asite genome projects, there is an urgent need for molecular techniques that can replace traditional time-consuming chromosomal gene "knockout" methodologies and enable rapid screening of identified open reading frames (ORFs) for functional significance. One promising approach is that of RNA interference (RNAi), which has been used to systematically analyze predicted ORFs on two different chromosomes of *Caenorhabditis.elegans*[6,7]. RNAi has been shown to work for several genes in *T. brucei*[8,9] and will likely prove useful for analysis of other genes in other protozoan parasites. Antisense PNA oligomer technology, as demonstrated by Stock *et al.*, may also be an effective strategy for rapid functional analysis of parasite genes. With protozoan disease and drug resistance an ever-present global problem, the more technologies we have at our disposal for unraveling parasite biology, the better.

1. Stock, R.J. *et al. Nat. Biotechnol.* **19**, 231–234 (2001).
2. WHO/PAHO/UNESCO. *Report of a consultation of experts on amoebiasis. Weekly Epidemiological Report of the WHO 72*, Vol. 14, pp. 97–99 (WHO, Geneva; 1997).
3. Spath, G.F. *et al. Proc. Natl. Acad. Sci. USA* **97**, 9258–9263 (2000).
4. Hayward, R.E. *et al. Mol. Microbiol.* **35**, 6–14 (2000).
5. Mamoun, C.B. *et al. Mol. Microbiol.* **39**, 26–36 (2001).
6. Fraser, A.G. *et al. Nature* **408**, 325–330 (2000).
7. Gonczy, P. *et al. Nature* **408**, 331–336 (2000).
8. Ngo, H. *et al. Proc. Natl. Acad. Sci. USA* **95**, 14687–14692 (1998).
9. LaCount, D.J. *et al. Mol. Biochem. Parasitol.* **111**, 67–76 (2000).
10. Gilchrist, E. A. *et al. J. Biol. Chem.* (2001), in press.

A rapid coming of age in tree biotechnology

Introduction of two genes from *Arabidopsis* significantly accelerates the maturation of citrus trees.

Marcos Egea-Cortines and Julia Weiss

Trees are characterized by an extended adolescence. In fact, the juvenile phase in certain species sometimes can last over 20 years. This is particularly important for commercial fruit/nut tree growers and breeders, because prolonged juvenile periods delay harvesting and the evaluation/breeding of new strains. In this issue, Martiñez-Zapater *et al*[1]. exploit existing knowledge of the genetic control of flower development in *Arabidopsis* to engineer orange trees that reach sexual maturity at least four years earlier than the wild type. Their results could have significant economic and scientific implications for the tree fruit industry.

The breeding of new strains of fruit/nut trees is tremendously important both in economic terms and for human nutrition. Until now, however, our ability to manipulate fruit/nut tree strains through genetics has been limited by the extended maturation period of these plants. In human terms, this has meant that a fruit tree breeder might have to convince his/her grandchildren to help analyze (or harvest) work started decades before. As an example, seeds from almond trees selected for self-compatibility in Murcia (Spain) in 1972, were available for crossing only in 1989 and released as new varieties 10 years later after evaluation of the F1 generation (L. Egea, personal communication). During the juvenile period, it is not possible to measure yield capacity, flowering time, mature tree architecture, branching, or resistance. Trait analysis often requires between three and five years after the first flowers appear, when trees become fully productive[2]. As a result, tree breeding research is among the slowest moving fields in plant biotechnology.

Now Martiñez-Zapater *et al*[1]. have shown that basic knowledge obtained in the plant model system *Arabidopsis* can be exploited to change and maybe to create a whole new field in plant biotechnology. Using transgenic approaches, they demonstrate that the *Arabidopsis* genes *APETALA 1* (*AP1*) and *LEAFY* (*LFY*) complementary DNAs, under the control of the commonly used cauliflower mosaic virus 35S promoter, can significantly alter the juvenile period of orange trees. As expected for normal orange trees, transgenic control plants flower in five or six years. In contrast, transgenic trees expressing either *LFY* or *AP1* show no such delay and flower the spring following transformation. This accelerated juvenile period (characterized by decreased thorn production and leaf shape) is heritable in crosses with nontransformed plants and in plants raised from self-pollination. In all cases, plants harboring either 35S:*LFY* or 35S:*AP1* flower within a year after germination. Notably, plants expressing the *AP1* gene always formed flowers at the proper time of the year (spring).

