Increased outcrossing in hothead mutants


Lolle et al. 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 revision 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-fertilizing). 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 transgene, 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) ecotype1 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 stigmas, or because of changes in cuticle composition. This tendency to outcross may provide an alternative explanation for the apparent genetic instability of hothead mutants.

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We described two experiments that were inconsistent with outcrossing: 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., 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 F1 populations, similar to those we originally described (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 hypothesis 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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