

female was exposed to them in turn. A female was placed in the centre of the female compartment within a plastic bag for 5 min, then released and the number and duration of visits (approaches within one body length) to each male was recorded for 5 min (a common index of mate choice^{25,26}, which correlates with mating probability in the goodeid *Girardinichthys multiradiatus*). She was then transferred to her home tank and a new female was tested until all had been exposed to that male pair. This was repeated on consecutive days until all females had been exposed to all male pairs. Position (left or right) of the TYB + male changed between pairs. Female preference in *X. captivus* (faint TYB) and *X. eiseni* and *C. audax* (no TYB) was assessed by painting an artificial TYB to one male of each pair using nail varnish. We proceeded as above but after completing the trials with a male pair, the treatment was reversed and all females were tested again. We used 10 females and 4 male pairs. This protocol was repeated, painting the tails of different species with conspicuous TYB (*A. splendens*, *C. pardalis* and *X. variata*).

Feeding responsiveness

Fish were kept without food for 24 h in the presentation tank, then exposed to two fish kept in green plastic boxes (4 × 2.5 × 2.5 cm) with adjustable walls that enabled only their tails to protrude. Boxes were hung at opposite sides of the tank, and gently rocked with a motor to stimulate tail movement. Before each trial, a partition hid the presentation boxes from the focal fish. Stimuli fish were one male with a conspicuous TYB and one female *X. variata* lacking a TYB (focal *C. pardalis* and *A. splendens* were exposed to *A. splendens* as stimuli). To minimize stress, stimuli fish were briefly exposed to a weak dilution of benzocaine and placed in the presentation boxes. The motor was then started and the partition was removed. Frequency and duration of approaches and frequency of bites to each tail were recorded for 10 min, then both focal and stimuli fish were replaced by new pairs, and the procedure was repeated until all fish had been tested. We also exposed *X. variata* to stimuli fish of species with more conspicuous TYBs (*A. splendens* and *C. pardalis*). Feeding responsiveness of *C. audax* (10 males and 10 females, tested in groups) to TYBs and damselfly larvae was assessed with a similar procedure, except that the male *X. variata* was presented alongside a damselfly larva (*Argia* sp.) of the same length as the male's TYB. We recorded which stimulus was approached first and which was bitten first.

Cost of the TYB

Digital images of tail fins of 11 male and 11 female *A. splendens* from outdoor ponds were measured using Paint Shop Pro v. 7. We traced TYB area, both bridging the gaps ('intact' band), and following the contour of tissue present (actual band). The difference measured the amount of tissue lost. Females lack a TYB but have a discontinuous black sub-terminal band seemingly homologous to the black sub-terminal band that limits the male TYB (Fig. 1). This was used in tracing the area of the female terminal band. The effect of fin regeneration on weight loss was evaluated by removing sections ($23 \pm 7.4\%$) of fin tail in 12 anaesthetized males. These were kept in individual 40-litre aquaria under controlled conditions (including equal feeding opportunity and antibiotics), and were measured, photographed and weighed weekly for two months.

Received 29 November 2004; accepted 14 January 2005; doi:10.1038/nature03363.

1. Fisher, R. A. *The Genetical Theory of Natural Selection* 146–156 (Dover Publications, New York, 1958).
2. Andersson, M. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* **40**, 804–816 (1986).
3. Hamilton, W. D. & Zuk, M. Heritable true fitness and bright birds: a role for parasites? *Science* **218**, 384–387 (1982).
4. Milinski, M. & Bakker, T. C. M. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344**, 330–333 (1990).
5. Holland, B. & Rice, W. R. Chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* **52**, 1–7 (1998).
6. Ryan, M. J. Sexual selection, sensory systems and sensory exploitation. *Oxf. Surv. Evol. Biol.* **7**, 157–195 (1990).
7. Ryan, M. J. & Keddy-Hector, A. Directional patterns of female mate choice and the role of sensory biases. *Am. Nat.* **139**, S4–S35 (1992).
8. Dawkins, M. S. & Guilford, T. Sensory bias and the adaptiveness of female choice. *Am. Nat.* **148**, 937–942 (1996).
9. Endler, J. A. & Basolo, A. L. Sensory ecology, receiver biases and sexual selection. *Trends Ecol. Evol.* **13**, 415–420 (1998).
10. Rodd, F. H., Hughes, K. A., Grether, G. F. & Baril, C. T. A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proc. R. Soc. Lond. B* **269**, 475–481 (2002).
11. Kokko, H., Brooks, R., McNamara, J. M. & Houston, A. I. The sexual selection continuum. *Proc. R. Soc. Lond. B* **269**, 1331–1340 (2002).
12. Kokko, H., Brooks, R., Jennions, M. D. & Morley, J. The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. B* **270**, 653–664 (2003).
13. Cordero, C. & Eberhard, W. G. Female choice of sexually antagonistic male adaptations: a critical review of some current research. *J. Evol. Biol.* **16**, 1–6 (2003).
14. Webb, S. A. *et al.* Molecular phylogeny of the live-bearing Goodeidae (Cyprinodontiformes). *Mol. Phylogenet. Evol.* **30**, 527–544 (2004).
15. LeBas, N., Hockham, L. R. & Ritchie, M. G. Nonlinear and correlational sexual selection on 'honest' female ornamentation. *Proc. R. Soc. Lond. B* **270**, 2159–2165 (2003).
16. Kodric-Brown, A. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav. Ecol. Sociobiol.* **25**, 393–401 (1989).
17. Olson, V. A. & Owens, I. P. F. Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol. Evol.* **13**, 510–514 (1998).
18. Partridge, L. & Parker, G. A. in *Evolution of Biological Diversity* (eds Magurran, A. E. & May, R. M.) 130–159 (Oxford Univ. Press, Oxford, 1999).
19. Basolo, A. L. Congruence between the sexes in preexisting receiver responses. *Behav. Ecol.* **13**(6), 832–837 (2002).

