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1 **TITLE**

2 Comparison of environmental and mutational variation in flowering time in Arabidopsis

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22

23

1 **ABSTRACT**

2 Developmental dynamics can be influenced by external and endogenous factors in a
3 more or less analogous manner. To compare the phenotypic effects of (1) environmental
4 (i.e. standard (stPhP) and extended (exPhP) photoperiods) changes in *Arabidopsis* wild
5 types and (2) endogenous genetic variation in *eav1* to *6l* early flowering mutants, we
6 analyze two temporal indicators, the time to bolting (DtB) and the number of leaves
7 (TLN). We find that DtB and TLN are differentially affected in different environmental
8 and genetic contexts and identify some factors of dynamic convergence. The
9 quantitative response to photoperiod is markedly contingent on the phototrophic input
10 for DtB but less so for TLN. To discriminate the light quantity and period components
11 in DtB, we determine two novel temporal indicators, LtB (photosynthetic time to
12 bolting) and PChron (DtB per hour of photoperiod) respectively. The use of PChron
13 results in a coincidence of the variation profiles across stPhP and exPhP, interpreted as a
14 buffering of the trophic response. Unlike natural accessions and later flowering mutants,
15 the variation profiles across stPhP and *eav* mutants are significantly divergent pointing
16 to differences in environmental and genetic variation in flowering time. Yet, phenocopy
17 effects and dynamic convergence between wild type and mutant profiles are detected by
18 using exPhP and the LtB indicator. Additional analyses of the cauline leaf number
19 (CLN) show that the apical and basal boundaries of the primary inflorescence vary
20 coordinately. The finding that the correlativity between CLN and TLN changes across
21 photoperiods suggests that different states of intra-connectedness are involved in
22 ontogenetic specification of flowering time and embodied in the primary inflorescence.

23

24 **KEY WORDS**

25 *Arabidopsis*, Bolting, Correlativity, Developmental dynamics, Flowering time, Early flowering
26 mutant, Phase change, Phenocopy, Phenotypic plasticity, Photoperiodic response

1 INTRODUCTION

2 Because plants are sessile organisms characterized by extended and sequential mode of
3 development, the adjustment of their endogenous developmental dynamics and ontogenetic
4 trajectory to seasonal time is crucial for their adaptation to the environment (Battey, 2000;
5 Roberts and Summerfield, 1987). This is achieved through phenotypic plasticity, the capacity to
6 express different predictable phenotypes in reaction to external changes, i.e. norms of reaction.
7 This capacity to vary relies on the constitutive mode of variation in living organisms, i.e. growth
8 forces that allow ontogenetic transformations and development (Debat and David, 2001;
9 Pigliucci, 1996; Sultan, 2004). The ontogenetic trajectory can be defined as a progression
10 through a series of growth phases, each one characterized by the production of morphological
11 structures with constant or gradually changing features, interspaced with discrete, critical
12 phases, or phase changes, in which new morphological organization occurs (Diggle, 1999;
13 Haughn et al., 1995; Poethig, 2003). Besides phenotypic plasticity in response to environmental
14 changes, variation in ontogenetic trajectory and in the timing of developmental processes, i.e.
15 heterochrony, may also be the consequence of endogenous changes at the chromatin or DNA
16 level, i.e. epigenetic or genetic changes, that in turn may lead to altered reactions to the
17 environment (Finnegan, 2001; Freeling et al., 1992; Haughn et al., 1995; Sung and Amasino,
18 2004; van Tienderen et al., 1996; Diggle, 1999; Wiltshire et al., 1994).

19 There are evidence that environmental and mutational variation may converge into analogous
20 phenotypes. Indeed, mutant phenocopies can often be obtained in the wild type by applying
21 adequate external constraints (Mitchell and Lipps, 1978; Waddington, 1942). Phenocopy and
22 the functional versatility reported for many genes suggest that mutant phenotypes reflect not
23 only specific functional defects but also distortions in wild type intra-connectedness of network
24 biological systems (Amzallag, 2000; 2001; Edelman and Gally, 2001; Espinosa-Soto et al.,
25 2004; Finnegan, 2001; Greenspan, 2001; Luscombe et al., 2004; Wagner, 2005). An important
26 question is to understand how this intra-connectedness is linked to the regulation of endogenous
27 dynamics and eventually to the adaptability to the environment. One possible approach to

1 address this question is to compare the phenotypic impact of environmental and mutational
2 ontogenetic variation.

3 Central in plant ontogenetic dynamics, flowering time is a key life history trait that is both
4 extremely plastic and sensitive to genetic variation (Bernier and Périlleux, 2005; Clarke et al.,
5 1995; Kuittinen et al., 1997; Searle and Coupland, 2004; Sung and Amasino, 2004; Zhang and
6 Lechowicz, 1994). Photoperiod, the only fully reliable seasonal signal, is one essential factor of
7 variation in flowering time which contributed to crop domestication and species adaptation, or
8 acclimation to different habitats (Borchert et al., 2005; Garner and Allard, 1920; Roberts and
9 Summerfield, 1987; Searle and Coupland, 2004). Commonly described as a quantitative long
10 day (LD) species, *Arabidopsis* flowers earlier under LD than under short days (SD) in
11 agreement with its latitudinal distribution, mostly in temperate areas (Koornneef et al., 2004).
12 However, natural accessions isolated at low latitudes show only weak quantitative response to
13 photoperiod (Alonso-Blanco et al., 1998). Most flowering time mutants, whether late or early,
14 are characterized by a modified SD to LD flowering time ratio, and several of them have been
15 described as day-neutral (Gaudin et al., 2001; Koornneef et al., 1991; Pouteau et al., 2004).

16 The transition to flowering, or floral switch, is a most critical phase change in plant ontogeny
17 leading from vegetative to reproductive growth. For practical reasons, its actual timing is rarely
18 determined as such. Flowering time is thus usually recorded at later stages based on
19 macroscopic morphogenetic changes. It can be measured by direct temporal indicators such as
20 the time to first floral bud opening or the number of days to bolting (DtB) in rosette species like
21 *Arabidopsis*. In the latter case, bolting is commonly used to divide the vegetative phase into
22 rosette leaf (RL) and primary inflorescence bearing cauline leaves (CL) sub-phases (Haughn et
23 al., 1995). Flowering time can also be estimated by indirect morphometric indicators such as the
24 total number of nodes bearing leaves (TLN), i.e. the sum total of RLN and CLN below the
25 secondary inflorescence bearing flowers without bracts.

26 The variation in the two types of temporal indicators seem essentially correlated across natural
27 accessions of *Arabidopsis* and late flowering mutants (Bagnall, 1993; Clarke et al., 1995;

1 Karlsson et al., 1993; Koornneef, 1991; Kuittinen et al., 1997; Stratton, 1998) suggesting that
2 the ontogenetic timing is tightly regulated possibly due to biophysical and/or physiological
3 constraints on the rate of growth. However, in an extensive survey of early flowering mutants
4 possible uncoupling between DtB and TLN suggested that they are not surrogates of each other
5 but correspond to differentially regulated temporal components of plant ontogeny. The notion
6 that earliness may impose or reveal greater developmental constraints than delayed flowering
7 was supported by the finding that early flowering mutants exhibit a high level of pleiotropy and
8 multiple changes in phenotypic plasticity (Pouteau et al., 2004).

