Genomic Mutation in Lines of *Arabidopsis thaliana* Exposed to Ultraviolet-B Radiation

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ABSTRACT

Studies that have attempted to estimate the rate of deleterious mutation have typically been conducted under low levels of ultraviolet-B (UV-B) radiation, a naturally occurring mutagen. We conducted experiments to test whether the inclusion of natural levels of UV-B radiation in mutation-accumulation (MA) experiments influences the rate and effects of mildly deleterious mutation in the plant *Arabidopsis thaliana*. Ten generations of MA proved insufficient to observe significant changes in means or among-line variances in experimental lines maintained either with or without supplemental UV-B radiation. Maximum-likelihood estimates of mutation rate for total flower number revealed a small but significant rate of mutation for MA lines propagated under supplemental UV-B exposure, but not for those in which supplemental UV-B was omitted. A fraction of the flower number mutations under UV-B (e~25–30%) are estimated to increase flower number. Results from the application of transposon display to plant materials obtained after MA, in both the presence and absence of supplemental UV-B, suggest that the average rate of transposition for the class I and II transposable elements (TEs) surveyed was no more than 10⁻³. Overall, the estimates of mutation parameters are qualitatively similar to what has been observed in other MA experiments with this species in which supplemental UV-B levels have not been used. As well, it appears that naturally occurring levels of UV-B do not lead to detectable increases in levels of transposable element activity.

Mildly deleterious mutation has been invoked as an important driving force in the evolution of many basic features of life, including sexual reproduction, genome size, sex chromosomes, sexual selection, molecular polymorphism, and senescence (Lynch et al. 1999). Despite extensive theoretical development in these areas, there have been relatively few attempts to obtain direct estimates of the rates and effects of deleterious mutations, and controversy remains over what constitutes a representative range of mutation parameters and effects in natural populations (Peck and Eyre-Walker 1997; Keightley and Lynch 2003; Shaw et al. 2003; Charlesworth et al. 2004; Fry 2004).

The classical approach to estimating mutation parameters has been to assay progeny fitness obtained from mutation-accumulation (MA) experiments. While this approach should allow accumulation of much of the mildly deleterious mutational load, fitness assays of MA lines may be limited in their power to detect changes in performance brought about by mutations of small effect. That this is so has been suggested by studies that have employed EMS mutagenesis to produce lines with expected numbers of mutations for fitness assay, measurements of mutation rate in DNA repair-deficient mutants, by direct sequencing of MA lines to detect all mutations and by using molecular-phylogenetic approaches that take advantage of mutational changes occurring over many thousands of generations (Kondrashov and Crow 1993; Davies et al. 1999; Denver et al. 2004; Estes et al. 2004).

Apart from the problem of limited detection power in fitness assays of MA lines, it is important to consider other aspects of the MA approach that could lead to underestimation of the rate of mutation in natural populations. One potential and underappreciated problem is the nature of the environment under which MA lines are propagated in the first place. While many mutations are likely to arise because of inborn errors of replication, another class of mutations is those induced by naturally occurring mutagenic features of the environment, features that may be absent or poorly reproduced under laboratory conditions. Most notable among these is ultraviolet-B (UV-B) radiation. UV-B causes mutations by promoting the formation of cyclobutane pyrimidine dimers between adjacent pyrimidine bases (Britt 1996). Direct exposure of pollen to UV-B has also been implicated in enhancing the activity of transposable elements in maize (Walbot 1999). To date, all MA experiments with plants and animals have been conducted either indoors under fluorescent and incandescent...
lamps or in glass houses, conditions under which levels of UV-B are well below those encountered in their natural environments. In fact, specially outfitted growth chambers are typically required to attain UV-B levels characteristic of open, natural environments (Sisson and Caldwell 1975).

While a number of studies have documented potentially damaging genomic changes brought about by UV-B exposure (e.g., Rousseaux et al. 1999; Ries et al. 2000), no analyses have been conducted with the aim of determining the influence of ambient UV-B on the overall rate and average effect of spontaneous mutation affecting quantitatively varying traits; this is spite of the fact that exposure of Arabidopsis thaliana plants to UV-B, under conditions of low visible light conditions (where DNA-repairing phytolase enzymes exhibit low activity—i.e., conditions intended explicitly to induce severe mutation), has been shown to lead to fitness reduction in progeny (Whittle and Johnson 2003).