20. Jayasooriya, A. P., Weisinger, R. S., Weisinger, H. S., Mathai, M. L. & Sinclair, A. J. Attraction to orange: sexiness, not gluttony. *Science* **296**, 847–848 (2002).
21. Grether, G. F., Hughes, K. A. & Rodd, F. H. Response to Jayasooriya *et al.* *Science* **296**, 847–848 (2002).
22. Burton, B. G. & Laughlin, S. B. Neural images of pursuit targets in the photoreceptor arrays of male and female houseflies *Musca domestica*. *J. Exp. Biol.* **206**, 3963–3977 (2003).
23. Córdoba-Aguilar, A. Sensory trap as the mechanism of sexual selection in a damselfly genital trait (insecta: calopterygidae). *Am. Nat.* **160**, 594–601 (2002).
24. Holland, B. & Rice, W. R. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc. Natl Acad. Sci. USA* **96**, 5083–5088 (1999).
25. Ryan, M. J. & Wagner, E. Asymmetries in mating preferences between species: female swordtails prefer heterospecific males. *Science* **236**, 595–597 (1987).
26. Basolo, A. L. Evolutionary change in a receiver bias: a comparison of female preference functions. *Proc. R. Soc. Lond. B* **265**, 2223–2228 (1998).

Supplementary Information accompanies the paper on www.nature.com/nature.

Acknowledgements We Thank the Lunáticos behaviour discussion group for constructive discussion throughout, and M. Ritchie, J. Graves and H. Kokko for useful comments on previous versions of the manuscript. A. Gaona and M. Molina helped with some of the behavioural records, and E. Ávila Luna provided logistical aid with fish husbandry and data collection. This project was partly supported with a grant from CONACyT to C.M.G. and is based on E.R.'s BSc thesis (Biology), which was supervised by C.M.G.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to C.M.G. (maciasg@servidor.unam.mx).

Genome-wide non-mendelian inheritance of extra-genomic information in *Arabidopsis*

Susan J. Lolle*, Jennifer L. Victor, Jessica M. Young & Robert E. Pruitt*

Department of Botany and Plant Pathology, Purdue University, 915 W. State Street, West Lafayette, Indiana 47907-2054, USA

*These authors contributed equally to this work

A fundamental tenet of classical mendelian genetics is that allelic information is stably inherited from one generation to the next, resulting in predictable segregation patterns of differing alleles¹. Although several exceptions to this principle are known, all represent specialized cases that are mechanistically restricted to either a limited set of specific genes (for example mating type conversion in yeast²) or specific types of alleles (for example alleles containing transposons³ or repeated sequences⁴). Here we show that *Arabidopsis* plants homozygous for recessive mutant alleles of the organ fusion gene *HOTHEAD*⁵ (*HTH*) can inherit allele-specific DNA sequence information that was not present in the chromosomal genome of their parents but was present in previous generations. This previously undescribed process is shown to occur at all DNA sequence polymorphisms examined and therefore seems to be a general mechanism for extra-genomic inheritance of DNA sequence information. We postulate that these genetic restoration events are the result of a template-directed process that makes use of an ancestral RNA-sequence cache.

We have recovered 11 point mutations at the *hth* locus⁶ that share the unusual property that they segregate phenotypically wild-type plants at a high frequency when homozygous mutant plants are allowed to self-fertilize. The frequency with which these 'revertant' plants are recovered varies, but is generally in the range of 10^{-1} to 10^{-2} revertants per chromosome per generation (Table 1). This is in stark contrast to most point mutations, which are completely stable (for example *erecta* (*er*) in Table 1). Because of the high frequency

with which *hth* phenotypic revertants were observed, we suspected that they might reflect either incomplete penetrance of the mutant phenotype or an epigenetic change that masked the mutant genotype. To determine the genotype of the phenotypic revertants we designed allele-specific polymerase chain reaction (PCR)-based markers. These molecular markers show clearly that the phenotypically wild-type plants observed are heterozygous for the parental *hth* allele (Fig. 1a, Table 1), indicating that the nucleotide sequence of the *hth* gene had been altered in about 10% of the progeny plants. This was confirmed by allowing the revertant plants to self-fertilize and examining the segregation of the *hth* phenotype in the next generation. In every case the mutant phenotype was observed in about 25% of the progeny plants (data not shown).

