QTL for survival to UV-C radiation in Drosophila melanogaster

Journal:	*International Journal of Radiation Biology*
Manuscript ID:	TRAB-2012-IJRB-0098.R2
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	n/a
Complete List of Authors:	Gomez, Federico; Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires, Departamento de Ecología, Genética y Evolución Norry, Fabian; Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires, Departamento de Ecología, Genética y Evolución Loeschcke, Volker; Aarhus University, Department of Bioscience, Integrative Ecology and Evolution
Keywords:	ultraviolet radiation, multiple trait mapping, environmental stress, thermotolerance

QTL for survival to UV-C radiation in *Drosophila melanogaster*

Federico H. Gomez [a], Volker Loeschcke [b], Fabian M. Norry [a]

[a] Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. (C-1428-EHA) Buenos Aires, Argentina

[b] Department of Bioscience, Integrative Ecology and Evolution, Aarhus University, Ny Munkegade 114, Bldg. 1540, DK-8000 Aarhus C, Denmark

Corresponding author: Fabian M. Norry, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. (C-1428-EHA) Buenos Aires, Argentina. Fax: (54-11) 4576-3354.

E-mail: fnorry@ege.fcen.uba.ar / fabian.norry@hotmail.com

Abstract

Purpose: The aim of this study is to investigate tolerance to UV-C (ultraviolet C, 280 nm – 100 nm) radiation in *Drosophila melanogaster*, implementing a quantitative trait locus (QTL) mapping approach. This is of interest to test for genetic variation in survival to UV radiation.

Materials and methods: We performed a QTL scan in *D. melanogaster* recombinant inbred lines (RIL) constructed from parental stocks derived from a crossing between northern and southern hemisphere populations that segregated substantial genetic variation in thermal resistance in a previous study. Here, two experimental treatments were implemented: continuous and cyclic UV-C radiation.

Results: Significant QTL were detected on all three major chromosomes. Among these, multiple trait composite interval mapping revealed a significant QTL in the pericentromeric region of chromosome 2, a genome region consistently implicated in thermotolerance in previous studies.

Conclusions: This study shows substantial genetic variation for UV-C radiation resistance in *D. melanogaster*, with QTL for survival to UV-C radiation generally overlapping with major thermotolerance QTL. The genetic architecture of UV-C radiation resistance appears to be more complex in continuously irradiated individuals.

Keywords: ultraviolet radiation; multiple trait mapping; environmental stress; thermotolerance.

Introduction

Many forms of environmental stress affect the distribution, abundance and evolution of organisms. Genomes may be exposed to damage by environmental, chemical and physical agents, such as UV (ultraviolet) and ionizing radiation, chemical mutagens and toxins. UV-B (ultraviolet B, 315 nm – 280 nm) light is a ubiquitous environmental stress source and a potent DNA damaging agent. UV-radiation levels reaching the planet's surface have increased in the last decades, mainly at medium and higher latitudes and primarily as a result of decreasing stratospheric ozone, although this increasing tendency has apparently levelled off recently (Herman 2010). Life-history traits have evolved in an environment where UV-radiation stress is present and where cell protection against it is crucial for adaptation. In the current global scenario it is a matter of interest to investigate the genetic basis of UV radiation resistance.

The sun emits UV radiation in form of UV-A (ultraviolet A, 400 nm –315 nm), UV-B (315nm-280nm) and UV-C (ultraviolet C, 280 nm – 100 nm) rays. UV-C rays have the highest energy and are absorbed by the ozone layer. UV-radiation directly causes DNA damage mostly in the form of pyrimidine dimer lesions, alterations that are both mutagenic and toxic (Setlow and Setlow 1972). The ring structures in DNA bases contain conjugated bonds, making DNA a prominent absorber of UV-radiation, and neighboring pyrimidine bases in DNA strands are preferentially damaged by both UV-C and UV-B (De Gruijl et al. 2001; Sinha and Häder 2002). Pyrimidine dimers block the transcription and replication processes, and avoidance or repair mechanisms are needed to deal with the UV-induced DNA damage that accumulates in an organism. Most important in removing UV-induced damage are the evolutionarily conserved nucleotide excision repair (NER) mechanisms and an enzymatic photoreactivation repair pathway

implicated in the elimination of the two major classes of pyrimidine dimer lesions caused by UV-radiation (i.e., the pyrimidine [6–4] pyrimidone photoproducts and cyclobutane pyrimidine dimers or CPDs) (Kittler and Løber 1977; Friedberg 1985; Thoma 1999). In addition, UV-radiation energy can be absorbed *in vivo* and a positive correlation exists between UV radiation and the formation of reactive oxygen species (ROS) (Black 1987). Coenzymes and pigments are important for absorbing UV-radiation *in vivo*, transferring the excitation energy to H_2O molecules and forming ROS (e.g., superoxide anions, hydrogen peroxide, hydroxyl radicals). Generally, there is a positive correlation between the level of UV-radiation and temperature in contemporary terrestrial environments. Both are higher during the summer periods and the association is stronger in the southern hemisphere (Nozawa et al. 2007).

