Ancient relaxation of an obligate short-day requirement in common bean through loss of CONSTANS-like gene function

Highlights

- The common bean gene COL2 specifies global differences in photoperiod sensitivity
- COL2 represses flowering and FT expression under non-inductive (long-day) conditions
- Independent sequential loss of COL2 and PHYA3 occurred in both domesticated lineages
- Near fixation of col2 mutations implies an important role during Andean domestication

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In Brief

González et al. show that the CONSTANS-like gene COL2 represses flowering under non-inductive (long-day) conditions and provides global adaptation to photoperiod in common bean, a major legume crop. Parallel loss of photoperiod sensitivity occurred independently in both domesticated lineages through sequential loss of COL2 and PHYA3 function.
Ancient relaxation of an obligate short-day requirement in common bean through loss of CONSTANS-like gene function

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SUMMARY

Common bean (Phaseolus vulgaris L.) is a major global food staple and source of dietary protein that was domesticated independently in Mexico and Andean South America. Its subsequent development as a crop of importance worldwide has been enabled by genetic relaxation of the strict short-day requirement typical of wild forms, but the genetic basis for this change is not well understood. Recently, a loss of photoperiod sensitivity was shown to result from mutations in the phytochrome photoreceptor gene Ppd/PHYA3 that arose independently within the two major domesticated lineages. Here, we define a second major photoperiod sensitivity locus, at which recessive alleles associate with deleterious mutations affecting the CONSTANS-like gene COL2. A wider survey of sequence variation in over 800 diverse lines, including wild, landrace, and domesticated accessions, show that distinct col2 haplotypes are associated with early flowering in Andean and Mesoamerican germplasm. The relative frequencies and distributions of COL2 and PHYA3 haplotypes imply that photoperiod adaptation developed in two phases within each gene pool: an initial reduction in sensitivity through impairment of COL2 function and subsequent complete loss through PHYA3. Gene expression analyses indicate that COL2 functions downstream of PHYA3 and may function in parallel with PvE1, the bean ortholog of a key legume-specific flowering repressor. Collectively, these results define the molecular basis for a key phenological adaptation, reveal a striking convergence in the naturally replicated evolution of this major crop, and further emphasize the wider evolutionary lability of CONSTANS effects on flowering time control.

INTRODUCTION

Common bean (Phaseolus vulgaris L.) has major significance as a staple food crop and source of dietary protein in many cultures around the world.1 The species provides a well-known illustration of patterns and processes in plant domestication1–4 and was domesticated twice, from divergent groups of wild germplasm in Mexico and the central Andes ~8,000 years ago.5,6 The parallel history of these two main Mesoamerican and Andean lineages offers a valuable opportunity for insight into evolutionary convergence.

As in many crop species, genetic relaxation of environmental constraints on flowering has been an important key to broad adaptation in common bean.1 Wild forms and many landraces are strongly photoperiod sensitive and flower only under short daylengths, but latitudinal expansion and global dissemination has been dependent on the emergence of variants in which this sensitivity is reduced or has been lost, enabling plants to flower under longer photoperiods.8 Insight into the genetic basis of this variation is therefore useful in tracing the history of common bean domestication, for guiding breeding efforts, and in gaining a better mechanistic understanding of photoperiod sensitivity and its evolution.

Studies in diverse plant species are revealing how flowering time adaptation arises through modification of genetic pathways that integrate information from light and the circadian clock to regulate the production of florigens, a family of mobile, hormone-like signaling proteins. Although the participation of phytochrome photoreceptors and clock components appears broadly conserved, the mechanism of integration seems to involve distinct transcriptional regulators in different plant groups. For example, in the long-day (LD) plant Arabidopsis, the CONSTANS gene has a central role in florigen activation and the induction of flowering under long days.9 In the short-day (SD) plant rice,
adaptive differences in flowering time derive partly from variation in the CO ortholog \( Hd1 \) but more prominently from other lineage-specific components, such as the B-type response regulator \( Ehd1 \).

