The Evolution of Vernalisation in Flowering Plants

For submission to trends in Plant Science – NB: Mendeley referencing style for submission is IEEE

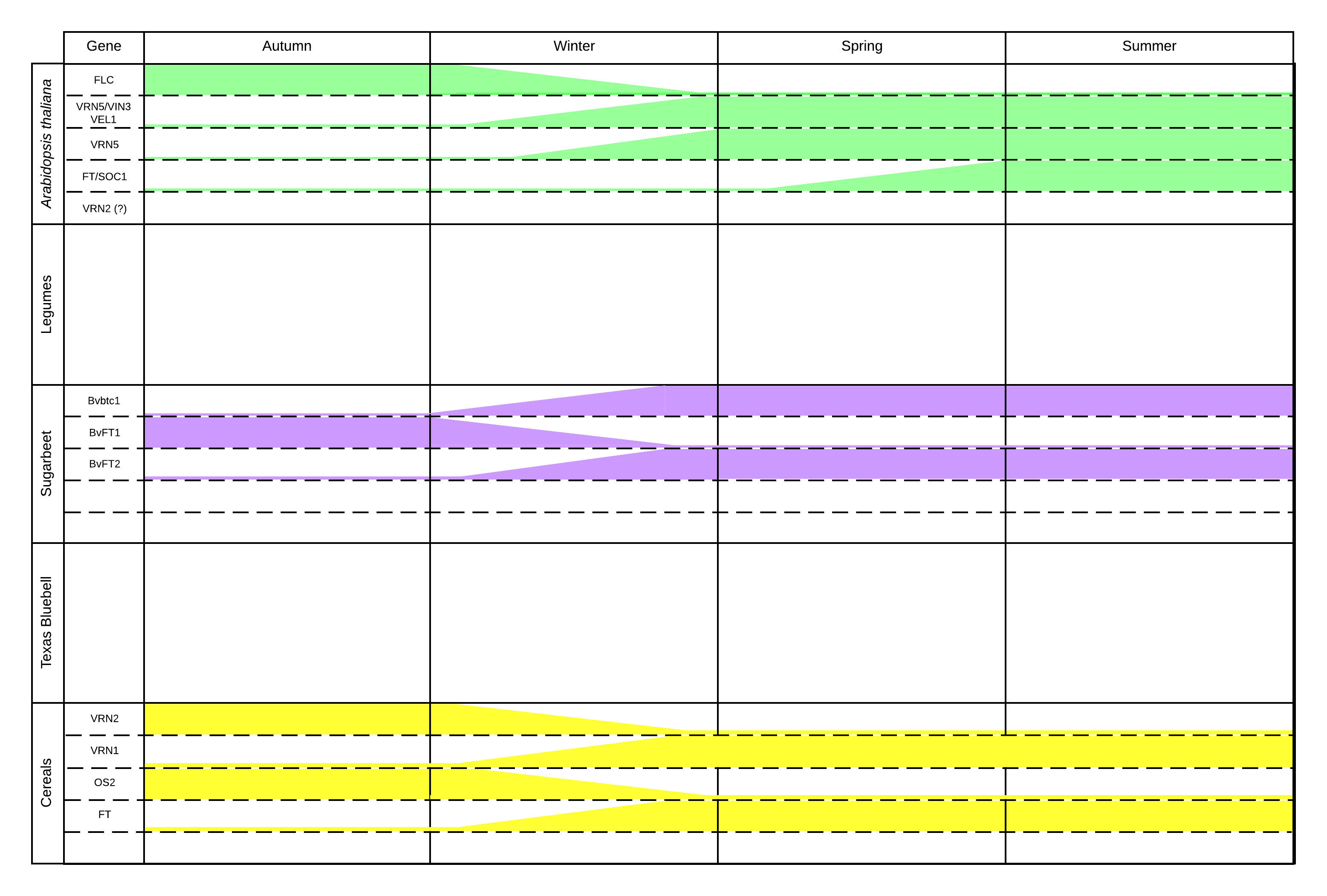
* Physiological response of vernalisation
* Effects of vernalisation
* Defined as “SEED cold treatment”

