

RNA back up rules out (?) the rule that ruled for long....

R. S. Sangwan and N. S. Sangwan

Mendel's laws¹ of genetics have been absolute, dictating the pattern of inheritance from one generation to the next all across plants and animals. Mendelism is the benchmark to infer results of inheritance of gene(s) across generations, be it breeding of genotypes or genetic/metabolic engineering. Mendelism or DNA model of transmission of genes/traits teaches that once homozygosity of introduced/ altered gene is achieved through recurrent self-breeding and selection or otherwise, the altered/mutated genotypes completely leave the genetic connection (of the trait/gene under reference) with the wild type ancestors. There is no chance or reason for the genotypes to recapture the ancestral wild type gene so long as the progeny is fully inbred. However, absolute belief in this notion has now been watered down, at least in plants, by recent researches by Lolle *et al.*². Their study compels one to think at a parallel inheritance system outside DNA in plants. The speculations favour existence of a 'RNA genome' that could be inherited as a back-up copy of genes to bring them in for genetic rescue/emergency gene transplant (intra), although sufficient clues are lacking so far.

A team led by Bob Pruitt has been working on *HOTHEAD* (*HTH*) gene of *Arabidopsis*. The gene is responsible for wax cuticle production that plays an important role in abiotic and biotic interactions between the plant and its environment, protection from water-loss, radiation and pathogens³ and ensuring proper separation and expansion of organs at the growing points of the plant^{3,4}. *HTH* is a member of a small gene family that encodes an enzyme related to FAD-containing oxidoreductases and is involved in regulating post-genital organ fusion³. The researchers developed 11 point mutations at the *hth* locus³ that had the mutation-associated organ fusion (clumped flowers with floral parts fused to form tight balls)³. In selfing experiments on the mutants homozygous for the recessive allele of the gene (*hth/hth*), some of the F1 plants had only one flawed allele (*hth*) of the *HOTHEAD* out of the two (i.e. were genotypically heterozygous, *hth/HTH*) and thus 'reverted' to wild-type phenotype. This was in stark contrast to the tenets of Mendelian

genetics (Figure 1). When these 'revertants' were inbred, the next generation followed Mendelian law of segregation, i.e. one-fourth of the offspring had both alleles of the gene mutated (homozygous, *hth/hth*), whereas rest three-quarter had either one mutated (*hth/HTH*) or none mutated (*HTH/HTH*) alleles of the gene [typical Mendelian genotypic ratio of 1:2:1 and phenotypic ratio of 3:1 (wild type: hothead) for a heterozygote].

Most surprisingly, revertants were encountered at a high frequency (10^{-1} to 10^{-2} per chromosome per generation) in the progeny. Molecular analysis of the phenotypic revertants for allele-specific polymerase chain reaction (PCR)-based markers revealed that these plants were genotypically heterozygous (*hth/HTH*) and had acquired a copy of the ancestral wild type allele (*HTH*). Thus, revertants did not originate due to epigenetic effects or incomplete penetrance of the mutant phenotype. Rather, nucleotide sequence of the gene had altered from *hth* to *HTH*. Possibilities of contamination by seeds of wild type and/or out-crossing with pollens of wild type were excluded as the revertants were heterozygous for the allele and allele-specific PCR analysis detected *HTH/hth* embryos developing on *hth/hth* parent plants.

Origin of *HTH/hth* trait during inbreeding of *hth/hth* was confirmed by the team elucidating that *HTH* allele in the revertants could be transmitted through pollen from a *hth/hth* plant across the accessions using another point mutation of the gene (*hth-4*). When *hth-4/hth-4 Landsberg erecta* background was reciprocally crossed with wild type Columbia plants, most of the embryos were *hth-4/HTH-4* (Mendelism), but when male parent was *hth-4/hth-4*, 8 out of 64 embryos were homozygously wild type (*HTH-4/HTH-4*; Figure 1). Accession-specific DNA marker analysis proved that pollen bearing *HTH* allele originated from *hth/hth* (as a true genetic event), rather than any error of selfing within Columbia parents.

DNA sequence analysis of *HTH* gene ruled out involvement of either transposons and/or repeated sequences in the phenomenon. High rates of random mutations in this particular region (*hth*) of the

genome were excluded by discerning that complete DNA sequence of the coding region of the *HTH* gene in revertants was exactly the same as in the *L. erecta* wild type, i.e. the reverted *HTH* allele lacked silent sequence changes. This, together with the high frequency of mutants reverting to wild type, led researchers to strongly propose it as a template-directed process rather than a random sequence-change associated one. Further, the team has excluded the possibility of gene conversion in the phenomenon through PCR and genomic hybridization results with *Arabidopsis* Columbia, *L. erecta* and *Wassilewskija* accessions. According to current concepts of genetics, in plants and animals, replicable and transmittable genetic (allelic) information is present only as DNA sequences and is stably inherited from one generation to the next, resulting in predictable segregation pattern of differing alleles. The physical and chemical nature of *HTH* allele (gene sequence) resource silently existing in *hth/hth* genotype, 'cryptically inherited' from ancestors and the mechanism of bringing it into genotypic and phenotypic functionality are moot points. Although more appropriately, so far that it connotes 'non-DNA inheritance', the phenomenon is envisaged more as a 'functional back-up RNA genome inheritance'.