Among several legume crops that share the SD habit of common bean, insight into the control of flowering is greatest in soybean. In this species, phytochrome and circadian clock components coordinate under long days to activate the key legume-specific inhibitor of flowering \( E1 \), a B3/Related to AB13/VP1 (RAV) family transcription factor that acts through direct transcriptional repression of several flowering (\( FT \)) genes.\(^{11,12} \) Loss of \( E1 \) function has had an important post-domestication role in adaptation to shorter growing seasons at higher latitudes.\(^{13} \)

In common bean itself, early photoperiod-insensitive flowering has arisen separately in Mesoamerican and Andean gene pools through distinct loss-of-function mutations in the phytochrome \( A \) gene \( Ppd \).\(^{14} \) Here, we show this was likely preceded by a partial loss of sensitivity conferred by mutations in the \( CONSTANS \)-like gene \( COL2 \) that are also specific to each germplasm group. In addition, we examine the functional interaction of \( COL2 \) with \( Ppd \) and present evidence that it may act in parallel with the legume-specific \( E1 \) gene to repress flowering (\( FT \)) gene expression and delay flowering under long photoperiods. Our study identifies a \( CONSTANS \) ortholog as an inhibitor of flowering and the key molecular route for the reduction in photoperiod sensitivity associated with broad latitudinal adaptation in both evolutionary lineages of this important SD crop species.

**RESULTS**

A major locus on chromosome 4 contributes to photoperiod sensitivity in common bean

We previously identified the molecular basis for the photoperiod sensitivity locus \( Ppd \) in a study of \( F_2 \) progeny from a cross between the Andean domesticated cultivar Midas and the Mesoamerican wild line G12873 (MG population)\(^{14} \) and reported additional genetic variation flowering under \( LD \) conditions.\(^{15} \) From this population, we generated \( F_3 \) and \( F_4 \) families homozygous for wild alleles at \( Ppd \) and assessed flowering under extended \( LD \) in a temperature-limited glasshouse. The shoot determinacy locus \( Fin/TFL1y \)\(^{16} \) is also segregating in this population, but all families selected were homozygous for the wild \( Fin \) allele and showed indeterminate growth. Among 13 families, approximately 2/3 (62%) segregated intermediate versus non-flowering in a 1:3 ratio (35:108; \( p = 0.88 \)), indicating a novel Mendelian locus conferring recessive early flowering, which we termed \textit{days to flower 4} (\( DTF4 \)). Mapping relative to candidate genes across the genome, including bean orthologs of soybean \( E1, E2/GIANTEA \) (\( GI \)), and \( E4/PHYA1 \) genes, localized \( DTF4 \) to a region on chromosome 4 = 20 cm from the \( PvGI2 \) gene (Phvul.004G088300). Within this region, we observed tight linkage to a \( CONSTANS-LIKE \) (\( COL \)) gene (\( PvCOL2; \) Phvul.004G046601) belonging to the group Ia COL subclade orthologous to \( CONSTANS \) (Figure 1E).\(^{17,18} \)

In parallel, we also used a recombinant inbred line (RIL) population Bolita \( \times \) Nuña (BN population),\(^{19} \) previously shown to segregate under summer (\( LD \)) field conditions for a major flowering time quantitative trait locus (\( QTL \)) in a broadly overlapping region on chromosome 4.\(^{20} \) A segregating population (\( n = 386 \)) derived from a single RIL family retaining heterozygosity across the \( QTL \) region showed a clear trimodal segregation for flowering time under \( LD \) (Figure 1A), which correlated with the genotype of the \( PvCOL2 \) marker, confirmed its strong effect, and revealed haploinsufficiency of the Nuña allele (Figure 1B). Marker segregation deviated from the expected 1:2:1 ratio, with a deficiency of Nuña (late) homozygotes (\( n = 64 \)) and an excess of Bolita homozygotes (\( n = 131 \)). Nevertheless, fine mapping narrowed \( DTF4 \) to a \( = 0.4 \) cM/360 kb interval (Figure 1C) containing 19 annotated genes in the reference genome (\textit{Phaseolus vulgaris} v2.1; https://phytozome-next.jgi.doe.gov), among which \( PvCOL2 \) was the only gene with any clear functional relationship to flowering time and the only obvious candidate gene (Table S1; Data S1).

Sequence differences identify \( COL2 \) as a strong candidate for \( DTF4 \)

Comparison of \( COL2 \) sequences in the BN parents revealed only two non-synonymous polymorphisms: one specifying a conservative substitution (P107L) and another introducing a stop codon at C66 in Bolita (\( col2-1 \) mutation). This site lies in the N-terminal half of the \( COL2 \) protein (Figure 1D), and the resulting truncation would thus remove the second B-box and the C-terminal \( CONSTANS \), CO-like, and TOC1 (\( CCT \)) domains thought to be critical for the DNA binding and protein-protein interactions.\(^{21,22} \) Interestingly, this nonsense mutation was also present in the G19833 reference genome\(^{23} \) and appears to have led to misannotation of \( Phvul.004G046601 \) (Figure S1) with the coding region indicated to commence only at the next potential start codon downstream of this SNP.

