

## LETTERS

## Transgeneration memory of stress in plants

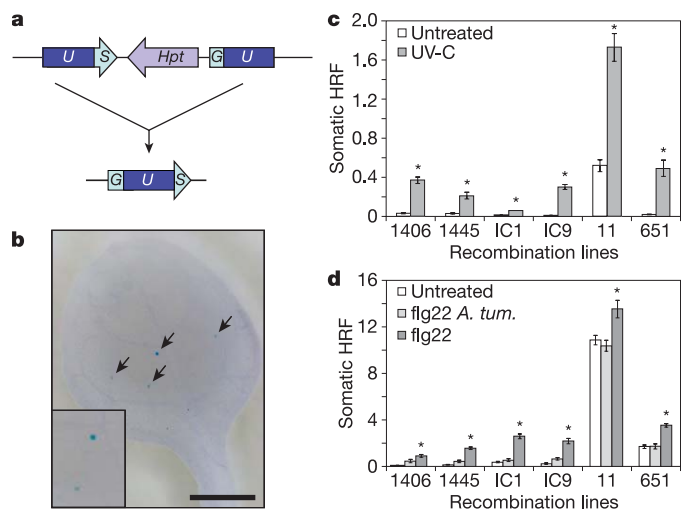
Jean Molinier<sup>1</sup>†, Gerhard Ries<sup>1</sup>†, Cyril Zipfel<sup>1</sup>† & Barbara Hohn<sup>1</sup>

Owing to their sessile nature, plants are constantly exposed to a multitude of environmental stresses to which they react with a battery of responses. The result is plant tolerance to conditions such as excessive or inadequate light, water, salt and temperature, and resistance to pathogens. Not only is plant physiology known to change under abiotic or biotic stress, but changes in the genome have also been identified<sup>1–5</sup>. However, it was not determined whether plants from successive generations of the original, stressed plants inherited the capacity for genomic change. Here we show that in *Arabidopsis thaliana* plants treated with short-wavelength radiation (ultraviolet-C) or flagellin (an elicitor of plant defences<sup>6</sup>), somatic homologous recombination of a transgenic reporter is increased in the treated population and these increased levels of homologous recombination persist in the subsequent, untreated generations. The epigenetic trait of enhanced homologous recombination could be transmitted through both the maternal and the paternal crossing partner, and proved to be dominant. The increase of the hyper-recombination state in generations subsequent to the treated generation was independent of the presence of the transgenic allele (the recombination substrate under consideration) in the treated plant. We conclude that environmental factors lead to increased genomic flexibility even in successive, untreated generations, and may increase the potential for adaptation.

Plants are influenced by abiotic and biotic environmental factors on several levels; apart from changes in plant physiology and the mounting of resistance responses, the dynamics of the genome can also be altered. Examples include the activation of transposable elements by abiotic and biotic stress conditions<sup>7–9</sup>, induction of mutations by chemical and physical agents<sup>10</sup>, and enhancement of homologous recombination by elevated temperatures<sup>11</sup> or ultraviolet-B (UV-B) (ref. 2). Especially interesting is the genomic flexibility shown by plant genomes in response to pathogen attack<sup>3,4,7</sup>. Whenever possible, such changes were monitored at the level of the sequence of affected genes. The influence these changes have in evolutionary terms, however, remained poorly understood, because most changes were detected in somatic tissue and not considered in further generations. In plants, the reproductive cell-lineage emerges from somatic tissue late in development<sup>12</sup>, thus some genomic changes acquired during the life of a plant can be transmitted to the next generation. Indeed, with progeny of UV-B- or pathogen-treated plants, the frequency of occurrence of genetically fixed mutation (in this case, homologous recombination) was reproducibly elevated<sup>2,4</sup>. The degree of genomic change in the offspring of the stressed population was expected to return to the basal level. We show here that increased levels of homologous recombination persist for several generations in the lineage from the original parent plants that were exposed to stresses, including ultraviolet radiation or flagellin.