The chromosomal regions containing the relevant genes with substantial effects on the phenotypic variation in a quantitative trait are identified as quantitative trait loci (QTL). QTL mapping provides a useful tool for the identification of chromosome regions underlying the genetic variation in resistance to environmental stresses including stress by UV radiation. In QTL mapping, associations between the phenotypic trait of interest and molecular markers are assessed on the basis of a linkage map of markers in recombinant populations (Falconer and Mackay 1996; Lynch and Walsh 1998). Because markers are neutral, the QTL approach has the advantage of not imposing any *a priori* predictions on the function of candidate genes in a genome scan, although nearly all QTL contain multiple genes (Morgan and Mackay 2006).

In this study we implemented a QTL mapping approach to investigate tolerance to UV-C radiation in an inter-continental set of *Drosophila melanogaster* recombinant inbred lines (RIL). These lines segregated QTL for thermal tolerance in a previous study

(Norry et al. 2008), but were not tested for resistance to UV-radiation. Experimental flies were subjected to one of either two different UV-C radiation treatments: continuous or cyclic. In the continuous treatment flies were irradiated until time of death. In contrast, the cyclic treatment allowed a recovery period between UV-C radiation events. Phenotypic data were analysed by composite interval mapping, and also the implication of considering the two treatments as different UV-C radiation resistance traits was explored by performing a multiple trait mapping analysis.

Materials and Methods

Recombinant inbred lines

The lines used here were constructed from two parental stocks highly divergent for thermotolerance. One of the stocks, denoted SH2, derived from a southern hemisphere population originating from temperate Melbourne, Australia (originally selected by McColl et al. 1996). The other stock, denoted D48, derived from a northern hemisphere population sampled in the colder eastern Jutland, Denmark. These two parental populations, SH2 and D48, were chosen from a total of 23 inbred lines with high knockdown resistance to high temperature (KRHT) and 42 inbred lines with low KRHT, respectively. SH2 and D48 flies were dramatically divergent for thermotolerance and were used for the construction of RIL as described in Norry et al. (2008). As in Norry et al. (2008), two sub-sets of RIL were used in the present study, RIL-SH2 and RIL-D48, which were derived from backcrosses to the respective parental line (i.e., SH2 and D48, respectively).

The microsatellite loci used as molecular markers allowed a genetic map with a total of 36 markers spread throughout all major *D. melanogaster* chromosomes. For

details on the genetic map associated to these RIL see Norry et al. (2008). All RIL stocks were maintained in replicated 2 x 10-cm standard vials containing 6 mL of instant culture medium with nipagin (Parafarm, Buenos Aires, Argentina) RIL were expanded for one generation from our stocks using 125-mL standard glass bottles containing 40-mL of dehydrated potato-based culture medium (Unilever Bestfoods Argentina, Buenos Aires) with water, nipagin and yeast (Lesaffre Argentina, Virrey del Pino, Buenos Aires, Argentina). Two standard bottles were set up per RIL at low-medium density (20 males plus 20 females). After 48 hours all individuals were removed from the bottles. Experimental flies were adult individuals eclosed from standard bottles at 25°C under a 12h light:12h dark cycle.

UV-C radiation treatment

Experimental individuals subjected to the UV-C radiation treatments were placed within a box equipped with a 250 nm UV-C lamp on the inner side of the lid as radiation source. Two different radiation regimes were used in this study**.** Experimental individuals were subjected to either a continuous or non-continuous (cyclic) UV-C radiation regime, using a 50x50x100-cm temperature-regulated chamber equipped with a 250 nm UV-C lamp on the inner side of the lid as radiation source. This UV-radiation chamber was placed within a walk-in room at 18°C. Temperature within the UV-radiation chamber was regulated at 25±1 °C for each radiation regime. Temperature within experimental vials was monitored by using a digital thermometer, ranging between 24 and 26°C for the whole experiment. During the continuous radiation treatment, experimental individuals were only briefly taken out of the UV-C radiation chamber once a day to check for dead flies. In the less harsh, cyclic UV-C radiation

treatment, experimental flies were irradiated for one hour every two days. To avoid possible variations due to circadian rhythms, all 1h radiation exposures were performed in the afternoon between 13:00 and 14:00 hours. Two replicate plastic vials (2 x 10 cm) containing 40-60 individuals (approximately 1:1 sex ratio) were set up per RIL and per treatment. All vials contained instant medium with nipagin and were covered with a thin net as lid, to avoid the obstruction of UV-C radiation by the vial material. The continuous radiation treatment was replicated once. All experimental flies were 1 day-old at the onset of the experiment and no anesthesia was used in their manipulation. Vials were periodically checked for deaths (see above) and living individuals transferred to new vials with fresh culture medium. The lifespan of experimental individuals was used as index of UV-C radiation resistance. For each radiation regime, mean lifespan values of replicates were averaged to obtain the final estimate of UV-C radiation resistance for each RIL. We used the two UV-C treatments (cyclic and permanent UV-C radiation) described above because we verified that survival of flies was strongly affected by UV-C in both treatments when compared to (not irradiated) controls. As controls we also used two replicated vials containing 40-60 individuals (with 1:1 sex ratio). All these control vials were completely covered with black paper to avoid UV-C radiation.