It has been speculated that mutant *hth* alleles were able to revert to wild type because recent ancestors of the *hth/hth* organ fusion mutant plants⁴ had a wild type copy (*HTH*) of the gene, and this was a template-directed process. Unable to detect a DNA template by either genomic DNA blotting or PCR, a putative alternative template provided by an RNA molecule has been rationalized/envisaged. The proposed model invokes a type of stable (possibly double-stranded), replicable and heritable RNA that under some circumstances might be modifying nuclear genome DNA sequences to restore sequence information cached from previous generations. However, circumstances that might have steered catching of the ancestral wild type back-up copy of the gene are not visible. The strongest support in favour of the RNA model is that silencing induced by double-stran-

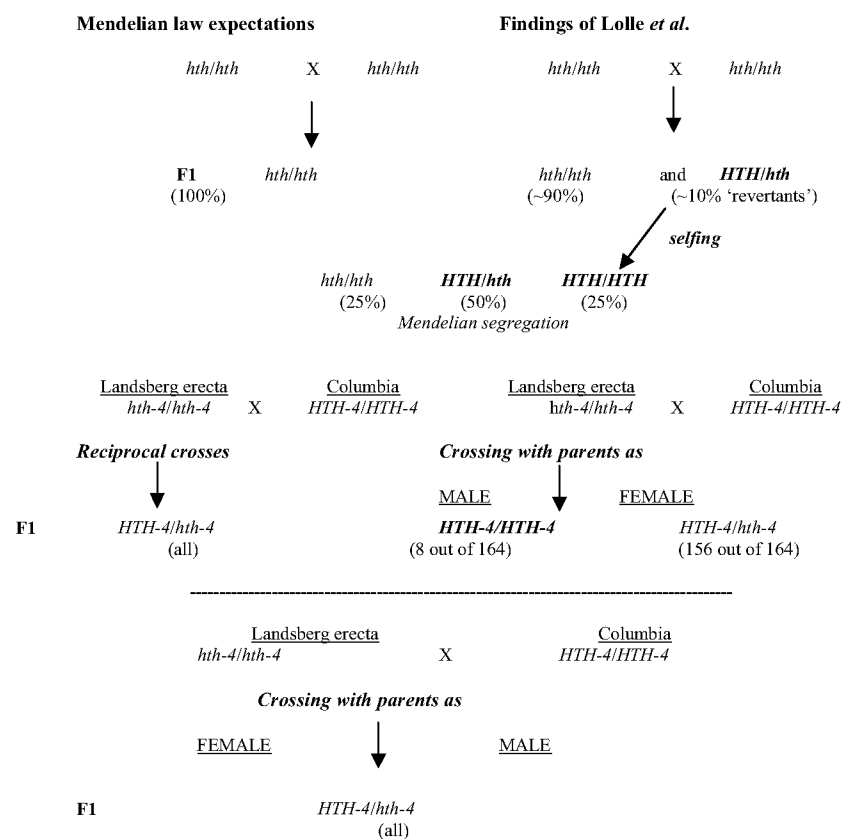


Figure 1. Observed pattern of trait (HOTHEAD mutations) inheritance in *Arabidopsis* deviated from Mendelian rules that leads to non-DNA (speculatively RNA) backed-up view of inheritance. Deviated genotypes are shown in bold.

ded RNA can persist for several generations in *Caenorhabditis elegans* and can spread through the organisms in plants, animals and fungi⁵.

If proved, RNA would get a significant role in inheritance in plants, but some of the aims of gene-knockouts of plant metabolism through metabolic engineering could be tough to achieve. Particularly, blocking of some function(s)/steps may involve stress on the plant beyond its threshold to invite fight-back from the 'cryptic RNA genome'. Of course, the phenomenon may help understand some of the interesting results on transgenic plants like those with tissue-specific plant co-suppression or gene-knockouts of metabolism⁶. Fundamentally, questions regard-

ing organization, size, intracellular localization, bio-interactions, etc. of replicable and heritable back-up genome of RNA become due concomitantly. Some take the theory as 'unbelievably true', whereas others prefer to wait till more results roll up in favour. According to Pruitt, 'Mendel's laws are fundamentally correct but not absolute'.

1. Mendel, G., *Naturforsch. Ver. Brunn*, 1866, **4**, 3–47.
2. Lolle, S. J., Jennifer, L. V., Jessica, M. Y. and Pruitt, R. E., *Nature*, 2005, **434**, 505–509.
3. Krolkowski, K. A., Victor, J. L., Nussbaum, W. T., Lolle, S. J. and Pruitt, R. E., *Plant J.*, 2003, **35**, 501–511.

4. Lolle, S. J., Hsu, W. and Pruitt, R. E., *Genetics*, 1998, **149**, 607–619.
5. Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C., *Nature*, 1998, **391**, 744–745.
6. Gottlob-McHugh, S. G. *et al.*, *Plant Physiol.*, 1992, **100**, 820–825.

*R. S. Sangwan** and *N. S. Sangwan*¹ are in the Central Institute of Medicinal and Aromatic Plants, Lucknow 226 015, India; ¹Present address: BOYSCAST Fellow at Samuel Roberts Noble Foundation, 2510 Noble Parkway, PO Box 2180, Ardmore, OK 73402, USA

*e-mail: rs.sangwan@cimap.res.in