Report

Current Biology

Vernalization and Floral Transition in Autumn Drive Winter Annual Life History in Oilseed Rape

Highlights

- Levels of the key floral repressor *FLC* fall during October rather than during winter
- Warming field plots in October delays flowering in spring
- Plants overwinter as an inflorescence meristem in a manner resembling perennials

Authors

Carmel M. O'Neill, Xiang Lu, Alexander Calderwood, ..., Rachel Wells, Richard Morris, Steven Penfield

Correspondence

steven.penfield@jic.ac.uk

In Brief

Vernalization has been traditionally understood as a response of plants to prolonged winter temperatures, which enable plants to flower. However, here, O'Neill et al. use a plot warming system to show that vernalization is an autumn phenomenon in the field.

O'Neill et al., 2019, Current Biology *29*, 4300–4306
December 16, 2019
https://doi.org/10.1016/j.cub.2019.10.051

CellPress

CellPress

Current Biology
Report

Vernalization and Floral Transition in Autumn Drive Winter Annual Life History in Oilseed Rape

Carmel M. O'Neill,[1] Xiang Lu,[1] Alexander Calderwood,[1] Eleri H. Tudor,[1] Philip Robinson,[1] Rachel Wells,[1] Richard Morris,[1] and Steven Penfield[1,2,*]

[1]Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK
[2]Lead Contact
*Correspondence: steven.penfield@jic.ac.uk
https://doi.org/10.1016/j.cub.2019.10.051

SUMMARY

Plants with winter annual life history germinate in summer or autumn and require a period of prolonged winter cold to initiate flowering, known as vernalization. In the Brassicaceae, the requirement for vernalization is conferred by high expression of orthologs of the *FLOWERING LOCUS C* (*FLC*) gene, the expression of which is known to be silenced by prolonged exposure to winter-like temperatures [1]. Based on a wealth of vernalization experiments, typically carried out in the range of 5°C–10°C, we would expect field environments during winter to induce flowering in crops with winter annual life history. Here, we show that, in the case of winter oilseed rape, expression of multiple *FLC* orthologs declines not during winter but predominantly during October when the average air temperature is 10°C–15°C. We further demonstrate that plants proceed through the floral transition in early November and overwinter as inflorescence meristems, which complete floral development in spring. To validate the importance of pre-winter temperatures in flowering time control, we artificially simulated climate warming in field trial plots in October. We found that increasing the temperature by 5°C in October results in raised *FLC* expression and delays the floral transition by 3 weeks but only has a mild effect on flowering date the following spring. Our work shows that winter annuals overwinter as a floral bud in a manner that resembles perennials and highlights the importance of studying signaling events in the field for understanding how plants transition to flowering under real environmental conditions.

RESULTS AND DISCUSSION

Plants use environmental cues to monitor seasonal progression and to optimize the timing of reproductive development to specific seasonal windows [2]. Winter annual plants are characterized by germination in the autumn and require the experience of a prolonged period of winter cold, known as vernalization, in order to promote flowering in spring [3]. In temperate regions, winter crop varieties are preferred where cultivation is climatically favorable because of higher yields and the presence of soil-stabilizing root systems in winter [4].

In *Arabidopsis* and other members of the Brassicaceae family, the sensing of vernalizing temperatures is performed primarily by the *FLOWERING LOCUS C* (*FLC*) locus [1]. *FLC* is highly expressed in young plants and epigenetically silenced by prolonged exposure to winter cold, in a process that targets polycomb repressive complex 2 to the first intron [5]. However, although major described flowering time genes are highly conserved and have documented large effects under laboratory conditions, their loss does not always translate to large effects on flowering date in the field [6]. The strong laboratory and weaker field phenotypes remain unexplained. Some phenotypes observed in constant laboratory temperatures are less distinct in more realistic temperature regimes [7]: this raises the question of other processes affecting flowering time in the field, in addition to passage through the floral transition. The timing of key molecular events in the field is only now starting to be understood [8].

Winter oilseed rape is an important oil crop in Europe and is sown in late summer, remaining vegetative in the field until flowering the following spring and exhibiting a classical winter annual phenology (Figure 1A). In the United Kingdom, bolting most commonly occurs at the end of winter, with flowering and seed set in spring and early summer. Many genes shown to be important for *Arabidopsis* flowering time control are known to share conserved functions in *Brassica napus* [9–14], including the key role of *FLC* in conferring a vernalization requirement.

To understand how and when major molecular processes that underpin the transition to flowering take place in the field, we first generated an RNA sequencing (RNA-seq) data series of apical meristems and surrounding tissue (STAR Methods) from the winter oilseed rape variety Cabriolet at 2 weekly intervals between the end of seedling establishment in September through to just before the appearance of first flowers in the following March (Figure 1D). Principal component analysis (Figure 1B) suggested that the major factor causing the variance in the dataset (PCA1; explaining 25.1% of the variation) was aligned to developmental time because PCA1 separates samples in approximate chronological order. PCA2 explained 17.9% of the variation and was low in late November and in winter and higher at other times of the year, suggesting a seasonal origin. Other principle components explained less than 10% of the total variation between samples. Because of the key role of transcription factors in reproductive development, we focused analysis initially on identifying key patterns of transcription factor