For analysis, a three-way ANOVA (analysis of variance) was performed using line, sex and radiation regime as fixed factors. Because of significant interactions between RIL line and UV-C treatment, ANOVAs were also performed separately for each radiation regime, using line and sex as fixed factors.

QTL analysis

Marker genotypes were number of SH2 alleles (0 or 2, i.e., the number of alleles from the parental SH2 line) for both RIL-SH2 and RIL-D48. Composite interval mapping (CIM; Zeng 1994) was used to test the hypothesis that an interval flanked by two adjacent markers contains a QTL. The test was performed using model 6 in QTL-Cartographer Windows Version 2.5 (Wang et al. 2010) for the Ri2 design (sib-mated RIL design). Additionally, multiple trait mapping was used to explore the results of considering the two radiation treatments, continuous and cyclic, as two different UV-C radiation resistance traits. Starting with 5 control markers and a window size of 10 cM, we explored the effects of altering the initial combination of parameters. Significant QTL peaks were consistent across a range of parameter combinations, but the QTL profiles reported here are those found by using 10 cM as window size and 5 control markers. All composite interval mapping and multiple trait mapping significance thresholds were determined by 1000 random permutations ($P < 0.05$). Ninety-five percent confidence intervals were calculated for significant QTL in accordance to the procedure suggested by Dupuis and Siegmund (1999). Epistatic interactions between pairs of markers within significant QTL regions were tested by using a linear model of $y = m_x + m_y + m_x m_y + e$, where m_x and m_y are the genotypes of markers x and y and e is the random error (Morgan and Mackay 2006).

Results

Both regimes implemented showed harmful effects on survival. Lethality was significantly higher when flies were continuously irradiated, with very few lines showing mean longevity values higher than 4 days (Figure 1). A three-way ANOVA with (1) line (RIL-SH2 vs. RIL-D48), (2) sex and (3) radiation time (continuous

radiation vs. cyclic radiation) showed a significant interaction between line and radiation time (Table I). Despite the interaction, the tendency is the same for both radiation treatments, as shown by two-way ANOVAs performed separately for each regime (Table 1). UV-C resistance was higher in the SH2 derived lines and in females, both in continuously- and cyclically-irradiated flies (Figure 1; Table I). Lifespan was much longer in control flies (non-irradiated) than in irradiated flies ($P < 0.001$) and, as in Defays et al. (2011), RIL-D48 flies lived longer than RIL-SH2 in the control (non-irradiated) conditions (Figure 1; ANOVA not shown).

CIM analysis of pooled SH2 and D48 RIL (51 lines) revealed a QTL for survival under continuous UV-C radiation more consistently in the pericentromeric region of chromosome 2 in males (denoted Q2 in Figure 2 and Table II), as well as two other QTL in females on chromosomes X and 3, respectively (denoted Q1 and Q3 in Figure 2 and Table II). No significant QTL in cyclically-irradiated flies were detected when pooling RIL. However, when performing a multiple trait mapping analysis considering the observed lifespan data from the two radiation treatments as different UV-C radiation resistance traits, in the joint-trait the pericentromeric chromosome 2 QTL remained significant in male flies (MT1 in Figure 2 and Table II), and was also significant in females, and an additional chromosome 3 QTL was detected (MT2 in Figure 2 and Table II). The genetic correlation between radiation resistance traits was estimated from the among-RIL covariance between continuous radiation resistance and cyclic radiation resistance. The correlation was significant in both males and females when assaying the RIL pool (51 lines), with males showing a higher correlation between traits. Spearman´s rank correlations for data shown in Figure 1 were 0.39** for males and 0.32* for females (* $P < 0.05$; ** $P < 0.005$).

A number of significant QTL were also detected when analysing SH2- and D48-derived RIL separately. Co-localization of a number of QTL peaks with the QTL detected in the RIL pool was evident. In both continuously irradiated male and female RIL-D48, two significant QTL detected on chromosomes X and 3 co-localized with the two QTL found in the female RIL pool (Figure 2; Table II). The correlation between continuous radiation resistance and cyclic radiation resistance was non-significant when assaying only RIL-SH2 or RIL-D48. Spearman´s rank correlations for RIL-SH2 were 0.22 for males and -0.09 for females. For RIL-D48, correlations were -0.27 for males and -0.06 for females.