9 To approach the question of how character intra-connectedness and ontogenetic dynamics may
10 be related, we were interested to compare different causes of variation in flowering time. In this
11 work, we examine the following issues. Firstly, what is the respective impact of photoperiodic
12 and mutational variation in flowering time on the relation between TLN and DtB ? Secondly,
13 can the evaluation of light quantity and period components in the wild type response to
14 photoperiod reveal similarity with mutant dynamic features ? Thirdly, can an ontogenetic basis
15 for the relation between DtB and TLN be found by analyzing changes in character correlativity
16 in the primary inflorescence ?

17 **MATERIALS AND METHODS**

18 **Plant Material**

19 The natural accessions Wassilewskija (Ws) and Columbia (Col-0) were used. The 61 T-DNA
20 insertion lines in the Ws background, *eav1* to *eav61*, were obtained from the Versailles
21 collection, INRA, France (Bechtold et al., 1993; Pouteau et al., 2001; 2004).

22 **Growth conditions for flowering time assays**

23 Mutant and wild type seeds were sown on soil (Stender A240, Bluemendenwerk Stender
24 GmbH, Germany) and grown in Sanyo Gallenkamp SGC660 growth cabinets at 20 +/- 0.2 °C
25 and 70 % +/- 2 % RH. The soil was kept moist by application of nutrient solution three times a

1 week. Under stPhP, the light during the whole day period was provided with mixed fluorescent
2 and incandescent tubes and the Photon Flux Density measured at soil level was 230 ± 20
3 $\mu\text{E}/\text{m}^2/\text{s}$ and $2 \pm 0.2 \mu\text{E}/\text{m}^2/\text{s}$, respectively. Under exPhP, the photosynthetically active light
4 quantity was maintained at a constant level by providing mixed fluorescent and incandescent
5 light during a 8h period and incandescent light only during extension periods promoting no
6 photosynthetic activity. Developmental uniformity was obtained by selecting the 10 most
7 uniform plants on average about 12 days after sowing, bringing the plant density to one plant
8 per pot, and rotating the trays three times a week.

9 **Measurement of flowering time indicators**

10 Bolting time (DtB) was measured as the number of days from sowing to the first elongation of
11 the floral stem at 0.1 cm height. The number of true leaves (RLN, CLN, TLN) produced by the
12 apical meristem was recorded on bolted plants. No major variation was observed in 2 to 4
13 independent repeats for the mutants. The LtB and PChron conversions of DtB were calculated
14 as follows: LtB (days) = DtB x hours under photosynthetically active light / 24; PChron
15 (days/hour) = DtB / Photoperiod. Where appropriate, linear regressions of the relative variation
16 of TLN with DtB were determined. The R dynamic index (day^{-1}) corresponding to the slope of
17 the linear regression was derived from the corresponding equation: $\text{TLN} = a + R \times \text{DtB}$. R
18 indices were analyzed by linear regression slope comparison based on a t test. Influential points
19 were sought by calculating Cook's distances with the SAS software package (SAS Institute
20 2000). Values of Cook's distance were lower than 0.5 except for one case at 1.04 (see Table 1).
21 For each independent regression across one variation factor (photoperiod in Ws, mutants, or
22 natural accessions), low outlying values when present were usually distributed among the latest
23 samples.

24 **Measurement of ontogenetic correlativity**

25 Variability and character correlation were measured by transforming coefficients into
26 quantitative variables according to Amzallag (2001). The quantity of variability was calculated

1 by the coefficient of variation ($CV = \text{standard deviation}/\text{average}$). The coefficients of correlation
2 (r' -values) were normalized with respect to the median degree of freedom (df) at all
3 photoperiods: $r^2 = (r')^2 \times (\text{median df}/\text{df})$. The quantity of correlation or connectance was
4 estimated by transforming non-normally distributed r -values into normally distributed z -values:
5 $z = 0.5 \times \text{Ln}[(1 + |r|)/(1 - |r|)]$.

6 **RESULTS**

7 **Analysis of phototropic and true photoperiodic variation in flowering time in Ws**

8 Seasonal changes in photoperiod affect simultaneously two factors: the light period and the
9 quantity of light available for photosynthesis. These two factors are also affected by increasing
10 photoperiod under constant light intensity (standard photoperiod or stPhP) in controlled
11 environments. Under these conditions, the variation in DtB in the Ws natural accession of
12 *Arabidopsis* is approximately linear for photoperiods ranging from 6 h to 14 h (Fig. 1A). The
13 conversion of DtB into the corresponding number of days with photosynthetically active lights
14 on (LtB) shows little variation across this range of photoperiod (Fig. 1A) suggesting that DtB is
15 mainly determined by the quantity of light available for photosynthesis. Above a photoperiod of
16 about 14 h, DtB reaches a minimum and is not influenced by further increases in light quantity
17 (Fig. 1A). This is commonly referred to as the critical photoperiod (P_c ; Roberts and
18 Summerfield, 1987), i.e. the photoperiod below which flowering is delayed. The variation in
19 flowering time as measured by RLN and CLN reveals similarities and discrepancies compared
20 to DtB (Fig. 1B). Under LD, the variation in both RLN and CLN parallels that of DtB, with a P_c
21 lying between 14 h and 16 h. Below the P_c however, the variation in RLN and CLN, unlike that
22 of DtB, is linear only within a limited window of photoperiod and a saturation is observed
23 below a photoperiod of about 8 h and between 12 h and 14 h, respectively. This is defined as the
24 ceiling photoperiod (P_{ce} ; Roberts and Summerfield, 1987), i.e. the photoperiod at and above
25 which the greatest delay in flowering occurs.

1 To circumvent the additional photosynthetic effects of stPhP, the photoperiod can be artificially
2 increased without modifying the total quantity of light available for photosynthesis by extending
3 a reference photoperiod of 8 h with periods at low light intensity (extended photoperiods or
4 exPhP; Bagnall et al., 1995; Karlsson et al., 1993; Lee and Amasino, 1995; Martinez-Zapater
5 and Somerville, 1990). However, a side-effect of the exPhP conditions in Arabidopsis is that
6 they trigger a typical shade avoidance response characterized by elongated hypocotyls, petioles
7 and limbs indicating that light signaling is modified (data not shown; Karlsson et al., 1993; Lee
8 and Amasino, 1995; Smith and Whitelam, 1997). Besides this shade avoidance response, a
9 number of quantitative changes are observed in the flowering response of Ws to exPhP
10 compared to stPhP (Fig. 1 and 2). Firstly, the variation in DtB below 14 h is non linear and
11 shows a gradual saturation with a Pce at approximately 10 h. Secondly, the DtB response curve
12 is shifted towards longer exPhP and a shift interval of approximately 2 h is observed for the Pc
13 of all temporal indicators and for the Pce of CLN. Thirdly, the maximum level of CLN and
14 minimum level of RLN are modified: under 10 h and 12 h exPhP, CLN exhibits a significant
15 increase above the highest level under short stPhP ($t = 5.75$ and 5.35 , $P \ll 0.1 \%$) and under
16 long exPhP, RLN decreases below the minimum value under stPhP ($t = 21.76$, $P \ll 0.1 \%$).
17 The flowering responses under stPhP and exPhP also share common features: the minimum DtB
18 and CLN under LD and the RLN response curve between 8 h and 16 h are little altered.

