

## **Traces of early Native Americans -- in sunflower genes**

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Sunflower (*Helianthus annuus*) is among the first wild plants cultivated by Native Americans. Credit: Nolan Kane

New information about early Native Americans' horticultural practices comes not from hieroglyphs or other artifacts, but from a suite of four gene duplicates found in wild and domesticated sunflowers.

In an upcoming issue of *Current Biology*, Indiana University Bloomington biologists present the first concrete evidence for how gene duplications can lead to functional diversity in organisms. In this case, the scientists learned how duplications of a gene called FLOWERING LOCUS T, or FT, could have evolved and interacted to prolong a flower's time to grow. A longer flower growth period means a bigger sunflower -- presumably an attribute of great value to the plant's first breeders.



"Our paper shows how <u>gene duplication</u> creates potential for evolutionary innovation not just through creating new gene content but also through new interactions among duplicates," said Ben Blackman, the report's lead author.

Blackman conducted the research as an IU Bloomington Ph.D. student. He is now a postdoctoral fellow at Duke University.

Biologists have long thought the accidental duplication of <u>genetic</u> <u>material</u> provides important fodder for evolution. Less risky than modifying an existing, possibly important gene, duplicates offer an out -one copy can continue its normal activities while the other copy acquires new functions. That's a hypothesis, anyway. The <u>Current Biology</u> paper suggests reality may be a little more complex.

FT genes play a role in sensitizing <u>flowering plants</u> to seasons, and their expression is usually triggered by changes in day length. Some flowering plants express FT genes early in the growing season as days get longer. Sunflower FT genes are expressed toward the end of the growing season when days are getting shorter. As far as biologists know, all flowering plants have at least one FT gene.

Blackman and his colleagues identified four FT genes in sunflower, *Helianthus annuus*, which are known as HaFT paralogs. Each of the paralogs, HaFT1 through HaFT4, has a unique genetic sequence, but is similar enough to the others to conclude three of them were the result of DNA duplication events in sunflower's distant past.

"Based on the level of divergence between the various HaFTs and the presence of a single FT copy in lettuce, we inferred that one copy became two during a whole genome doubling event that occurred roughly 30 million years ago," Blackman said. "One of those copies proliferated further through two small-scale duplications that we infer



occurred much more recently."

The scientists examined each paralog's expression patterns within sunflower, and by strategically cloning variants of the HaFT genes into the model plant Arabidopsis thaliana, discerned the paralogs' physiological properties in one another's presence.

One of the paralogs, HaFT3, has lost function and is no longer expressed. Countless genome surveys show "non-functionalization" is a common fate for gene duplicates in plants and other eukaryotes, possibly because the extra dose of genetic expression can be wasteful or overtly harmful to the organism.

Two of the paralogs, HaFT2 and HaFT4, are structurally similar to each other and have retained normal function. The proteins they encode are produced in leaves in response to day length. It is believed the HaFT2 and HaFT4 proteins travel down to the stem and up to the shoot tip, where they compel meristem cells to develop into flower buds, but this has yet to be shown conclusively for *Helianthus annuus*.

HaFT1 isn't produced in the leaves but at the site of HaFT2 and HaFT4's target -- the shoot tip and the green bracts that will radiate out from the flower itself. There are two basic versions of the HaFT1 called alleles. The domesticated HaFT1 allele is distinguished from the wild allele by the omission of a single nucleotide. But what a difference that nucleotide makes. The protein produced from the domesticated HaFT1 is larger than its wild cousin and has a novel domain.

Only two of the 23 wild populations surveyed possess both types of the HaFT1 allele.

That is not the case for domesticated sunflower populations, for which the domestic version of HaFT1 completely (or almost completely)



dominates. Modern domesticated sunflowers used in farming are homogeneous for domesticated HaFT1. The scientists also examined "landraces," Native Americans' own domesticated cultivars, some of which are quite old. These too are dominated by domesticated HaFT1.

By comparing the activity of domesticated and wild HaFT1, the scientists learned it is the domesticated version of HaFT1 that lengthens the time period during which flowers grow and mature. This can have a wide variety of effects, from increasing the size of the sunflowers' seed disk to increasing the flowers' total seed mass.

Despite its name, domestic HaFT1 isn't the result of domestication -- its origin likely precedes human cultivation. It is called domestic, because it is the version of HaFT1 that caused traits early Native Americans seem to have preferred as they bred the plants for horticulture. Genetic evidence the scientists collected from a broad survey of domesticated and wild HaFT1 genes suggests domesticated HaFT1 experienced a "selective sweep" around the time early Native Americans would have begun cultivating sunflower.

"Our study is the first to provide both strong functional evidence and strong evolutionary evidence that a particular nucleotide variant in this one gene -- HaFT1 -- was critical for early sunflower domestication," Blackman said.

How HaFT1 was exerting its flower-delaying effects was not clear until the scientists cloned HaFT1, HaFT2 and HaFT4 into Arabidopsis thaliana in different combinations. A. thaliana's own FT gene had been removed. Cloning genes in this way can eliminate complicating factors when scientists are interested in knowing how a few genes (and the proteins they encode) interact.

Domesticated HaFT1 had no impact on flowering in the presence of



HaFT2. But HaFT1 did delay A. thaliana flowering in the presence of HaFT4. The scientists concluded the newer HaFT1 and older HaFT4 are interacting, possibly directly, in such a way to interfere with HaFT4's function, thereby delaying flowering.

"In the sunflower story, what is most interesting in my view is how evolution has exploited both recent and ancient gene duplicates in the same gene family to achieve shifts in flowering time and photoperiod sensitivity," said IU Bloomington plant evolutionary biologist Loren Rieseberg, the study's principal investigator.

**More information:** "The Role of Recently Derived FT Paralogs in Sunflower Domestication," Current Biology, by Benjamin K. Blackman, Jared L. Strasburg, Andrew R. Raduski, Scott D. Michaels, and Loren H. Rieseberg (unpub.; early access)

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