The main objective of this study was not to reexamine the DNA damaging effect of UV-B, which is well documented, but instead to test whether the current practice of conducting MA experiments without natural levels of UV-B could lead to underestimation of the rates and effects of mutation parameters—accordingly, the UV-B levels used in the work described below were intended to mimic natural rather than high levels of exposure predicted under various scenarios of ozone thinning (Kerr and McElroy 1993). Specifically, here we report the results of a MA experiment with A. thaliana involving 10 generations of UV-B exposure, at intensities that fall within the range of summer levels found at low elevations in middle latitudes. In addition to measuring the effects of UV-B on mutation rate for two fitness components, we have also examined the genomes of several of the UV-B-exposed MA lines in an attempt to determine whether there is evidence of enhanced transposon mobility in the germ lines. The rationale for doing so arises from the observation that transposon-induced mutation may represent a significant fraction of fitness decline arising from mildly deleterious mutation (Mackay et al. 1992; Lyman et al. 1996).

MATERIALS AND METHODS

Lighting conditions: A single plant growth chamber (Conviron PGW 36) of ~4-m² surface area, housed in the McGill University Phytotron, was used for all MA line propagation. This chamber was designed to deliver UV-B radiation (wavelength 280–320 nm) equivalent to that encountered on a cloudless, midsummer day at 45° N latitude, or ~7 kJ/m²/day (biologically effective units; see below), although fluctuations in UV-B intensity due to day-to-day differences in cloud cover, as expected in natural environments, were not simulated in the chamber.

To achieve this level of exposure, the chamber was equipped with a canopy of 12 ultraviolet lights (ultraviolet lamp no. UV-B 313-EL, Q-Panel Laboratory Products) suspended at a height of 50–70 cm above the plants. The UV lights were “burned in” for a 72-hr period before the plants were placed in the chamber. As the power output of the lamps diminished over time, the lamps were replaced twice, once after generation four and again after generation eight. To avoid artificially high levels of DNA damage when exposing plants to UV-B, it is necessary to ensure adequate levels of phytosynthetically active radiation (PAR) required to activate the normal DNA-repair enzymes. To provide PAR (~800–1000 μm/m²/sec), the chamber was equipped with eight evenly spaced 1000-W metal halide UV-B lamps. While metal halide lamps do not deliver the exact spectral balance of outdoor PAR, they are often used in experiments involving UV-B exposure to provide the PAR required to ameliorate UV-B-induced injury through activation of repair enzymes (Krizek et al. 1997).

A. thaliana grows as a winter annual in some locations and thus may not always be subjected to the summer levels of UV-B employed in this experiment. Thus, the levels of UV-B used here may be somewhat higher than those encountered by A. thaliana in the field. But since our main objective was to determine whether the omission of UV-B from MA experiments leads to underestimation of the rate and effects of deleterious mutation, the use of exposure levels near the upper range of what this plant might normally encounter in the field is justifiable.

Plants exposed to UV-B radiation (henceforth referred to as UV-B-exposed lines) were grown in trays placed in aluminum-lined, wooden boxes and covered with cellulose acetate film. Cellulose acetate (CA) allows the transmission of UV-A, UV-B, and PAR, but blocks UV-C. Plants protected from UV-B radiation (henceforth referred to as UV-B protected lines) were grown in similar boxes, but were shielded with Mylar film, which allows penetration of PAR and UV-A, but not of UV-B or UV-C. Filters were replaced weekly because these plastics darken over time. Following the completion of this work, a report was published that indicates that CA filters induce chlorosis and epinasty in lower leaves of cucumber plants (Krizek and Mirecky 2004). These effects, if present, were not pronounced in the plants grown here, and, to date, no evidence suggests that CA is genotoxic.