19 The use of the LtB conversion reveals a differential requirement for photosynthetically-active
20 light across exPhP, unlike stPhP, with apparent Pc and Pce at 16 h and 10 h, respectively. This
21 difference may account for true photoperiodic contribution to flowering below a mean baseline
22 of 15.7 days of photosynthesis. By using an additional time indicator, the DtB per hour of
23 photoperiod, or "photochron" (PChron), a more similar response can be obtained for the two
24 types of photoperiodic conditions (Fig. 2A). This suggests that PChron can be useful to
25 discriminate the period versus light quantity component in the variation of DtB with
26 photoperiod. Interestingly, a linear representation can be tentatively obtained with the reciprocal
27 of PChron (Fig. 2B) and could be used for the prediction of flowering time in Ws at different
28 photoperiods.

1 **Analysis of the photoperiodic variation in the relative progression to flowering in Ws**

2 The variation in the progression to flowering was visualized by plotting TLN against DtB (Fig.
3 3). Variation profiles were tentatively compared by calculating a linear regression where
4 appropriate and by using the regression slope as an estimate of the dynamic variation, hereafter
5 called the R dynamic index (Table 1). The results show differential dynamic variation across
6 photoperiods. In Ws across stPhP, the relative variation of TLN with DtB is linear within a large
7 photoperiod window (Fig. 3A) and the corresponding R index is not significantly different from
8 the one determined across exPhP (Table 1; Supplementary Table T1). Yet, the variation across
9 exPhP appears continuously linear whilst the DtB and TLN temporal indicators are gradually
10 uncoupled under short stPhP leading to a plateau above 50 DtB (corresponding to a 8 h stPhP).
11 Similar results were observed in the Col-0 natural accession (data not shown) indicating that
12 they are not contingent on the deficiency in phytochrome D and light signaling in Ws
13 (Auckerman et al., 1997).

14 The LtB and PChron conversions provide further indications on the respective contribution of
15 light quantity and period on the dynamic variation in Ws (Fig. 4). For obvious reasons, the LtB
16 conversion does not modify the profile across exPhP. In contrast, the profile across stPhP is Z-
17 shaped with ceiling and basal plateaus below 10 h and above 16 h, respectively (Fig. 4A). This
18 may point to the need for a higher photosynthetic input for flowering under stPhP than under
19 exPhP, especially in LD. In contrast to the LtB conversion, the PChron conversion results in an
20 almost complete coincidence of the profiles across stPhP and exPhP (Fig. 4B). Therefore,
21 PChron may be a useful tool to examine true photoperiodic response. The results also indicate
22 that period effects on the relative progression to flowering are essentially unaltered by
23 additional light quantity and signaling effects associated with the stPhP and exPhP conditions,
24 respectively.

1 **Comparison of mutational and photoperiodic variation in the progression to flowering**

2 Heterochrony in the *eav1* to *eav61* early flowering mutants reflects common alterations in
3 endogenous connectedness expressed at the organism level of organization. For this reason,
4 although these mutants probably exhibit different molecular genetic alterations at a lower level
5 of organization, we consider that they can be analyzed together as a coherent population. We
6 compared their variation profile under SD and LD to that of Ws across photoperiods. Under SD,
7 the *eav* mutant profile is separate from that of Ws across stPhP (Fig. 3A; Pouteau et al., 2004)
8 whilst under LD a consistent overlap is observed (Fig. 3B). Conversely, many mutants under
9 SD localize near the variation profile of Ws across exPhP (Fig. 3A) whilst under LD the overlap
10 is only marginal (Fig. 3B). Yet, the corresponding R indices are significantly different in all
11 cases (Table 1; Supplementary Table T1).

12 To explore further to what extent some mutants may phenocopy the dynamic behavior of the
13 wild type in a different environment, the variation of TLN with LtB and PChron was compared
14 in Ws and the mutants (Fig. 4). The LtB and PChron conversions have contrasting impacts on
15 the overlap between mutant and Ws distributions compared to the DtB response and point to the
16 importance of light response changes in the mutant dynamics. Under LD, the overall
17 distribution of the mutants is expanded in the LtB conversion whilst it is compressed near the
18 Ws values in the PChron conversion, indicating that trophic effects on true photoperiodic
19 response may be buffered in the mutant as in Ws (see above). Under SD, the LtB conversion
20 results in a coincidence between the least precocious mutants and Ws at a 12 h stPhP (Fig. 4A)
21 whilst the PChron responses of the mutants and Ws across stPhP and exPhP remain separate
22 (Fig. 4B), suggesting that the mutant variation involves both light response phenocopy effects
23 and period perception changes.

24 To examine the potential impact of different sources of genetic variation, we analyzed the
25 variation of TLN with DtB in a large collection of natural accessions and in a set of later
26 flowering mutants based on data recently published by Lempe et al. (2005; Supplementary Fig.
27 S1 and S2, Table 1, Supplementary Table T1). This study shows that the R index across natural

1 accessions is conserved under different temperatures in LD but significantly different in SD
2 compared to LD (Supplementary Fig. S1, Supplementary Table T1). Interestingly, the variation
3 profiles across natural accessions and later flowering mutants overlap and the R index is
4 conserved under SD. Similarities are also found with the R index in Ws across photoperiods but
5 not with the R index across *eav* mutants (Supplementary Fig. S2, Supplementary Table T1).
6 These comparisons can only be provisional since the environmental conditions used in the work
7 by Lempe et al. (2005) and ours differ in a number of factors, in particular temperature. But they
8 seem to support the notion that early and late flowering mutants have a different dynamic
9 behavior.

10 **Link between flowering time indicators and the specification of the primary inflorescence**

11 The bolting node marks the beginning of the primary inflorescence characterized by the
12 presence of CL subtending secondary inflorescences, or coflorescences, so that the size of the
13 primary inflorescence, or CL zone, visualizes to some extent the relation between the DtB and
14 TLN temporal indicators. The analysis of the variation of CLN with DtB and TLN in Ws across
15 photoperiods and in mutants under SD and LD (Fig. 5) reveals a number of important features.

16 Firstly, the variation of CLN with DtB in Ws across stPhP shows a saturation above 35 DtB
17 (corresponding to the Pce of CLN at 12 h) whilst the saturation is more limited for the variation
18 of CLN with TLN (Fig. 5). These results raise the possibility that the measure of flowering time
19 by the DtB indicator is overestimated for late flowering times and that TLN is potentially a
20 more accurate temporal indicator. Secondly, under exPhP, the saturation in the variation of CLN
21 with DtB is less pronounced and more CLN are produced with short extension periods (2 h and
22 4 h) than under a 8 h stPhP (Fig. 5A). In addition, the CLN to TLN ratio is globally higher
23 across exPhP than across stPhP (Fig. 5B). This indicates that the global light regime and not
24 only the photoperiod or flowering time per se influences the size of the CL zone. Thirdly,
25 except for a few cases, the variation of CLN seems loosely connected to DtB in the mutants
26 under both SD and LD compared to Ws (Fig. 5A), suggesting that no strong ontogenetic
27 correlation exists between the DtB indicator and the specification of the CL zone. In contrast,

1 the variation in the CLN to TLN ratio is grossly conserved between the mutants under SD and
2 Ws across stPhP (Fig. 5B). Even under LD, most mutants localize in the continuity of the
3 distribution observed for Ws across photoperiods. These results may indicate that the leaf ratio
4 reflects an intrinsic developmental correlativity.