The work clearly demonstrates that the juvenile phase in trees can be manipulated by floral meristem identity genes[3,4] from model organisms like *Antirrhinum* or *Arabidopsis*. To date, *Squamosa* (*SQUA*)/*AP1*[5,6] orthologs have been cloned in several plants and ectopic expression of *AP1* in *Arabidopsis* shown to induce early flowering[7]. The work of Martiñez-Zapater *et al.* confirms these findings, demonstrating that floral primordia are produced sooner than expected in *AP1/LFY* transgenic plants, even though environmental control of the process imposes a flowering period coinci-

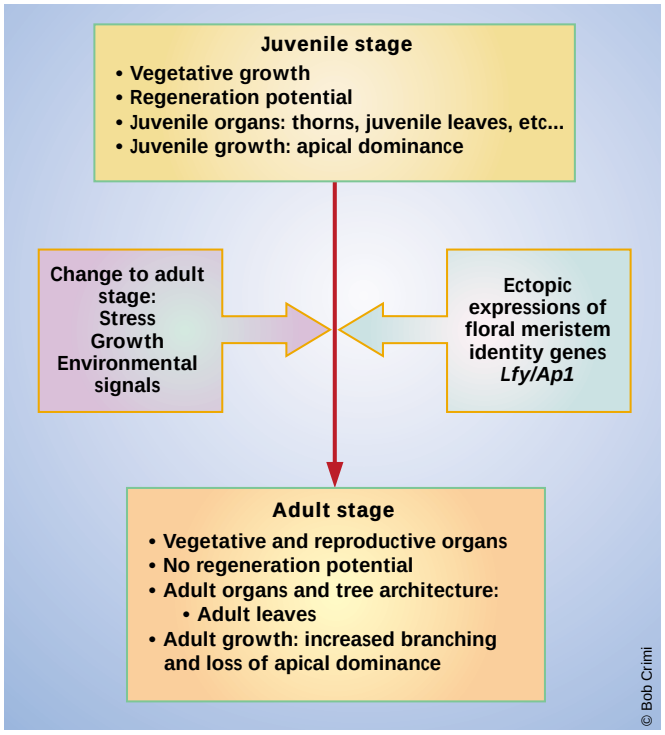

Figure 1. Reaching the next stage. Martiñez-Zapater have shown that ectopic expression of *LFY/AP1* can significantly shorten the juvenile phase in the development of orange trees.

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dent with spring. Ectopic constitutive expression of *AP1* or *LFY* appears important in determining juvenile-phase length, although neither gene plays such a role in *Arabidopsis* when expressed from its natural promoter.

Identification of conserved biological functions across species that can be manipulated by transgene strategies is one aspect of functional genomic research that relies on genetic analysis in model plants like *Arabidopsis*. Determining whether the pathway is conserved in other plants, however, will become increasingly important as knowledge is transferred from such model plants into crops. One notable finding in the present work is the effectiveness of *AP1* in manipulating orange tree development, despite its inability to effect juvenility in aspen. This suggests that we might have to identify conserved pathways suitable for manipulation in individual species.

The ability of genes from *Arabidopsis* to have such profound effects on plants with such different growth habits and distance in evolution is remarkable. Experiments using *Drosophila*[8] as a model suggest that master genes recruited to control a certain pathway during evolution may be conserved in structure and function, whereas peripheral genes under their control may diversify. Perhaps the ancestor of *SQUA/AP1* may have been a master gene controlling floral meristem identity, which can also fortuitously influence time to flower if misexpressed. Different plants have acquired different sets of elements regulating both the onset of gene activation of *SQUA/AP1* and their partners, but *FLO/LFY* still control *SQUA/AP1*, and if the proper partners are there, flowers will form. In orange trees, those partners seem to be recruited, appearing in the spring. It is still not clear, however, what makes juvenile phases fade and allow adult growth habit to take over the whole architecture.

The ability of genes from *Arabidopsis* to have such profound effects on plants with such different growth habits and distance in evolution is remarkable.

As a final point, although the prolonged juvenile period may be a drawback for tree breeding progams, it is also the only stage from which explants can be used for *in vitro* regeneration or simple vegetative propagation—approaches important for maintaining germplasm in conifers or trees that are many centuries in age. The work of Martiñez-Zapater *et al*. suggests that we now have at our disposal a means of creating experimental models to study juvenility in trees both to facilitate tree breeding and biodiversity efforts. It has been a long time coming.

1. Martiñez-Zapater J.M. *et al. Nat. Biotechnol.* **19**,263–267 (2001).
2. Asins, M.J., Maestre, P., García, J.E., Dicenta, F. & Carbonell, E.A. *Theoret. Applied Genet*. **89**, 358–364 (1994).
3. Coen, E.S. *et al. Cell* **63**, 1311–1322 (1990).
4. Weigel, D., Alvarez, J., Smyth, D.R., Yanofsky, M.F. & Meyerowitz, E.M. *Cell* **69**, 843–859 (1992).
5. Huijser, P *et al. EMBO J*. **11**, 1239–1249 (1992).
6. Mandel, M.A., Gustafson-Brown, C., Savidge, B. & Yanofsky, M. *Nature* **360**, 273–277 (1992).
7. Mandel, M.A. & Yanofsky, M. *Nature* **377**, 522–524 (1995).
8. Gehring W.J. & Kazuho I. *Trends Genet*. **15**, 371–377 (1999).