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 ± 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.

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

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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 alleles[1]. 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 yeast[2]) or specific types of alleles (for example alleles containing transposons[3] or repeated sequences[4]). 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* locus[6] 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

Table 1 **Phenotypic and genotypic reversion of *hth* alleles**

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

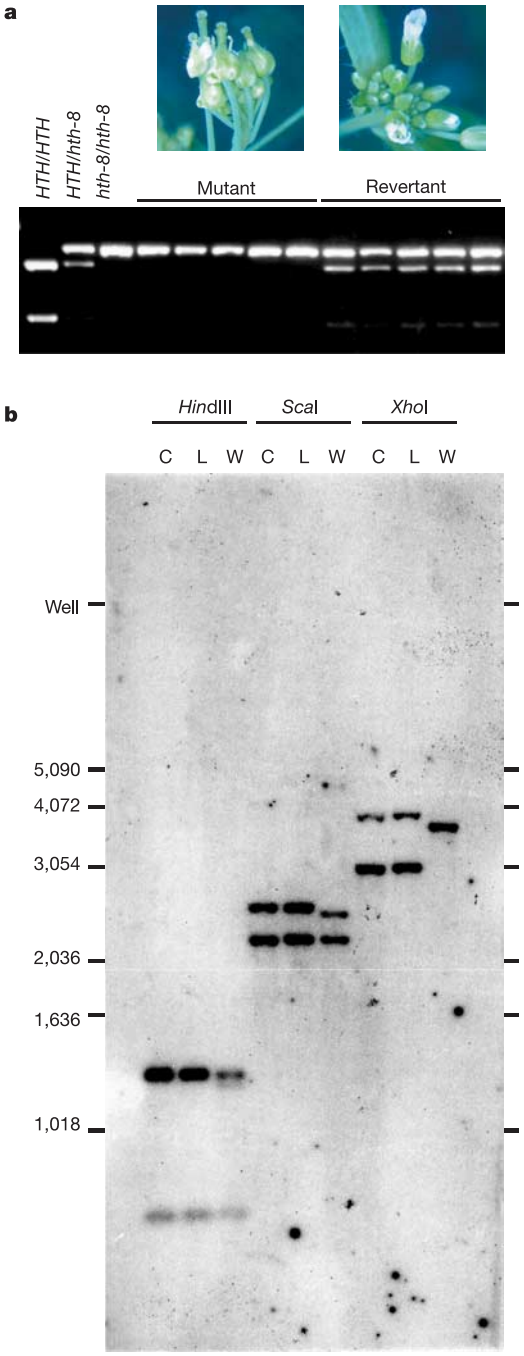

Figure 1 Molecular genetic analysis of *HTH*. **a**, PCR-based assay demonstrating genetic reversion of *hth-8*. DNA was extracted from mutant and revertant progeny that were derived from the self-fertilization of a homozygous *hth-8* parent, amplified with gene-specific primers and digested to reveal 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 *Hin*dIII: Col 1,285, 1,253, 664; Ler 1,275, 1,253, 664; Ws 1,253, 1,204, 666; for *Sca*I: Col 2,537, 2,187; Ler 2,527, 2,187; Ws 2,466, 2,188; for *Xho*I: 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.

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*) genes[6]. 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

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

Mutation	Genotype of embryos*			Reversion frequency†
	hth/*hth*	*hth*/*HTH*	*HTH*/*HTH*	
hth-4	174 (75)	57 (24)	2 (1)	1.3×10^{-1}
hth-8	67 (62)	41 (38)	0 (0)	1.9×10^{-1}
hth-10	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.

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.

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 population[7]. It has also been reported that random amplified polymorphic DNA (RAPD) markers can in rare instances be transmitted from stock to scion after grafting[8]. 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 gene[9]. 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 attack[27] 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
	hth/*hth*	*HTH*/*HTH*	
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 F_3 progeny derived by the self-fertilization of F_2 plants with the genotype indicated at the top of the table. Numbers in parentheses indicate the percentage of non-parental alleles observed in the F_3 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 previously[5]. Genomic DNA sequences were determined by amplifying portions of the *hth* gene and directly sequencing the PCR product[30]. 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.

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Independent recruitment of a conserved developmental mechanism during leaf evolution

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Vascular plants evolved in the Middle to Late Silurian period, about 420 million years ago[1]. 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-transcriptional[12], 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 tobacco[18], 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 ago[19]. 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