We measured the rate of homologous recombination in the untreated offspring of plants exposed to conditions of environmental

stress. We used *A. thaliana* plants harbouring  $\beta$ -glucuronidase (*GUS*)-based constructs in which truncated but overlapping parts of the gene allow quantification of somatic homologous recombination. The results of this event are visualized as blue spots on a white background following histochemical staining of plants (Fig. 1a, b). Previous molecular analyses of the plant DNA confirmed that the blue spots, which represent *GUS* activity, indeed symbolize bona fide recombination events<sup>13,14</sup>. Using this assay, the influence of ultraviolet-C (UV-C) was tested in six independent transgenic lines that carried the recombination reporter in different relative orientations of the *GUS* sequence fragments: ‘*GU*’ and ‘*US*’ (ref. 15). The basal levels of homologous recombination, indicated as numbers of recombination sectors per plant, varied among the six lines; the degrees of stimulation were also different, but in all cases the treatment with UV-C stimulated the level of homologous recombination (Fig. 1c). UV-C induction of homologous recombination together with variation between independent transgenic lines is consistent with previous reports<sup>2</sup>.

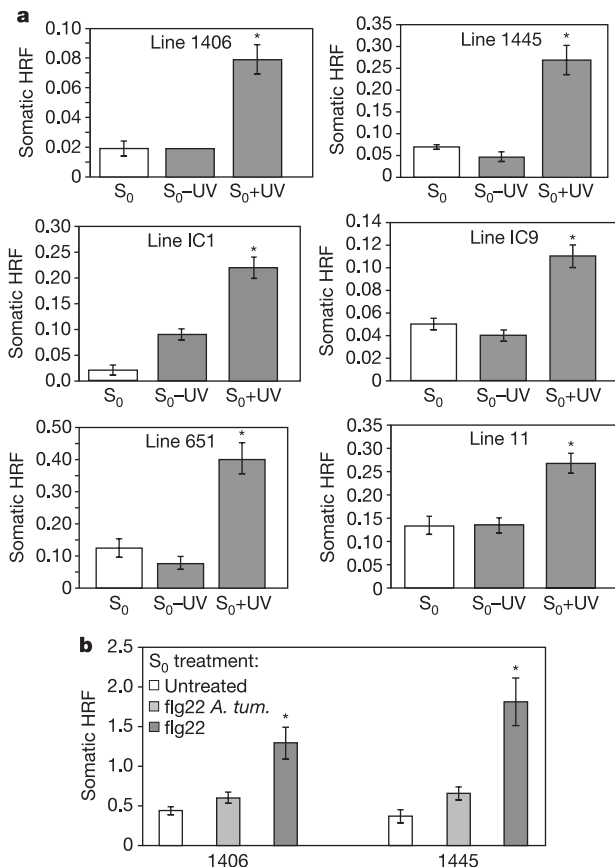


**Figure 1 | Somatic homologous recombination in UV-C- and flg22-treated plants.** **a**, Schematic representation of a recombination substrate used for monitoring somatic homologous recombination (lines IC1 and IC9). *GUS*,  $\beta$ -glucuronidase gene; *Hpt*, hygromycin-resistance gene. Homologous recombination region is shown in dark blue. **b**, Recombination events (blue spots highlighted by black arrows) giving a measure of homologous recombination frequency (HRF; see Methods) in line IC1 after flg22 treatment. Scale bar, 1 mm; inset,  $\times 3$  original magnification. **c**, Somatic HRF in untreated and UV-C-treated  $S_0$  plants. Results are means  $\pm$  s.e.m. ( $n > 50$  plants;  $t$ -test  $*P < 0.05$ ). **d**, Somatic HRF in either untreated plants, plants treated with flg22 *A. tum.*, or treated with flg22. Results are means  $\pm$  s.e.m. ( $n > 40$  plants;  $t$ -test  $*P < 0.05$ ).

<sup>1</sup>Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland. †Present addresses: Institut de Biologie Moléculaire des Plantes, 12 Rue du Général Zimmer, F-67084 Strasbourg Cedex, France (J.M.); BioMedinvestor AG, Elisabethenstrasse 23, CH-4051 Basel, Switzerland (G.R.); The Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK (C.Z.).