5 **Changes in ontogenetic correlativity across photoperiods**

6 Because the transition to flowering involves drastic morphogenetic changes including the
7 cessation of leaf production and start of flower organogenesis, we addressed the question as to
8 whether extensive variation in this transition across photoperiods in Ws is accompanied by
9 changes in ontogenetic correlativity. This can be estimated by measuring actual correlations
10 between characters based on their r coefficient of correlation and z quantity of correlation or
11 connectance (see Material and Methods). In addition, the quantity of character fluctuations, i.e.
12 variability measured by the coefficient of variation (CV), can also provide an estimate of a loose
13 or tight connection with other characters (Amzallag, 2001).

14 On average, the CV within independent experiments shows no significant variation for DtB
15 across photoperiods. But a significant increase is observed for RLN and CLN from 10 h to 12 h
16 and from 12 h to 14 h respectively ($t = 3.88$ and $t = 3.37$, $P < 1.5 \%$; Fig. 6A) resulting in higher
17 mean CV under LD than under SD. The CV between independent experiments is more
18 variable, especially under intermediate photoperiods, with a peak at 12 h and a trough at 14 h
19 which can be discriminated by variance comparison (risk = 1 % ; Fig. 6B). These findings
20 indicate that the susceptibility of RLN and CLN to changes in initial conditions and/or
21 experimental microvariation is highest under photoperiods inducing largest variation in
22 flowering time (see Fig.1). Strikingly, the correlation between RLN and CLN is low at all
23 photoperiods except for a major peak ($r = 0.69$) at 12 h (Fig. 6C), corresponding to a high
24 connectance ($z = 0.86$). The coincidence of this peak with the major peak of variability suggests
25 that stronger correlativity for the leaf ratio is associated with increased susceptibility to the
26 environment and/or reduced connection with other characters. Conversely, a relaxed leaf
27 correlativity under SD and LD, in particular at 14 h, seems to coincide with lesser

1 environmental influence and/or tighter connection with other characters. An interpretation for
2 the apparently higher variability above 16 h could be that an excess of light supply generates
3 additional developmental instability.

4 **DISCUSSION**

5 Based on extensive comparison between mutational and environmental changes in flowering
6 time, a number of conclusions on photoperiodic regulation in *Arabidopsis* wild types and early
7 flowering mutants can be discussed.

8 **Discrimination of light period and phototrophic effects on flowering time**

9 In modeling for the prediction of flowering time in annual crops under natural and therefore
10 variable environments, the daily contribution of photoperiod to flowering time can be treated as
11 additive increments, i.e. photoperiodic time, in the same way as the thermal time (Roberts and
12 Summerfield, 1987). Yet, the photoperiodic time is a measure not only of true photoperiodic
13 response but also of phototrophic effects. Here we show that under standard photoperiods
14 (stPhP) the bolting date (DtB) of the *Ws* natural accession is a linear function of the light sum
15 total available for plant photosynthesis below the critical photoperiod rather than photoperiod
16 per se. In contrast, the use of extended photoperiods (exPhP) that maintain a constant
17 phototrophic input leads to a typical quantitative variation in the DtB function characterized by
18 critical (P_c) and ceiling (P_{ce}) photoperiods. Yet, plants also exhibit a strong shade avoidance
19 response (Karlsson et al., 1993; Lee and Amasino, 1995; Smith and Whitelam, 1997; this work)
20 unlike other species with a SD habit such as *Impatiens* (Battey and Lyndon, 1984; Pouteau et
21 al., 1997). In addition, the P_c and P_{ce} values, hence the notions of SD and LD, prove
22 contingent, not only upon temperature as reported for various species (Roberts and
23 Summerfield, 1987), but also upon the phototrophic input. Other natural accessions of
24 *Arabidopsis* probably share this phototrophic contingency, e.g. *Landsberg erecta* and *Col-0*
25 under different irradiances (Corbesier et al., 1996).

1 Although both stPhP and exPhP present caveats, respectively photosynthetic and light signaling
2 modifications, that may interfere with the interpretation of flowering time, our results suggest
3 two ways to address true photoperiodic responses. Firstly, the RLN and TLN norms of reaction
4 appear more robust against environmental variation in the phototrophic input. It may thus be
5 concluded that the leaf number indicator is a more operational measurement of true
6 photoperiodic responses in Ws than the bolting date. Secondly, we derived two new temporal
7 indicators from DtB, a photochron index corresponding to the DtB per hour of photoperiod
8 (PChron) and the photosynthetic time to bolting (LtB), that prove useful to discriminate the
9 period and trophic components of photoperiod. Indeed, LtB shows little variation across stPhP
10 whilst the reciprocal of PChron is a linear function of photoperiod. Most strikingly, the
11 variations of DtB with TLN across stPhP and exPhP, which are parallel but distinct, coincide
12 when using the PChron conversion. Despite the fact that sugars are an important component of a
13 multifactorial, mobile inductive signal for flowering (Bernier et al., 1993), it can be concluded
14 that the trophic input necessary for flowering does not interfere with light signaling in true
15 photoperiodic response. This confirms the conclusions reached in analyses of flowering
16 responses to modified photoassimilate and phytochrome levels (Bagnall et al., 1995; King and
17 Evans, 1991). Similarly, Roberts and Summerfield (1987) showed that the responses to both
18 temperature and photoperiod are linear and without interaction, this allowing to simplify the
19 prediction of photothermal responses based on the measurement of a small number of genetic
20 coefficients.

21 **Differential impact of photoperiodic and mutational changes on ontogenetic dynamics**

22 The analysis of the variation profiles of DtB with TLN suggests that the dynamic impacts of
23 environmental (external) and genetic (endogenous) changes operate through distinct processes.
24 Indeed, the variation profiles across photoperiods and early flowering mutants are different. Yet,
25 the use of exPhP contributes to reduce these differences, leading to a response profile that
26 phenocopies to a large extent the mutant variation under SD. In addition, the LtB conversion
27 also reveals convergent dynamic behavior between Ws under a 12 h stPhP and some mutants

1 under SD. These results and the various alterations in hypocotyl elongation observed under LD
2 and/or SD and in the dark (Pouteau et al., 2004) suggest that changes in light quantity
3 perception and signaling have an important part in the heterochronic modifications exhibited by
4 the early mutant population. The finding that the mutant and Ws distributions remain separate
5 with the PChron conversion also points to more profound ontogenetic changes, possibly
6 associated to perturbations in global correlativity of developmental networks and explaining the
7 high level of pleiotropy observed in the *eav1-61* mutants (Pouteau et al., 2004).

8 The previous observations of a conserved linear variation between DtB and TLN in natural
9 accessions and in mostly late flowering mutants were re-examined based on a large set of data
10 recently reported by Lempe et al. (2005). In spite of a significant difference in the variation
11 profiles under LD, the behavior of these mutants was globally similar to that of the natural
12 accessions. Insofar as the different environmental conditions used by Lempe et al. and in our
13 work can be compared, this analysis thus brings support to the notion that ontogenetic dynamics
14 of early and late flowering mutants are different. Heterochronic changes in the timely onset,
15 duration and dynamics of the different ontogenetic phases (Diggle, 1999; Wiltshire et al., 1994)
16 seem more likely to impose uncoupling between morphogenetic events and physiological
17 processes in early than in late flowering mutants.

