Maize Transposon Storm Kicks up a *White Cap*

Thomas Peterson[1]
Department of Genetics Development and Cell Biology, and Department of Agronomy, Iowa State University, Ames, Iowa 50011
ORCID ID: 0000-0002-9933-7556 (T.P.)

KEYWORDS hAT transposable; elements; tandem duplications; carotenoids; maize endosperm

In this commentary, Thomas Peterson discusses Tan et al. (2017), "Structure and origin of the White Cap Locus and its role in evolution of grain color in maize," published in this issue of GENETICS.

THE dominant maize *White Cap* (*Wc*) factor acts like a genetic toothbrush for corn: it converts yellow kernels to white, and plain off-white kernels to pearly white. Although *Wc* has been an important factor in corn breeding and genetics for nearly a century, its molecular structure and mode of action are only now revealed in a paper published in this issue (Tan *et al.* 2017). The *Wc* locus contains an impressive tandem array of up to 23 copies of a 28-kb repeat sequence. Each 28-kb repeat includes three genes, and one of them (*Ccd1*) encodes a carotenoid cleavage dioxygenase. This enzyme degrades yellow carotenoids, thus explaining the dominant effect of *White Cap* in changing kernel endosperm from yellow to white (Figure 1).

Of course, tandem segmental duplications are nothing new. The original and most famous example is that of the *Drosophila Bar* locus (Wolfner and Miller 2016). In this classic textbook case, the Bar eye phenotype is associated with a tandem duplication encompassing seven bands in the 16A section of the *Drosophila* X chromosome (Bridges 1936; Sutton 1943). Alfred Sturtevant showed that subsequent unequal crossing over among the duplicated segments could produce more severe *double-Bar* flies with three copies, as well as reciprocal single-copy non-*Bar* revertants (Sturtevant 1925). While the Bar phenotype was described in 1915, only in 1989 was it shown that the original *Bar* duplication was produced by unequal crossing over between two Transposable Elements (TEs) located at the proximal and distal *Bar* duplication boundaries in the progenitor alleles (Tsubota *et al.* 1989).

In the case of *Bar*, the TE sequences likely served as passive substrates of an out-of-register homologous recombination event resulting in unequal cross-over between the two parental chromosomes. In contrast, the initial *White Cap* duplication was generated directly by a series of transposition events involving multiple copies of *Tam3L*, members of the *hAT* TE family. The story begins with a shower of *Tam3L* insertions in and around the vicinity of the progenitor *Ccd1* locus, producing a macrotransposon composed of *Tam3L* elements flanking *Ccd1* and two other genes. Transposition of the macrotransposon to a nearby site generated the incipient *Wc* locus containing a single extra copy of *Ccd1*. A subsequent aberrant transposition event involving an additional *Tam3L* copy generated a 28-kb tandem duplication of *Ccd1* within the macrotransposon. This original *White Cap* duplication was then further amplified by unequal crossing over to generate the extant alleles containing up to 23 repeats per haplotype. The structural evidence supporting the model is strong: The putative macrotransposon is flanked by 8-bp Target Site Duplication sequences, which would have been generated by the macrotransposon insertion. Within the macrotransposon, the internal *Tam3L* transposon termini are found precisely at the breakpoints of the individual repeat units, as would be predicted by the aberrant transposition step. Some steps of the origin model are obscure and may never be resolved if key intermediates are lacking; nevertheless, the proposed events are plausible and entirely consistent with the extant *White Cap* structure.