Two trivial explanations could account for the observed revertant progeny: contamination of our stocks with wild-type seed or outcrossing of the *hth* mutant plants with wild-type pollen. Seed contamination was an unlikely explanation because the apparent revertants are all heterozygous for the specific *hth* allele carried by their parents. To rule out all possibility of seed contamination, individual embryos were dissected out of fruits developing on selfed plants that were genotypically confirmed to be *hth/hth* by using allele-specific PCR-based markers. The genotypes of these embryos with respect to *HTH* were then determined with the same markers. The results in Table 2 show that we can detect frequent *HTH/hth* embryos developing on *hth/hth* parent plants, eliminating the possibility that the observed high frequency of reversion is due to seed contamination. In fact, in this experiment we also detected rare double revertant *HTH/HTH* embryos, which must have inherited one of their two wild-type *HTH* genes from the maternal parent and therefore could not have been the result of outcrossing. The frequency of apparent reversion events observed in embryos is somewhat higher than that observed phenotypically. We believe this probably reflects somatic reversion events occurring in embryonic tissues that do not contribute to the adult plant.

To demonstrate directly that *hth/hth* mutant plants can be a source of wild-type *HTH* alleles, we tested whether it was possible to transmit an *HTH* allele through pollen derived from an *hth/hth* plant. Plants homozygous for *hth-4* in the Landsberg *erecta* (Ler) background were reciprocally crossed with wild-type Columbia (Col) plants and the embryos arising from the crosses were genotyped with respect to *HTH*. Most embryos were heterozygous as expected, but in the crosses where the male parent was homozygous for *hth-4*, 8 of 164 embryos were observed that were of the genotype *HTH/HTH* (reversion frequency 4.9×10^{-2}). Because the Col and Ler accessions differ at numerous DNA sequence polymorphisms, we used accession-specific DNA markers to verify that the *HTH/HTH* embryos were not due to self-pollination of the Col parent. No *HTH/HTH* embryos were detected when the female parent was homozygous for *hth-4* (0 of 230 embryos). These data indicate that *hth/hth* plants can be a source of pollen bearing an *HTH* allele and furthermore that there is a strong bias for genetic changes to occur in the male reproductive system. Taken together, the results show that the wild-type *HTH* alleles observed

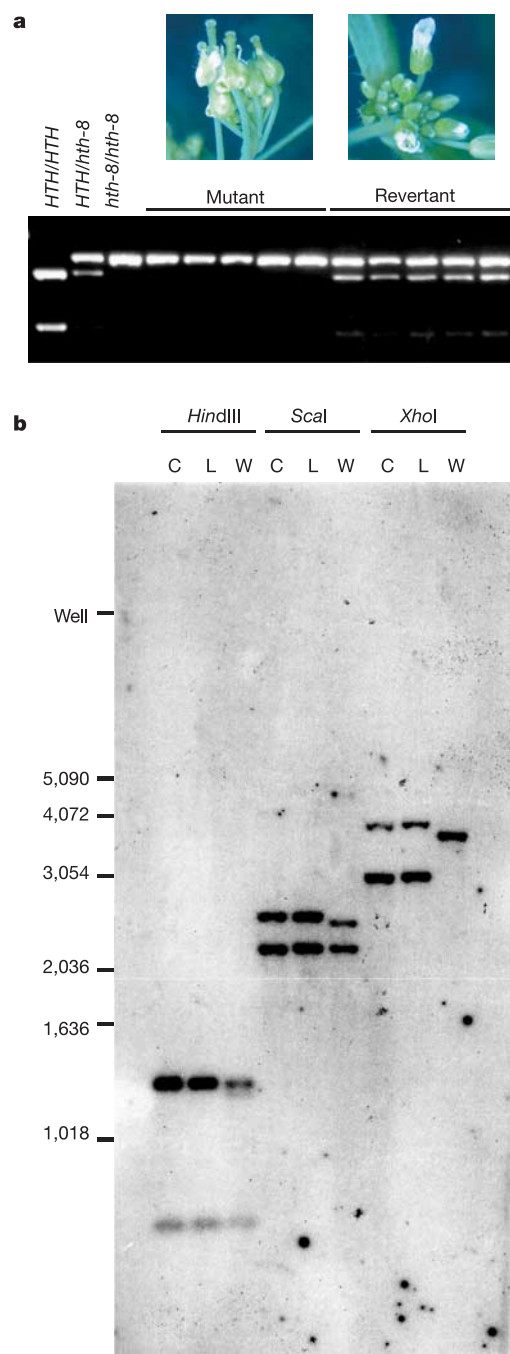


Figure 1 Molecular genetic analysis of *HTH*. **a**, PCR-based assay demonstrating genetic reversion of *hth-8*. DNA was extracted from mutant and revertant progeny that were derived from the self-fertilization of a homozygous *hth-8* parent, amplified with gene-specific primers and digested to reveal the presence or absence of a polymorphic restriction site. Controls in the left three lanes show digest patterns derived from wild-type (*HTH/HTH*), heterozygous (*HTH/hth*) and homozygous (*hth/hth*) mutant plants. Photos above mutant and revertant lanes show the *hth/hth* mutant phenotype and the revertant phenotype. **b**, Genome blot showing that *HTH* is present in a single copy in Col (C), Ler (L) and Ws (W). Genomic DNA was digested as indicated and probed with a labelled fragment of Col DNA corresponding to the *HTH* coding sequence. Expected fragment sizes in base pairs are as follows: for *HindIII*: Col 1,285, 1,253, 664; Ler 1,275, 1,253, 664; Ws 1,253, 1,204, 666; for *Scal*: Col 2,537, 2,187; Ler 2,527, 2,187; Ws 2,466, 2,188; for *XhoI*: Col 3,928, 3,016; Ler 3,918, 3,016; Ws 3,669. Differences in hybridization intensity occur because portions of the Ws sequence have diverged significantly from the Col sequence.