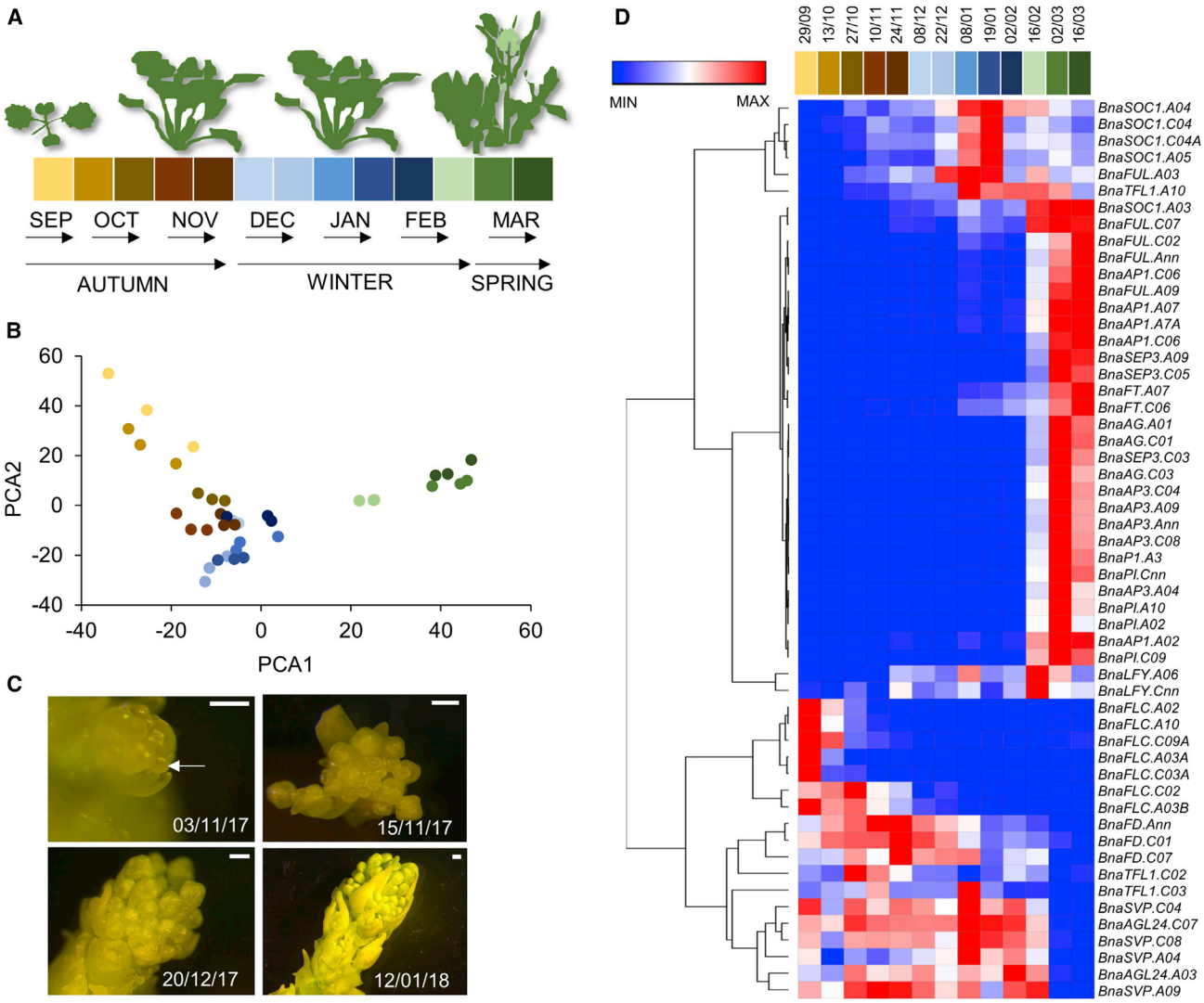


Figure 1. Winter Oilseed Rape Proceeds through the Floral Transition in Mid-Autumn in Norwich, UK

(A) Schematic to show the growth cycle of winter oilseed rape in United Kingdom. Each color block represents sampling time of shoot apices at 2-week intervals in the 2016/2017 growing season.

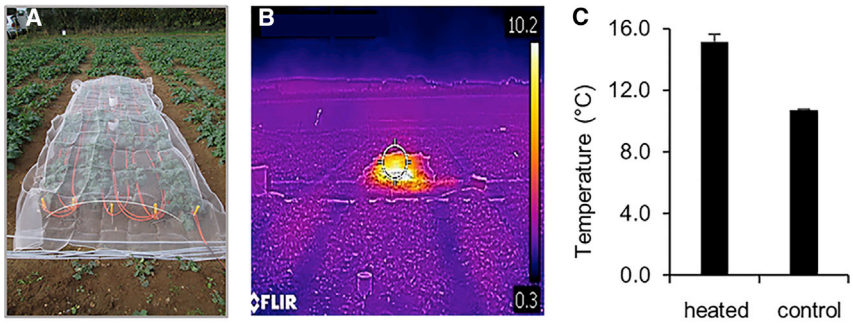
(B) Principal-component analysis to show the relatedness of three biological replicate samples for each time point of the transcriptome sample series, with colors to match those in (A).

(C) Dissected shoot apical meristems of variety Cabriolet during the floral transition with sampling dates in the 2017/2018 growing season. The first image shows presence of floral organ primordia (white arrow). Scale bars represent 200 μM.

(D) Hierarchical clustering of gene expression of selected key regulators of plant reproductive development in winter oilseed rape. Mean of three replicates is shown, centered on the mean for each gene. A complete dataset dendrogram of all transcription factors is shown in Figure S1.