We also attempted to compare \( COL2 \) sequences in the MG parents. The \( COL2 \) coding sequence from the wild parent G12873 did not differ from that of Nuña, but we could not amplify \( COL2 \) from Midas despite using multiple primer combinations and positive controls. We were also unable to amplify the downstream gene \( Phvul.004G046400 \) from Midas, but the next gene upstream \( Phvul.004G046700 \) (\( RPL9 \)) was present in both parents, suggesting the existence of a major sequence variant in Midas involving deletion of \( COL2 \) and at least 3.1 kb of downstream flanking sequence. A marker for \( RPL9 \) cosegregated perfectly with the \( DTF4 \) phenotype in \( F_3 \) and \( F_4 \) progenies (\( n = 177 \)), providing independent confirmation of close linkage between the deletion and flowering time in the MG population.

\( COL2 \) sequence diversity survey identifies several mutant alleles

We next examined \( COL2 \) diversity by amplicon resequencing from a diverse selection of 128 wild accessions, landraces, and cultivars variously held in the collection at Misión Biológica de Galicia or obtained from other collections (MBG panel; Data S1). Among these, we identified five other Andean accessions (race Nueva Granada) with an apparent deletion of \( COL2 \), similar to Midas. Analysis of the 683-accession diversity panel from the Chinese Academy of Agricultural Sciences (CAAS panel) recently described by Wu et al.\(^{25} \) identified an additional 26 mostly Andean accessions carrying a large deletion of \( \approx 100 \) kb. This deletion included \( COL2 \) and the two adjacent gene models \( Phvul.004G046400 \) and \( Phvul.004G046500 \) (Figure 1D), which respectively encode a leucine-rich repeat disease-resistance-like protein and a hypothetical, putative transmembrane
The nature of this deletion (col2-2 mutation) was confirmed by sequencing of a deletion-spanning fragment in cv. Midas and the five other accessions in the MBG panel (Data S1). This large deletion is thus present in 10% of domesticated material across both panels. Initial resequencing from MBG accessions identified a third functionally significant variant (col2-3) featuring a single-nucleotide deletion in the first exon directing a frameshift at codon H122 and introducing a premature stop codon 3 residues later (Figure 1 D). This variant open reading frame (ORF) retains the two B-box domains but predicts truncation of the CCT domain, implying complete loss of function. This variant was almost entirely restricted to Mesoamerican domesticated material, in which it occurred in 120/338 or 36% of accessions across both panels. It was also present in the BAT93 reference genome, resulting in misannotation of the corresponding gene model (PHAVU_004G046600 in Ensembl Plants) with an extra intron annotated within the first exon (Figure S1 A). Analysis of sequence from the CAAS panel also identified one further significant variant, an 8-bp deletion at the end of the first B-box domain (col2-4), present in only five Mesoamerican landraces (Figure 1D).

Molecular evolution of COL2
Overall, we observed 60 distinct sequence variants and 40 COL2 haplotypes within the MBG panel (Table S2; Data S1). The panel included four wild accessions from Peru and Ecuador, representing a distinct group (the so-called Phaseolin I or PhI group) that has a basal relationship to the two major domesticated lineages. As expected, these accessions formed a distinct group of COL2 haplotypes (H5–H7). Also, as expected from previously characterized differences in genetic diversity between these lineages, greater sequence diversity for COL2 was found within Mesoamerican (27 haplotypes) than in Andean accessions (ten haplotypes). Among the Andean haplotypes (H31–H40), three were found in wild accessions (Figure 2 A; Table S2). Of these, one (H32) was
present only in a single accession (PHA0897). The other two (H31 and H33) were found both in wild accessions, including one Phi accession, and our parental line Nuniña (PHA1037), a weedy landrace. The single haplotype carrying the col2-1 (Bolita) mutation (H39) was found mainly in landraces and appeared to be directly derived from H31 (Nuniña) by a single SNP. Five other functional haplotypes (H34–H38) occurred at low frequency and only in landraces and were apparently derived from Nuniña independently of the mutant haplotypes.