At the beginning of the experiment the spectral irradiance at plant level was measured using a UV spectroradiometer (model OL 754-O-PMT, Optronic Laboratories). These measurements, combined with a DNA damage action spectrum (Setlow 1974), were used to calibrate a Solar Light PMA 2100 Detector (dose meter) employed in weekly chamber monitoring to ensure that the proper UV-B dosage was maintained as the lights aged and the plants grew taller and closer to the lights. Adjustment of the height of the lamps above the plant canopy and/or the number of hours of exposure to UV-B allowed the dosage to be held at a constant daily level. At the conclusion of MA generations, UV-B exposure measurements were again taken with a UV-spectroradiometer (OL 754-O-PMT, Optronic Laboratories), confirming that concordance had been maintained between the UV-B dose measured with the highly accurate, but time-consuming, spectroradiometer and the quicker dose meter method.

Mutation-accumulation experiments: A single, highly inbred individual of the Columbia ecotype of A. thaliana, grown from seed provided by Ruth Shaw (University of Minnesota), served as the progenitor of the MA lines used in this experiment. Two separate sets of 120 MA lines were derived from the self-fertilized seed of this individual. One set of lines was subjected to UV-B radiation (see above) throughout the MA process, while the other was protected from UV-B, but grown in the same growth chamber in the Phytotron of McGill University. The two sets of lines were propagated by single-seed descent (SSD), which shields mild-effect mutations from removal by selection (Schultz et al. 1999; Shaw et al. 2000).
Plants were grown in 7 × 7-cm square pots, filled with a 1:1 mixture of ProMix, perlite, and vermiculite. To promote synchronized germination, for 1 week following each planting the growth chamber was kept dark and the temperature held at 4°C. For the remainder of the growth phase, the temperature of the chamber was maintained at 22°C with 70% relative humidity. Plants were bottom watered twice a week, with an additional watering once a week with a fertilizer solution, alternating between half-strength 20-20-20 and Hoagland’s solution. Plants were allowed to reach maturity and were then harvested and placed in labeled envelopes each generation. Subsequent generations of each MA line were founded by randomly planting two to four seeds and thinning after germination to one plant per pot. Remaining seeds were then stored at 4°C. This procedure was repeated until the lines had undergone 11 generations of MA.

**Progeny testing:** Seeds stored at the beginning (generation 1, control) and end (generation 11, MA Treatment) of MA, from both the UV-B exposed and UV-B protected lines, were sown in a common environment to produce seed of the same age for progeny testing. Two chambers were used, each with one replicate of the 105 surviving lines of both the UV-B exposed and UV-B protected lines at generations 1 and 11. All plants were sown individually in plastic cones 4 cm in diameter by 20 cm long, filled with the soil mix described above. Each plant was randomly assigned a position in 1 of the 14 supporting trays in each chamber, with each tray containing 30 plants positioned in a checkerboard pattern. Immediately after sowing, plants were subjected to 1 week in the dark at 4°C (to promote germination) and were then grown at 70% humidity, 22°C, and 800 μm/sec of photosynthetically active radiation (but no UV-B source). Trays were randomized within each chamber every week to reduce the effects of microenvironmental variation within the chambers. At senescence, the infructescense was collected from each plant. The seeds released from these plants were used to sow the final progeny test.

Due to space limitations, we were unable to grow all lines in the progeny test. Thus, regenerated seeds from 86 UV-B exposed and 85 UV-B protected MA lines were used in the final progeny test. To achieve replication of progeny from the different MA lines, five growth chambers were used. Each chamber housed one plant from each UV-B treatment-generation combination from each of the two common growth (maternal) chambers, randomly assigned a position within the chamber. These plants were again grown in tubes. Each chamber contained 14 trays, each with 49 plants arranged in a checkerboard pattern for a total of 684 plants in each chamber.

Plants that had not germinated after 1 week of growth under the lights were replaced with new seed (84 plants). This delay in planting, however, had a strongly negative effect on the growth of these replants, which became suppressed by neighboring plants, and it was decided to exclude them from all analyses. Another 115 plants were unusually small and shriveled, suggesting problems with water delivery to the tubes. In no case was there more than a single such plant per MA line (indicating that mutation was not the cause of their poor performance), and these plants were distributed nearly identically among the UV-B protected and UV-B exposed MA and control lines (χ² = 1.13, d.f. = 3, NS). Because there was strong evidence that these plants had succumbed to problems induced by poor maintenance and handling during growth, they were not used in the analysis.