See also Figure S2.

expression, using a hierarchical clustering approach (Figure S1A). This analysis suggested that the seasonal expression of transcription factors could be divided into a small number of major classes that varied in expression through the growing season and coincided with the expression of key known regulators of the floral transition (Figures S1 and 1D). One cluster had high expression at the start of the time series, which then declined sharply during mid-autumn (Figure S1, clade 3). A second class started the series with undetectable or low expression, was strongly induced only at the end of winter and spring (Figure S1, clade 5), and contained the well-known floral homeotic genes. In the 3 months between the expression of these two gene groups, two further principle gene expression patterns could be observed: one of roughly stable expression until spring when expression suddenly declined (Figure S1, clade 2), which contained homologs of *Arabidopsis SHORT VEGETATIVE PHASE* (*SVP*) and *AGAMOUS-LIKE 24* (*AGL24*). A final more complex pattern with peaks of gene expression during winter was observed (Figure S1, clade 1). Studying the genes within each class, the October-expressed cluster contained all *Brassica napus FLC* isoforms, of which there are 9 in *Brassica napus* [10]. Seven *FLC* genes were highly expressed in Cabriolet shoot



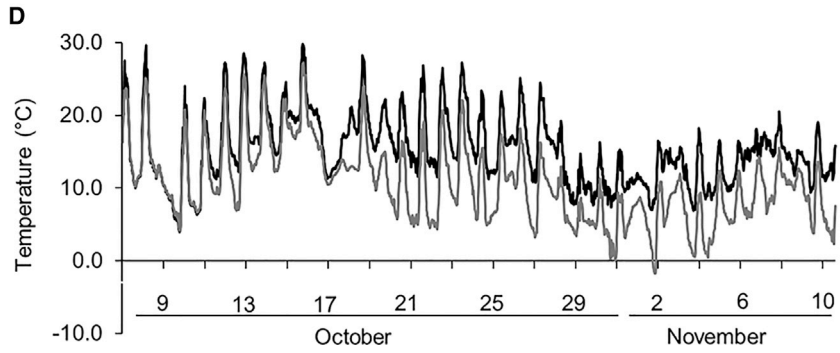


Figure 2. A Plot-Warming Experimental Setup for the Interruption of Vernalization

(A) Image to show a single warmed plot with surface-heated cables running either side of winter oilseed rape rows in October 2018.

(B) Thermal camera image from 2017 showing the relative temperature differential between warmed plot and surrounding trial plots.

(C) Chart to show the mean temperature differential for control and heated plots between October 8, 2018 and November 9, 2018, when warming ceased.

(D) Real-time temperature data of control (gray line) and warmed (black line) of Cabriolet plots between October 8, 2018 and November 9, 2018.

apices (Figure 1D), and five of these had declined in gene expression to very low levels by the beginning of November. All *FLC* gene expression decreased to low levels by the end of November, when the mean daily air temperature was between 10°C and 15°C (Figure S1B). Homologous genes known from *Arabidopsis* to be highly expressed in inflorescence meristems at the floral transition, such as *LEAFY* [15] and *SUPPRESSOR OF CONSTANS 1* (*SOC1*) [16], were detected in Cabriolet during November for the first time. In *Arabidopsis*, *SOC1* expression is repressed by the FLC protein [17]. The detection of *SOC1* expression in Cabriolet, which coincided with a reduction in the expression of *FLC*, is consistent with low FLC protein activity at the shoot apex. Finally, well-known floral meristem and floral organ identity genes, such as *AP1*, *FUL*, and *SEP3*, were more highly expressed in late winter and spring, indicating a transition to flowering (Figure 1D). Taken together, these data suggested that, in the *Brassica napus* variety, Cabriolet *FLC* repression occurred predominantly in October (mid-autumn) and not during winter, as is most commonly assumed. Furthermore, subsequent gene expression changes were consistent with an autumn entry into an inflorescence meristem stage, which persisted until late winter, when the final transition to flowering coincided with observed bolting of the plants. This saw high expression of *FRUITFULL* (*FUL*) and floral organ identity genes, coupled with loss of *SVP* and *AGL24* expression, consistent with a transition from inflorescence to floral organ meristem identity [18]. Interestingly, two *Brassica* homologs of *Arabidopsis FLOWERING LOCUS T* (*FT*) (*BnFT.A07* and *BnFT.C06*) were expressed in the shoot apex itself in late winter, in line with observations during bud burst in perennial species [19].

To test the hypothesis that life history strategy in winter annual *Brassica napus* requires autumn floral transition, we dissected shoot apical meristems of variety Cabriolet for microscopic analysis in the 2017/2018 growing season (Figure 1C; similar data are shown for 2018–2019 in Figure 3). By the 3rd November, the apex had developed some floral characteristics, including floral meristems and a domed appearance of the apical meristem itself. By 15th November, the apical meristem had clearly transitioned to inflorescence development, and this continued throughout winter (Figure 1C). Our observation that the transition from vegetative to inflorescence development in Cabriolet by November is consistent with vernalization, as sensed by *FLC*, being an autumn-determined phenomenon, as has previously been described for a Northern Swedish *Arabidopsis* accession [20]. This raised the prospect that autumn floral transition is the default winter annual life history strategy in *Brassica napus*. We tested this hypothesis by following a panel of current and recent European winter oilseed rape commercial varieties. Each of 9 varieties tested displayed reproductive meristem characteristics in November (Figure S2), from which we concluded that this is a general feature of winter *Brassica napus* phenology. In *Arabidopsis*, sequential aspects of the floral transition occur over a timescale of hours [18, 21]; however, our analysis shows that this process can require several weeks to months in the field for winter *Brassica napus*.