Table 1 Phenotypic and genotypic reversion of *hth* alleles

Mutation	Phenotype*		Genotype of revertants†			Reversion frequency‡
	Mutant	Revertant	<i>hth/hth</i>	<i>hth/HTH</i>	<i>HTH/HTH</i>	
<i>hth-4</i>	491 (92)	43 (8)	0 (0)	38 (100)	0 (0)	4.0×10^{-2}
<i>hth-8</i>	184 (84)	36 (16)	0 (0)	24 (100)	0 (0)	8.2×10^{-2}
<i>hth-10</i>	251 (88)	35 (12)	0 (0)	30 (100)	0 (0)	6.1×10^{-2}
<i>er</i>	1,000 (100)	0 (0)	—	—	—	0

Numbers in parentheses are the percentage of individuals with a given phenotype or genotype.
 *Observed numbers of phenotypically mutant and wild-type progeny derived from the self-fertilization of plants homozygous for the mutant alleles shown in the first column.
 †Genotype of phenotypically wild-type plants determined by PCR analysis. Not all revertant individuals were genotyped.
 ‡Reversion frequency (reversion events per chromosome per generation) calculated on the basis of the observed number of phenotypic revertants and the observed genotypes of the revertants.

in progeny of selfed *hth/hth* mutant plants are not the result of seed contamination or cross-pollination but represent true genetic events manifested at the DNA sequence level.

We considered several conventional explanations for the genetic instability observed at *hth*. DNA sequence analysis of the *HTH* gene rules out the involvement of transposons and repeated sequences as possible explanations. Two additional explanations that we considered were a high rate of random mutation in this particular region of the genome and correction of the gene through a gene conversion mechanism.

If reversion of *hth* to *HTH* were due to a high rate of random mutation at this locus, we would expect to find additional silent sequence changes in the reverted *HTH* allele. Given the frequency with which the mutant nucleotide is observed to revert to wild-type and the 1,226 possible silent nucleotide substitutions in the *HTH* coding sequence, we would expect to see between 49 and 100 other changes on average in the coding sequence of the reverted alleles, assuming that all types of nucleotide substitutions are equally likely. To test this we determined the complete DNA sequence of the coding region of the *HTH* gene after three independent reversion events in each of three independently derived mutant *hth* alleles (*hth-4*, *hth-8* and *hth-10*). In every case the sequence of the reverted *HTH* allele matched the Ler wild-type sequence exactly. Given the very high frequency with which the mutant nucleotide reverts to the wild-type sequence, this result rules out a mechanism dependent on random sequence changes and strongly indicates a mechanism that is template-directed.

In considering the possibility of gene conversion we needed to exclude two types of source template. First, it was possible that the *Arabidopsis* Ler and Wassilewskija (Ws) accessions contained a second copy of the *HTH* gene that was not present in the Col genome sequence. This is unlikely because we have designed many PCR-based genetic markers that allow genotyping of mutant *hth* alleles and none revealed a cryptic wild-type copy of the gene in homozygous *hth* plants. To examine this possibility further, we performed a DNA blotting experiment with genomic Col, Ler and Ws DNA. We were able to detect all of the expected *HTH* fragments in this experiment, and no additional hybridizing sequences were identified (Fig. 1b). Taken together, the PCR and genomic hybridization results effectively rule out the existence of a second cryptic copy of the *HTH* gene.

The second class of template we considered was the family of *HOTHEAD-LIKE* (*HTL*) genes⁶. For a variety of reasons this possibility could also be discounted. For *hth-4*, none of the *HTL* sequences contained the appropriate nucleotide to correct the mutant sequence to wild-type (Fig. 2). For *hth-8* and *hth-10*, some of the *HTL* sequences did contain the relevant nucleotide to correct the mutant sequence, but if any of these related sequences had been used as a corrective template this would have resulted in the incorporation of other nearby DNA sequence changes, unless the conversion tract were limited to just a few nucleotides (Fig. 2). Because no other DNA sequence changes were observed, it seems unlikely that conventional gene conversion is the explanation for the high level of genetic change seen at the *HTH* locus.