Tray positions were again randomized each week to reduce microenvironmental effects. After 48 days of growth, the number of flowers, buds, and seed pods on each plant was counted as a measure of the total flower number. Plants were harvested after 56 days of growth by cutting the plant just below the rosette. Harvested plants were placed in labeled envelopes and were then dried in a forced-air drying oven at 65°C for 48 hr (±2 hr), and the dry weight of all aboveground parts was then determined.

**Statistical analysis:** Data from 3192 plants in total were available for analysis (n = 1588 from UV-B protected progeny, and n = 1604 UV-B exposed progeny, split evenly among control and MA treatments). Data were not transformed, as inspection of Q-Q plots and histograms indicates that they met normality assumptions. A mixed linear model was used to analyze the flower number and weight data, with family and maternal parent nested within family treated as random effects and assay growth chamber, tray position (edge vs. center), and observer (for flower number only) treated as fixed effects. The components of variance between lines (V_L), between seed parents within lines (V_M, maternal variance), and within replicates (V_e, environmental variance) were estimated by restricted maximum likelihood (REML) (LYNCH and WALSH 1997). Analyses were conducted using SAS Version 8.0.

Change in mean number of flowers per plant and plant dry weight (∆M) per generation, along with changes in among-line variance per generation (∆V_L), were estimated from the per generation change observed between generations 1 and 11. Bootstrapping of the original data set, at the level of family, was carried out 1000 times, and ~95% confidence intervals for means and variance components were constructed from the estimates of the bootstrap variances (HILBORN and MANGEL 1997; MANLY 1997).

Estimates of the mutation rate and mutational effects were obtained using maximum-likelihood (ML) methods developed by KEIGHTLEY (1984). The program MLGENOMEU (version 2.08) written by Peter Keightley (University of Edinburgh) was used for all ML estimates. Generation 1 data were used as the control, while generation 11 data were used to obtain phenotype data for MA treatment. Phenotypic values were transformed by adjusting for chamber, edge, and observer estimates estimated using the linear model approach described above.

The ML method assumes that phenotypes are normally distributed with mean M and environmental variance V_e, and that mutations act to alter phenotypic values. The method also assumes that the gamma distribution, with shape and scale parameters β and α, respectively, is assumed to provide a reasonable distribution of mutational effects. The diploid rate of mutation (U_diploid) is presented below, and the mean of the gamma distribution is assumed to estimate the average mutation effect, E(α) = β/α. Leptokurtic distributions of mutational effects are implied by small values of β (β = 1, an exponential distribution of effects), while near infinite values of β imply a variance near zero and equivalence with the equal-effects model assumed in the Bateman-Mukai approach (DRAKE et al. 1998; LYNCH et al. 1999). Mutation parameter estimates were carried out assuming a reflected gamma distribution, which (in addition to the other mutation parameters) allows the estimation of the proportion of mutations (R) that increase the trait value (KEIGHTLEY and OHNISHI 1998). In implementing the ML approach, the parameters M, V_e, U_diploid, β, α, and R were estimated from the data. Ninety-five percent confidence limits for the parameter estimates were estimated from the curvature of the profile-likelihood functions.

**Transposon display:** Transposon display (TD) (KORSWAGEN et al. 1996; VAN DEN BROECK et al. 1998) is an AFLP-based technique that can be applied to genomic DNA to examine the changes in genomic positions of transposable elements (TEs). In TD, genomic DNA is digested with a frequent-cutting restriction enzyme, and adaptors (vectorrettes) are ligated to the digested fragments. Labeled PCR amplification of the digestion-ligation template is carried out using TE-specific and
adaptor-specific primers. Individual element insertions can be resolved and visualized on a polyacrylamide gel, due to the fact that restriction sites are located at variable distances from the TEs. Variation among plant genomic DNA samples suggests variation in genomic positions of TEs. In the present case, where all DNA samples are descended from a single inbred progenitor, and propagated subsequently by SSD, it is assumed that variable band positions arise from the mobilization (insertion or deletion) of TEs during propagation of the MA lines.