Other *hAT* family transposons, including the *Ac/Ds* system first described by McClintock, are known to induce genome rearrangements via alternative transposition reactions. These reactions have been shown to directly generate a variety of rearrangements, including deletions (Zhang and Peterson

doi: https://doi.org/10.1534/genetics.116.199638
[1]Address for correspondence: Iowa State University, 2258 Molecular Biology, Ames, IA 50011-3260. E-mail: thomasp@iastate.edu

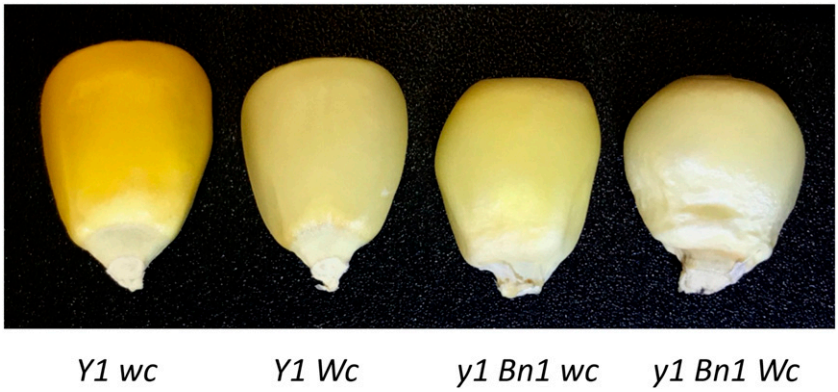

Figure 1 *White cap* (Wc) enhances white grain-color in two ways. In a high-carotenoid *Yellow1* (*Y1*) background, (pair of kernels on the left) *Wc* produces a dominant white phenotype by elevating expression of the CCD1 carotenoid cleavage dioxygenase that degrades carotenoids. The pair of kernels on the right show that in addition, *Wc* enhances the white endosperm phenotype conditioned by recessive *y1* by removing an unidentified yellow brown pigment attributed to presence of the *Brown aleurone 1* (*Bn1*) gene.

2005), inversions and translocations (Zhang *et al.* 2009), and duplications (Zuo *et al.* 2016). Similarly, a recently characterized large (1.17-Mb) paracentric inversion in *Arabidopsis* originated from aberrant transposition of a *Vandal* TE ~5000 years ago. The inversion is associated with fecundity in drought conditions, and is now found in over 170 accessions from Europe and North America (Fransz *et al.* 2016). Together, these findings add to the growing body of evidence that TEs play a direct role in genome evolution.

The *White Cap* locus represents the latest example in a growing number of tandem gene duplications. In maize, a variety of multi-copy tandem arrays have been described, including the *p1-wr* locus which specifies cob color and contains 11 nearly identical copies of a 12-kb sequence encoding a *Myb*-homologous transcription factor. Extensive structural analyses indicate that the *p1-wr* cluster was generated through a series of events including transposition, unequal crossing over/Nonallelic Homologous Recombination, and gene conversion (Goettel and Messing 2009, 2010). The formation of multi-copy tandem direct repeats remains an interesting question: many cases are attributed to repeated unequal crossing over events reminiscent of the exchanges at the *Drosophila Bar* locus, while in other cases a rolling-circle-type mechanism has been invoked (Zhang *et al.* 2014).

The effects of tandem arrays on gene expression can differ dramatically. In the case of the *p1-wr* complex described above, the tandem array appears to result in an epigenetic silencing of kernel pericarp pigmentation (Goettel and Messing 2010). In contrast, *White Cap* exhibits a direct gene dosage effect on endosperm whitening. The authors show that the number of *Ccd1* copies can vary among different *White Cap* alleles, and that *Ccd1* expression levels are directly proportional to *Ccd1* copy number. Together these results indicate that the dominant effects of *Wc* are due to the dosage-proportional expression of the *Ccd1* gene. In contrast, the *Drosophila* Bar phenotype is not an effect of gene dosage *per se*; rather, the duplication breakpoint appears to induce misregulation of a pair of nearby Hox-related *Bar1* and *Bar2* genes (Higashijima *et al.* 1992).