Pairwise epistatic interactions were tested between markers linked to all QTL. It is interesting that several significant interactions are suggested for the two pericentromeric markers linked to the QTL on chromosome 2 (Table III).

Discussion

The pericentromeric region of chromosome 2 has been implicated consistently with thermotolerance in *D. melanogaster* (Norry et al. 2004, 2007, 2008; Morgan and Mackay 2006; Loeschcke et al. 2011). The significant QTL for UV-C radiation resistance detected in this study overlapped with previously reported QTL for thermotolerance in the middle of chromosome 2 (Morgan and Mackay 2006; Norry et al. 2007, 2008) as well as with an X-linked QTL for thermotolerance identified by Rand et al. (2010).

In *D. melanogaster,* several genes linked to the centromere of chromosome 2 (e.g. *trap1, catsup, Ddc*) have been implicated in thermotolerance (Baden et al. 1996; Sabban and Kvetnansky 2001; Carbone et al. 2006; Norry et al. 2009). The

pericentromeric chromosome 2 QTL was detected in this study by composite interval mapping (CIM) in continuously irradiated males when pooling SH2- and D48-RIL (Figure 2). Results from multiple trait mapping analysis were consistent with CIM results in continuously irradiated males and suggest that the QTL on chromosome 2 may be important in females as well (Figure 2). This QTL was also found to be significant in continuously irradiated RIL-pooled females and RIL-pooled males when three or less control markers were used in the CIM analysis, and also when simple interval mapping was performed (results not shown). Further, epistasis was also apparent from markers linked to the QTL for survival to UV-C radiation in the middle of chromosome 2 (Table III). Co-localization with previously found QTL for thermal stress (Morgan and Mackay 2006; Norry et al. 2007, 2008) suggests that genes implicated in thermal-stress resistance may also be important for survival to UV-radiation. Thermal-stress resistance genes may be either closely linked to UV resistance genes in this chromosome 2 region, or otherwise pleiotropic for both traits. The pericentromeric chromosome 2 region appears to be pleiotropic for diverse stress-resistance traits in adult flies of *D. melanogaster* under laboratory conditions (Morgan and Mackay 2006; Norry et al. 2007, 2008; Gomez and Norry 2012). For this QTL, two QTL genotypes were recently observed to determine performances of adult flies at high and low temperatures in field-release experiments (Loeschcke et al. 2011).

All of the UV-C resistance QTL include candidate genes (Table II). The photoreactivation pathway enzymes, or photolyases, are specific for either cyclobutane pyrimidine dimers (CPDs) or the [6–4] photoproducts, the most frequently UV-induced lesions in DNA. Genes encoding the photolyase activity are widely distributed among species and both CPD- and [6–4]-photolyase genes are present in *D. melanogaster*

(Yasui et al. 1994; Todo et al. 1996, 1997) in the pericentromeric region of chromosome 2 and may be important for UV-C radiation resistance in adult flies. Other possible candidates may include genes implicated in the nucleotide excision repair (NER) pathway. Both NER and photoreactivation are efficient repair mechanisms for UV-induced DNA damage and among higher eukaryotes genes involved in photorepair have also been implicated in NER, suggesting a mechanistic link between the two pathways (Yamamoto et al. 1983, 1984). However, whereas NER is a complex mechanism requiring a large protein complex, photoreactivation only requires a single enzyme to eliminate from DNA the pyrimidine dimer lesions caused by UV-radiation. Moreover, the use of light from the same source that induces DNA damage to repair it suggests that photolyases are proteins with an important evolutionary role in environments where UV-radiation is present.

DNA, among other cellular components, is also damaged by UV-induced reactive oxygen species (ROS) (Jurkiewicz and Buettner 1994; Shindo et al. 1994). Recently, Suzuki et al. (2009) observed reduced mortality in diapausing females of the spider mite *T. urticae* when exposed to UV-C and UV-B radiation, possibly as a result of carotenoid accumulation, which act as scavengers for ROS. Several candidate genes implicated in the oxidative stress response, with antioxidant activity or involved in cuticular pigmentation map within the significant QTL regions found in this study (Table II). Additionally, apoptosis of post-mitotic cells triggered by sensitivity to UV radiation could have an impact on adult lifespan. Important cell death genes controlling apoptotic pathways (White et al. 1994; Grether et al. 1995; Chen et al. 1996; Nordstrom et al. 1996; Zimmermann et al. 2002) and high temperature stress response genes, like Hsp70 (heat-shock protein 70), map to band 75C, within the significant chromosome 3

QTL revealed by multiple trait mapping in both male and female flies (Table II, Figure 2). Mosser et al. (2000) reported protective effects of Hsp70s against high temperature stress-induced apoptosis in human cell lines.

