Correspondence

Genome expansion in three hybrid sunflower species is associated with retrotransposon proliferation

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The origin of new diploid species through inter-specific hybridization may be facilitated by rapid genomic reorganization. There is evidence that this process was involved in the independent origins of three annual sunflower species in the genus *Helianthus*. The three hybrid taxa, *H. anomalus*, *H. deserticola* and *H. paradoxus*, are products of ancient hybridization events between the same two parental taxa, *H. annuus* and *H. petiolaris* [1]. The hybrid species have geographically restricted ranges and occupy habitats that are abiotically extreme relative to other *Helianthus* species; *H. anomalus* and *H. deserticola* are found in desert environments, whereas *H. paradoxus* is restricted to saline marshes [2]. In addition to several novel karyotypic rearrangements [3], each hybrid taxon has a nuclear genome at least 50% larger than that of either parental species [4]. These genome size differences occur in spite of the fact that the hybrid and parental species are diploids and all possess the same number of chromosomes (n = 17). Because both inter-specific hybridization and abiotic stress have played important roles in the evolutionary history of the hybrid taxa, and because both have been implicated as natural agents of retrotransposon activation and proliferation [5,6], we sought to determine whether the genome size differences associated with hybrid speciation in these sunflowers could be attributable to proliferation of mobile genetic elements in the hybrid taxa.

While multiple categories of transposable elements exist in eukaryotic genomes, the class I elements known as long terminal repeat (LTR) retrotransposons most often have been associated with genome size variation in plants [7]. Based on a previously reported *Ty3/gypsy*-like LTR retrotransposon sequence in *Helianthus* [8], we developed a PCR probe (887 base pairs from the integrase-domain-encoding region) to use in Southern blot experiments comparing element abundance in the hybrid and parental taxa. The probe was amplified from genomic DNA of the parental species *H. annuus*. Southern blots revealed a considerably stronger hybridization signal for the hybrid species relative to their parental species (Figure 1), indicating a higher relative abundance of *Ty3/gypsy* sequences in the hybrid species' genomes. This remained the case whether loadings were standardized by genome equivalents, standardized to 1 μg, or standardized to 500 ng without a restriction digest (Figure 1).

To gain better quantitative estimates of element copy numbers in these sunflower genomes, we used a quantitative PCR strategy. Examination of 6–10 individuals per species and two different populations of each parental taxon confirmed a stunning increase of *Ty3/gypsy* sequences in all hybrid taxa (Figure 2), with 5.6 to 23.6-fold increases in copy number in the hybrid species. Statistically significant differences were not observed among different populations of parental species. Assuming a size of 5.2 kb for a *Ty3/gypsy* element in *Helianthus* [9] we estimate an additional 1330 Mb, 1162 Mb and 909 Mb of DNA in *H. anomalus*, *H. deserticola* and *H. paradoxus*, respectively, that is attributable to *Ty3/gypsy* proliferation.

These estimates account for ~73%, ~79%, and ~62% of the differences in genome size between the parental species *H. annuus* (3000 Mb) [10] and *H. anomalus*, *H. deserticola* and *H. paradoxus*, respectively [4]. Retrotransposons represent ancient lineages that often exhibit considerable sub-lineage diversity in plant genomes. So although estimates reported in Figure 2 accurately reflect real differences between the hybrid

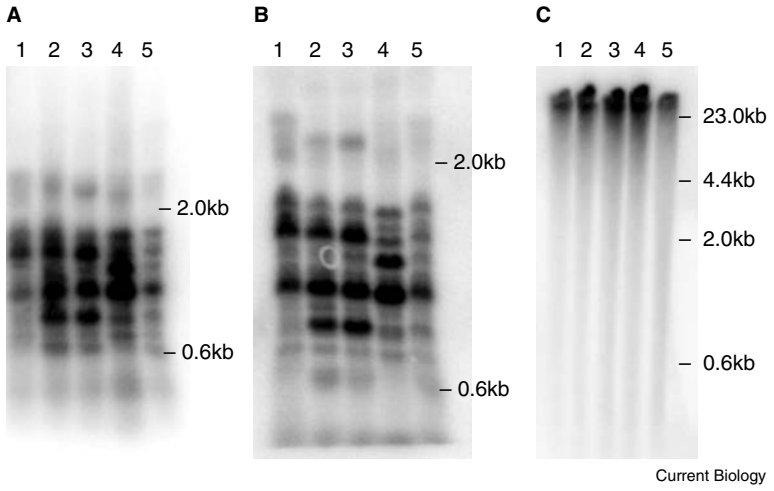

Figure 1. Southern blots probed with an 887 base pair region of the *Ty3/gypsy* integrase domain.