Within Mesoamerican material, we identified two major haplogroups characterized by differences in the intron. One group (H22–H30) showed close affinity to the Andean haplotypes, with one wild haplotype H30 (PHA2306) distinguished from the main Andean wild H31 by a single SNP (A1383C). The main Mesoamerican haplotype featuring the col2-3 frameshift mutation (H22) was found only in domesticated accessions and was closely related to H30, consistent with having been derived directly from it (Figure 2A; Table S2). The second major Mesoamerican haplogroup (H13–H21) featured haplotypes that were either exclusively wild (H14 and H15) or domesticated. Surprisingly, two of the domesticated haplotypes associated with this group (H20 and H21) also carried the col2-3 mutation, suggesting either an independent origin of this mutation in a distinct sub-pool of Mesoamerican germplasm or more likely an instance of intragenic recombination between haplotypes H19 and H22 (Figure 2A; Table S2). Numerous other diverse Mesoamerican wild haplotypes, including H1 (G12873), appeared to be intermediate between these two major haplogroups, with varying degrees of divergence across the intron.

To examine the potential origin of the Andean deletion allele (col2-2), we also analyzed diversity across 20 kb of sequence spanning the deletion in the CAAS panel. The accessions carrying the deletion were most similar to those in haplotype H39 (col2-1; Figure 2B), indicating that the deletion most likely arose in a genetic background in which COL2 function had already been lost. This dataset also revealed that, for approximately 8% to 9% of accessions, the COL2 haplotype did not align with the gene pool affinity, consistent with a history of infrequent admixture and introgression likely driven by the transfer of adaptive traits between gene pools. Finally, we analyzed association of the COL2 genotype with flowering in the CAAS panel using a dataset from three field seasons in two different locations: a
high-latitude LD site in Harbin (HB) (46° N; daylength 15.1 h at sowing date) and a low-latitude SD site in Hainan (HN) (18° N; daylength 11.2 h at sowing date). The col2 mutant haplotypes were robustly associated with earlier flowering in the HB site, but not in the HN site (Figure 3A), emphasizing the importance of these mutations for photoperiod sensitivity and latitudinal adaptation.

Co-occurrence and interaction of COL2 and PPD/PHYA3 mutations indicate a sequential loss of photoperiod sensitivity

Our previous study showed that, within both Andean and Mesoamerican germplasm groups, ppd mutations were present in only a minority of accessions and were largely restricted to cultivars, suggesting a relatively recent origin. In contrast, col2 mutations overall were more widespread, occurring in landraces and cultivars. Across 648 domesticated accessions from both panels in which COL2 could accurately be genotyped, col2 mutations were present in 37% of Mesoamerican domesticated accessions but 90% of Andean domesticated accessions (Figure 3).

To gain a more comprehensive picture of the frequency of ppd/phyA3 mutations and their coincidence with col2 mutations, we also examined PHYA3 sequence diversity within the CAAS panel. This analysis showed that the major loss-of-function mutations A534frameshift (phyA3-1) and E783X (phyA3-2) described previously were present at 40% and 22% in Andean (n = 250) and Mesoamerican (n = 392) gene pools, respectively. We also identified additional deleterious mutations at much lower frequency in Mesoamerican germplasm (phyA3-3; frameshift at H256; 6%) than in Andean (phyA3-4; Q67X; 1%; Figure S1). Analysis of the subset of CAAS accessions with unambiguous genotypes and uniformly Mesoamerican or Andean haplotypes for both genes (n = 469) showed that the majority of phyA3 mutations were found in combination with a col2 mutation, both in Andean (n = 86/86; 100%) and Mesoamerican material (n = 56/69; 81%; Figure 3C). Together, these results indicate that ppd/phyA3 mutations arose more recently than col2 mutations in both gene pools and most likely occurred in genetic backgrounds already carrying col2 mutations.

Our earlier analysis in the MG population gave a strong indication that the effect of ppd was epistatic to dtf4/col2 under greenhouse extended LD conditions, and we confirmed this by genotyping (Figure S2). We also examined how PHYA3 and COL2 allelic variants interacted in their association with flowering time in the CAAS diversity panel under field conditions. In this analysis, we further categorized the material as indeterminate or determinate according to genotype at the main determinacy locus Fin/TFL1y. Regardless of growth habit, at the HB site, accessions carrying phyA3 or col2 mutations were significantly earlier than accessions with functional copies of both genes (Figure 3B). In addition, in one of the three seasons (2015), accessions carrying mutant phyA3 alleles either alone or in combination with col2 were significantly earlier than those carrying only col2. In contrast, at the HN site, no effect of mutations at either locus was observed. These results indicate that loss-of-function COL2 and PHYA3 alleles made a major contribution to high-latitude adaptation in common bean and emphasize the epistasis of phyA3 over the col2 effect.