Previously, we have identified that, in the United Kingdom, warmer Octobers have been associated with higher yields the following summer [22]. The observation that *FLC* expression declines predominantly in October led us to hypothesize that warm temperatures in October might delay flowering. This hypothesis is consistent with later flowering being associated with higher yields [13, 23]. To test plant responses to climate change, warming cables have been used to raise temperatures in natural grasslands to simulate winter warming [24]. We employed a similar approach to warm field plots in Norwich, UK (Figure 2; STAR Methods). Use of soil-surface-warming cables could raise the temperature of field plots by between 4°C and 8°C, depending on weather conditions (Figures 2C and 2D). We hypothesized that, if vernalization is occurring during October, then warming in October would lead to later rather than earlier flowering and that this would be accompanied by higher *FLC* gene expression levels. To test this, warming cables were switched on at the beginning of October and switched off when plants in control

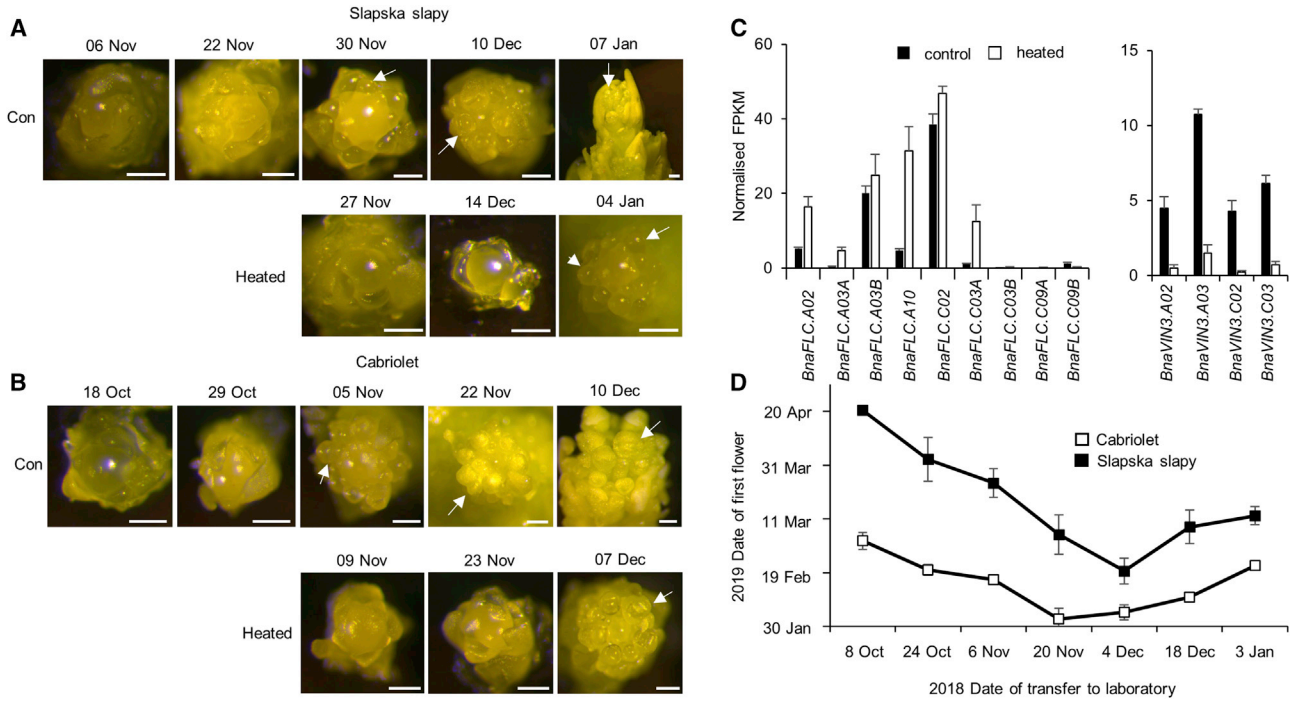


Figure 3. Warming Field Plots in October Delays the Floral Transition by at Least 20 Days and Increases *FLC* Expression in November

(A and B) Dissected shoot apices of Cabriolet (A) and Slapska Slapy (B) plants on the indicated dates in control and warmed plots. White arrows indicate the presence of floral organ primordia, indicating floral transition.

(C) Gene expression of *B. napus FLC* and *VIN3* orthologs in Cabriolet apices (from control and heated plots November 9, 2018), as derived by RNA-seq. Data indicate the mean and SE of three biological replicates.

(D) Plant transfer experiment showing that vernalization occurs in the field during October in Norwich, UK. Plants were removed from the field at the indicated dates in 2018 to 2019 on the x axis and placed in a controlled environment room, with flowering times in 2019 recorded on the y axis. The delay to flowering date caused by early transfer from the field conditions indicates that plants that remained in the field were vernalizing. Data indicate mean and SE of 5 plants per time point per genotype.

See also Figure S3.

plots proceeded through the floral transition at the shoot apex, as determined by microscopy. This experiment was performed using two winter *B. napus* varieties, Cabriolet and Slapska Slapy. Cabriolet shows a weaker vernalization requirement (but which is nevertheless standard for modern varieties grown in the UK; Figure S2), whereas laboratory experiments revealed that Slapska slapy has a requirement of more than 12 weeks in the cold to promote all plants reliably into flower, compared to 6 weeks for Cabriolet (Figure S3A). The vernalization requirement phenotype of Slapska Slapy is therefore comparable with *Arabidopsis* accessions, such as Var2-6 and Lov-1 from N. Sweden, described as having long cold duration requirements for vernalization [25]. Interestingly, previously published transcriptome data suggest the total *BnaFLC* expression level in leaves before vernalization is approximately three-fold higher in Slapska Slapy compared with Cabriolet (Figure S3B) [26].