The experimental procedures used to implement TD follow our earlier studies (Wright et al. 2001), except that genomic DNA was extracted using the DNeasy plant mini kit (QIAGEN, Mississauga, ON). Sequence information for a subset of class I and II TEs (supplementary Table S1 at http://www.genetics.org/supplemental/) was obtained from the A. thaliana transposable element database (http://www.tebureau.mcgill.ca; Lè et al. 2000), as well as from additional BLAST searches of GenBank [National Center for Biotechnology Information (NCBI); http://www.ncbi.nlm.nih.gov/blast]. Genomic data for the elements surveyed were aligned using the program PILEUP (University of Wisconsin Genetics Computing Group, Version 10.0), and conserved subterminal regions were identified. For each element examined, two overlapping degenerate primers were designed for nested PCR using TD (supplementary Table S1). The restriction enzyme BfaI (New England Biolabs, Beverly, MA) was used, as no sites were present between the primer sequence and terminus of the elements. The BfaI adaptor sequences used were as follows: 5′-BfaI, 5′-TAGCAAGGAGAGGACGCTGTCTGTCGAAGGTAAGG AACGGACGAGAAGGGAGA-3′ and 3′-BfaI, 5′-TCTTCCCTTCTCGAATCGTAACCGTTCGTACGAGAATCGCTGTCCTCTCCTTGTCG-3′. Two nested, adaptor-specific primers were used: ap1 (preselective: 5′-CGAATCGTAACCGTTCGTACGAGAATCGCTGTCCTCCTTGTCGCTTGCCATTGG-3′) and ap2 (selective: 5′-GTACGAGAATCGCTGTCCTTGTCGCTTGCCATTGG-3′). Details of genomic DNA extraction, restriction enzyme digestion, vectorette ligation, PCR, and electrophoresis are as described in Wright et al. (2001).

To examine whether there had been any TE mobility, genomic DNA was extracted from plants in 13 separate UV-B exposed and 3 UV-B protected lines produced after six consecutive generations of SSD. TD patterns were recorded and examined for evidence of TE mobility from differentiation among samples in band positions and presence/absence patterns. As well, the number of visible bands revealed by TD for each type of TE was used as an estimate of the number of copies detectable with the technique.

The total number of copies of TEs revealed in TD multiplied by the number of generations of MA propagation and the number of MA lines sets an upper limit for the detection of TE activation in this experiment—this assumes that each element detected has the capability to become mobilized during line propagation.

As a positive control for detection of past TE mobility using TD, we applied TD to reveal the banding patterns of several separate ddm1 (decrease in DNA methylation) mutant plants of A. thaliana. Germline mobilization of transposons has been shown to occur in these ddm1 mutants (Miura et al. 2001). As this material was provided to us after an unspecified number of generations of propagation from the ancestral, ddm1-bearing stock, we could not use the pattern to calculate transposition rate.

RESULTS

Changes in means and variances between control and MA generations: Germination rates were ~98% in both the UV-B exposed and UV-B protected lines. Survival rates to flowering were >99% in both sets of lines.

Calculation of trait means and among-line variances for the control (1 generation of MA) and MA treatments (11 generations of MA) were carried out separately within each of the two UV-B exposures (Figures 1 and 2, Tables 1 and 2). In the case of the flower number in the UV-B exposed lines (Figure 1), there was a small decrease in the mean between the control and the MA treatment, coupled with an increase in among-line variances. But these changes were not significantly different from zero due to large sampling variance (Table 2). Mutational heritability for flower number under the experimental conditions was 0.74 ± 0.27.

Figure 1.—Frequency distribution of least-squares means of flower number per plant in UV-B protected and UV-B exposed control and MA lines of Arabidopsis thaliana.

Figure 2.—Frequency distribution of least-squares means of plant dry weights (milligrams) in UV-B protected and UV-B exposed control and MA lines of Arabidopsis thaliana.
UV-B treatment was estimated to be ~0.001, but was not significantly different from zero due to large sampling variance.

Under UV-B protected conditions, no among-line variance for flower number was detected in either the control or the MA lines, and the small increase in mean between the control and MA treatments was not significant (Table 1).