The results of this study show genetic variation for UV-C radiation resistance in *D. melanogaster*. QTL may often be the result of interacting gene networks (Coffman et al. 2005; Norry et al. 2009), and our results showed that the genetic architecture of UV-C radiation resistance appears to be more complex in continuously irradiated individuals. Overall, QTL for survival to UV-C radiation generally overlapped with major thermotolerance QTL previously identified. Fine scale mapping using complementation tests with mutant alleles will be helpful to determine if the same genes affect both traits or whether both traits are associated by linkage rather than pleiotropy.

Declaration of interest

This research was supported by grants from the University of Buenos Aires, ANPCyT-Argentina (Agencia Nacional de Promoción Científica y Tecnológica) and CONICET-Argentina (Consejo Nacional de Investigaciones Científicas y Técnicas) to FMN and by frame grants from the Danish Natural Sciences Research Council to VL.

References

Baden HP, Kollias N, Anderson RR, Hopkins T, Raftery L. 1996. *Drosophila melanogaster* larvae detect low doses of UVC radiation as manifested by a writhing response. Archives of Insect Biochemistry and Physiology, 32: 187-196.

Black HS. 1987. Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. Photochemistry and Photobiology, 46: 213-221.

Carbone MA, Jordan KW, Lyman RF, Harbison ST, Leips J, Morgan TJ, et al. 2006. Phenotypic variation and natural selection at catsup, a pleiotropic quantitative trait gene in *Drosophila*. Current Biology, 16: 912-919.

Chen P, Nordstrom W, Gish B, Abrams JM. 1996. grim, a novel cell death gene in *Drosophila*. Genes & Development, 10: 1773-1782.

Coffman CJ, Wayne ML, Nuzhdin SV, Higgins LA, McIntyre LM. 2005. Identification of co-regulated transcripts affecting male body size in *Drosophila*. Genome Biology, 6: R53.

Defays R., Gómez FH, Sambucetti P, Scannapieco AC, Loeschcke V, Norry FM. 2011. Quantitative trait loci for longevity in heat-stressed *Drosophila melanogaster*. Experimental Gerontology, 46: 819-826.

De Gruijl FR, Van Kranen HJ, Mullenders LHF. 2001. UV-induced DNA damage, repair, mutations and oncogenic pathways in skin cancer. Journal of Photochemistry and Photobiology, 63: 19-27.

Dupuis J, Siegmund D. 1999. Statistical methods for mapping quantitative trait loci from a dense set of markers. Genetics, 151: 373-386.

FlyBase Consortium. 2003. The FlyBase database of the *Drosophila* genome projects and community literature. Nucleic Acids Research, 31: 172-175 http://flybase.org.

Friedberg EC. 1985. DNA Repair. New York: Freeman.

Gomez FH, Norry FM. 2012. Is the number of possible QTL for asymmetry phenotypes dependent on thermal stress? Journal of Thermal Biology, 37: 1-5.

Grether ME, Abrams JM, Agapite J, White K, Steller H. 1995. The head involution defective gene of *Drosophila melanogaster* functions in programmed cell death. Genes & Development, 9: 1694-1708.

Herman JR. 2010. Global increase in UV irradiance during the past 30 years (1979–2008) estimated from satellite data. Journal of Geophysical Research, 115: D04203.

Jurkiewicz BA, Buettner GR. 1994. Ultraviolet light-induced free radical formation in skin: an electron paramagnetic resonance study. Photochemistry and Photobiology, 59: 1-4.

Kittler L, Løber G. 1977. Photochemistry of the nucleic acids. In: Smith KC, editor. Photochemical and Photobiological Reviews. New York: Plenum Press. Vol. 2, pp. 39-131.

Falconer DS, Mackay TFC. 1996. Introduction to Quantitative Genetics. Longman Group Limited, Essex, UK.

Loeschcke V, Kristensen TN, Norry FM. 2011. Consistent effects of a major QTL for thermal resistance in field-released *Drosophila melanogaster*. Journal of Insect Physiology, 57: 1227-1231.

Lynch M, Walsh B. 1998. Genetics and Analysis of Quantitative Traits. Sinauer Associates, Inc., MA.

McColl G, Hoffmann AA, McKechnie SW. 1996. Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. Genetics, 143 1615-1627.

Morgan TJ, Mackay TFC. 2006. Quantitative trait loci for thermotolerance phenotypes in *Drosophila melanogaster*. Heredity, 96: 232-242.

Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI, Massie B. 2000. The chaperone function of hsp70 is required for protection against stress-induced apoptosis. Molecular and Cellular Biology, 20: 7146-7159.

Nordstrom W, Chen P, Steller H, Abrams JM. 1996. Activation of the reaper gene during ectopic cell killing in *Drosophila*. Developmental Biology, 180: 213-226.