Figure 3. Phenotypic associations, genotype frequencies, and interactions of COL2 and PHYA3 mutations in a diverse collection of domesticated common bean accessions.

(A) Flowering time for accessions carrying COL2 or col2 haplotypes from the CAAS panel grown in field conditions in short-day (Hainan; 19.6° N) and long-day (Harbin; 45.8° N) environments over multiple seasons.

(B) Interaction of putative functional and non-functional COL2, PHYA3, and TFL1y haplotypes in the control of flowering time in the long-day environment of Harbin over three seasons. In both (A) and (B), whiskers indicate the 5–95 percentile range, and statistically significant differences within the same season (p ≤ 0.05), as determined by one- or two-way ANOVA, respectively, are indicated by different lowercase letters.

(C) Observed frequencies of putative functional and loss-of-function haplotypes for COL2 and PHYA3. The COL2 data represent combined data from the CAAS and MBG panels, whereas data for PHYA3 and the COL2/PHYA3 interaction are derived only from the CAAS panel. See also Figure S1.
COL2 may act in parallel with E1 to mediate the suppression of FT genes by Ppd/PHYA3

We next explored effects and interactions of COL2 and Ppd/PHYA3 genes more directly, using independent near-isogenic line (NIL) pairs developed from the MG population. Confirming our earlier observation, 11,13,26 NILs carrying wild-type (WT) alleles at both loci flower extremely late or not at all under LD but show a strong promotion of flowering by SD, whereas the ppd/phyA3 NILs flower early under both LD and SD and is effectively insensitive to photoperiod (Figure 4A). In comparison, the col2 NIL showed only partial loss of sensitivity, with an intermediate flowering time under LD and no substantial difference from the COL2 NIL under SD (Figure 4A).

We previously showed that three of the five FT genes in common bean (FTa3, Ftb1, and FTC) were expressed at higher levels in a ppd NIL relative to a Ppd NIL. 14 Similar analysis showed the same genes were also expressed at a higher level in a col2 NIL compared to a COL2 NIL and confirmed the effect of Ppd (Figure 4B). Although the two sets of NILs were derived independently and should not be directly compared, it is notable that the effect of the ppd allelic difference was substantially stronger than that of col2, consistent with the observed differences in flowering time (Figure 4A). This could suggest that COL2 may act downstream of PHYA3 and in parallel with other factors to repress FT expression under LD.

To further explore this possibility, we examined rhythmic expression of COL2, PHYA3, and several FT genes in the Ppd and COL2 NILs grown under LD. We found that the COL2 gene is expressed with two distinct peaks: one at zeitgeber time 12 (ZT12) (i.e., 12 h after lights on) and the other early in the night, at ZT20 (Figure 4C). However, in the ppd mutant, the expression level of COL2 was lower than the corresponding Ppd NIL across the daily cycle with substantially lower expression at the two peaks. This suggests that Ppd/PHYA3 may act upstream of COL2, in part to promote its transcription. Consistent with this, there was no clear effect of the COL2 allelic difference on PHYA3 expression (Figure 4C). As expected, there was no detectable COL2 expression in the col2 NIL (carrying the col2-2 deletion allele from Mudas), and PHYA3 expression was somewhat reduced in the ppd NIL. One potential explanation for the intermediate photoperiod response phenotype of the col2 genotypes might be partial functional redundancy of COL2 with a second group Ia helix transcription factor E1 is a key factor mediating the repression of PHYA genes. 17,18 Our result shows that, in the two WT (i.e., Ppd/PHYA3) NILs, PvE1 is expressed with three distinct peaks of expression. Two of these coincide with COL2 peaks at ZT12 and ZT20, but a third morning peak at ZT4 is also apparent. Expression of PvE1 was significantly weaker at all three peaks in the ppd NIL, but not in the col2 NIL relative to their corresponding WT NILs (Figure 4C). This shows that, in bean, E1 is also regulated by Ppd/PHYA3 but also indicates that this effect is not mediated through an effect on COL2 expression. Consistent with the single-time-point experiment shown in Figure 4B, the
**DISCUSSION**

Common bean was featured in the first systematic study of plant photoperiodism by Garner and Allard, who reported the strict SD dependence of tropical accessions and subsequently documented variation in sensitivity across a wider range of material. Evaluation of >3,000 accessions by White and Laing revealed a trimodal distribution for photoperiod sensitivity, with distinct insensitive and “intermediate” categories in both Mesoamerican and Andean germplasm. Genetic analysis by Coyne and subsequently Kornegay et al. obtained strong evidence for two major loci (A and B), respectively specifying the complete and partial loss of photoperiod sensitivity across both gene pools. We recently identified the “A” locus, commonly known as *Ppd*, as a phytochrome A gene (*PHYA3*) orthologous to the soybean maturity gene *E3*. Here, we identify the *CONSTANS*-like gene *COL2* as a compelling candidate for the “B” locus and show that partial loss of photoperiod sensitivity has arisen independently in the Mesoamerican and Andean lineages through different mutations unambiguously specifying loss of *COL2* function.