Warming began on 8th October 2018 and continued until control plants progressed through the floral transition for each variety (9th November for Cabriolet and 27th November for Slapska Slapy; Figure 3A). By dissecting apices, we found that warming during October delayed the floral transition by between 3 and 4 weeks for both varieties (Figures 3A and 3B), with Cabriolet delayed from the first week of November until the beginning of December. Surprisingly, we found in control plots that Slapska Slapy proceeded through the floral transition only 3 weeks after Cabriolet in Norwich, UK (Figure 3), and morphological evidence showed that warming delayed the floral transition at the apex by a similar length of time to Cabriolet. Next, we compared *FLC* gene expression in control and warmed Cabriolet plots as the control plants began the floral transition (Figure 3C). We used RNA-seq to ensure we captured the behavior of all homeologous gene copies in *B. napus*. We found that at least three copies of *FLC* exhibited higher expression under warm conditions. Warming also reduced the cold-induced increase in *VERNALISATION INDEPENDENT 3* (*VIN3*) gene expression known from *Arabidopsis* to be necessary for vernalization [27]. Together, our data showed that warm weather during October delayed the floral transition and results in increased *FLC* expression compared to control plants at the same time of year.

To further confirm that field conditions in October led to vernalization, we removed plants from field plots and placed them in controlled environment chambers in spring-like conditions at 2 weekly intervals during autumn and early winter. With increasing vernalization in the field, *Arabidopsis* plants flower more rapidly when transferred to controlled, warm conditions [20]. We found that Cabriolet and Slapska Slapy plants that remained in the cooler field conditions for longer flowered earlier compared to those plants brought into controlled laboratory

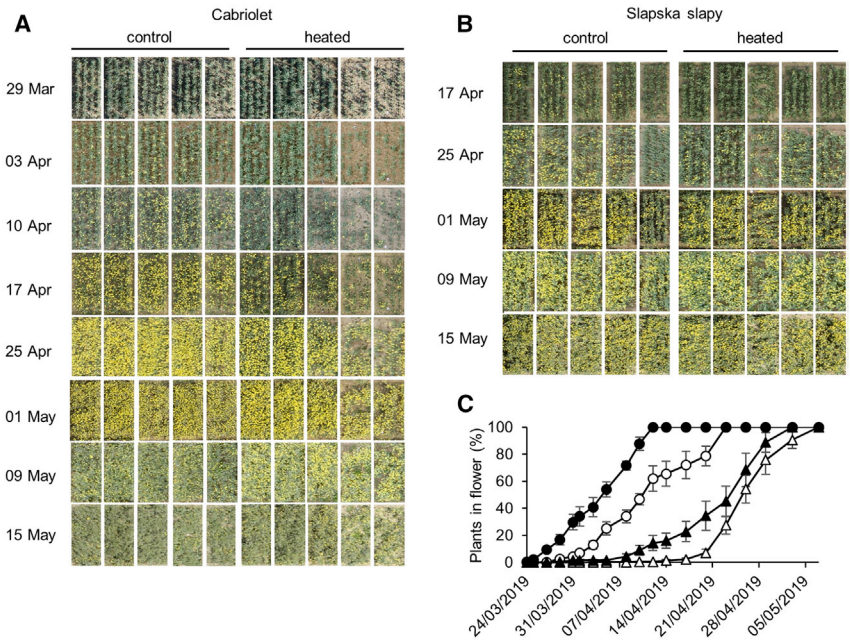


Figure 4. October Warming Produces a Mild Delay to Flowering Date the following Spring

(A) Drone flyover images of Cabriolet control and heated plots, showing the timing of peak flowering through the appearance of yellow color.

(B) Drone flyover images of Slapska slapy control and heated plots, showing the timing of peak flowering through the appearance of yellow color.

(C) Manual counts of the proportion of plants in flower in control and warmed plots on the indicated dates. Filled symbols, control plots, open symbols, and warmed plots. Circles indicate Cabriolet plots and triangles Slapska Slapy. Data are the mean and SE of 5 plots per treatment.

See also Figure S4.

conditions earlier in autumn (Figure 3D). This effect continued up to and beyond the date of the floral transition at the apex (compare Figures 3A, 3B, 3D, and 4) and was extended for the later vernalizing Slapska Slapy variety compared to Cabriolet. Interestingly, for plants remaining in the field 20 days or more after the floral transition, field temperatures began to delay flowering compared to controlled conditions, suggesting that, after the floral transition, warm temperatures accelerate flowering.

In spring, flowering was scored in control and warmed plots by visible appearance of yellow flowers, captured by drone flyovers, and by counting the frequency of plants with at least one open flower in each plot. Dissecting apices in late February revealed that the unheated Cabriolet plants had begun to bolt before the heated plants (Figure S4), showing that the effects of October warming remained. For both varieties, we found that October warmth delayed time to 50% flowering by 5–7 days (Figures 4A–4C). Although the floral transition was delayed by 3 to 4 weeks, flowering was only changed by 5 days (Figure 3), showing that other processes in inflorescence meristems affect flowering date, in addition to the timing of the floral transition at the apex [28].