In the experiments described above we examined the process of

DNA sequence change at the *hth* locus in an *hth* mutant background. To determine whether DNA sequence changes also occur elsewhere in the genome, we crossed *hth-4*, *hth-8* and *hth-10* mutant plants (in the Ler genetic background) to wild-type Col plants. The *F*₁ hybrids were allowed to self-fertilize and individual *F*₂ plants were genotyped with the use of PCR-based markers to identify those that were homozygous for the mutant or wild-type allele of *HTH*. These *F*₂ individuals were then genotyped for several molecular markers that are polymorphic between Col and Ler, to identify individuals that were homozygous for one parental allele or the other. Randomly chosen *F*₃ progeny from these plants were in turn examined for the stable inheritance of those DNA sequence polymorphisms. In progeny of *hth/hth* *F*₂ plants we observed a high rate of sequence change in both directions (Col to Ler, and Ler to Col), indicating that the mechanism of genetic instability observed at *hth* seems to apply globally to other sequences in the genome (Table 3). Although the polymorphisms examined are all within genes, their locations are in exons, introns and the 3' untranslated region of those genes (Table 3), indicating that presence in the mature mRNA is not required for extra-genomic transmission of sequence information. We also detected phenotypic reversion of the *er* mutation in the progeny of *hth/hth er/er* *F*₂ plants at similar frequencies, demonstrating that the absence of *HTH* leads to the destabilization of *er*, which is in contrast to the stability seen in an *HTH/HTH* background (Table 1). Similarly, inheritance of molecular markers in the progeny of the *HTH/HTH* *F*₂ plants was also completely stable, indicating that the instability observed is dependent on the absence of *HTH* gene function.

Mutation of the *Arabidopsis* *HTH* gene reveals an unusual pattern of genetic transmission in which progeny plants inherit, at a relatively high frequency, DNA sequences different from those

```

HTH      ATTCGGCCGTGTCACACCGC
hth-4     ATTCGGCCGTGTCACACCGC
HTL1      caaCGGCaaTaGACACACCGC
HTL2      gTgtGGgaaaGacATACatC
HTL3      gTgtGGgaaaGacATACatC
HTL4      tactGGgaGgaGACACAGctC
HTL5      ggatGGtCaTaGACACAGGC
HTL6      tgctGGtCaTaGACATACCGC
HTL7      tgctGGtCaTaGACACAGGC

```

```

HTH      CGAGTCTCCAGGAACCAACCC
hth-8     CGAGTCTCCAGGAACCAACCC
HTL1      ttAtTgtCCCGgACaAatCC
HTL2      aGaaTcaCCAGGAACaAACCC
HTL3      aGaaTcaCCAGGAACaAACCC
HTL4      tatcTCTCCAGgActAatCC
HTL5      CaAGTCTCCGGgActAatCC
HTL6      CaAGTCTCCGGgActAatCC
HTL7      CaAGTCTCCGGgActAatCC

```

```

HTH      CAGACTGTTGGAATTACAAAG
hth-10    CAGACTGTTGGAATTACAAAG
HTL1      gAGgtTGTGGgATTACggg
HTL2      gAtcCaccTcaAgTTgtAgcG
HTL3      gAGcCaccTcaAgTTgCAGca
HTL4      CAagtCgTgCGgtTTActgAG
HTL5      CAagCcGTaGGAATcACcAAG
HTL6      CAagCTGTGGgATcACAAAG
HTL7      CAagtCgTGGaATcACAAAG

```

Figure 2 DNA sequences of *HTH* and *HTL* genes around the sites of mutation in *hth-4*, *hth-8* and *hth-10*. The location of the mutant nucleotide is boxed, and the wild-type nucleotide at this position is shown in blue; differences from the *HTH* sequence are shown in red lower-case letters. For *hth-4* none of the related sequences contain the wild-type nucleotide at the site of the mutation, for *hth-8* all of the other sequences contain the appropriate wild-type nucleotide, and for *hth-10* some of the other sequences contain the relevant wild-type nucleotide. In all cases there are sequence differences between the *HTL* sequences and *HTH* within a few nucleotides of the site of the mutation.

Table 2 Genotypic reversion of embryonic progeny of *hth/hth* parents

Mutation	Genotype of embryos*			Reversion frequency†
	<i>hth/hth</i>	<i>hth/HTH</i>	<i>HTH/HTH</i>	
<i>hth-4</i>	174 (75)	57 (24)	2 (1)	1.3×10^{-1}
<i>hth-8</i>	67 (62)	41 (38)	0 (0)	1.9×10^{-1}
<i>hth-10</i>	222 (84)	43 (16)	0 (0)	8.1×10^{-2}

Numbers in parentheses are the percentage of embryos with a given genotype.

* Observed numbers of embryos with each genotype derived from the self-fertilization of plants homozygous for the mutant alleles shown in the first column.

† Reversion frequency (reversion events per chromosome per generation) calculated on the basis of the observed frequencies of embryonic genotypes.

carried by their parents. Although this was initially observed as a result of the instability of nucleotide substitution mutations in the *hth* gene itself, we have subsequently shown that loss of *HTH* gene function also results in the genetic instability of DNA sequences located throughout the *Arabidopsis* genome. However, the instability observed is not random but seems to be confined to the restoration of sequence information in progeny plants that, although absent from the parent genome, was present in the genome of an earlier ancestor. Thus, we believe that mutant *hth* alleles are able to revert to wild type because recent ancestors of the *hth/hth* mutant plants had a wild-type copy of the *HTH* gene and, similarly, that we can identify sequence polymorphism changes in plants whose parents were homozygous for one form of the polymorphism because they had grandparents that were heterozygous for the same polymorphism. Sequencing of multiple independently reverted *hth* alleles revealed no DNA sequence changes other than the restoration of the mutant nucleotide to the wild type, also indicating that the process of sequence change is non-random.