Inoculation of homologous-recombination-tester plants with pathogens was previously shown to increase homologous recombination frequencies<sup>3,4</sup>. Here we use flagellin, a bacterium-derived elicitor, to activate plant defences. In *A. thaliana*, perception of flagellin, the building block of the bacterial flagellum, occurs by recognition of the most conserved domain on the flagellin amino terminus, represented by the peptide flg22 (ref. 16). Treatment of plants with this peptide has been shown to trigger resistance to pathogenic bacteria<sup>6</sup>, unlike treatment with the inactive analogue from *Agrobacterium tumefaciens* (flg22 *A. tum.*). In all six transgenic lines analysed, we detected an increase of homologous recombination on treatment with flg22, but not with the inactive flg22 *A. tum.* peptide (Fig. 1d). Thus, flagellin represents an agent mimicking a pathogen in inducing both a pathogenic response<sup>6</sup> and elevated levels of homologous recombination.

Progeny of UV-treated and self-pollinated plants grown under non-stress conditions were analysed for homologous recombination. In all six lines, the frequency of homologous recombination was greater than that of plants from untreated progenitors (Fig. 2a). As the location of the transgene in the different lines varies, the transgenerational effect of increased frequencies of somatic homologous recombination is most likely independent of genome position. Homologous recombination frequencies in the  $S_1$  selfed generation of treated plants were 2–4-fold higher than those of  $S_1$  plants derived from untreated parents. The effect was also independent of ecotype and orientation



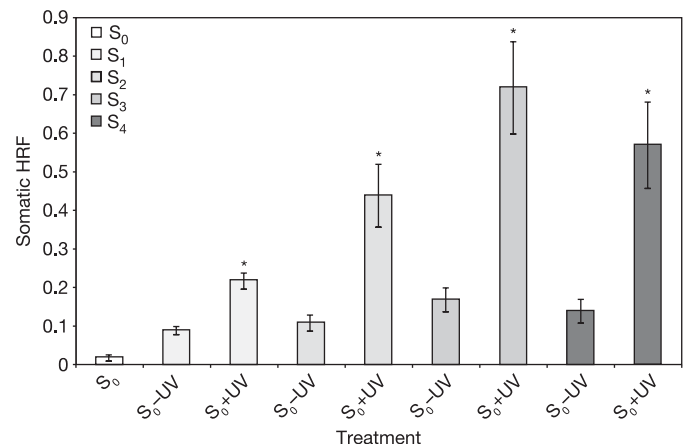
**Figure 2 | Somatic HRF in  $S_0$  lines and their respective  $S_1$  progenies.** **a**, Somatic HRF in six different untreated  $S_0$  lines (white bars) and in untreated  $S_1$  plants (grey bars) derived from those same  $S_0$  lines treated with UV-C ( $S_0 + UV$ ) or untreated ( $S_0 - UV$ ). Results are means  $\pm$  s.e.m. ( $n > 40$  plants;  $t$ -test  $*P < 0.05$  compared with  $S_1$  plants from  $S_0 - UV$ ). **b**, Somatic HRF in  $S_1$  plants (lines 1406 and 1445) from  $S_0$  plants that were untreated, treated with the inactive flagellin analogue (flg22 *A. tum.*), or treated with the active flagellin (flg22). Results are means  $\pm$  s.e.m. ( $n > 40$  plants;  $t$ -test  $*P < 0.05$  compared with  $S_1$  treated with flg22 *A. tum.*).

of the recombination substrate (see Methods for details), as lines 1406, 1445, IC1 and IC9 were in the Columbia ecotype, whereas lines 11 and 651 were in C24. Similarly, the progeny of flg22- and flg22 *A. tum.*-treated plants, and of untreated plants, were analysed for persistence of elevated levels of homologous recombination; again, the plants ‘memorized’ their previous exposure or reaction to the biologically active peptide flg22 and exhibited a constitutively elevated level of somatic recombination (Fig. 2b). This response was specific, as treatment with the inactive peptide did not lead to the described transgenerational effect.

The basis for the described transgenerational effect must be epigenetic because the whole population changes its behaviour, whereas a mutation would affect only very few plants. Epigenetic change can be described as mitotically and/or meiotically heritable, potentially reversible chromatin alteration, which occurs in the absence of change in the DNA sequence. Heritable changes of epigenetic traits may last for several generations. We tested whether the homologous-recombination-enhancing epigenetic change acquired through the environmental stimulus of ultraviolet radiation can be detected in generations succeeding  $S_1$ . Somatic homologous recombination was persistently found to be increased up to the selfed  $S_4$  progeny of plants that were treated with UV-C only in  $S_0$  (Fig. 3). The epigenetic change leading to enhanced homologous recombination frequencies is thus stable for at least four generations.