It is generally assumed that vernalization is a cold response that takes place during winter in temperate climates. However, our analysis shows that, instead, vernalization takes place predominantly during October, during which the mean air temperature is approximately 10°C–12°C in the United Kingdom. The floral transition then occurs at the apex in November. Previous research shows that the upper temperature limit for vernalization is at least 14°C in both *Arabidopsis thaliana* and 17°C for *Brassica napus* [20, 29, 30], consistent with the idea that, in the Brassicaceae family, vernalization is not a response to winter temperatures alone. We observed that this field behavior was stable over at least three growing seasons in 2016 to 2017 (Figure 1D), 2017 to 2018 (Figure 1C), and 2018 to 2019 (Figures 3 and S2). Furthermore, we found that all varieties tested shared a similar life history (Figure S2), indicating that this result is not due to unconventional behavior of our chosen experimental variety. Warming plots by an average of 5°C in October delayed the floral transition by at least 3 weeks but delayed flowering itself by only 5–7 days (Figure 4C). Warm Octobers are also historically associated with higher yields [22], suggesting that premature vernalization is a candidate mechanism for weather-related yield reductions in the United Kingdom. This indicates that other processes in addition to the timing of the floral transition control flowering date. This may explain the weak correlation between *FLC* expression level and flowering time previously observed [7]. *Brassica napus* plants in the field exhibited inflorescence meristem characteristics from November through to February, where strong induction of well-known floral organ identity genes takes place (Figure 1D). Over timescales of hours, a similar multi-step floral transition has been described previously at the *Arabidopsis* shoot apex [21]. In *Arabis alpina*, stable silencing of the *FLC* ortholog *PERPETUAL FLOWERING 1* (*PEP1*) is observed only when the floral transition takes place during vernalization [31]. This may be evidence that this species is also adapted to a life history with an autumn or winter floral transition. Overwintering as an inflorescence meristem is similar to the life history of conventional perennials, where chilling and subsequent warming are necessary to promote dormancy loss and out-growth of floral buds [32]. Indeed, a further similarity is the observation that two of the four *B. napus FT* orthologs are expressed in the apex itself (Figure 1D), as has been observed for hybrid aspen *FT1* before bud break [19, 33]. The transition from warm delaying flowering to warm promoting earlier flowering is also reminiscent of the perennial transition from endodormancy to ecodormancy. Our data show that winter annual behavior in the Brassicaceae is more similar to perennial life history than previously realized.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- LEAD CONTACT AND MATERIALS AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.10.051.

ACKNOWLEDGMENTS

This work was funded by BBSRC grant funding to the John Innes Centre, project numbers BB/P013511/1, BB/R004196/1, and BB/P003095/1. The authors would like to thank members of the John Innes Centre field trials team for their work on the project and Dr. Judith Irwin for constructive advice on experimental design.

AUTHOR CONTRIBUTIONS

Experiments were designed by S.P., R.W., and C.M.O. C.M.O., X.L., E.H.T., R.W., and P.R. conducted experimental work. Bioinformatics was conducted by A.C., R.M., and X.L. Further data analysis was conducted by S.P. and C.M.O. S.P. wrote the manuscript with assistance from co-authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: September 10, 2019
Revised: October 24, 2019
Accepted: October 25, 2019
Published: December 5, 2019