Norry FM, Dahlgaard J, Loeschcke V. 2004. Quantitative trait loci affecting knockdown resistance to high temperature in *Drosophila melanogaster*. Molecular Ecology, 13: 3585-3594.

Norry FM, Gomez FH, Loeschcke V. 2007. Knockdown resistance to heat stress and slow recovery from chill coma are genetically associated in a central region of chromosome 2 in *Drosophila melanogaster.* Molecular Ecology, 16: 3274-3284.

Norry FM, Scannapieco AC, Sambucetti P, Bertoli C, Loeschcke V. 2008. QTL for the thermotolerance effect of heat hardening, knockdown resistance to heat and chill-coma recovery in an intercontinental set of recombinant inbred lines of *Drosophila melanogaster*. Molecular Ecology, 17: 4570-81.

Norry FM, Larsen PF, Liu Y, Loeschcke V. 2009. Combined expression patterns of QTL-linked candidate genes best predict thermotolerance in *Drosophila melanogaster*. Journal of Insect Physiology, 55: 1050-7.

Nozawa H, Yamamoto H, Makita K, Schuch NJ, Pinheiro DK, Carbone S, et al. 2007. Ground-based observation of solar UV radiation in Japan, Brazil and Chile. Revista Brasileira de Geofísica. 25 (suppl. 2): 7-5.

Rand DM, Weinreich DM, Lerman D, Folk D, Gilchrist GW. 2010. Three selections are better than one: Clinal variation of thermal QTL from independent selection experiments in Drosophila. Evolution, 64: 2921-2934.

Sabban EL, Kvetnansky R. 2001. Stress-triggered activation of gene expression in catecholaminergic systems: dynamics of transcriptional events. Trends in Neurosciences, 24: 91-98.

Setlow RB, Setlow JK. 1972. Effects of radiation on polynucleotides. Annual Review of Biophysics and Bioengineering, 1: 293-346.

Shindo Y, Witt E, Han D, Packer L. 1994. Dose–response effects of acute ultraviolet radiation on antioxidants and molecular markers of oxidation in murine epidermis and dermis. Journal of Investigative Dermatology, 102: 470-475.

Sinha, RP, Häder DP. 2002. UV-induced DNA damage and repair: a review. Photochemical and Photobiological Sciences, 1: 225–236.

Suzuki T, Watanabe M, Takeda M. 2009. UV tolerance in the two-spotted spider mite, *Tetranychus urticae*. Journal of Insect Physiology, 55: 649-654.

Thoma F. 1999. Light and dark in chromatin repair: repair of UV-induced DNA lesion by photolyase and nucleotide excision repair. EMBO Journal, 18: 6585-6598.

Todo T, Ryo H, Yamamoto K, Toh H, Inui T, Ayaki H, et al. 1996. Similarity among the Drosophila (6-4)photolyase, a human photolyase homolog, and the DNA photolyase-blue-light photoreceptor family. Science, 272: 109-112.

Todo T, Kim ST, Hitomi K, Otoshi E, Inui T, Morioka H, et al. 1997. Flavin adenine dinucleotide as a chromophore of the Xenopus (6-4)photolyase. Nucleic Acids Research, 25: 764-768.

Wang SC, Basten J, Zeng Z-B. 2010. Windows QTL Cartographer 2.5. Department of Statistics. North Carolina State University, Raleigh, NC.

White K, Grether ME, Abrams JM, Young L, Farrell K, Steller H. 1994. Genetic control of programmed cell death in *Drosophila*. Science, 264: 677-683.

Yamamoto K, Satake M, Shinagawa H. 1984. A multicopy phr- plasmid increases the ultraviolet resistance of a recA strain of *Escherichia coli*. Mutation Research, 131: 11-18.

Yamamoto K, Fujiwara Y, Shinagawa H. 1983. Evidence that the phr+ gene enhances the ultraviolet resistance of *Escherichia coli* recA strains in the dark. Molecular and General Genetics, 192: 282-284.

Yasui A, Eker AP, Yasuhira S, Yajima H, Kobayashi T, Takao M, Oikawa A. 1994. A new class of DNA photolyases present in various organisms including aplacental mammals. EMBO Journal, 13: 6143-6151.

Zeng Z-B. 1994. Precision mapping of quantitative trait loci. Genetics, 136: 1457-1468.

Zimmermann KC, Ricci JE, Droin NM, Green DR. 2002. The role of ARK in stress-induced apoptosis in Drosophila cells. Journal of Cell Biology, 156: 1077-1087.

Table I. Results of ANOVA using line, sex and radiation regime as fixed factors (a). Results of ANOVA performed separately for each radiation regime are also shown, using line and sex as fixed factors (b, c).