So far, data on homologous recombination in successive generations following treatment were generated by analysing self-pollinated plants. It was of interest to test whether the epigenetic changes could be preferentially transmitted through the male or the female germline. To test this, plants were irradiated with UV-C and crossed to untreated plants. In offspring of treated and self-pollinated plants of line 1406, the frequency of homologous recombination was about one blue spot per plant as opposed to untreated plants, which gave rise to about 0.4 spots per plant in the next generation (Fig. 4a). However, recombination in the progeny of plants from a cross where only one parent was UV-treated was as high as in the treated, self-pollinated plants, irrespective of the direction of the cross. Using line 1445, a similar picture emerged (Fig. 4a). Comparable results were obtained in an experiment in which only one crossing partner was treated with flagellin; again, recombination was elevated to a similar level in the offspring of UV-treated outcrossed and selfed plants (Fig. 4b). We conclude that the epigenetic ‘memory’ could be inherited through both gametes in a dominant manner.

The epigenetic change revealed may be inscribed on the entire genome, on a particular locus, or on the transgene of the treated

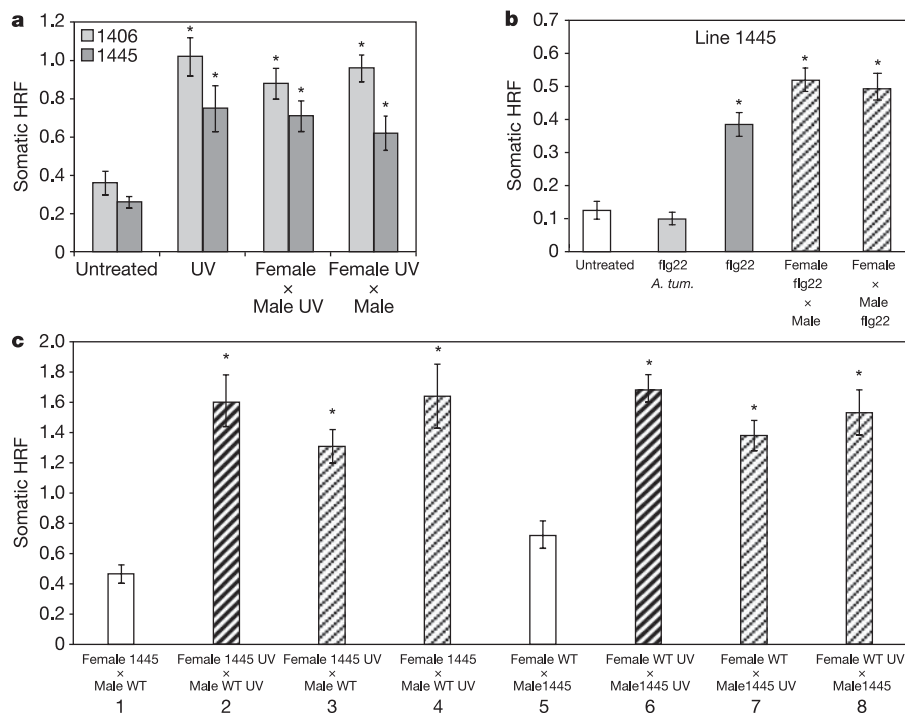


**Figure 3 | Somatic HRF in  $S_0$  plants and in the next four generations.**  $S_0$  plants (line IC1) were either untreated or UV-treated. Somatic HRF was measured in untreated  $S_1$ ,  $S_2$ ,  $S_3$  and  $S_4$  plants. Results are means  $\pm$  s.e.m. ( $n > 50$  plants;  $t$ -test  $*P < 0.05$  compared with the corresponding  $S_0 - UV$  generation).