Plant weight data followed a pattern broadly similar to that of flower number (Figure 2 and Table 2); that is, the among-line variances were not significantly different from zero in either the control or the MA treatments, and the changes in mean between the control and MA treatments were not significant (Table 2).

**ML estimates of mutation parameters:** ML estimation of mutational parameters makes more efficient use of data than methods based solely on changes in phenotypic means and among-line variances (Keightley 1994). ML estimation was conducted assuming an underlying reflected gamma distribution of mutational effects (i.e., mutational effects that are both positive and negative) (Keightley and Ohnishi 1998). For the UV-B protected lines the estimate of mutation rate for flower number is not significantly different from zero (Table 3). For the UV-B exposed lines, however, a significant genomic mutation rate ($U \approx 0.02-0.06$) was detected. Average effects of mutations are significantly different from zero, ranging from 13 to 34% depending upon the assumption about the shape of the effect distribution (i.e., the value of $\beta$). There is insufficient information to distinguish between an equal-effect distribution and leptokurtic distribution of mutational effects.

There is also evidence that ~25% of the mutations have effects that increase the phenotypic value of the trait. The estimate of $R$ is significantly different from zero (log-likelihood-ratio test, $P < 0.001$) for all values of $\beta$; e.g., for $\beta = \infty$, $R = 0.255$, while for $\beta = 1$, $R = 0.357$.

### TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UV-B exposed</th>
<th>UV-B protected</th>
</tr>
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<tbody>
<tr>
<td>$M$ (control)</td>
<td>99.86 (95.29, 104.43)</td>
<td>101.90 (97.71, 106.09)</td>
</tr>
<tr>
<td>$V_L$ (control)</td>
<td>15.41 (~24.51, 55.34)</td>
<td>0 (~6.31, 6.31)</td>
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<td>$V_M$ (control)</td>
<td>55.15 (~1.89, 106.26)</td>
<td>0 (~45.38, 45.38)</td>
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<tr>
<td>$V_E$ (control)</td>
<td>984.09 (880.93, 1087.24)</td>
<td>961.82 (860.58, 1063.05)</td>
</tr>
<tr>
<td>$M$ (MA)</td>
<td>99.59 (94.10, 105.08)</td>
<td>102.49 (98.78, 106.19)</td>
</tr>
<tr>
<td>$V_L$ (MA)</td>
<td>35.46 (~20.48, 91.40)</td>
<td>0 (~15.82, 15.82)</td>
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<tr>
<td>$V_M$ (MA)</td>
<td>113.27 (35.84, 190.69)</td>
<td>27.31 (~28.92, 82.54)</td>
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<tr>
<td>$V_E$ (MA)</td>
<td>1084.30 (967.68, 1200.00)</td>
<td>945.28 (820.60, 1069.96)</td>
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<tr>
<td>$\Delta M$</td>
<td>~0.05 (~0.44, 0.38)</td>
<td>0.06 (~0.27, 0.39)</td>
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<tr>
<td>$k^2_m \times 10^3$</td>
<td>1.02 (~2.66, 4.71)</td>
<td>0 (~2.00, 2.00)</td>
</tr>
</tbody>
</table>

Parameters are as follows: $M$, mean; $V_L$, among-line variance; $V_M$, maternal variance; $V_E$, environmental variance; $\Delta M$, rate of decline in flower number per generation; and $k^2_m = (\Delta V_L/2)/V_L$ (control) = mutational heritability, where $\Delta V_L$ is the change per generation in among-line variance estimated from the difference between $V_L$ (control) and $V_L$ (MA).

