literature (about one new recessive sex-linked lethal mutation in 1000 X chromosomes) (Mason *et al.*, 1985). The rate of UV-induced mutations for the gonad-only treatment is significantly higher than the spontaneous controls (P = 0.04). The UV rate is not significantly higher than the controls, however, for the whole-body treatments or for the total of the two treatments. These results support UV as a germ-cell mutagen in *D. melanogaster* and support the use of a DNA transilluminator as an appropriate source of mutagenic UV.

A class discussion of the results of these crosses could include the following topics: 1) Would one expect UV to cause mutations in gametes of humans? 2) Go to the literature and determine if UV has been observed to cause chromosome breakage and nondisjunction in *Drosophila*. 3) What is the main cause of DNA damage by UV? 4) Would increased skin pigmentation reduce genetics damage by UV?

References: Friedberg, E.C., G.C. Walker, and W. Siede 1995, *DNA Repair and Mutagenesis*. ASA Press, Washington, D.C.; Greaves, M., 2000, *Cancer The Evolutionary Legacy*. Oxford University Press, Oxford; Klug, Cummings, and Spencer 2007, *Essentials of Genetics*; Mackenzie, K., and H.J. Muller 1940, Proc. Royal Soc. B 129: 491-517; Mason, J.M., R. Valencia, R.C. Woodruff, and S. Zimmering 1985, Environ. Mutagenesis 7: 663-676; Mason, J.M., C.S. Aaron, W.R. Lee, P.D. Smith, A. Thakar, R. Valencia, R.C. Woodruff, F.E. Wurgler, and S. Zimmering 1987, Mutation Research 189: 93-102.

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