Radiation	Source of variation	d.f.	*F* ratio
(a) Both	(1) Line	1	217.1***
	(2) Sex	1	27.7***
	(3) Radiation	1	1477.3***
	(1) x (2)	1	0.053
	(1) x (3)	1	120.8***
	(2) x (3)	1	0.381
	(1) x (2) x (3)	1	0.154
	Error	200	
(b) Continuous	(1) Line	1	29.8***
	(2) Sex	1	45.9***
	(1) x (2)	1	0.057
	Error	98	
(c) Cyclic	(1) Line	1	192.9***
	(2) Sex	1	10.1**
	(1) x (2)	1	0.113
	Error	102	

d.f. is degrees of freedom.
*** $P < 0.001$, ** $P < 0.005$

Table II. QTL for UV-C radiation resistance identified by composite interval mapping in RIL-SH2, RIL-D48 and the RIL pool, and by multiple trait mapping in the RIL pool.

QTL	Range	Radiation	Line	*a*	% Var	Candidate genes within the QTL range
Q1	7B3-10A1	Continuous	RIL-D48, males	0.093	10	*dhd, Trxr-1, TrxT, fh, Pink1, Corp, CG4078, CG12728, CG32756, Ogg1, XRCC1, Gclc, Mcm3, raptor, Btd, CG2887, CG32727, Hsp60, iav, tan*
			RIL-D48, females	0.093	16	
			RIL-pooled, females	0.065	22	
Q2	34C4 – 42A	Continuous	RIL-pooled, males	0.064	30	*phr6-4* (38D2), *CG10211, p38b, Cul-3, Hsp60D, Ku80, CG17331, CG10336, CG10700, cact, CG31742, CycE, RpII33, Cyt-c-d, Hr39, Top3α, tos, CG9272, Dif, grp, lok, Tango6, Top2, Ddc, catsup, ninaD*
Q3	64D-66D10	Continuous	RIL-D48, males	0.094	14	*Txl, CG6673, ntc, MED24, Gen, mus312, RecQ4, Pole2, S6k, CG7182, DnaJ-1, ple, P450*
			RIL-D48, females	0.107	13	
			RIL-pooled, females	0.063	17	
MT1	34C4 – 42A	Multiple-trait	RIL-pooled, males			as above for this QTL range.
			RIL-pooled, females			
MT2	73B – 90B1	Multiple-trait	RIL-pooled, males			*Cat, Trxr-2, Su(P), CG7484, CG7439, CG6852, CG31559, park, TORC, grim, rpr, skl, hid, CG6812, DNApol-η, Mms19, mus304, Rad9, RAD6, p54, elF4AIII, gfzf, gig, Hph, rept, Rga, Rpb8, CG7130, CycT, GstD2, GstD8, Itp-r83A, SdhC, abs, agt, Caf1, CG10898, DNApol-ι, Irbp, lig3, mus308, Snm1, Hus1-like, mus309, CG9588, Kap-α3, MBD-R2, pont, Prosβ3, Prosβ7, Rpt3R, timeout, RhoL RpA-70, CG11035, CG14650, Droj2, Hcs, unc-45, ninaG, CG4009, CG5873, Irc, Pxd, Prx3, abd-A, Cas-3, Rad17, spn-B, foxo, CycC, pr-set7, SF2, wah, RpII15, RpII18, Hsc70-2, Hsp70Aa, Hsp70Ab, Hsp70Ba, Hsp70Bb, Hsp70Bbb, Hsp70Bc, ninaB*
			RIL-pooled, females			

The QTL range is based on the closest markers. Additive effect (*a*) is given in √ (days), with positive values indicating that the SH allele increases resistance to UV-C radiation (marker genotypes were number of SH2 alleles (0 or 2) for both RIL-D48 and RIL-SH2, as in Norry et al. 2008). Candidate genes are implicated in the following biological processes, as reported in the Flybase ontology database (FlyBase Consortium 2003): DNA repair, response to oxidative stress, thermal stress response, response to DNA damage stimulus, apoptosis and pigmentation.

Table III. Analysis of epistatic interactions which were significant before correcting for multiple comparisons between QTL-linked markers. Markers AC004759 and AC006203 are linked to the pericentromeric region of chromosome 2 (Norry et al. 2008). Significant interactions after Bonferroni correction for multiple comparisons within this table are in boldface. Reported *P*-values are those associated with the AC004759 x AC006203 interaction term.

Interaction (marker x marker)	Sex	*F* ratio
AC004759 x DS06577	Males	4.82*
AC004759 x DROT	Males	4.49*
AC004759 x DMEHAB	Males	4.50*
AC006302 x DMU56661	Females	4.44*
AC006302 x DMU96440	Males	6.43*
AC006302 x DMU96440	Females	6.49*
AC006302 x DROSEV	Males	4.71*
AC006302 x DROSEV	Females	4.14*
AC006302 x 3L5235154gt	Males	6.20*
AC006302 x 3L5235154gt	Females	5.30*
AC006302 x AC008198	Males	6.20*
AC006302 x AC008198	Females	5.30*
AC006302 x AC004576	**Males**	**10.2****
AC006302 x AC004576	Females	6.89*

* $P < 0.05$, ** $P < 0.005$.