### TABLE 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UV-B exposed</th>
<th>UV-B protected</th>
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<tbody>
<tr>
<td>$M$ (control)</td>
<td>192.31 (187.88, 196.74)</td>
<td>192.65 (187.31, 197.95)</td>
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<tr>
<td>$V_L$ (control)</td>
<td>45.33 (~61.53, 152.19)</td>
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<td>$V_M$ (control)</td>
<td>10.08 (~155.99, 166.07)</td>
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<tr>
<td>$V_E$ (control)</td>
<td>2644.80 (2375.75, 2913.85)</td>
<td>2837.31 (2534.29, 3140.33)</td>
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<tr>
<td>$M$ (MA)</td>
<td>186.92 (182.20, 191.64)</td>
<td>192.84 (187.75, 197.93)</td>
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<tr>
<td>$V_L$ (MA)</td>
<td>42.38 (~52.05, 136.81)</td>
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<tr>
<td>$V_M$ (MA)</td>
<td>0 (~172.13, 172.03)</td>
<td>0 (~113.70, 113.70)</td>
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<tr>
<td>$V_E$ (MA)</td>
<td>2832.30 (2516.80, 3147.80)</td>
<td>2602.63 (2285.11, 2920.15)</td>
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<tr>
<td>$\Delta M$</td>
<td>~0.539 (~1.167, 0.0882)</td>
<td>0.020 (~0.498, 0.537)</td>
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<tr>
<td>$k^2_m \times 10^3$</td>
<td>1.02 (~2.66, 4.71)</td>
<td>0 (~2.00, 2.00)</td>
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</table>

See Table 1 legend for parameter abbreviations. Estimates of means are multiplied by $10^3$ and those of variances by $10^6$. 
ML estimation of mutation rate for dry weight shows no evidence that mutations affecting this character occurred in either the UV-B protected or the UV-B exposed lines (Table 3).

**Transposon display:** TD provides estimates of the number of copies of each type of element examined (Table 4). These estimates are smaller than the actual numbers of elements present in the genome, as some elements plus their flanking regions yield band products that lie outside the region of the gel where resolution is good (e.g., 50–500 bp)—nevertheless, consideration of the expected frequency distribution of BfaI fragment sizes on the basis of the published Arabidopsis genome sequence data (Q. H. Le, unpublished results) shows that these represent a relatively small proportion of elements, and our interest here is in detecting mobility of any element, as opposed to every element in the genome.

No evidence of TE mobility was uncovered using TD in either UV-B exposed or UV-B protected lines. The TD gels containing materials from all lines showed uniformly consistent banding patterns across all lanes (Figure 3). This is in contrast to the significant TE mobility observed among ddm1 lines of A. thaliana, where past mobility was detectable for MULE-45 and Cac1 elements (Figure 3).

This lack of detectable TE movement may be put into perspective by calculating the probability of not observing a transposition event for any of /C24200 different TE copies observed in the 13 diploid MA lines, each propagated for six generations. That is, given a rate of transposition per element per generation of 10/2554, the Poisson probability of not observing a transposition event is /243%. Given a transposition rate of 10/2555, this probability is /2470%. Thus, the rate of transposition is likely no more than 10/2554.

**DISCUSSION**

MA experiments have previously been conducted with A. thaliana by other researchers, each under conditions without supplemental UV-B. SCHULTZ et al. (1999) compared several fitness components for MA lines

<table>
<thead>
<tr>
<th>TE</th>
<th>No. of bands observed</th>
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<tbody>
<tr>
<td>Class I</td>
<td></td>
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<tr>
<td>gypsy</td>
<td>46</td>
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<td>copia</td>
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<td>TRIM</td>
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<td>SINE</td>
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<td>Class II</td>
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propagated for 10 generations with those of controls. Changes in means of the fitness components were observed to be small, and significant among-line variance was not detected for any trait. Bateman-Mukai estimates of mutation rates and average effects for mutations affecting single fitness components ranged from 0.002 to 0.054 and from 0.23 to 0.62, respectively. Shaw et al. (2000) propagated MA lines for 17 generations and conducted progeny tests at generations 0 (controls), 8, and 17. Significant changes in means and among-line variances for the fitness components were detectable only after 17 generations, and there was evidence that mutational effects were approximately symmetrically distributed around zero. Further analysis of these data, employing Markov chain Monte Carlo maximum likelihood to estimate the mutation rate under a model involving a displaced gamma distribution, indicated a mutation rate between 0.1 and 0.2, again with mutational effects spanning zero (Shaw et al. 2002).