REFERENCES

1. Sheldon, C.C., Rouse, D.T., Finnegan, E.J., Peacock, W.J., and Dennis, E.S. (2000). The molecular basis of vernalization: the central role of FLOWERING LOCUS C (FLC). Proc. Natl. Acad. Sci. USA *97*, 3753–3758.
2. Springthorpe, V., and Penfield, S. (2015). Flowering time and seed dormancy control use external coincidence to generate life history strategy. eLife *4*, e05557.
3. Hepworth, J., and Dean, C. (2015). Flowering locus C's lessons: conserved chromatin switches underpinning developmental timing and adaptation. Plant Physiol. *168*, 1237–1245.
4. Thomas, C.L., Graham, N.S., Hayden, R., Meacham, M.C., Neugebauer, K., Nightingale, M., Dupuy, L.X., Hammond, J.P., White, P.J., and Broadley, M.R. (2016). High-throughput phenotyping (HTP) identifies seedling root traits linked to variation in seed yield and nutrient capture in field-grown oilseed rape (Brassica napus L.). Ann. Bot. *118*, 655–665.
5. Qüesta, J.I., Song, J., Geraldo, N., An, H., and Dean, C. (2016). Arabidopsis transcriptional repressor VAL1 triggers Polycomb silencing at FLC during vernalization. Science *353*, 485–488.
6. Wilczek, A.M., Roe, J.L., Knapp, M.C., Cooper, M.D., Lopez-Gallego, C., Martin, L.J., Muir, C.D., Sim, S., Walker, A., Anderson, J., et al. (2009). Effects of genetic perturbation on seasonal life history plasticity. Science *323*, 930–934.
7. Burghardt, L.T., Runcie, D.E., Wilczek, A.M., Cooper, M.D., Roe, J.L., Welch, S.M., and Schmitt, J. (2016). Fluctuating, warm temperatures decrease the effect of a key floral repressor on flowering time in Arabidopsis thaliana. New Phytol. *210*, 564–576.
8. Hepworth, J., Antoniou-Kourounioti, R.L., Bloomer, R.H., Selga, C., Berggren, K., Cox, D., Collier Harris, B.R., Irwin, J.A., Holm, S., Säll, T., et al. (2018). Absence of warmth permits epigenetic memory of winter in Arabidopsis. Nat. Commun. *9*, 639.
9. Tadege, M., Sheldon, C.C., Helliwell, C.A., Stoutjesdijk, P., Dennis, E.S., and Peacock, W.J. (2001). Control of flowering time by FLC orthologues in Brassica napus. Plant J. *28*, 545–553.
10. Zou, X., Suppanz, I., Raman, H., Hou, J., Wang, J., Long, Y., Jung, C., and Meng, J. (2012). Comparative analysis of FLC homologues in Brassicaceae provides insight into their role in the evolution of oilseed rape. PLoS ONE *7*, e45751.
11. Raman, H., Raman, R., Coombes, N., Song, J., Prangnell, R., Bandaranayake, C., Tahira, R., Sundaramoorthi, V., Killian, A., Meng, J., et al. (2016). Genome-wide association analyses reveal complex genetic architecture underlying natural variation for flowering time in canola. Plant Cell Environ. *39*, 1228–1239.
12. Schiessl, S., Iniguez-Luy, F., Qian, W., and Snowdon, R.J. (2015). Diverse regulatory factors associate with flowering time and yield responses in winter-type Brassica napus. BMC Genomics *16*, 737.
13. Shi, J., Li, R., Qiu, D., Jiang, C., Long, Y., Morgan, C., Bancroft, I., Zhao, J., and Meng, J. (2009). Unraveling the complex trait of crop yield with quantitative trait loci mapping in *Brassica napus*. Genetics *182*, 851–861.
14. Hou, J., Long, Y., Raman, H., Zou, X., Wang, J., Dai, S., Xiao, Q., Li, C., Fan, L., Liu, B., and Meng, J. (2012). A Tourist-like MITE insertion in the upstream region of the BnFLC.A10 gene is associated with vernalization requirement in rapeseed (Brassica napus L.). BMC Plant Biol. *12*, 238.
15. Blázquez, M.A., Soowal, L.N., Lee, I., and Weigel, D. (1997). LEAFY expression and flower initiation in Arabidopsis. Development *124*, 3835–3844.
16. Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F., and Coupland, G. (2000). Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. Science *288*, 1613–1616.
17. Lee, H., Suh, S.S., Park, E., Cho, E., Ahn, J.H., Kim, S.G., Lee, J.S., Kwon, Y.M., and Lee, I. (2000). The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in Arabidopsis. Genes Dev. *14*, 2366–2376.
18. Yu, H., Ito, T., Wellmer, F., and Meyerowitz, E.M. (2004). Repression of AGAMOUS-LIKE 24 is a crucial step in promoting flower development. Nat. Genet. *36*, 157–161.
19. Rinne, P.L., Welling, A., Vahala, J., Ripel, L., Ruonala, R., Kangasjärvi, J., and van der Schoot, C. (2011). Chilling of dormant buds hyperinduces FLOWERING LOCUS T and recruits GA-inducible 1,3-beta-glucanases to reopen signal conduits and release dormancy in Populus. Plant Cell *23*, 130–146.
20. Duncan, S., Holm, S., Questa, J., Irwin, J., Grant, A., and Dean, C. (2015). Seasonal shift in timing of vernalization as an adaptation to extreme winter. eLife *4*, e06620.
21. Wagner, D. (2017). Key developmental transitions during flower morphogenesis and their regulation. Curr. Opin. Genet. Dev. *45*, 44–50.
22. Brown, J.K.M., Beeby, R., and Penfield, S. (2019). Yield instability of winter oilseed rape modulated by early winter temperature. Sci. Rep. *9*, 6953.
23. Guo, Y., Hans, H., Christian, J., and Molina, C. (2014). Mutations in single FT- and TFL1-paralogs of rapeseed (Brassica napus L.) and their impact on flowering time and yield components. Front. Plant Sci. *5*, 282.
24. Grime, J.P., Brown, V.K., Thompson, K., Masters, G.J., Hillier, S.H., Clarke, I.P., Askew, A.P., Corker, D., and Kielty, J.P. (2000). The response of two contrasting limestone grasslands to simulated climate change. Science *289*, 762–765.
25. Li, P., Filiault, D., Box, M.S., Kerdaffrec, E., van Oosterhout, C., Wilczek, A.M., Schmitt, J., McMullan, M., Bergelson, J., Nordborg, M., and Dean, C. (2014). Multiple FLC haplotypes defined by independent cis-regulatory

variation underpin life history diversity in Arabidopsis thaliana. Genes Dev. *28*, 1635–1640.

26. Harper, A.L., Trick, M., Higgins, J., Fraser, F., Clissold, L., Wells, R., Hattori, C., Werner, P., and Bancroft, I. (2012). Associative transcriptomics of traits in the polyploid crop species Brassica napus. Nat. Biotechnol. *30*, 798–802.

27. Sung, S., and Amasino, R.M. (2004). Vernalization in Arabidopsis thaliana is mediated by the PHD finger protein VIN3. Nature *427*, 159–164.

28. Torti, S., Fornara, F., Vincent, C., Andrés, F., Nordström, K., Göbel, U., Knoll, D., Schoof, H., and Coupland, G. (2012). Analysis of the Arabidopsis shoot meristem transcriptome during floral transition identifies distinct regulatory patterns and a leucine-rich repeat protein that promotes flowering. Plant Cell *24*, 444–462.