18 **Coordinate specification of the primary inflorescence boundaries**

19 The analysis of the primary inflorescence, or CL zone, in Ws across photoperiods and in
20 mutants provides further indication on the link between the two types of temporal indicators,
21 DtB and TLN. Indeed, the basal and apical boundaries of the CL zone are determined by bolting
22 and cessation of leaf differentiation, respectively. Our results suggest that the two boundaries
23 vary coordinately in wild types and mutants, possibly due to biophysical and/or homeostatic
24 constraints. Their timely specification, which is conserved in stPhP and exPhP, is altered in
25 mutants. This heterochrony may result from the shortening of developmental phases with a
26 different growth rate and/or photoperiod sensitivity. Indeed, early stages in Arabidopsis are
27 characterized by a slower growth rate (Groot and Meicenheimer, 2000). It was also shown in

1 soybean that more sensitive genotypes exhibit longer phases of photoperiod insensitivity
2 (Upadhyay et al., 1994).

3 Alternatively, uncoupling between the bolting node and time may be a consequence of variation
4 in leaf and flower specification relative to the time and nodal position at which the floral switch
5 occurs. Indeed, due to flexible organ specification the position of the apical boundary proves
6 contingent on the potency of the inductive treatment since it can coincide with the switch node
7 (Hempel and Feldman, 1994) or be specified at a lower node under more potent inductive
8 conditions (Hempel et al., 1998). Likewise, in *Impatiens* the first axillary flowers can be moved
9 above or below the switch node by applying inductive conditions at early or late developmental
10 time, respectively (Pouteau et al., 1998). It is thus likely that the position of the apical boundary
11 fluctuates relative to the switch node due to environmental and genetic variation in the quantity
12 and/or diffusion of the floral inductive signal and in plant age or ontogenetic stage when the
13 floral switch occurs.

14 Similarly, internode specification may be gradual and susceptible to modification in the course
15 of differentiation in response to environmental and/or genetic conditions and lead to fluctuations
16 in the position of the bolting node. Internode elongation is first detected 52 h after the floral
17 switch in *Arabidopsis* meristem microscopic analyses (Jacqumard et al., 2003) and becomes
18 macroscopically visible at a much later stage. This implies that the basal boundary is specified
19 either at the same time or after the apical boundary. The morphological discontinuity introduced
20 by bolting, leading to the adoption of an erect bearing common to most flowering plants, is
21 mediated by phytochromes and gibberellins (Devlin et al., 1998; King et al., 2001; Koornneef et
22 al., 1995). Yet, the specification of the bolting node itself is still poorly understood. We show
23 here that this ontogenetic specification is differentially regulated across photoperiods in *Ws*.
24 The correlation between RLN and CLN appears highest under strongest photoperiodic
25 influence, i.e. an intermediate photoperiod of 12 h, whilst it is low under SD and LD. Because a
26 high variability is also observed under a 12 h photoperiod and possibly reflects a reduced
27 correlativity with other characters, this correlation peak may be interpreted as an endogenous

1 compensation to maintain ontogenetic integrity. In conclusion, we propose that the CL zone
2 may represent an important mediating zone in which environmental and endogenous
3 fluctuations are ontogenetically integrated and possibly buffered through variation in its
4 boundaries. This raises new questions for future work as to how this transition zone is
5 established and whether it may be involved in developmental correlativity and possibly more
6 robust to mutational perturbations.

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12 **SUPPLEMENTARY MATERIAL**

13 Supplementary material consisting in two figures S1 and S2 and one table T1 are available
14 online.

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1

Table 1. R dynamic index in different genotype and photoperiod contexts					
Genotype	Photoperiod interval	Temperature (°C)	Name of condition	R index	r ²
Ws	10 h to 24 h stPhP	20	20stP	1.33	0.94
	10 h to 24 h exPhP	20	20exP	1.32	0.96
<i>eav1</i> to <i>eav60</i>	8 h	20	20SD	0.85	0.74
	16 h	20	20LD	0.50	0.55
NA*	8 h	23	23SD	1.20	0.62
	16 h	23	23LD	1.61	0.83
	16 h	16	16LD	1.56	0.78
Mutants*	8 h	23	23SD	1.14	0.68
	16 h	23	23LD	1.24**	0.84**
	16 h	16	16LD	1.33	0.83

The R dynamic index was determined based on the relative variation of TLN with DtB (see Materials and methods) between the corresponding genotypic lines and/or photoperiod intervals. r² : coefficient of determination of the corresponding linear regressions.

*Data from Lempe et al. (2005): comparison of 122 natural accessions (NA) and 30 mutants in three different environmental conditions. Light intensity was 125-175 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$, i.e. about two thirds of the light intensity used in this work (see Materials and Methods, Supplementary Fig. S1 and S2).

**Cook's distance analysis revealed one outlier with a value at 1.04 in the Mutants 23 LD set of data. The corresponding data was deleted in the regression analysis. Cook's distances were below 0.5 for other genotypes and/or conditions.

2

1 **FIGURE LEGENDS**

2 **Figure 1.** Variation in flowering time across standard and extended photoperiods in Ws. A)
3 Number of days to bolting (DtB) and corresponding number of days with photosynthetically
4 active light (LtB). B) Number of rosette leaves (RLN) and cauline leaves (CLN). DtB and RLN:
5 filled diamonds (stPhP) and blue circles (exPhP). LtB and CLN: crosses (stPhP) and orange
6 circles (exPhP). The corresponding critical photoperiod (Pc) and ceiling photoperiod (Pce) for
7 DtB (A) and RLN and CLN (B) are indicated. Blue arrows point to the shifts in Pc and Pce
8 under exPhP. Lines are traced following the mean values at each photoperiod. Independent
9 experimental repeats each comprising 10 individuals on average are presented.

10 **Figure 2.** Variation in the photochron indicator across photoperiods in Ws. A) Number of days
11 to bolting per hour of photoperiod or photochron (PChron). B) Reciprocal of PChron. Lines in
12 A) are traced following the mean values at each photoperiod. Lines in B) correspond to linear
13 regressions. Continuous line (stPhP): $y = 0.061x - 0.313$; $r^2 = 0.98$. Dotted line (exPhP): $y =$
14 $0.065x - 0.487$; $r^2 = 0.99$. Independent repeats each comprising 10 individuals on average are
15 presented.

16 **Figure 3.** Variation in the relative progression to flowering across photoperiods and mutants. A)
17 Relation between the total number of leaves (TLN) and DtB for Ws between 6 h and 24 h
18 photoperiods (stPhP: black-filled diamonds; exPhP: blue-filled circles) and *eav1* to *eav61* T-
19 DNA insertion mutants under 8 h stPhP (red crosses). The corresponding linear regressions are
20 shown (black alternate dotted line for Ws between 10 h and 24 h stPhP: $y = 1.33x - 14.62$; red
21 thick line for *eav* mutants: $y = 0.85x - 8.82$; blue thick line for Ws between 10 h and 24 h
22 exPhP: $y = 1.32x - 21.05$). B) Relation between TLN and DtB for Ws between 16 h and 24 h
23 photoperiods (stPhP: black-filled diamonds; exPhP: blue-filled circles) and *eav* mutants (red
24 crosses) under 16 h stPhP. The linear regression for *eav* mutants (red thick line: $y = 0.50x +$
25 1.85) is shown. Black alternate dotted line and blue thick lines: same as A). The r^2 coefficient

1 for all linear regressions are presented in Table 1. Independent repeats each comprising 10
2 individuals on average are presented.