plants. Although the first two alternatives are difficult to address, we tested whether the irradiated plants have to contain the transgene in order to transmit their epigenetic status to the descending generation. We therefore crossed irradiated or untreated *A. thaliana* wild-type plants lacking the transgene locus with treated or untreated transgenic plants and analysed the somatic homologous recombination frequency of the offspring. When both parental plants were exposed to UV-C, recombination was elevated by a factor of about four in comparison with untreated parents (Fig. 4c, columns 1 and 2, 5 and 6). When an irradiated female transgenic plant was crossed to a non-irradiated male wild-type plant, homologous recombination was increased to a similar extent (column 3). Therefore, the irradiated plant does not require the presence of the transgene in the crossing partner in order to be dominant (this also confirms and extends the data shown above, in which only one parent was irradiated, but both were transgenic; Fig. 4a). Column 4 in Fig. 4c shows the reciprocal experiment: homologous recombination was measured in the offspring of plants in which only the non-transgenic crossing partner was exposed to irradiation, whereas the other partner carried the transgene. Comparison to columns 1, 2 and 3 allows the conclusion that the epigenetic change, measured as increased potential to undergo homologous recombination, can be transmitted through crossing with an irradiated plant lacking the recombination-substrate locus. Therefore, irradiation does not directly change the epigenetic status of the transgene locus, and activation (whatever this 'activation' may be) of the recombination potential of the transgene locus can be accomplished in *trans*. Again, the outcome of this experiment is independent of the direction of performed crosses: columns 5–8 show levels of homologous recombination measured in offspring of crosses in which the female parent was non-transgenic and the pollen-donor carried the transgene.

Influences of the environment on the plant genome have been documented, and include the activation of transposition in maize<sup>7,8</sup>

and Solanaceae<sup>9</sup>; genomic rearrangement following changes in the nutrition of flax<sup>5</sup> and climatic conditions in *Tradescantia*<sup>17</sup>; and alterations in the frequency of homologous recombination following exposure of plants to a variety of agents<sup>2–4,11,18</sup>. These influences have been interpreted as 'genomic shock' (refs 1, 19, 20). Here we demonstrated that environmental influences, specifically ultraviolet radiation and a bacterial elicitor, change the flexibility of the plant genome in somatic tissue of treated plants and in somatic tissue of their progeny. As these influences persist in the entire population of plants, the basis for the change is epigenetic rather than genetic. Plants carrying the transgene locus do not have to face the environmental challenges themselves in order to transmit the epigenetic change to the offspring; the stimulus for an increase of recombination can be imposed in *trans* by a single treated parent. It is unclear, however, whether this stimulation is exerted by activation of a component of the homologous recombination machinery or by rendering the recombination locus more accessible, for instance by chromatin restructuring. Transcriptome analyses of  $S_0$  and  $S_1$  plants originating from untreated or treated (UV-C or flg22) plants did not reveal significant changes in gene expression (data not shown). This indicates that a global transcriptional stimulation of key players of the homologous recombination machinery may not be responsible for the observed phenomenon. However, it cannot be excluded that this functional genomic approach failed to reveal subtle transcriptional changes or induction of microRNAs responsible for the observed transgenerational 'memory' effect. A mechanism resembling paramutation in which one allele changes the epigenetic state of the other can be excluded; the enhancement of recombination can be exerted by a stressed crossing partner that lacks the transgene. The demonstrated epigenetic change is heritable for at least four generations. In this respect, it resembles epimutation such as that in *Linaria vulgaris* affecting floral symmetry<sup>21</sup>, or that in *A. thaliana*, called *SUPERMAN*, also affecting flower development<sup>22</sup>. Because of



**Figure 4 | Somatic HRF in either self-pollinated or outcrossed plants.**

**a**, Somatic HRF in offspring of either self-pollinated untreated plants, UV-treated plants, or plants in which one of the parents was UV-treated ( $n > 40$  plants;  $t$ -test  $*P < 0.05$ ). **b**, Somatic HRF in offspring of either self-pollinated untreated plants (white bar), flg22-treated plants (grey bars), or plants in which one of the parents was flg22-treated (hatched bars;  $n > 35$

plants;  $t$ -test  $*P < 0.05$ ). **c**, Somatic HRF in offspring of plants in which one parent was wild type (WT) and the other harboured the recombination substrate ( $n > 40$  plants;  $t$ -test  $*P < 0.05$ ). White bar, both parents untreated; dark hatching, both parents UV-treated; light hatching, one parent UV-treated. Refer to the main text for the lines used. All the results are means  $\pm$  s.e.m.

the genetic stability of the epigenetic change in recombination potential, it is conceivable that meiotic recombination is affected as well.

We have demonstrated an induced, epigenetic, heritable change of a molecularly defined and quantitatively measured trait. However, an adaptive value of the observed changes cannot easily be evaluated at this point. We propose that the environmental influences that lead to increased genomic dynamics even in successive, untreated generations may increase the potential for adaptive evolution<sup>23</sup>. This work should help in elucidating the underlying molecular mechanism that causes the observed transgenerational 'memory'.