Lanes 1 and 5: parental species *H. annuus* and *H. petiolaris*, respectively. Lanes 2–4: hybrid species *H. anomalus*, *H. deserticola* and *H. paradoxus*, respectively. Blots depict standardized loadings of (A) genome equivalents (*H. annuus*, 1.10 μg; *H. anomalus*, 1.76 μg; *H. deserticola*, 1.63 μg; *H. paradoxus*, 1.63 μg; *H. petiolaris*, 1.00 μg); (B) 1 μg DNA; and (C) 500 ng uncut genomic DNA.

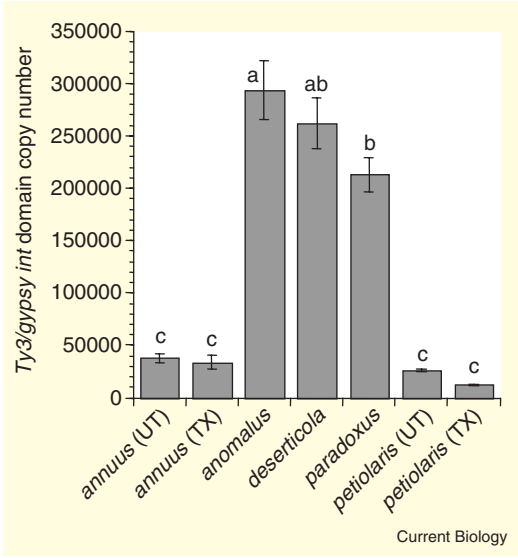

Figure 2. Copy number estimates (per genome) of the *Ty3/gypsy* integrase domain based on quantitative PCR.

Two different populations of the *Helianthus* parental species (*H. annuus* and *H. petiolaris*) and one population each of the three diploid hybrid species (*H. anomalus*, *H. deserticola* and *H. paradoxus*) were assayed. Shown are means with one SE. The number of individuals assayed from each species are as follows: *H. annuus* (UT), n = 9; *H. annuus* (TX), n = 10; *H. anomalus*, n = 10; *H. deserticola*, n = 10; *H. paradoxus*, n = 6; *H. petiolaris* (UT), n = 9; *H. petiolaris* (TX), n = 9. Different lowercase letter above histogram bars indicate that means are significantly different (Tukey-Kramer HSD).

and parental taxa, they may not reflect global estimates of element copy number because of the PCR-based nature of this assay and the potential for sequence variation at priming sites.

Alternative explanations for these observations such as preferential probe hybridization/PCR amplification in the hybrid species and/or extra-chromosomal DNA (retroelement cDNA) in the hybrid species seem untenable. Preferential probe hybridization/PCR amplification in the hybrid species relative to the parental species is implausible because the development of our Southern blot probe and quantitative PCR primers was based on a *Ty3/gypsy* sequence derived from the parental species *H. annuus* [8]. Extra-chromosomal DNA in the hybrid species is ruled out by our Southern blots: a Southern blot of undigested genomic DNA probed with a *Ty3/gypsy* integrase fragment also exhibits stronger hybridization signal in the hybrid species (Figure 1C), and fails to exhibit distinct, faster migrating cDNA transposition intermediates [11].

Future work will explore the potential impact of the proliferation of retrotransposons on the establishment and differentiation of the hybrid species. Given the prominent roles played by hybridization and abiotic stress in the evolutionary history of all three of the hybrid taxa, it is tempting to conclude that one or both of these factors may have been involved in these proliferations. Further experiments, however, will be necessary in order to address this question explicitly. Therefore, in addition to their established role as a model system for investigating homoploid hybrid speciation in plants [1,2,12], these hybrid sunflower species will likely emerge as an excellent group for studying the ecological and evolutionary dynamics of LTR retrotransposon activation and proliferation.

Supplemental data

Supplemental data including experimental procedures are available at http://www.current-biology.com/cgi/content/full/16/20/R872/DC1/

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