The Vernalisation response

Vernalisation (from the Latin *vernum*, meaning *spring*), and its effect on harvest time and crop yield, has been a central research focus of the plant biology community for over 150 years (Klippart 1857). Vernalisation is characterised by a prolonged (i.e. greater than ten days) exposure to low but non-freezing temperature (Burn et al. 1993), with an effect of early flowering in susceptible plant varieties. Furthermore, the resultant time to flowering is directly proportional to the length of cold exposure (Sheldon et al. 2000). During vegetative growth, cells in the shoot apical meristem actively divide and differentiate into vegetative growth tissues (Fletcher 2002). Once *FLOWERING TIME* (*FT*) is expressed in leaf tissue in response to external environmental cues, such as vernalisation conditions, FT travels through the phloem and into meristem tissues (Corbesier et al. 2007). Once there, FT triggers floral induction by transitioning meristem cells to a reproductive state. These then divide and differentiate into reproductive tissues (REFERENCE). It has been demonstrated that after exposure to vernalisation conditions, a vernalised shoot tissue can be grafted to another plant, effectively transferring the effect to the new root stock (REFERENCE). This observation has been corroborated in both *facultative* (where vernalisation decreases time to flowering, but is not essential for flowering), and *absolute* (where vernalisation is required to progress from vegetative growth to flowering) vernalisation sensitive species (REFERENCE). Contemporary research has shown that the regulatory pathways and genetic mechanisms of vernalisation are species-specific (Reeves et al. 2007). Being epigenetic in nature, the response pathway of all vernalisation responsive plant species is mechanistically related, i.e. environmental cues can modify gene expression between varieties of the same species, resulting in expression of different phenotypes (REFERENCE). These epigenetic changes are reset after each generation (REFERENCE).

<Phylogenies – ½ page>

* What is the evolutionary timeline for flowering plants?
* Phylogenetic Tree
* <combine phylogenies with physiological response – Fig 1>



Vernalisation in Arabidopsis <1 page>

* NB: Finnegan 2005 says DNA methylation does NOT contribute to vernalisation and repression of FLC! Countered by Lee et al 2015 (expression of DNA methyltransferase and timing of vernalisation?)

(Boss et al. 2004)

VIN3 – Loss of function mutant, tends to directly relate to detection of cold (Sung & Amasino 2004)

The MADS-box transcription factor FLC mediates the transition of *Arabidopsis* to its reproductive state (Sheldon et al. 2000)*.* While research has shown that both genetic and epigenetic mechanisms contribute to *FLC* repression during this transition, the exact nature of the epigenetic mechanisms are still in question (Finnegan et al. 2005; De Lucia et al. 2008).

*FRIGIDA* (*FRI*), *FRIGIDA-LIKE 1* (*FRL1*) and *FRIGIDA-LIKE 2* (*FRL2*) promote *FLC* expression,. This in turn represses the expression of *FLOWERING TIME* (*FT*), the FT homolog *TWIN SISTER OF FT* (*TSF*), and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). This, in turn, promotes expression of *LEAFY* (*LFY*) and *APETALA1* (*AP1*), the two primary promoters of floral apical meristem growth (Amasino 2004).

Upon induction of FLC expression, the VERNALISATION 2 (*VRN2*)/Plant Homeo domain Polycomb Repression Complex 2 (PHD-PRC2) consisting of *VRN2* and PHD-PRC2 proteins, *CURLY LEAF* (*CLF*), *SWINGER* (*SWN*) and *FERTILIZATION-INDEPENDENT ENDOSPERM* (*FIE*) (Köhler & Villar 2008) is constitutively bound to the *FLC* locus, whereas VERNALISATION 5 binds to a specific locus. The binding of the VRN2/PHD-PRC2 complex to *FLC* maintains the locus in an open conformational shape, allowing transcriptional machinery to access the *FLC* regulatory sequence and promote *FLC* expression via H3 acetylation (De Lucia et al. 2008). During vernalisation, *VERNALISATION INSENSITIVE 3 (VIN3)* levels increase. VIN3, along with *VERNALISATION 5/VIN3-LIKE* (*VEL1*) and *VERNALISATION 5* (*VRN5*), bind to the PHD-PCR2 complex to promote histone H3 deacetylation and *VRN2*-directed methylation of H3K9 and H3K27. Methylation of the *FLC* locus closes the open conformation of *FLC*, blocking the transcription machinery from accessing *FLC*, thereby repressing *FLC* expression (Finnegan & Dennis 2007). This epigenetic repression of *FLC* is stable and irreversible, ensuring the transition to a flowering state is permanent (Levy et al. 2002; Sung & Amasino 2004). Reduced levels of FLC leads to increased expression of *SOC1* and *FT*. Increased SOC1 and FT levels in turn enhance the expression of the floral promoters *LFY* and *AP1*.

