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A Proposed Natural Genetic Solution to the Long Juvenility Problem in Apple

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Abstract

From a tree fruit breeder's perspective, long juvenility represents a significant challenge. Because breeders can only make crosses once trees reach maturity and start to flower, they must often wait through a multi-year juvenile phase before evaluating fruit and making further crosses. Within wild relatives of apple, much useful natural variation exists in length of juvenility. However, the genes and their allelic variation governing the transition from the juvenile to adult phase are not as well elucidated as other valued traits for breeding. Some cross-compatible apple wild relatives transmit short-juvenility alleles to offspring. To identify these genetic factors and their underlying genes, derived families exhibiting variation for length of juvenile phase are needed for QTL analyses. The existing natural genetic variation might then be exploited for apple breeding. Combining genetic factors associated with short juvenility from several distinct sources holds promise for achieving ultra-short juvenility naturally to overcome the problem of long juvenility in apple.

Juvenility duration in apple trees. To enjoy flowers or fruit of hardwood perennials such as apple (*Malus × domestica*), humans must endure the juvenile phase of plants. All plants pass through a juvenile phase to prepare for maturity and the concomitant production of flowers, fruit, and seeds. The juvenile phase can be defined as the extended period of post-germination vegetative growth in which flowering is suppressed even under otherwise favorable environmental conditions (van Nocker and Gardiner, 2014). In this phase, a plant focuses its energy on vegetative growth and development to reach an optimal size and architecture for photosynthesis, water and nutrient uptake, withstanding environmental stresses, and physically supporting reproductive organs (Apple et al., 2002; Bernier et al., 1981; Huijser and Schmid, 2011). Perennial hardwood plants can have juvenile phases up to 40 years (Robinson and Wareing, 1969; van Nocker and Gardiner, 2014). In high-value crops such as apple, long juvenility is a major problem from a breeder's perspective. Seeds arising from crossing must be germinated and seedlings raised through juvenility before their (hopefully superior) fruit can be evaluated (Luby and Shaw, 2001). A short juvenile phase allows earlier fruit evaluation and creation of the next generation (Liebhard et al., 2003)

Much variation exists in duration of the juvenile period across and within apple (*Malus*) species. In apple, the juvenility period is typically 5-12 years (Flachowsky et al., 2012; van Nocker and Gardiner, 2014) and such variation is caused by environmental or genetic differences (Hanke et al., 2007) as the transition to maturity is a complex process. Environmental factors that influence flowering can include temperature, water, light, and nutrients (Kurokura et al., 2013; van Nocker and Gardiner, 2014). Growing tree fruit seedlings under conditions that promote rapid and continuous growth, such as in a greenhouse, might help shorten juvenility (van Nocker and Gardiner, 2014; Volz et al., 2009; Zhang et al., 2007).

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Existing genetic variation in apple enables some plants to flower in less than four years, which is considered a short juvenility period. Certain dessert and ornamental apple cultivars, when used as parents, generated a higher proportion of seedlings flowering in four years after crossing than others, especially with use of precocious rootstocks (Fig. 1). In wild *Malus* species, the evolutionary leap to short juvenility can be rationalized. For example, *M. sieversii* evolved in the dense forests of Kazakhstan and neighboring countries (Volk et al., 2013). Becoming reproductive sooner to avoid years of intense competition and produce the next generation quickly could be a successful evolutionary strategy to ensure optimal representation in future generations. It has also been argued that long juvenility indicates tree establishment has priority over fruit and seed production; perhaps evolutionarily priority was given to tree survival over many years to allow seed production in years of optimal weather and minimal stresses (Lakso and Goffinet, 2017). Certain accessions of apple wild relatives such as *M. sieversii*, *M. baccata*, *M. prunifolia*, and the ornamental *M.* × *zumi* pass short juvenility to their offspring