## METHODS

**Plant material.** Homozygous *A. thaliana* lines, IC1 and IC9 (ref. 14), 1406 and 1445 (ref. 24) (ecotype Columbia), 11 and 651 (ref. 25) (ecotype C24), each carrying a single copy of a recombination substrate were used for monitoring somatic homologous recombination frequency (HRF). The recombination substrate consists of a non-functional chimaeric *uidA* (*GUS*) gene containing partially overlapping homologous sequence (Fig. 1a). Lines IC1 and IC9 carry an intermolecular recombination substrate; lines 1406, 1445, 11 and 651 carry an intramolecular recombination substrate. Wild-type *A. thaliana* plants (ecotype Columbia) were also used for crosses. S<sub>1</sub> plants are defined as the progeny of either untreated or treated S<sub>0</sub> plants. S<sub>2</sub>, S<sub>3</sub> and S<sub>4</sub> plants correspond to the subsequent generations.

**Growth conditions.** For soil-cultured plants, seeds were sown (2 per pot) and put at 4 °C in the dark for 3 days. The pots were transferred to a phytotron (70% humidity) and kept under a cycle of 16-h-light (20 °C) and 8-h-dark (16 °C). For *in vitro* culture, plants were germinated on GM medium (MS salts (Duchefa), 1% sucrose, 0.8% agar, pH 5.8). Plants were grown in a culture chamber under a 16-h-light (20 °C) and 8-h-dark (16 °C) photoperiod. Three-week-old plants were transferred to soil and grown for seed production (self-pollination) or crosses.

**Treatment with UV-C and flagellin.** For all experiments, plants were grown *in vitro* on solid GM medium (MS salts (Duchefa), 1% sucrose, 0.8% Agar-agar ultrapur (Merck), pH 5.8) in a culture chamber under a 16-h-light (20 °C) and 8-h-dark (16 °C) photoperiod for 13 days before being subjected to the specific preculture condition established for each treatment. For UV-C treatment, plants were transferred to large Petri dishes (160-mm diameter) containing solid GM medium, to a density of 1 plant per cm<sup>2</sup>. For the UV-C irradiation (254 nm, 6 kerg cm<sup>-2</sup>), a Mineral light-lamp (UV-Products) was used. For the flagellin treatment with either the active peptide (flg22) or the inactive peptide (flg22 *A. tum.*), plants were subcultured for 24 h in 300 µl of liquid GM medium in 24-cell plates. Flg22 or flg22 *A. tum.* (1 µM) diluted in liquid GM medium was applied. Four days after each treatment, plants were transferred to soil and grown for seed production (self-pollination) or crosses.

**Monitoring of somatic homologous recombination events.** For monitoring the somatic homologous recombination events, the histochemical GUS assay<sup>26</sup> was performed on 3-week-old *in vitro* germinated plants. The HRF corresponds to the average number of blue spots (equivalent to the number of homologous recombination events) per plant ± s.e.m., in a population. For each recombination line, experiments were at least duplicated. The *t*-test was used for statistical analyses with *P* = 0.05. Note that somatic HRF can only be compared within experimental series due to the specific culture conditions used for different treatments: solid culture medium and liquid culture medium for UV-C and flg22, respectively.

Received 29 March; accepted 29 June 2006.

Published online 6 August 2006.