Similar patterns of inheritance have been observed previously under various exceptional circumstances. It has been reported that molecular markers absent from several generations of a population of synthetic polyploid plants can reappear subsequently in the population⁷. It has also been reported that random amplified polymorphic DNA (RAPD) markers can in rare instances be transmitted from stock to scion after grafting⁸. In addition to reports of aberrant inheritance of molecular markers, there are also numerous reports of 'replicating instabilities', which seem to propagate both wild-type and mutant alleles in a single copy of a gene⁹. All of these occurred at much lower frequencies than we have observed in the *hth* mutant background, but could be explained by a common mechanism involving the non-mendelian inheritance of sequence information maintained outside the conventional DNA genome.

On the basis of the high degree of specificity seen in the observed DNA sequence changes, we propose that this is a template-directed process. Because we have been unable to detect a DNA template by either genomic DNA blotting or PCR, a logical alternative is that the template is instead provided by an RNA molecule. We propose a model in which a type of stable RNA, possibly in a double-stranded form, can be replicated and transmitted over multiple generations. This template must under some circumstances be capable of modifying the DNA sequence of the nuclear genome to restore sequence information cached from previous generations. Given that this type of sequence modification occurs globally throughout the genome, we think it likely that the postulated genome-wide sequence cache also exists in wild-type plants and that the *hth* mutation simply increases the frequency with which DNA sequences are modified.

Although the model proposed above might represent an extraordinary view of inheritance, each of the processes required by the model has been demonstrated previously. Research performed over the past few years in post-transcriptional gene silencing or RNA interference has shown that silencing induced by double-stranded

RNA can persist for several generations in *Caenorhabditis elegans*¹⁰ and can spread through the organism in plants, fungi and animals^{11,12}. This strongly implies that some form of nucleotide sequence information must be stably replicated and transmitted outside the DNA genome, which is consistent with a requirement for an RNA-directed RNA polymerase in post-transcriptional gene silencing^{13–15}. Replication of the sequence cache could allow it to persist for several generations; this is consistent with the fact that *hth* mutations retain their ability to revert even after multiple generations in the homozygous state (S.J.L. and R.E.P., unpublished observations). Experiments to detect changes in the reversion frequency after several generations of homozygous propagation are in progress. Both double-stranded RNA and microRNA have been implicated in the sequence-specific modification of the nuclear genome by DNA methylation^{16–18} and it has been possible to perform site-directed mutagenesis by the introduction of chimaeric RNA–DNA oligonucleotides in both plant and animal cells^{19–21}. We propose that a mechanism combining these individual steps could explain the exceptional inheritance we see in *hth* mutants as well as the unusual results observed by earlier workers.

It is tempting to speculate on the function of this unusual mechanism of inheritance in the wild-type organism. On the basis of our observation that progeny of *hth* mutant plants seem to inherit a wide variety of sequence information missing from the DNA genomes of their parents, one possibility is that this mechanism provides a cache of genetic variation beyond that carried in the chromosomes. If this information can then be copied back into the DNA genome it could allow self-fertilizing species such as *Arabidopsis* to avoid the negative consequences of inbreeding. Perhaps even more intriguing is the possibility that the inheritance of non-genomic templates occurs in wild-type and *hth* plants, but the rate at which those templates are used to modify genomic sequences is elevated in *hth* as a result of an indirect 'stress' put on the plant by the absence of the HTH gene product. Under these circumstances one could envisage a mechanism in which additional allelic information is maintained outside the normal genomic context but could be used under conditions that compromised the continued functioning of the organism. The extra-genomic information used by the organism would not be random in this case but would contain a library of potential allelic sequences that were previously carried in the DNA of its ancestors, all of which must have survived to reproduce. These sequences would therefore represent a collection of alleles already known to be functional under at least some circumstances, and the restoration of these sequences to the DNA genome might represent a mechanism for the organism to recover from a genotype that was poorly adapted to its present environment. Any sequence changes that resulted in the elimination of the 'stress' would also decrease the rate of sequence alteration back to its normal background level, effectively fixing a favourable genotype. This is at least superficially similar to some models proposed to account for 'adaptive' mutation in micro-organisms^{22–24} and it is possible that recent work describing increased rates of genomic change in response to environmental stress^{25,26} and pathogen attack²⁷ reflect the action of such a mechanism. It is also possible that some of the cryptic genetic variation uncovered through the loss of Hsp90 function^{28,29} is actually present in the postulated ancestral sequence cache and is restored to the DNA genome in response to a lower level of Hsp90 activity.

Whatever molecular mechanism leads to the unusual pattern of genetic inheritance we have observed in *hth* mutants, the genetic results presented here indicate the existence of an extra-genomic mechanism that leads to the high-frequency modification of DNA sequences in a template-directed manner. The structure of the templates together with the mechanisms by which they are produced, inherited and used remain questions for future research. It will be of great interest to see how widely this mechanism of inheritance is distributed throughout the tree of life. □

Table 3 Genetic instability of molecular markers in the *hth* background

Marker	F ₂ genotype (parents)		Location in gene
	<i>hth/hth</i>	<i>HTH/HTH</i>	
AG	7/186 (4)	0/190 (0)	Intron
GAPC	9/242 (4)	0/190 (0)	Intron
GL1	1/90 (1)	0/196 (0)	3'-UTR
HTH	10/484 (2)	0/590 (0)	Exon
RGA	14/402 (3)	0/386 (0)	Exon
UFO	16/438 (4)	0/196 (0)	Exon

Fractions in the table represent the observed number of non-parental alleles/total number of chromosomes examined for a non-selected sample of F₃ progeny derived by the self-fertilization of F₂ plants with the genotype indicated at the top of the table. Numbers in parentheses indicate the percentage of non-parental alleles observed in the F₃ progeny. UTR, untranslated region. Further information on markers is available on TAIR (<http://www.arabidopsis.org>).