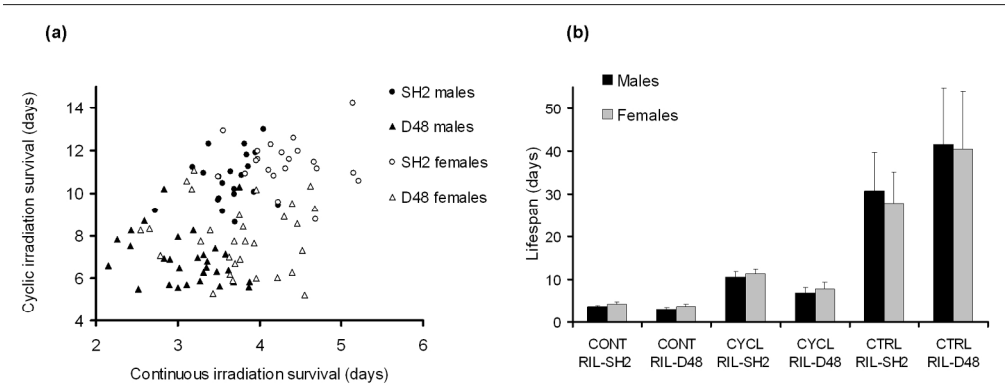

Figure 1. (a) Survival under UV-C radiation is scored as the mean lifespan (in days) for each RIL. Mean lifespan under continuous radiation is represented on the horizontal axis, while mean lifespan under cyclic radiation is represented on the vertical axis. (b) Mean lifespan (in days) is shown for averaged RIL-SH2 and averaged RIL-D48 populations used in this study. CONT RIL-SH2 is continoulsly irradiated SH2-RIL; CONT RIL-D48 is continoulsly irradiated D48-RIL; CYCL RIL-SH2 is cyclically irradiated RIL-SH2 lines; CYCL RIL-D48 is cyclically irradiated D48-RIL; CTRL RIL-SH2 is non-irradiated SH2-RIL; CTRL RIL-D48 is non-irradiated D48-RIL. Error bars correspond to the standard deviation of mean.
361x148mm (150 x 150 DPI)

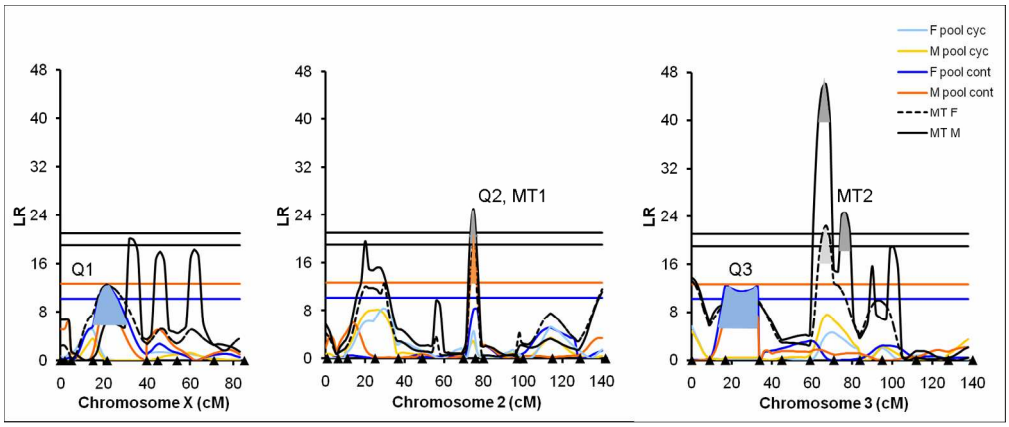

Figure 2. Likelihood Ratio (LR) as a function of genetic distance (cM) for composite interval mapping of UV-C radiation resistance in pooled RIL-D48 and RIL-SH2 populations. In flies continuously exposed to UV-C radiation, a significant QTL was detected by composite interval mapping in the central region of chromosome 2 in males. In flies subjected to the less severe cyclic radiation treatment, no significant QTL were detected in pooled RIL populations. Significance thresholds were determined by 1000 random permutations. Shaded areas under significant QTL peaks indicate a higher than 95% confidence interval for peak width, using 1.5 LOD = 6.9 LR (Dupuis and Siegmund 1999). Microsatellite marker positions are represented by triangles on the horizontal axis. All data was square-root transformed to improve normality. F: female, M: male, Cyc: cyclic radiation treatment, Cont: continuous radiation treatment, MT: multiple trait.
312x130mm (150 x 150 DPI)