The results of the MA experiments described here are qualitatively similar to those obtained in previous studies of mutation with this species. This is true of those conducted both with and without supplemental UV-B radiation. Most significantly, it appears that the inclusion of natural levels of UV-B light may at most only slightly elevate the rate of mutation, and therefore one may conclude that the absence of supplemental UV-B in past MA studies of this species is unlikely to have led to severe underestimation of the rate of mildly deleterious mutation. It is notable that an alternative approach to examining deleterious mutation in this A. thaliana, one based on sequence evolution of protein-coding regions over many thousands of generations in natural populations (Keightley and Eyre-Walker 2000; Wright et al. 2002), gave an estimate of U between 0.2 and 0.6, a value that is larger than that reported in any of the MA studies of this species, suggesting that phenotypic evaluation of progeny from MA experiments does not detect all of the deleterious mutations.

A second finding from this study is the evidence that a portion of mutations increase the phenotypic value of one of the traits (flower number) studied. This type of finding has also been reported in the case of several life-history traits in A. thaliana (Shaw et al. 2002). As has been pointed out elsewhere (Bataillon 2000; Keightley and Lynch 2003; Shaw et al. 2003), however, the evolutionary interpretation of such a result is not straightforward. Flower number is only one of several major life-history traits, and it is unknown how changes in this trait alone would affect total lifetime fitness. As well, evaluation of mutations influencing fitness traits under growth room conditions may reveal little about how these same mutations would affect the phenotype under natural conditions, and, as has been pointed out previously, MA experiments of short duration may be limited in their ability to detect changes in mean fitness (Keightley and Lynch 2003).

Third, the results from this investigation reveal no effects of UV-B on activation of TEs in the MA lines. UV-B light has been considered as an environmental stress by some researchers, and it is known that certain such stresses can activate transposable elements in plants (Hirochika et al. 1996; Wessler 1996). That UV-B activation of TEs was not observed in this study is not likely due to the inability of elements investigated here to become mobilized, as activation of both cac1 and MULE-45 (data not shown) elements has been observed in the ddm1 mutants examined as positive controls for

**Figure 3.**—Characteristic TD gel showing lack of detectable TE mobility in UV-B exposed SSD lines of Arabidopsis thaliana contrasted with detected TE mobility in ddm1 mutant lines of A. thaliana. (a) TD of cac1 elements in three UV-B protected MA lines (lanes 1–3) and eight UV-B MA exposed lines (lanes 4–11) following six generations of SSD. (b) TD of cac1 in 14 ddm1 mutant lines. Approximate band positions of molecular weight markers (base pairs) are on the right.
the TD methods. On the surface, these results would appear to contradict what has been reported for Mutator (Mu) elements in maize (Walbot 1999), but there are several important differences to consider between that study and this one. First, in Walbot’s (1999) study, pollen was exposed directly to UV-B, while in our own investigation entire plants were exposed. Pollen is known to be susceptible to DNA damage through UV-B exposure (Jackson 1987), and in A. thaliana pollen may be protected from much of the UV-B received as it is embedded within anthers that are themselves partially shielded by flower parts. Second, unlike maize, A. thaliana is highly self-pollinating. Theoretical models that encompass transposon dynamics and mating system variation suggest that selection to reduce TE mobility could be stronger in selfers than in outcrossers (Charlesworth and Langley 1986; Wright and Schoen 1999; Morgan 2001). Moreover, the TE complement comprises a larger fraction of the maize genome than of the Arabidopsis genome (Benetzen 2000; Le et al. 2000).

Given that UV-B has known DNA damaging effects, the question of why much larger genomic changes were not observed can be raised. There are several possible answers. First, as has been pointed out elsewhere, plants possess a number of features that may limit damage caused by UV-B exposure, especially structural (plant cell layers that inhibit penetration of UV-B) and biochemical (plant pigments that absorb UV-B) (Britt 2004). Second, single-seed descent, the method of MA used here, is not expected to allow accumulation of mutations of large deleterious effect. To the extent that UV-B-induced mutations are of this type, the methods used in the present investigation would underestimate their rate of production.

Finally, it is worth reiterating that the work described here involved approximately ambient levels of UV-B exposure, assuming normal protection provided by the ozone layer. As such, our work verifies the validity of MA experiments conducted under laboratory conditions without the effects of naturally occurring levels of UV-B irradiation, but may not be easily extrapolated to predict genomic damage arising from unnaturally high levels of UV-B exposure.

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LITERATURE CITED


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