Methods

DNA extraction, blotting, labelling and hybridization techniques were as described previously⁵. Genomic DNA sequences were determined by amplifying portions of the *hth* gene and directly sequencing the PCR product³⁰. DNA samples for the PCR genotyping of embryos were obtained by dissecting individual embryos from siliques and surrounding maternal seed coat tissue, crushing the embryo with a plastic pestle in a Microfuge tube and resuspending the resulting homogenate in 20 µl of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). A 1 µl portion of this sample was used in a standard PCR reaction.

Received 8 December 2004; accepted 21 January 2005; doi:10.1038/nature03380.

- Mendel, G. Versuche über Pflanzen Hybriden. *Verhandl. Naturforsch. Ver. Brünn* **4**, 3–47 (1866).
- Klar, A. J., Fogel, S. & Lusnak, K. Gene conversion of the mating-type locus in *Saccharomyces cerevisiae*. *Genetics* **92**, 777–782 (1979).
- McClintock, B. The origin and behavior of mutable loci in maize. *Proc. Natl Acad. Sci. USA* **36**, 344–355 (1950).
- Gondo, Y. *et al.* High-frequency genetic reversion mediated by a DNA duplication: the mouse pink-eyed unstable mutation. *Proc. Natl Acad. Sci. USA* **90**, 297–301 (1993).
- Lolle, S. J., Hsu, W. & Pruitt, R. E. Genetic analysis of organ fusion in *Arabidopsis thaliana*. *Genetics* **149**, 607–619 (1998).
- Krolkowski, K. A., Victor, J. L., Nussbaum Wagler, T., Lolle, S. J. & Pruitt, R. E. Isolation and characterization of the *Arabidopsis* organ fusion gene *HOTHEAD*. *Plant J.* **35**, 501–511 (2003).
- Song, K., Lu, P., Tang, K. & Osborn, T. C. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. *Proc. Natl Acad. Sci. USA* **92**, 7719–7723 (1995).
- Taller, J., Hirata, Y., Yagishita, N., Kita, M. & Ogata, S. Graft-induced genetic changes and the inheritance of several characteristics in pepper (*Capsicum annuum* L.). *Theor. Appl. Genet.* **97**, 705–713 (1998).
- Auerbach, C. & Kilbey, B. J. Mutation in eukaryotes. *Annu. Rev. Genet.* **5**, 163–218 (1971).
- Fire, A. *et al.* Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811 (1998).
- Cogoni, C. *et al.* Transgene silencing of the *al-1* gene in vegetative cells of *Neurospora* is mediated by a cytoplasmic effector and does not depend on DNA-DNA interactions or DNA methylation. *EMBO J.* **15**, 3153–3163 (1996).
- Palauqui, J. C., Elmayan, T., Pollien, J. M. & Vaucheret, H. Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. *EMBO J.* **16**, 4738–4745 (1997).
- Cogoni, C. & Macino, G. Gene silencing in *Neurospora crassa* requires a protein homologous to RNA-dependent RNA polymerase. *Nature* **399**, 166–169 (1999).
- Dalmay, T., Hamilton, A., Rudd, S., Angell, S. & Baulcombe, D. C. An RNA-dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. *Cell* **101**, 543–553 (2000).
- Mourrain, P. *et al.* *Arabidopsis* SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. *Cell* **101**, 533–542 (2000).
- Wassengger, M., Heimes, S., Riedel, L. & Sanger, H. L. RNA-directed de novo methylation of genomic sequences in plants. *Cell* **76**, 567–576 (1994).
- Mette, M. F., Aufsatz, W., van der Winden, J., Matzke, M. A. & Matzke, A. J. Transcriptional silencing and promoter methylation triggered by double-stranded RNA. *EMBO J.* **19**, 5194–5201 (2000).
- Bao, N., Lye, K.-W. & Barton, M. K. MicroRNA binding sites in *Arabidopsis* class III HD-ZIP mRNAs are required for methylation of the template chromosome. *Dev. Cell* **7**, 653–662 (2004).
- Cole-Strauss, A. *et al.* Correction of the mutation responsible for sickle cell anemia by an RNA-DNA oligonucleotide. *Science* **273**, 1386–1389 (1996).
- Beetham, P. R., Kipp, P. B., Sawicky, X. L., Arntzen, C. J. & May, G. D. A tool for functional plant genomics: chimeric RNA/DNA oligonucleotides cause *in vivo* gene-specific mutations. *Proc. Natl Acad. Sci. USA* **96**, 8774–8778 (1999).
- Zhu, T. *et al.* Targeted manipulation of maize genes *in vivo* using chimeric RNA/DNA oligonucleotides. *Proc. Natl Acad. Sci. USA* **96**, 8768–8773 (1999).
- Hall, B. G. Spontaneous point mutations that occur more often when advantageous than when neutral. *Genetics* **126**, 5–16 (1990).
- Foster, P. L. & Cairns, J. Mechanisms of directed mutation. *Genetics* **131**, 783–789 (1992).
- Galitski, T. & Roth, J. R. A search for a general phenomenon of adaptive mutability. *Genetics* **143**, 645–659 (1996).
- Ries, G. *et al.* Elevated UV-B radiation reduces genome stability in plants. *Nature* **406**, 98–101 (2000).
- Bjedov, I. *et al.* Stress-induced mutagenesis in bacteria. *Science* **300**, 1404–1409 (2003).
- Kovalchuk, I. *et al.* Pathogen-induced systemic plant signal triggers DNA rearrangements. *Nature* **423**, 760–762 (2003).
- Queitsch, C., Sangster, T. A. & Lindquist, S. Hsp90 as a capacitor of phenotypic variation. *Nature* **417**, 618–624 (2002).
- Rutherford, S. L. & Lindquist, S. Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342 (1998).
- Pruitt, R. E., Vielle-Calzada, J. P., Ploense, S. E., Grossniklaus, U. & Lolle, S. J. *FIDDLEHEAD*, a gene required to suppress epidermal cell interactions in *Arabidopsis*, encodes a putative lipid biosynthetic enzyme. *Proc. Natl Acad. Sci. USA* **97**, 1311–1316 (2000).