3 **Figure 4.** Contribution of the light sum and photochron to the relative progression to flowering.
4 Variation of TLN with LtB (A) and PChron (B) for *Ws* across photoperiods (stPhP: black-filled
5 diamonds; exPhP: blue-filled circles) and for *eav1* to *eav61* mutants (8 h stPhP: orange
6 triangles; 16 h stPhP: red crosses). The range of photoperiods shown for *Ws* is 6 h to 24 h (A)
7 and 8 h to 24 h (B). The lines are traced following the mean values at each photoperiod (black
8 plain line: stPhP; blue short-dotted line: exPhP). The purple area highlights the coincidence
9 between *Ws* at a 12 stPhP and some of the least precocious mutants under SD. Independent
10 repeats comprising 10 individuals on average are presented.

11 **Figure 5.** Influence of flowering time on the specification of the primary inflorescence. A)
12 Relation between CLN and DtB (A) and between CLN and TLN (B) for *Ws* across
13 photoperiods (stPhP: black-filled diamonds; exPhP: blue-filled circles) and for *eav1* to *eav61* T-
14 DNA insertion mutants (orange triangles) under 8h stPhP. Insets: *Ws* between 16h and 24 h
15 stPhP (filled diamonds) and *eav* mutants under 16 h stPhP (red crosses). Lines as in Figure 4.
16 Red thick line (inset in B): exponential regression for *eav* mutants. Independent repeats
17 comprising 10 individuals on average are presented.

18 **Figure 6.** Variation in the correlativity of the primary inflorescence across photoperiods.
19 Quantity of variability within experiment (A, mean CV) and between experiments (B, CV of
20 experiment averages) for DtB (filled diamonds), RLN (crosses), and CLN (open triangles). C)
21 Correlation between RLN and CLN.