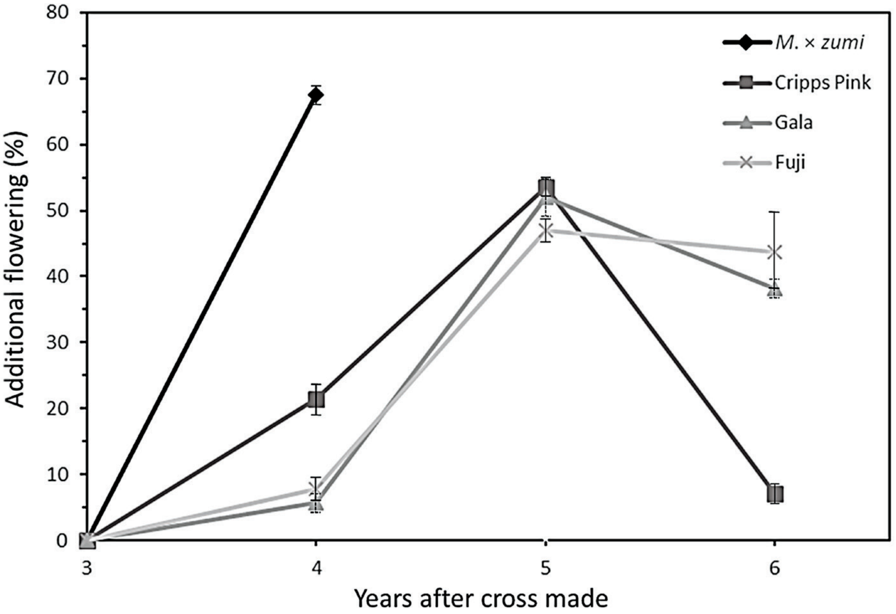

Fig. 1. Proportion of total number of seedlings from three cultivars and one wild relative observed to be flowering since the previous year, monitored four, five, and six years after crossing. Data is a subset of flowering observations for 35 parents and 107 families in the Washington State University Apple Breeding Program from 1998 to 2003 (courtesy of Bruce Barritt and Kate Evans); number of families included were 2 for *M.* × *zumi* 'Calocarpa', 19 for 'Cripps Pink', 19 for 'Gala', and 24 for 'Fuji', with an average number of seedlings in these families of 128 (B. Barritt, pers. comm.). "Typical" juvenility periods are displayed by the 'Gala' and 'Fuji' distributions, while seedlings of 'Cripps Pink' effectively had a juvenility period approximately one year shorter, and *M.* × *zumi*-derived juvenility was approximately one year shorter again. These seedlings had been bud-grafted onto M.9 rootstock two years after crosses were made (Evans, 2013), for the practical breeding purposes of reducing natural juvenility, compressing variation in juvenility period among seedlings, reducing tree sizes, and mimicking commercial practices of the U.S. Pacific Northwest region.

(Schmidt, 1994; Volk et al., 2013). For example, among seedlings of Roșu de Cluj (= *M.* × *domestica*) × *M. niedzwetzkyana* (= *M. sieversii*), average time to flowering was 7.0 years, whereas among seedlings of *M. prunifolia* × *M. niedzwetzkyana*, average time to flowering was 4.9 years (Cătălina et al., 2015). Rootstocks can also promote early fruit bearing of scions, known as precocity, and precocity was positively correlated with short juvenility (Janick and Moore, 1996). In an apple rootstock progeny derived from 'Ottawa 3' (ancestry includes *M. baccata*, *M. sylvestris*, and *M. prunifolia*) and 'Robusta 5' (*M.* × *robusta*), genetic factors *Eb1* and *Eb2* were associated with precocity (Fazio et al., 2014). To the extent that these various sources of short juvenility are from distinct species (e.g., *M.* × *zumi* is a hybrid of *M. baccata* × *M. sieboldii, and M.* × *robusta* is *M. baccata* × *M. prunifolia*; Rehder, 1951), such accessions likely have unique alleles at the same or different loci as they evolved under different circumstances.

Several genes have been characterized in other plants that appear to govern the vegetative-to-flowering phase change. Floral induction in Arabidopsis is influenced by multiple inputs that regulate expression of genes involved in the transformation of the vegetative meristem into an inflorescence meristem (Hanke et al., 2007; Poethig, 2013), possibly involving microRNA (An et al., 2018; Poethig, 2013). These genes are orthologous across many plant species (Endo et al., 2005; Hsu, 2006; Kotoda and Wada, 2005), including apple (Flachowsky et al., 2007). A *Juvenile-to-Adult transition* (*JAT*) gene was reported in olive (Fernández-Ocaña et al., 2010). Variation in such genes might underlie the allelic variation observed in apple.

A transgenic approach to shortening apple juvenility has been reported. The approach consists of the overexpression in apple of a birch (*Betula pendula*) MADS4 transcription factor and has been used for introgression of fire blight resistance from apple wild relatives (Schlathölter et al., 2018; Luo et al., 2020b). In the final generation, the transgene segregates, and null segregants are kept. In the U.S., these null segregants have been officially ruled non-transgenic (USDA, 2014), although public acceptance is far from unanimous (Ishii and Araki, 2016). In other regions such as Europe, null segregants have yet to be ruled as non-transgenic, and the approach has not been adopted in breeding. A detail not included in the Schlathölter et al. (2018) study was the length of time for null segregants to flower and fruit, which would be as long as a typical juvenile period. Combining this transgenic approach with use of natural variation in apple juvenility period could be advantageous, especially for introgression of other valuable alleles from wild sources (Luo et al., 2020a).