Acknowledgements We thank R. M. Lee and T. Nussbaum Wagler for technical assistance, and J. A. Banks, J. L. Bowman, U. Grossniklaus, R. A. Jorgensen, J. Ogas, D. R. Smyth, A. Steimer, V. Sundaresan and M. A. Webb for discussions. This work was supported by the National Science Foundation. Any opinion, findings, conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to R.E.P. (pruitt@purdue.edu).

Independent recruitment of a conserved developmental mechanism during leaf evolution

C. Jill Harrison*, Susie B. Corley*, Elizabeth C. Moylan†, Debbie L. Alexander†, Robert W. Scotland & Jane A. Langdale

Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK

* These authors contributed equally to this work

† Present addresses: BioMed Central, Middlesex House, 32–42 Cleveland Street, London W1T 4LB, UK (E.C.M.); Carnegie Institution of Washington, Department of Plant Biology, 260 Panama Street, Stanford, California 94305, USA (D.L.A.)

Vascular plants evolved in the Middle to Late Silurian period, about 420 million years ago¹. The fossil record indicates that these primitive plants had branched stems with sporangia but no leaves. Leaf-like lateral outgrowths subsequently evolved on at least two independent occasions^{2–4}. In extant plants, these events are represented by microphyllous leaves in lycophytes (club-mosses, spikemosses and quillworts) and megaphyllous leaves in euphyllophytes (ferns, gymnosperms and angiosperms). Our current understanding of how leaves develop is restricted to processes that operate during megaphyll formation. Because microphylls and megaphylls evolved independently, different mechanisms might be required for leaf formation. Here we show that this is not so. Gene expression data from a microphyllous lycophyte, phylogenetic analyses, and a cross-species complementation experiment all show that a common developmental mechanism can underpin both microphyll and megaphyll formation. We propose that this mechanism might have operated originally in the context of primitive plant apices to facilitate bifurcation. Recruitment of this pathway to form leaves occurred independently and in parallel in different plant lineages.

Microphylls and megaphylls are determinate organs produced on the flanks of indeterminate shoot apical meristems (SAMs)². The formation of all vascular plant leaves therefore involves the addition of a determinate growth programme to the indeterminate apical growth programme. Microphylls develop simply with a single vascular trace, whereas megaphylls develop complex vasculature and variable shape. The genetic basis of the developmental transition from indeterminate growth in the apex to determinate growth in the leaf has so far been studied only in euphyllophyte species with megaphyllous leaves. Indeterminate apical growth is marked by class I *knotted1*-like homeobox (*KNOX*) gene expression^{5–12}. Conversely, determinate leaf growth is marked by transcriptional^{5–10}, or possibly post-transcriptional¹², repression of *KNOX* activity. In *Arabidopsis*, maize and *Antirrhinum*, MYB orthologues (*ASYMMETRIC LEAVES1*, *ROUGH SHEATH2* and *PHANTASTICA* (*ARP*), respectively) maintain the *KNOX*-off state in leaves^{13–16}. *KNOX* and *ARP* genes are expressed in mutually exclusive domains. In loss-of-function *arp* and gain-of-function *KNOX* mutants, ectopic foliar *KNOX* expression leads to indeterminate growth such that simple leaves become lobed^{6,13–17}. Therefore, in these three species and also in tobacco¹⁸, *KNOX*–*ARP* interactions in the shoot apex regulate the balance between indeterminate and determinate growth.

KNOX–*ARP* interactions facilitate megaphyll formation in both monocots and eudicots, suggesting that aspects of leaf development are conserved between groups that diverged about 140 million years ago¹⁹. Involvement of the same mechanism in the formation of microphylls would imply the independent recruitment of identical processes in species that diverged more than 350 million years ago. To investigate the meristem-to-leaf transition in a lycophyte, we have examined *KNOX*–*ARP* interactions in *Selaginella kraussiana*, a

Copyright of Nature is the property of Nature Publishing Group and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.