Our survey of haplotype diversity and distribution in over 800 accessions indicates that *col2* mutations are relatively common in both major germplasm groups (Figure 2A) but occur at substantially higher frequency in Andean than in Mesoamerican domesticated material (Figure 3C). In particular, the high frequency in the Andean gene pool approaches the values reported for alleles that similarly confer reduced photoperiod sensitivity in maize (*ZCN8*) and soybean (*Tof12*) and suggests that loss of *COL2* function likely conferred a selective advantage early in the evolution of the Andean domesticated lineage. This might reflect improved productivity along local environmental gradients (e.g., in temperature/altitude) and/or a shorter growth cycle. Irrespective of the basis for the initial selection of *col2* mutations, their clear association with earlier flowering at high latitudes (Figure 3A) illustrates their importance for expansion of common bean from its sub-tropical centers of origin. One important caveat in interpretation of allele frequencies is that both of our panels have likely been subject to some degree of bias in selection, considering the somewhat arbitrary representation of landraces in the MBG panel, and the additional likelihood of adaptive filtering in the case of the China-focused CAAS panel. A more definitive assessment will require a focus on sympatric wild accessions and landraces in the proposed domestication centers.

Combined analysis of *COL2* and *PHYA3* haplotypes also indicates that *phyA3* mutations are less common than *col2* mutations and are predominantly found in *col2* accessions (Figure 3C). Together with the epistasis of *ppd* over *col2* (Figure S2), this indicates that complete insensitivity due to loss of *PHYA3* function most likely arose following an earlier partial reduction through *COL2* impairment. This was particularly clear within Andean material, where we did not identify a single accession with *COL2 phyA3* genotype.

Several recent analyses have also reported marker-trait associations for flowering and related traits in a region of *Pv04* potentially consistent with an identity as *COL2*. However, these loci are not particularly prominent, and it is also curious that, despite the importance and broad distribution of *col2* alleles, loci in this region have not been detected more clearly and more often. One explanation is simply that most studies have focused on agronomically relevant variation for specific environments, either at high latitudes, where functional *COL2* alleles might be relatively rare, or at low latitudes, where the effect of *col2* is likely to be smaller (Figure 3A). This is also reflected in the fact that the first two reference genomes for common bean both feature *col2* mutations. It is also intriguing that this genomic region has also been reported to influence yield-related traits under stress, hinting at the potential broad pleiotropic effects of genetic variation for phenology.

The CO gene is considered central to the mechanism for photoperiod response in *Arabidopsis* and participates in the direct activation of the florigen gene FT, binding to defined elements its promoter. Regulation at both transcriptional and post-transcriptional levels by light signaling and the circadian clock prevents CO protein accumulating under short photoperiods and thereby restricts its activity to LD. Broad conservation of CO function was initially indicated by the observation that the CO ortholog *Hd1* has a dual role promoting florigen expression under SD and inhibiting it under LD. *CO* genes have subsequently been shown to influence photoperiod sensitivity in several diverse species, primarily through activation of *FT* gene expression under inductive conditions. However, there is continuing uncertainty around the extent and nature of this conservation and a growing awareness that CO orthologs may also exhibit minor, context-dependent repressive effects on *FT* gene expression under non-inductive conditions.

Our results highlight a major role for a CO-like gene in the legume family and reveal its primary importance as a repressor rather than a promoter of flowering. The group la clade of *COL* genes that includes AtCO is represented in legumes by two distinct subclades, and of these, only one (*COL1/COla*) is present in most LD legumes, with the other having apparently been lost (Figure 1E). Results from the LD species pea and Medicago argue against any substantial role for the *COla* gene in flowering time control despite a conserved role for the GI/FLAVIN-BINDING KELCH REPEAT, F-BOX 1 (FKF1)/CYCLING DOF FACTOR (CDF) module. In soybean, the closest SD comparator of common bean, weak early-flowering phenotypes have subsequently been shown to influence photoperiod sensitivity in several diverse species, primarily through activation of *FT* gene expression under inductive conditions. However, there is continuing uncertainty around the extent and nature of this conservation and a growing awareness that CO orthologs may also exhibit minor, context-dependent repressive effects on *FT* gene expression under non-inductive conditions.
important at least in Arabidopsis, and the possibility cannot be excluded that PvCOL1 might have the same function as PvCOL2 but be regulated primarily at the protein level.