Harnessing natural genetic variation of juvenility in apple. To understand and exploit the natural variation of juvenility period in apple, a concerted effort is required. Specific regions in the genome with genetic variation associated with observed differences in a particular trait, i.e., quantitative trait loci (QTLs) (Xu et al., 2017), need to be discovered and characterized for juvenility, as they have for many other valuable apple traits (e.g., Costa et al., 2010; Guan et al., 2015; Sadok et al., 2015). Across traits, favorable alleles can be accumulated via breeding to develop superior new cultivars. The same strategy is used for some traits with multiple influencing QTLs, such as disease resistance (Baumgartner et al., 2015; Kellerhals et al., 2011, 2013). When favorable alleles from the various loci associated with a trait are combined into a single individual, each allele is expected to incrementally raise the trait level of the individual (Mundt, 2014). Once they are discovered and characterized, combining genetic factors for short juvenility from several species of apple holds the promise of achieving ultra-short juvenility in this important crop (Fig. 2). Another opportunity is aiding introgression. Apple's wild relatives also harbor many other alleles for valuable attributes such as disease resistance (Forsline and

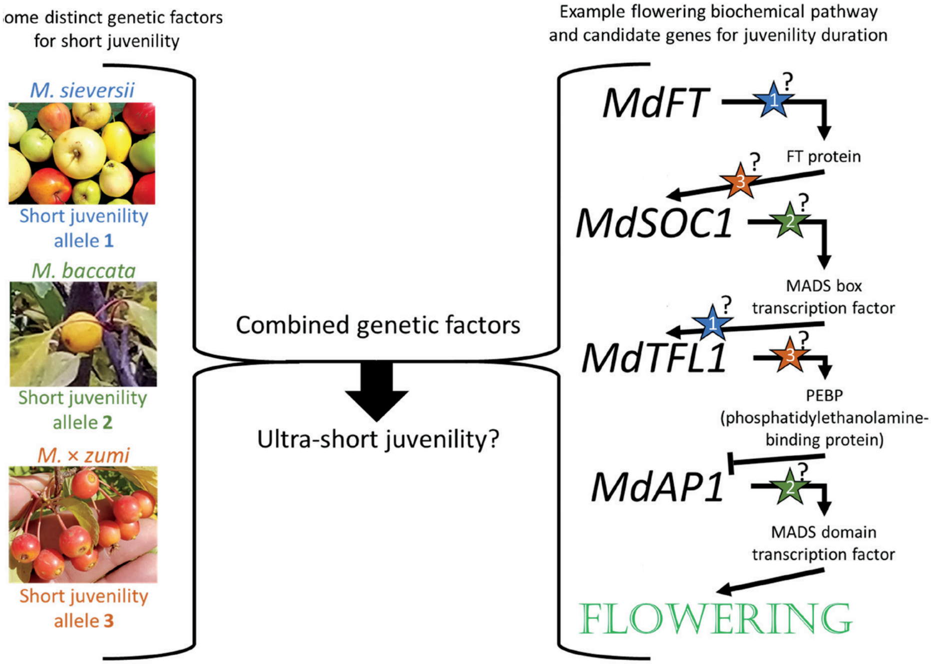

Fig. 2. Distinct sources of short juvenility alleles in apple (left) might represent alleles for genes in the well-described plant flowering pathway (right: *MdFT* (*FLOWERING LOCUS T*) proteins along with *MdSOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CO 1*) transcripts activate downstream floral meristem identity genes such as *MdAP1* (*APETALA 1*), while *MdTFL1* (*TERMINAL FLOWER 1*) products act antagonistically in the pathway; Mathieu et al., 2007; Mimida et al., 2013; Su et al., 2018) or other biochemical pathways. Combining multiple genetic factors for short juvenility in apple might result in ultra-short juvenility.

Aldwinkle, 2004), abiotic stress tolerance, and desirable productivity and fruit quality but their introgression into elite apple cultivars is hampered by long juvenile phases over multiple generations (Volk et al., 2015). Using parents with natural short juvenility during introgression efforts could speed the process by shaving off years at each generation.

Challenges to combining short juvenility alleles. Genetic architecture of juvenility, pleiotropy, and epistasis might represent significant challenges in exploiting short juvenility alleles in breeding. Alleles for short juvenility might be associated pleiotropically with undesirable phenotypes such as erratic bloom or insufficient vigor. Combining alleles over multiple loci could also result in unexpected and undesirable phenotypes. Short juvenility alleles might be tightly linked in coupling phase to alleles associated with undesirable phenotypes such as small fruit size, flesh astringency, biennial bearing, or disease susceptibility. Another challenge to harnessing natural variation would be if alleles at many, small-effect QTLs are required to achieve short juvenility. Genetically mapping the loci influencing juvenility and understanding how their alleles interact would help address the challenges.

Conclusion

Long juvenility represents a significant challenge from a tree fruit breeder's perspective. In apple, a large amount of variation in juvenile period exists, especially considering wild relatives. A concerted effort is required to identify and characterize the genetically variable loci underlying the transition to maturity so the knowledge can be used for breeding purposes. Short juvenility would enable apple breeders to efficiently evaluate fruit traits and reach next generations faster. Short juvenility would also empower the introgression of valuable alleles from wild species quickly and efficiently. Combining natural genetic factors associated with short juvenility from several distinct sources holds promise for achieving ultra-short juvenility to overcome the problem of long juvenility in apple.

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