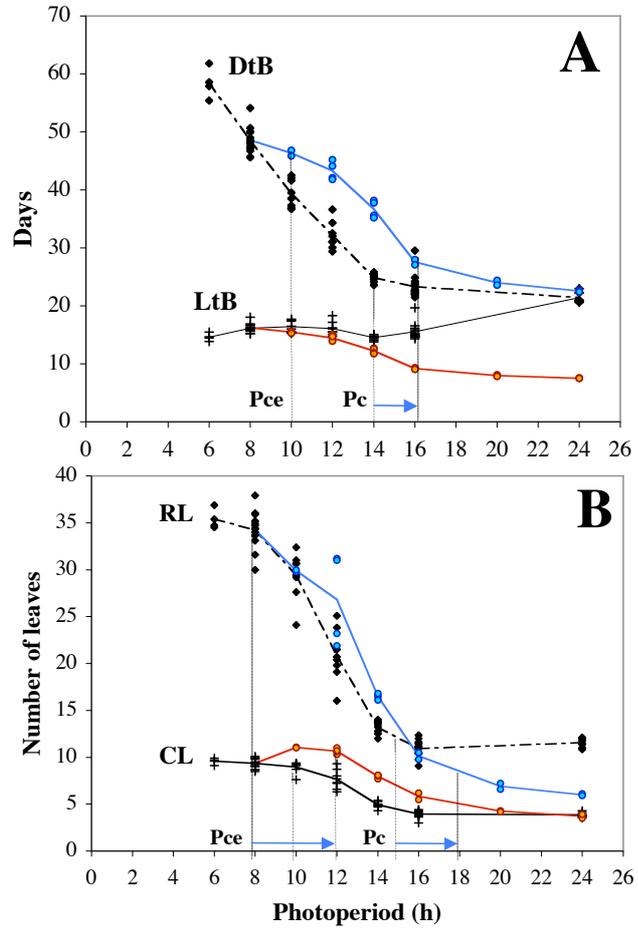


Figure 1

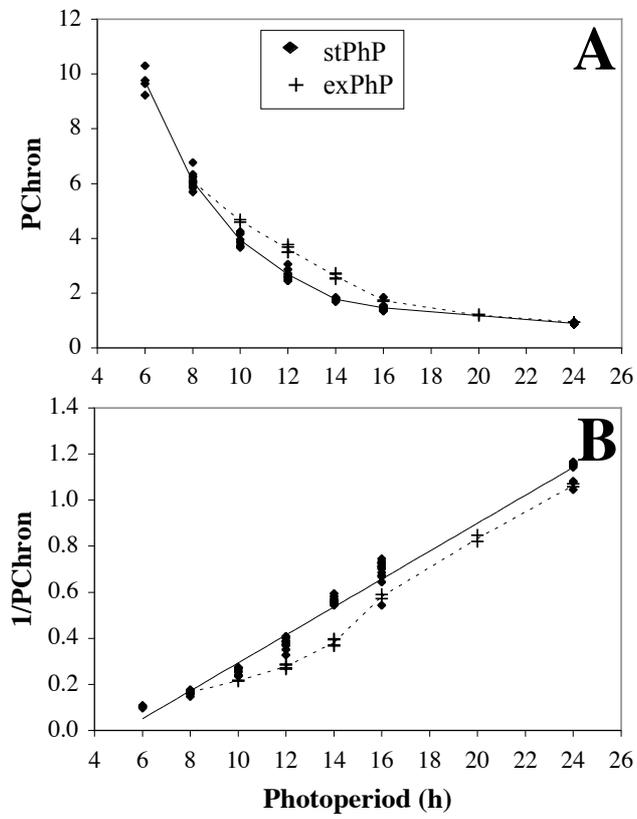


Figure 2

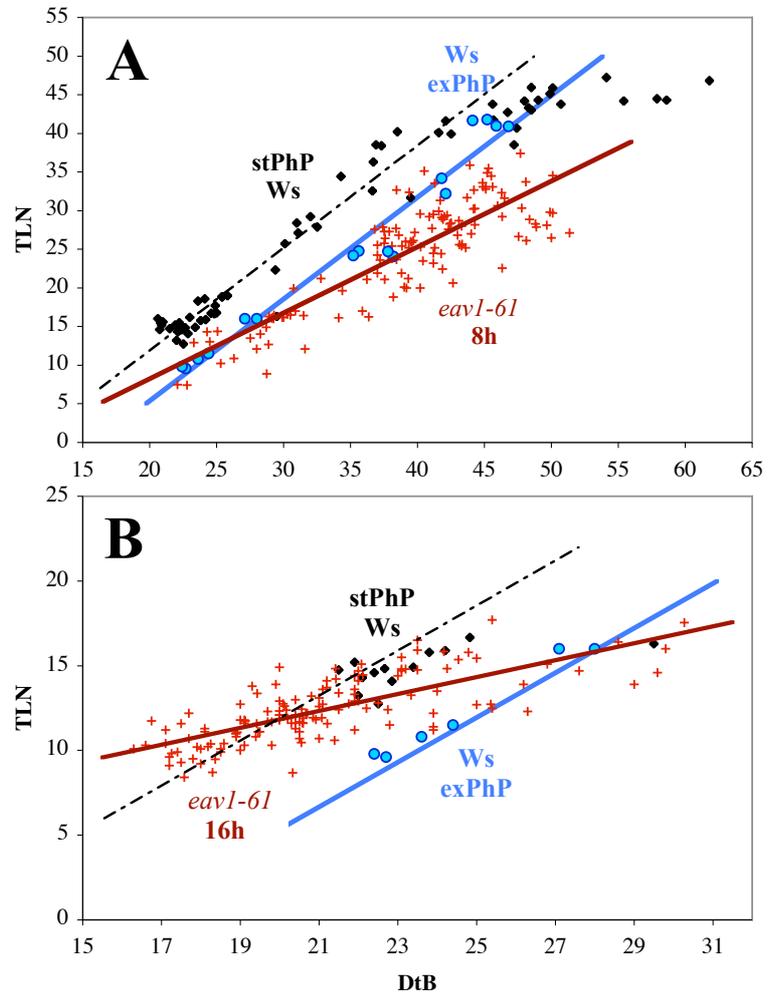


Figure 3

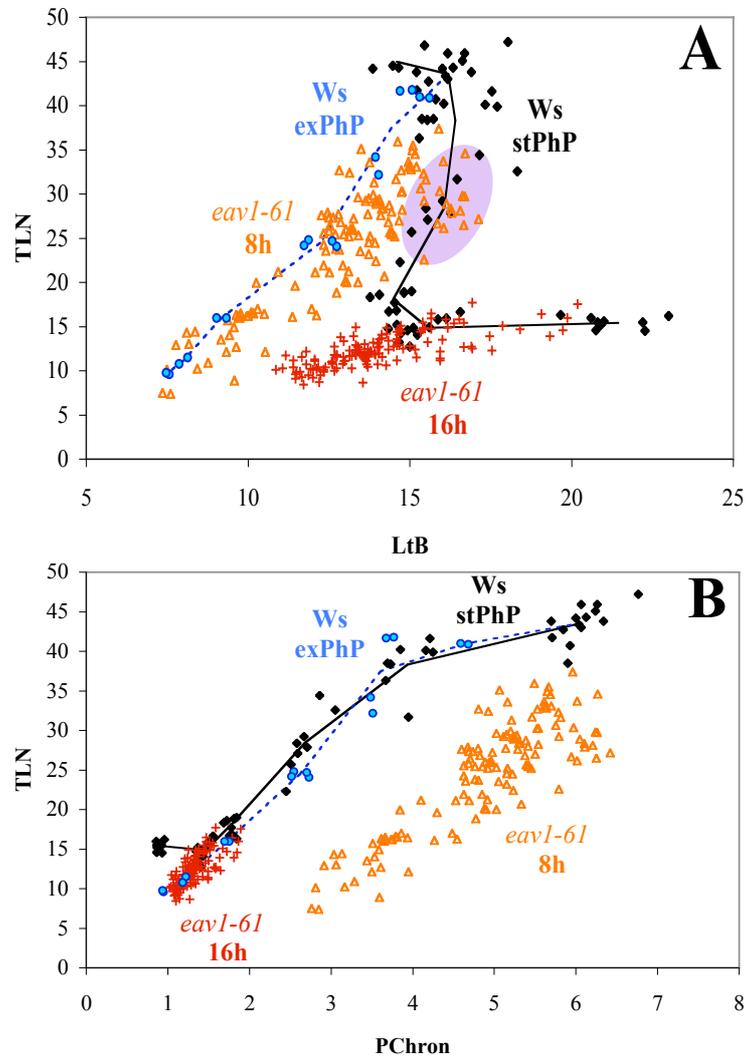


Figure 4

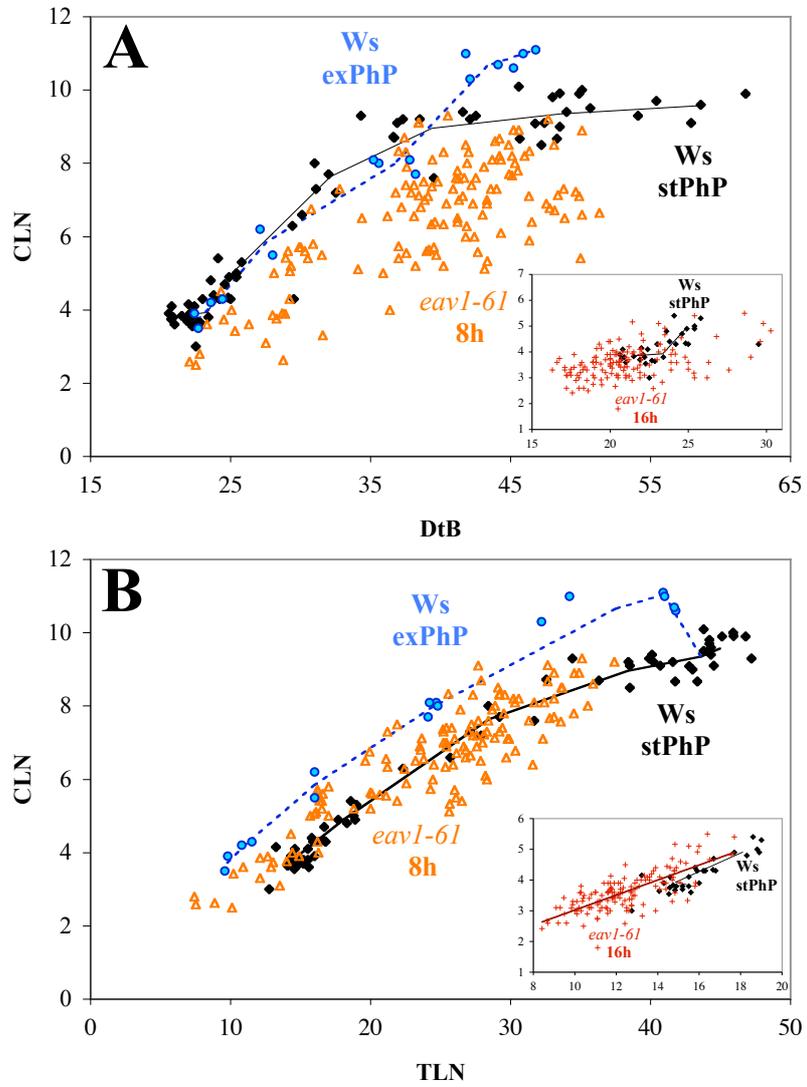


Figure 5

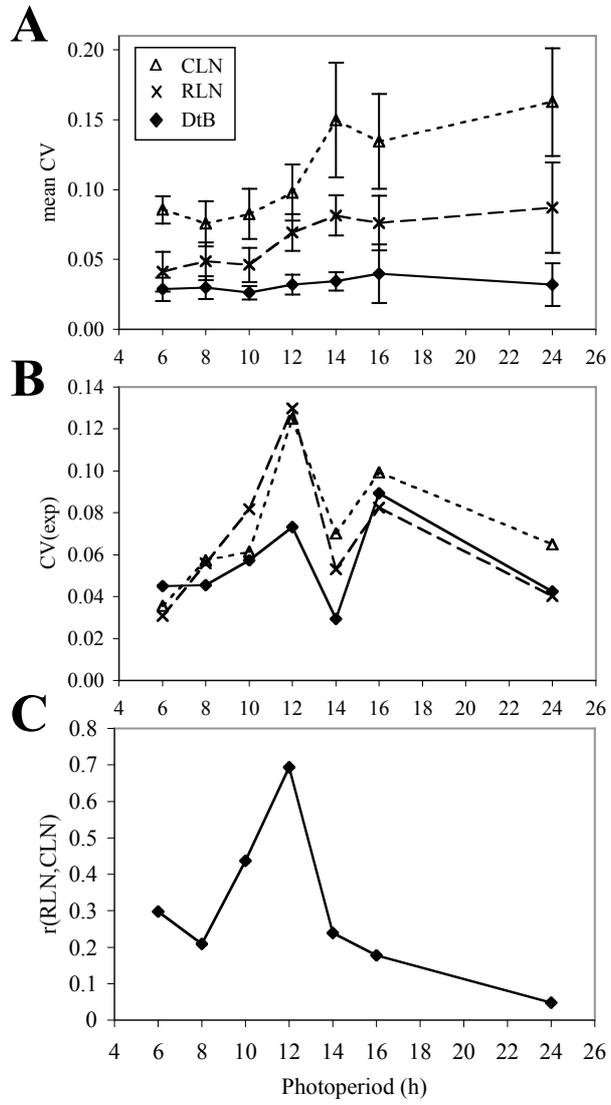
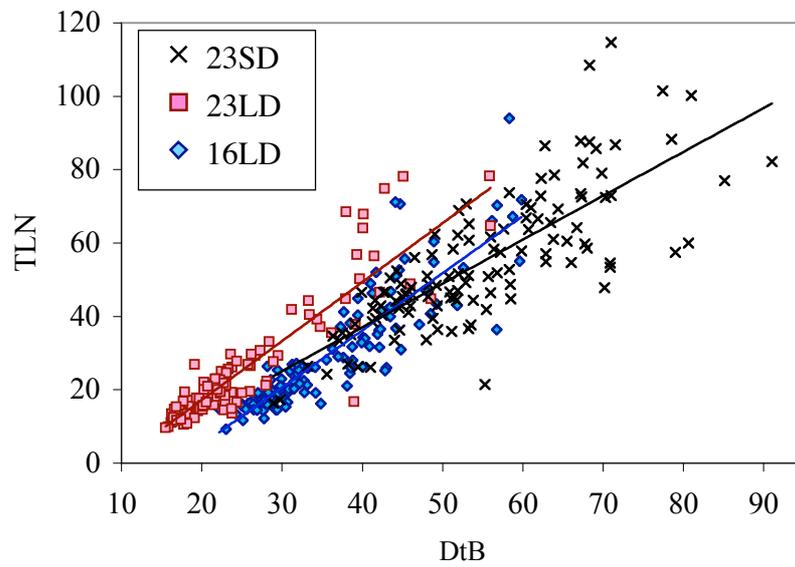


Figure 6

Supplementary Table T1. Comparison of R dynamic indices

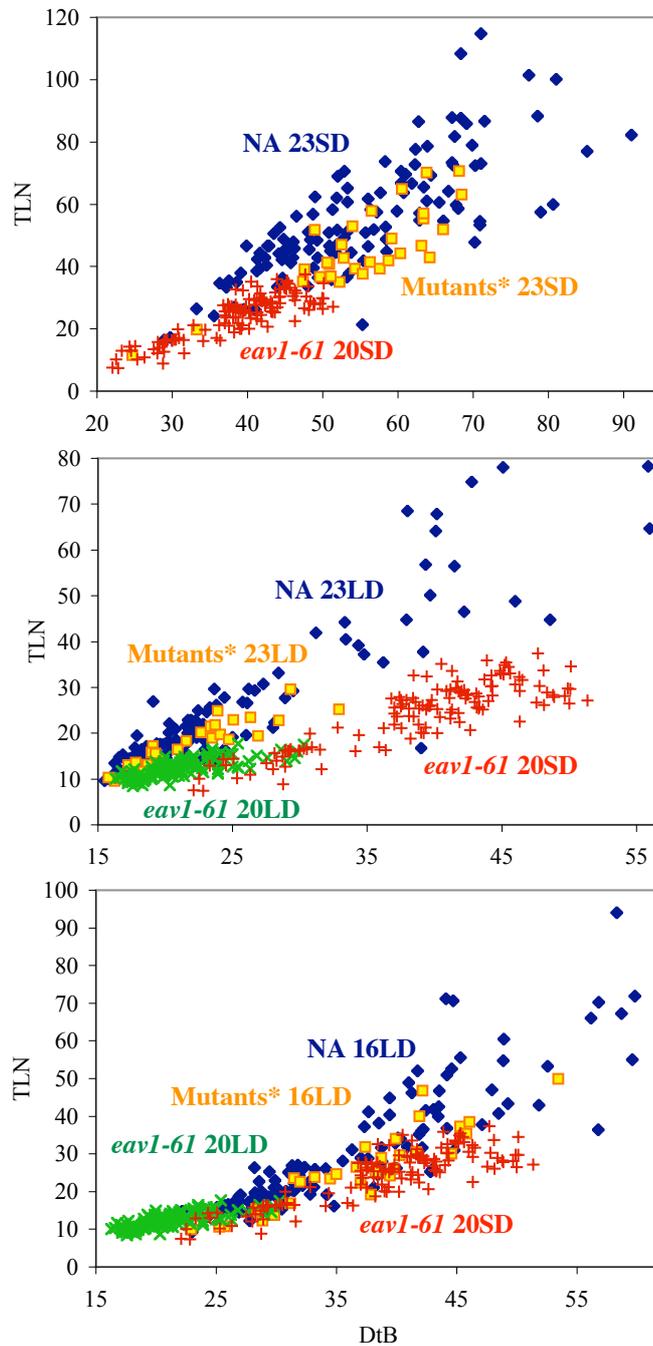
	Ws 20stP	Ws 20exP	eav 20SD	eav 20LD	NA 23SD	NA 23LD	NA 16LD	Mutants 23SD	Mutants 23LD
Ws 20exP	0.88 (t=0.16)	-	-	-	-	-	-	-	-
eav 20SD	<0.1‰	<0.1‰	-	-	-	-	-	-	-
eav 20LD	<0.1‰	<0.1‰	<1%	-	-	-	-	-	-
NA 23SD	0.58 (t=0.55)	0.55 (t=0.59)	<1%	<1%	-	-	-	-	-
NA 23LD	0.05	0.03	<0.1‰	<0.1‰	<1‰	-	-	-	-
NA 16LD	0.16	0.11	<0.1‰	<0.1‰	<1%	0.62 (t=0.49)	-	-	-
Mutants 23SD	0.21	0.24	<1%	<1‰	0.46 (t=0.74)	<1%	0.01	-	-
Mutants 23LD	0.47	0.47	0.13	<1‰	0.93 (t=0.09)	<0.1‰	0.30	0.91 (t=0.37)	-
Mutants 16LD	1.00	0.93	<0.1‰	<0.1‰	0.65 (t=0.65)	0.11	<1%	0.35 (t=0.94)	0.63 (t=0.49)

The potential significance of similarity between R dynamic indices shown in Table 1 was estimated by regression slope comparison. Corresponding probabilities based on a t test are presented. t values are indicated for significant similarities described in the text. Data and names of conditions are as in Table 1.



Supplementary Figure S1. Variation in the relative progression to flowering across natural accessions in different environments.

Data available from 121 natural accessions analyzed in parallel in several conditions by Lempe et al. (2005) are presented. Variation under SD at 23°C (23SD, crosses) and LD at 23°C (23LD, pink squares) and at 16°C (16LD, blue diamonds) are shown with their corresponding linear regressions.



Supplementary Figure S2. Comparison of mutant and natural variation in the progression to flowering.

Data available from 121 natural accessions (NA, blue diamonds) and 30 mutants (Mutants*, yellow squares) analyzed in parallel by Lempe. et al. (2005) under environmental conditions as in Supplementary Fig. S1 are presented. The distributions obtained for *eav1* to *eav61* early flowering mutants at 20°C under SD (20SD, red crosses) and LD (20LD, green crosses) are shown for comparison.