In soybean, analyses of photoperiod adaptation have not identified COL genes but instead emphasize the importance of the E1 gene. This gene is, in effect, the adaptive equivalent of common bean COL2, in the sense that loss-of-function variants are widespread, relatively old, and incompletely impair photoperiod sensitivity. Our evidence indicates that, in common bean, as in soybean, E1 transcription is strongly regulated by PHYA (Figure 4C). The fact that PvE1 is apparently not misregulated in the col2 mutant suggests that it might act in parallel with COL2 to inhibit FT expression. Alternatively, it could conceivably act upstream to mediate the positive effect of PHYA3 on COL2 expression. The regulatory relationship between the soybean COL and E1 genes is also not yet clear. Expression of GmCOL1a and COL1b is increased in plants ectopically expressing E1, but none of the four group Ia COL genes show markedly different expression in a near-isogenic comparison between E1 and e1 genotypes. There is even an indication that E1 expression may be weakly promoted by GmCOL2b. We favor a hypothesis in which PvCOL2 and PvE1 act in parallel, and to some extent redundantly, to repress multiple FT genes (Figure 4D). However, the identification of functional variants for PvE1 and PvCOL1 and comprehensive functional analysis of E1-like and group la COL genes in soybean will be needed to clarify these relationships and how they may differ between the two species. It is also notable that multiple daily expression peaks for both COL2 and E1 occur in both species (Figure 4C). This implies that they are subject to several different regulatory influences across the daily cycle. However, at least in bean, these complex patterns are not closely reflected in FT expression (Figure S3), implying that protein-level regulation may be an additional factor determining their ultimate activity.

Increasing understanding of flowering time adaptation across an ever-wider range of species is revealing the extent to which the associated genetic architectures, genes, and gene interactions are conserved or divergent and is beginning to provide insight into how these features may limit the adaptive solutions available for any given species. The situation we characterize for photoperiod sensitivity in common bean fits a broad pattern for photoperiod sensitivity. Our evidence indicates that, in common bean, as in soybean, E1 transcription is strongly regulated by PHYA (Figure 4C). The fact that PvE1 is apparently not misregulated in the col2 mutant suggests that it might act in parallel with COL2 to inhibit FT expression. Alternatively, it could conceivably act upstream to mediate the positive effect of PHYA3 on COL2 expression. The regulatory relationship between the soybean COL and E1 genes is also not yet clear. Expression of GmCOL1a and COL1b is increased in plants ectopically expressing E1, but none of the four group Ia COL genes show markedly different expression in a near-isogenic comparison between E1 and e1 genotypes. There is even an indication that E1 expression may be weakly promoted by GmCOL2b. We favor a hypothesis in which PvCOL2 and PvE1 act in parallel, and to some extent redundantly, to repress multiple FT genes (Figure 4D). However, the identification of functional variants for PvE1 and PvCOL1 and comprehensive functional analysis of E1-like and group la COL genes in soybean will be needed to clarify these relationships and how they may differ between the two species. It is also notable that multiple daily expression peaks for both COL2 and E1 occur in both species (Figure 4C). This implies that they are subject to several different regulatory influences across the daily cycle. However, at least in bean, these complex patterns are not closely reflected in FT expression (Figure S3), implying that protein-level regulation may be an additional factor determining their ultimate activity.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Experimental models: organisms/strains | | |
| Diverse *Phaseolus vulgaris* accessions | MBG, CIAT, USDA, CAAS | See Data S1 and Wu et al. 23 |
| *Phaseolus vulgaris* NILs | This paper | N/A |
| Oligonucleotides | | |
| Primer sequences used for genotyping and sequencing | This paper | See Table S3 |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jim Weller (Jim.Weller@utas.edu.au).

Materials availability
Near-isogenic lines for *COL2* and *Ppd PHYA3* alleles will be provided on request.

Data and code availability
Details of sequence variants characterized in this study are given in Figure S1 and Table S2.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

This study utilized common bean (*Phaseolus vulgaris* L.) as an experimental model. Genetically diverse accessions of common bean were obtained from collections at the Spanish national *Phaseolus* germplasm bank (MBG-CSIC, Spain), CIAT (Colombia), USDA (USA) and CAAS (China).