29. Tommey, A.M., and Evans, E.J. (2008). Temperature and daylength control of flower initiation in winter oilseed rape (Brassica napus L.). Ann. Appl. Biol. *118*, 201–208.

30. Wollenberg, A.C., and Amasino, R.M. (2012). Natural variation in the temperature range permissive for vernalization in accessions of Arabidopsis thaliana. Plant Cell Environ. *35*, 2181–2191.

31. Lazaro, A., Obeng-Hinneh, E., and Albani, M.C. (2018). Extended vernalization regulates inflorescence fate in *Arabis alpina* by stably silencing *PERPETUAL FLOWERING1*. Plant Physiol. *176*, 2819–2833.

32. Singh, R.K., Svystun, T., AlDahmash, B., Jönsson, A.M., and Bhalerao, R.P. (2017). Photoperiod- and temperature-mediated control of phenology in trees - a molecular perspective. New Phytol. *213*, 511–524.

33. Karlberg, A., Englund, M., Petterle, A., Molnar, G., Sjödin, A., Bako, L., and Bhalerao, R.P. (2010). Analysis of global changes in gene expression during activity-dormancy cycle in hybrid aspen apex. Plant Biotechnol. *27*, 1–16.

34. Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. *14*, R36.

35. Chalhoub, B., Denoeud, F., Liu, S., Parkin, I.A., Tang, H., Wang, X., Chiquet, J., Belcram, H., Tong, C., Samans, B., et al. (2014). Plant genetics. Early allopolyploid evolution in the post-Neolithic Brassica napus oilseed genome. Science *345*, 950–953.

36. Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. *7*, 562–578.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
E.N.Z.A. Plant RNA Kit	Omega Bio-tek	R6827-01
Deposited Data		
RNaseq raw Illumina reads	This study	GenBank: PRJNA578690
Experimental Models: Organisms/Strains		
Brassica napus var Cabriolet	[22]	N/A
Brassica napus var Slapska slapy	[22]	N/A
Software and Algorithms		
Tophat2 v.2.1.1	[33]	https://ccb.jhu.edu/software/tophat/index.shtml
Cufflinks v.2.02	[34]	http://cole-trapnell-lab.github.io/cufflinks/
Morpheus	Broad institute	https://software.broadinstitute.org/morpheus
Other		
Soil warming cable 50 m	Thermoforce	HD5036
Environmesh	Agralan	HA50/1

LEAD CONTACT AND MATERIALS AVAILABILITY

This study did not generate new unique reagents. Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Steven Penfield (steven.penfield@jic.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All materials studied were winter *Brassica napus* varieties Cabriolet or Slapska slapy, from the *Brassica napus* diversity fixed foundation set [26] and can be obtained from the John Innes Centre Germplasm Resource Unit (https://www.seedstor.ac.uk). Where plants were vernalised in the laboratory, 3 weeks old plants were placed at 5°C in 8 hours days for the indicated time period, before recording flowering time in a glasshouse heated to a minimum of 24°C. Where plants were transferred from the field to controlled 'spring-like' conditions in the lab, the precise growth conditions were 13 hours light, 11 hours darkness, 15°C day and 10°C night temperatures.

METHOD DETAILS

Field trials

Field trials were conducted at the John Innes Centre experimental farm located 3km West of Norwich, UK. On each growing occasion 5 replicate 6 m x 1.2 m plots per genotype were used in an alpha randomized experimental design. Variety Cabriolet was grown for 3 successive seasons in 2016/17, 2017/18 and 2018/19, and Slapska slapy in 2018/19. Seeds were drilled on prepared beds during the last 10 days of August. If necessary plots were irrigated to promote seedling emergence during dry conditions. Plots were heated for the indicated time period with 2 lengths of 50 m of warming cable as described previously, laid on the soil surface (Figure 2). Temperature differences were measured with Tinytag TGP-4017 environmental dataloggers (Gemini Data Loggers UK Ltd.,). Heated plots were covered in enviromesh (Agralan, UK) to improve heat retention. Aerial images were captured with a drone-mounted DJI Phantom 4 Pro v2.0 Plus camera (1'' CMOS sensor with 20MP). Flowering time was scored as the appearance of the first open flower on each plant in each plot, with % of plants in flower in each plot noted for each variety and treatment.

Gene expression analysis

RNA was harvested from single shoot apices with 3 biological replicates per time-point, developing organs were removed before freezing in liquid nitrogen RNA was extracted according to the manufacturer's instructions using the ENZA Plant RNA kit (Omega Bio-tek). RNaseq for 2016/17 was conducted using an Illumina Hiseq 4000 at Earlham Institute, Norwich, UK generating 150bp paired-end sequences with 44-53 million reads per sample. The 2018/19 RNA samples were processed at Novogene, Beijing using an Illumina Hiseq 6000 to construct strand-specific libraries, 250-300bp paired-end sequences with 23-36 million reads per sample were acquired.

Microscopy

Single shoot apices were manually dissected and photographed using a Leica M80 dissection microscope fitted with a Leica DFC295 digital camera.

Data analysis

Data was trimmed and aligned to *B. napus* Darmor reference genome (version 4.1) with reference gene annotation version 5 [35] by using Tophat v2.1.1 with default parameters [34]. The gene expression was measured in Reads Per Kilobase per Million mapped reads (RPKM) value by using cufflinks [36]. Hierarchical clustering was performed using the Morpheus tool (https://software.broadinstitute.org/morpheus).

DATA AND CODE AVAILABILITY

The RNaseq data is available in GenBank under the following project: PRJNA578690.