The Photoperiod Pathway, also referred to as the External Coincidence Model, mediates the transition to flowering by exposure to increasing day length without a vernalisation period (Hayama & Coupland 2004). Increased photoperiod promotes the expression of *CONSTANS* (*CO*), which in turn overrides the repressive effects of FLCvia CO-mediated activation of *FT* and *SOC1* expression (Golembeski et al. 2014). If the vernalisation or photoperiod pathways are not activated, eventually the Autonomous Pathway will trigger the transition of *Arabidopsis* to flowering(Simpson 2004).

<Vernalisation in Legumes – ½ page>

It has been known for some time that legumes such as *Lupinus albus*, *L. augustifolius* and   
*L. luteus* respond to vernalisation environmental cues, decreasing the time to bolt proportional to the time the seedlings have been exposed to the vernalisation conditions (Gladstones & Hill 1969; Landers 1995).

* Need some more information on the genes that regulate flowering in Legumes

<Vernalisation in Texas Bluebell? – ½ page>

Texas Bluebell (*Eustoma grandiflorum* and *Eustoma russelianum*) is an ornamental flowering plant native to the southern United States, Central America and the northern regions of South America. *Eustoma* have a similar vernalisation response to *Arabidopsis* and *L. augustifolius*, where the time to bolting is shortened in proportional to the amount of cold exposure (Pergola 1992). *Eustoma* contains homologs to FLC, FT and SOC1 (EgFLCL, EgFTL and EgSOC1L respectively) in *Arabidopsis*. While EgFTL and EgSOC1L appear to be functional homologs of their *Arabidopsis* counterparts, being lowly expressed until restoration of indiuctive photoperiod post vernalisation, when expression levels increase. However, EgFLCL appears to be lowly expressed during vegetative growth and only increases with the onset of vernalisation, the opposite of what is observed in *Arabidopsis* (Nakano et al. 2011). This may indicate that the function of EgFLCL is closer to that of MAF5 in *Arabidopsis* than other MADS-box containing transcripts such as FLC or MAF2-5 (Ratcliffe et al. 2003).

<Vernalisation in Sugarbeet – ½ page>

Sugar Beet (*Beta vulgaris* ssp. *Vulgaris*) is cropped for its large sucrose rich root organ. Early studies of demonstrated the necessity for photothermal induction (i.e. exposure to vernalisation conditions followed by increased day length) for flowering (Owen et al. 1940). Because of the absolute requirement for vernalisation for flowering, breeders have selected for phenotypes that maintain the vegetative and root growth state so as to maximise root yield. Recent studies have characterised the molecular mechanisms underpinning the vernalisation response in beet as distinct from both *Arabidopsis* and cereals (Pin et al. 2012). Two paralogous *FT* genes, *Flowering Time 1* (*BvFT1*) and *Flowering Time 2* (*BvFT2*), central to the regulation of flowering, are controlled by *BOLTING TIME CONTROL 1* *FLOWERING TIME 1* (*FT1*) is responsible for regulating *FLOWERING TIME 2* (*FT2*),(Pin et al. 2010). Rather than a single *FT* gene responsible for triggering flowering time controlled by FLC, as seen in *Arabidopsis* (*BvBTC1*). In biannual sugar beet, it is the recessive allele of *Bvbtc1* that produces a phenotype with an absolute vernalisation requirement. While the vernalisation pathway in *B. vulgaris* is distinct from *A. thaliana*, it also containsa number of homologous pathways, including the photoperiod pathway (Chia et al. 2008) and the autonomous pathway (Abou-Elwafa et al. 2011). However, these pathways are yet to be fully characterised in sugar beet.

<Vernalisation in Cereals – 1 page>

Cereals such as barley are regulated via expression of *VERNALISATION 2* (*VRN2*).