METHOD DETAILS

Experimental material
A population of 185 RILs (*F* 2:7), derived from a cross between a photoperiod-insensitive and early flowering cultivar from Spain (Bolita) and a late flowering photoperiod-responsive landrace from Bolivia (PHA1037) had been used previously for linkage map construction and QTL analysis of flowering time. 19 Individuals carrying residual heterozygosity for the QTL *DTF4* region were identified in progeny of a single line from this population (RIL104) using a dCAPs marker designed for *PvCOL2* (Table S3), and used to generate a near-isogenic segregating population (*n* = 400) for fine-mapping. This population was grown at MBG during the summer of 2019 with 14h light and 10h dark (latitude 42° 24’ N, longitude 8° 38’ W, and altitude 40 masl). The second population used was derived from a cross between the Mesoamerican wild accession G12873 and cv. Midas as previously described by Weller et al. 14 NILs for *PHYA3/phyA3* and *COL2/col2* in an indeterminate (*TFL1y*) background were developed from a progeny of *F* 2 individuals that did not flower in LD conditions, by marker-assisted recurrent selection of heterozygotes in subsequent generations and visual selection for phenotypic uniformity. Progeny were subsequently grown under either the same conditions or 12h short-day (SD) conditions in an automated phytotron, where they were transferred from day conditions in the glasshouse to night compartments. The MBG diversity panel (Data S1) was assessed in a greenhouse trial at Pontevedra, Spain (latitude 42° 24’ 17.99” N, longitude 8° 38’ 38.2” W, altitude 40 m above sea level) under LD (> 12 h light, 20–35° C night–day regime, relative humidity 50%–70%). Crop management followed local practices.

Allele frequency and phenotype association analyses were extended using resequencing and multi-environment phenotype data from the 683-accession CAAS diversity panel described previously by Wu et al. 23 Days to flowering (DTF) was recorded as the number of days from germination to the appearance of the first open flower. Total branching was measured as a sum of all the branches off the main stem at four weeks old (mm) and plant height was measured from node 1 (first opposite leaves) to the apex of the plant at four weeks old (mm).
Molecular and sequence analyses
Intron-spanning fragments of selected genes were generated by PCR and sequenced to identify suitable polymorphisms for genotyping (Table S3). dCAPS markers (Table S3) were designed using dCAPs Finder 2.0 (http://helix.wustl.edu/dcaps/dcaps.html) and linkage maps were constructed using JoinMap® 4.0 software.61 PCR from genomic DNA was used to amplify the full-length COL2 gene (2127 bp) in two overlapping fragments using primers indicated in Table S3. PCR products from the diversity panel were purified by the PCR Clean-Up Kit (Promega) and were sequenced by conventional Sanger technology using BigDye® Terminator v3.1 chemistry and the Applied Biosystems™ 3500 Series Genetic Analyzer. Sequence analysis and alignments were performed using Geneious V8.1.9 software (https://www.geneious.com).

To identify structural variation and SNPs in PvCOL2, PvPHYA, and PvRPL9 within the CAAS panel, paired-end reads from resequencing of the 683-accession diversity panel23 were mapped to the common bean reference genome (P.vulgaris v1.1). SNP and indel calling were performed using BCFtools and SAMtools software.62 VCF files were further filtered using the VCFtools software (v.0.1.16)63 with the following parameters: min-alleles 2, max-missing 0.5 and maf 0.002. Reads were mapped simultaneously to the sequence of the reference wild accession G12873 (Table S2) for confirmation as the reference genome contained a COL2 mutation. Only reads for which both sequences of a pair mapped were kept.

For expression experiments shown in Figures 4 and S3, PHYA3 and COL2 NILs were grown under the LD conditions described above for Hobart, and comparable leaf material was harvested for RNA isolation. RNA extraction, reverse transcription, and real-time PCR analysis were performed as previously described,18 using primers listed in Table S3.

Phylogenetic and haplotype network analysis
Sequences were aligned in Geneious 8.1.9 (https://www.geneious.com) and phylogenetic analysis was performed using the PhyML 3.0 maximum-likelihood algorithm64 within Geneious. The median-joining haplotype network (Figure 2A) was constructed using POPART.65

QUANTIFICATION AND STATISTICAL ANALYSIS
Statistical details of individual experiments can be found in the figures and figure legends.