1. McClintock, B. The significance of responses of the genome to challenge. *Science* **226**, 792–801 (1984).

2. Ries, G. *et al.* Elevated UV-B radiation reduces genome stability in plants. *Nature* **406**, 98–101 (2000).
3. Lucht, J. M. *et al.* Pathogen stress increases somatic recombination frequency in *Arabidopsis*. *Nature Genet.* **30**, 311–314 (2002).
4. Kovalchuk, I. *et al.* Pathogen-induced systemic plant signal triggers DNA rearrangements. *Nature* **423**, 760–762 (2003).
5. Cullis, C. A. Mechanisms and control of rapid genomic changes in flax. *Ann. Bot. (Lond.)* **95**, 201–206 (2005).
6. Zipfel, C. *et al.* Bacterial disease resistance in *Arabidopsis* through flagellin perception. *Nature* **428**, 764–767 (2004).
7. Mottinger, J. P., Johns, M. A. & Freeling, M. Mutations of the *Adh1* gene in maize following infection with barley stripe mosaic virus. *Mol. Gen. Genet.* **195**, 367–369 (1984).
8. Walbot, V. Reactivation of Mutator transposable elements of maize by ultraviolet light. *Mol. Gen. Genet.* **234**, 353–360 (1992).
9. Grandbastien, M. A. *et al.* Stress activation and genomic impact of Tnt1 retrotransposons in Solanaceae. *Cytogenet. Genome Res.* **110**, 229–241 (2005).
10. Kovalchuk, I., Kovalchuk, O. & Hohn, B. Genome-wide variation of the somatic mutation frequency in transgenic plants. *EMBO J.* **19**, 4431–4438 (2000).
11. Lebel, E. G., Masson, J., Bogucki, A. & Paszkowski, J. Stress-induced intrachromosomal recombination in plant somatic cells. *Proc. Natl Acad. Sci. USA* **90**, 422–426 (1993).
12. Walbot, V. Sources and consequences of phenotypic plasticity in flowering plants. *Trends Plant Sci.* **1**, 27–32 (1996).
13. Swoboda, P., Gal, S., Hohn, B. & Puchta, H. Intrachromosomal homologous recombination in whole plants. *EMBO J.* **13**, 484–489 (1994).
14. Molinier, J., Ries, G., Bonhoeffer, S. & Hohn, B. Interchromatid and interhomolog recombination in *Arabidopsis thaliana*. *Plant Cell* **16**, 342–352 (2004).
15. Schuermann, D., Molinier, J., Fritsch, O. & Hohn, B. The dual nature of homologous recombination in plants. *Trends Genet.* **21**, 172–181 (2005).
16. Felix, G., Duran, J. D., Volko, S. & Boller, T. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.* **18**, 265–276 (1999).
17. Klumpp, A., Ansel, W., Fomin, A., Schnirring, S. & Pickl, C. Influence of climatic conditions on the mutations in pollen mother cells of *Tradescantia* clone 4430 and implications for the Trad-MCN bioassay protocol. *Hereditas* **141**, 142–148 (2004).
18. Puchta, H., Swoboda, P. & Hohn, B. Induction of intrachromosomal homologous recombination in whole plants. *Plant J.* **7**, 203–210 (1995).
19. Jorgensen, R. A. Restructuring the genome in response to adaptive challenge: McClintock's bold conjecture revisited. *Cold Spring Harb. Symp. Quant. Biol.* **69**, 349–354 (2004).
20. Madlung, A. & Comai, L. The effect of stress on genome regulation and structure. *Ann. Bot. (Lond.)* **94**, 481–495 (2004).
21. Cubas, P., Vincent, C. & Coen, E. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* **401**, 157–161 (1999).
22. Jacobsen, S. & Meyerowitz, E. M. Hypermethylated *SUPERMAN* epigenetic alleles in *Arabidopsis*. *Science* **277**, 1100–1103 (1997).
23. Jablonka, E. & Lamb, M. J. *Epigenetic Inheritance and Evolution: The Lamarckian Dimension* (Oxford Univ. Press, Oxford, 1995).
24. Gherbi, H. *et al.* Homologous recombination in *planta* is stimulated in the absence of Rad50. *EMBO Rep.* **2**, 287–291 (2001).
25. Puchta, H., Swoboda, P., Gal, S., Blot, M. & Hohn, B. Somatic intrachromosomal recombination events in populations of plant siblings. *Plant Mol. Biol.* **28**, 281–292 (1995).
26. Jefferson, R. A. Assaying chimeric genes in plants: the GUS gene fusion system. *Plant Mol. Biol. Rep.* **5**, 387–405 (1987).

**Acknowledgements** We acknowledge the critical analysis of the manuscript by O. M. Scheid, D. Schuermann, R. Jorgensen, U. Grossniklaus, D. Schuebeler, L. Comai and T. Boller. We are grateful to the Novartis Research Foundation and the European Union project PLANTREC for financial support.

**Author Information** Reprints and permissions information is available at [www.nature.com/reprints](http://www.nature.com/reprints). The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to B.H. ([barbara.hohn@fmi.ch](mailto:barbara.hohn@fmi.ch)).