In monocotyledous species (monocots), such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and *Brachypodium distachyon* (a model monocot), the vernalisation pathway differs greatly from *Arabidopsis*. While there are many mechanisms conserved between monocots and *Arabidopsis,* such as the Photoperiod Pathway (Griffiths et al. 2003) and CO as a regulator of *FT* the most notable difference in cereals is the absence of a known homolog for AtFLC(Figure 6 below). Instead, HvVRN1 (a MADS-box transcription factor) and HvVRN2 (a zinc-finger motif with CCT domain, which in cereals is distinct from AtVRN2) (Yan et al. 2004), along with HvFT, are responsible for regulating flowering time in cereals. Before wintering, the cereal remains in a state of vegetative growth due to the presence of HvVRN2, which represses *HvFT* expression (Ream et al. 2014). The floral repressor, ODDSOC2 (OS2), also present at high levels in winter cereals prior to vernalisation, maintains the cereal’s vegetative growth state (Greenup et al. 2010). Once exposed to vernalisation conditions, winter barleys increase expression of *HvVRN1* via lower H3K27me3 and higher H3K4me3 levels in the *HvVRN1* locus. This opens the histone conformational shape, allowing *VRN1* expression (Oliver et al. 2009). Similar to *FLC* in *Arabidopsis*, this change is stable, but in winter barley, the shape change allows access (rather than restricting access) to the *HvVRN1* gene. Increased levels of HvVRN1 in turn repress expression of *HvVRN2*. Low HvVRN2 levels promote *HvFT* expression, triggering the transition of the winter barley to flowering (Trevaskis et al. 2006). Exposure to vernalisation conditions and the presence of HvVRN1 also stably inhibits the expression of *OS2*. Lower levels of OS2 promote the expression of *FPF1*, which in turn promotes the transition to a flowering state.

Due to its acclimatisation to tropical environments, the vernalisation response in rice (*Oryza sativa*) differs substantially to other cereals. Exposing rice to cold conditions during development, significantly warmer and for shorter periods than seen in vernalisation, damages the developing flower and seed heads, resulting in sterility and significant crop losses (Oliver et al. 2007).

<Whole Genome Sequencing – ½ page>

* Enabling technology for elucidating genes and gene pathways

<Vernalistion in Asteraceae>

<Figure 2 – Quantitative vernalisation response in lettuce>

While much research has been undertaken with regards to the vernalisation response in *Arabidopsis*, cereals and sugar beet, current research regarding the vernalisation response in safflower, and indeed, other members of the *Asteraceae* family is relatively scarce. Early research in lettuce (*Lactuca sativa*) observed that germinated seeds that were vernalised prior to planting responded by bolting up to four weeks earlier than unvernalised seeds (Gray 1942; Warne 1947; Rappapport et al. 1956). Later studies indicated that, similar to *Arabidopsis*, given the correct daylight exposure, lettuce bolts to flowering without the need for vernalisation, indicating a *facultative* vernalisation response in vernalisation sensitive lettuce species (Waycott 1995). However, all of this research focuses on the expressed phenotype and did not examine anything at the genetic level.

Chicory (*Cichorium intybus*), is an *Asteraceae* with an absolute vernalisation requirement. It has been demonstrated that when exposed to vernalisation conditions, chicory expresses CiFL1, a MADS-box transcription factor with significant sequence homology to AtFLC (Périlleux et al. 2013). To confirm this similarity, when *CiFL1* was transformed into *Arabidopsis* and over expressed, the mutants showed a significant delay in onset of flowering, regardless of vernalisation exposure, indicating a similar molecular pathway effect between CiFL1 and AtFLC.

Early studies in safflower indicate a relationship between photoperiod, vernalisation and a decrease in the time to flowering in some safflower varieties (Zimmerman 1973). However, as mentioned above, the limited availability of genetic resources for safflower and the *Asteraceae* make characterisation of molecular pathways and mechanisms in these species challenging. As further resources become available and are annotated, the mechanisms by which vernalisation, and other factors, affect flowering time will be better understood.

* Focus on Lettuce physiological response, but no quantitative/molecular data available for vernalisation

(Warne 1947)

<Conclusions>

* Reiterate the question “How do plants sense the cold?”
  + We thought discovering genes would reveal this, but not so
* Often easier to confirm activated genes than repressed ones

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