In addition to elucidating the *White Cap* structural features described above, Tan *et al.* (2017) include a very nice discussion of the role of *Wc* as a regulator of endosperm color, particularly in combination with alleles of the *Y1* and *Bn1* genes. The maize *Yellow1* gene induces the accumulation of yellow carotenoids in non-*Wc* stocks, while *Y1 Wc* kernels are white due to the action of the amplified *Ccd1*-encoded carotenoid cleavage dioxygenase. Interestingly, *Wc* is more often found together with *y1*, a curious juxtaposition since *y1* kernels lack most carotenoids and are already white. Tan *et al.* (2017) show that, even in the presence of *y1*, *Wc* has a whitening effect: ordinary off-white kernels become "whiter than white" (Figure 1). The authors argue that the prevalence of *Wc* in many historical maize landraces, including *y1* stocks, reflects positive selection by early farmers for white grain color; *i.e.*, early maize farmers used genetics to obtain ever-whiter varieties of maize. In contrast, consumer preference for white wheat flours is satisfied commercially by addition of chemical bleaching agents such as benzoyl peroxide to the milled flour. Although benzoyl peroxide-treated flour poses no documented hazards, one wonders whether a *White Cap* analog in wheat may provide a natural alternative to chemical bleaching. In any case, maize *White Cap* tells a fascinating story of genome and cultural coevolution.

Acknowledgments

This work is supported by the United States Department of Agriculture National Institute of Food and Agriculture Hatch project number IOW05282, and by State of Iowa funds.

Literature Cited

Bridges, C. B., 1936 The bar "gene" a duplication. Science 83: 210–211.

Fransz, P., G. Linc, C. Lee, S. A. Aflitos, J. R. Lasky *et al.*, 2016 Molecular, genetic and evolutionary analysis of a paracentric inversion in Arabidopsis thaliana. Plant J. 88: 159–178.

Goettel, W., and J. Messing, 2009 Change of gene structure and function by non-homologous end-joining, homologous recombination, and transposition of DNA. PLoS Genet. 5(6): e1000516.

Goettel, W., and J. Messing, 2010 Divergence of gene regulation through chromosomal rearrangements. BMC Genomics 11: 678.

Higashijima, S., T. Kojima, T. Michiue, S. Ishimaru, Y. Emori *et al.*, 1992 Dual Bar homeo box genes of Drosophila required in two photoreceptor cells, R1 and R6, and primary pigment cells for normal eye development. Genes Dev. 6(1): 50–60.

Sturtevant, A. H., 1925 The effects of unequal crossing over at the *Bar* locus in Drosophila. Genetics 10: 117–147.

Sutton, E., 1943 Bar eye in Drosophila melanogaster: a cytological analysis of some mutations and reverse mutations. Genetics 28: 97–107.

Tan, B. C., J.-C. Guan, S. Ding, S. Wu, J. W. Saunders *et al.*, 2017 Structure and origin of the *White Cap* locus and its role in evolution of grain color in maize. Genetics 206: 135–150.

Tsubota, S. I., D. Rosenberg, H. Szostak, D. Rubin, and P. Schedl, 1989 The cloning of the *Bar* region and the B breakpoint in Drosophila melanogaster: evidence for a transposon-induced rearrangement. Genetics 122: 881–890.

Wolfner, M. F., and D. E. Miller, 2016 Alfred Sturtevant walks into a *Bar*: gene dosage, gene position, and unequal crossing over in Drosophila. Genetics 204: 833–835.

Zhang, J., and T. Peterson, 2005 A segmental deletion series generated by sister chromatid transposition of *Ac* transposable elements in maize. Genetics 171: 333–344.

Zhang, J., C. Yu, V. Pulletikurti, J. Lamb, T. Danilova *et al.*, 2009 Alternative *Ac/Ds* transposition induces major chromosomal rearrangements in maize. Genes Dev. 23: 755–765.

Zhang, J., T. Zuo, D. Wang, and T. Peterson, 2014 Transposition-mediated DNA re-replication in maize. Elife 3: e03724.

Zuo, T., J. Zhang, A. Lithio, S. Dash, D. F. Weber *et al.*, 2016 Genes and small RNA transcripts exhibit dosage-dependent expression pattern in maize copy-number alterations. Genetics 3: 1133–1147.

Communicating editor: J. A. Birchler