

PLANT GENETICS

Increased outcrossing in hothead mutants

Arising from: S. J. Lolle, J. L. Victor, J. M. Young and R. E. Pruitt *Nature* 434, 505–509 (2005)

Lolle *et al.*¹ report that loss-of-function alleles of the *HOTHEAD* (*HTH*) gene in *Arabidopsis thaliana* are genetically unstable, giving rise to wild-type revertants. On the basis of the reversion of many other genetic markers in *hth* plants, they suggested a model in which a cache of extragenomic information could cause genes to revert to the genotype of previous generations. In our attempts to reproduce this phenomenon, we discovered that *hth* mutants show a marked tendency to outcross (unlike wild-type *Arabidopsis thaliana*, which is almost exclusively self-fertilizing²). Moreover, when *hth* plants are grown in isolation, their genetic inheritance is completely stable. These results may provide an alternative explanation for the genome wide non-mendelian inheritance reported by Lolle *et al.*

Initially, we constructed *hth-12 gl1-4* double-mutant plants in the Columbia ecotype, reasoning that *HTH* and *GL1* should revert independently because they are on different chromosomes. *hth-12* DNA carries a thymidine (T) insertion (SALK_024611) and *gl1-4* is a guanine-to-adenine (G-to-A) transition mutation (like that shown previously to revert¹) that changes the start codon of the trichome gene *GL1* (ref. 3) from ATG to ATA. Among 1,597 progeny of *hth-12 gl1-4* plants, 10 were phenotypically *GL1* (normal trichomes). Genotyping based on polymerase chain reaction showed that nine were heterozygous for *gl1-4*, and one was *GL1/GL1*. Surprisingly, the nine *GL1/gl1-4* plants were also heterozygous for *hth-12*, and the *GL1/GL1* homozygote was homozygous for *HTH*. These observations are most easily explained by pollen contamination (nine heterozygous plants) and seed contamination (one homozygous plant). We also found a single *hth-*

12 heterozygote that was still homozygous for *gl1-4*, which could be explained by pollen contamination from nearby *gl1-4* plants.

To test whether pollen contamination could be a source of apparent *hth* genetic reversion, we grew homozygous *hth-12* plants either in a mixed population (near to, but not touching, plants with varied genotypes) or in an isolated room containing only *hth-12* plants. In one experiment, the progeny of plants grown in the mixed-growth room showed 19/245 revertants (Table 1). Eighteen of nineteen revertants segregated the *erecta* phenotype in the next generation, suggesting that they arose from pollen contamination by nearby *erecta*-containing plants.

In a second mixed-population experiment, 18/415 plants were phenotypically *HTH*. All 18 contained a *BIN2-1::GFP* transgene⁴, which was present in other plants grown in the room (Table 1). In contrast, not a single revertant was found among 932 progeny of *hth-12* plants grown in isolation.

We repeated these experiments with the originally reported *hth-8* and *hth-5* alleles in the Landsberg *erecta* (*Ler*) ecotype^{1,5} and found that *hth-8* plants grown in mixed populations yielded 156/994 progeny with a *HTH* phenotype. Most were either *ERECTA*, or contained *BIN2-1::GFP* (Table 1). However, *hth-8* plants grown in isolation gave exclusively *hth* progeny, none of which was *ERECTA* (Table 1). Similar results were obtained with *hth-5*. Our results indicate that *hth* mutants are particularly susceptible to pollen contamination, possibly because the *hth* floral organ fusion defects lead to inefficient self-pollination and exerted stigmas¹, or because of changes in cuticle composition⁵. This tendency to outcross may pro-

Table 1 | Outcrossing in *hth* mutants

| Genotype | Number of phenotypically revertant plants | |
|---------------|---|---------------------|
| | Mixed population | Isolated population |
| <i>hth-12</i> | 19/245 (7.8%)* | 0/295 (0%) |
| <i>hth-12</i> | 18/415 (4.3%)† | 0/637 (0%) |
| <i>hth-8</i> | 156/994 (15.7%)‡ | 0/890 (0%)§ |
| <i>hth-5</i> | 22/1144 (1.9%) | 0/913 (0%)§ |

Homozygous *hth* plants were grown in a room with plants of mixed genotype (mixed population) or in isolation (isolated population). Progeny from these two populations were scored for plants with the wild-type *HTH* phenotype. (Plants were cared for by Yu Li, Shawn Cokus, Lynn Jacobsen, Zhongliang Peng and Suwen Wang.)

*Of 19 phenotypically *HTH* plants, 18 segregated *erecta* in the next generation.

†All 18 revertants segregated a *BIN2-1::GFP* transgene in the next generation.

‡Among 156 *HTH* revertants, 145 were *ERECTA*, four were dwarf plants containing the *BIN2-1::GFP* transgene, and seven seemed similar to *Ler* plants.

§All plants also retained an *erecta* phenotype.

||All 22 revertants were also *ERECTA*.

vide an alternative explanation for the apparent genetic instability of *hothead* mutants.

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Pruitt *et al.* reply

Replying to: P. Peng, S. W.-L. Chan, G. A. Shah and S. E. Jacobsen *Nature* 443, doi:10.1038/nature05251 (2006).

The results obtained by Peng *et al.*¹ are consistent with an increased amount of outcrossing in *hth* mutants of *Arabidopsis thaliana*. Some of the results, such as the acquisition of a novel transgene, would be difficult to explain by any other mechanism. Outcrossing was a possibility that we thoroughly explored early on in our investigation², however, but we discounted it as an explanation because it was inconsistent with many of our experimental results.

We described two experiments that were inconsistent with outcrossing²: one in which there was transmission of a wild-type *HTH* allele from a homozygous mutant (*hth/hth*) male parent, and another in which there was recovery of homozygous wild-type (*HTH/HTH*) embryos dissected from homozygous mutant (*hth/hth*) parents. In further experiments that were similar, but not identical, to those described by Peng *et al.*¹, we did see

reversion in *hth/hth* homozygotes grown in isolation (results not shown).

These results together indicate that the genetic events that we see in *hth* mutants cannot be explained solely by outcrossing, but they do not rule out the possibility that outcrossing could be increased relative to that occurring in the wild type. The lower frequency of reversion seen when pollen from an *hth/hth* parent is used to pollinate a wild-type female may reflect elimination of these outcrossing events.

We have also examined more extensive patterns of inheritance of single-nucleotide polymorphisms in F₂ populations, similar to those we originally described² (J.M.Y. and R.E.P., unpublished data). These patterns of inheritance are also inconsistent with an outcrossing

explanation because there was no single male parent present that could have provided the combination of non-parental alleles observed in the 'restored' progeny. Furthermore, the results indicate that genetic restoration of ancestral alleles can take place in *HTH/hth* heterozygotes; these plants have a floral morphology identical to wild type and therefore would not be expected to show increased outcrossing.

In summary, the outcrossing explanation proposed by Peng *et al.*¹ is a reasonable hypoth-

esis to explain some of the data associated with *hothead* genetics, and indeed is one of the first that we considered. Ultimately, we discarded this explanation because it was inconsistent with many of our experimental results. However, the results of Peng *et al.* show that, at least under some growth conditions, outcrossing in *hth/hth* plants remains an issue that needs to be taken into account.

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(S. J. L. declined to sign this reply)**

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