doi:10.1111/j.1558-5646.2012.01828.x

PLEIOTROPY IN THE WILD: THE DORMANCY GENE *DOG1* EXERTS CASCADING CONTROL ON LIFE CYCLES

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Received August 6, 2012
Accepted September 25, 2012
Data Archived: Dryad doi:10.5061/dryad.g792f

In the wild, organismal life cycles occur within seasonal cycles, so shifts in the timing of developmental transitions can alter the seasonal environment experienced subsequently. Effects of genes that control the timing of prior developmental events can therefore be magnified in the wild because they determine seasonal conditions experienced by subsequent life stages, which can influence subsequent phenotypic expression. We examined such environmentally induced pleiotropy of developmental-timing genes in a field experiment with *Arabidopsis thaliana*. When studied in the field under natural seasonal variation, an *A. thaliana* seed-dormancy gene, *Delay Of Germination 1* (*DOG1*), was found to influence not only germination, but also flowering time, overall life history, and fitness. Flowering time of the previous generation, in turn, imposed maternal effects that altered germination, the effects of *DOG1* alleles, and the direction of natural selection on these alleles. Thus under natural conditions, germination genes act as flowering genes and potentially vice versa. These results illustrate how seasonal environmental variation can alter pleiotropic effects of developmental-timing genes, such that effects of genes that regulate prior life stages ramify to influence subsequent life stages. In this case, one gene acting at the seed stage impacted the entire life cycle.

KEY WORDS: **Dormancy, flowering time, germination, life history, natural selection.**

Organisms in the wild develop under seasonally variable conditions. Genes that regulate developmental timing determine seasonal conditions of subsequent development and thereby can affect the expression of subsequent plastic phenotypes. Hence, allelic effects of early developmental genes can potentially be magnified when organisms develop in the wild, such that one gene affects multiple traits. This magnification of the effects of developmental-timing genes, via environmentally induced pleiotropy, could have important evolutionary and ecological consequences, but it has not been explicitly demonstrated. Using *Arabidopsis thaliana* in the field, we report that a single locus acting early in development alters the entire life cycle.

Phenology, the timing of biological events, is a primary determinant of life-history variation, and natural selection on the seasonal timing of development can be extremely strong (Donohue et al. 2005a,c; Huang et al. 2010). For this reason, plasticity of developmental timing in response to seasonal environmental factors is considered to be one of the most important

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INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

influences on organismal responses to environmental change and their subsequent fitness (Chuine and Beaubien 2001; Walther et al. 2002; Menzel et al. 2006; Parmesan 2006; Bradshaw and Holzapfel 2008; Willis et al. 2008). Genes that regulate phenology are therefore of particular interest for predicting how organismal life histories can respond to altered environments.

Plasticity of early life stages has been hypothesized to be especially important in sessile organisms, such as plants, that cannot seek alternative microenvironments (Donohue 2003). The timing of early developmental transitions, such as seed germination, has been shown to influence subsequent life-history expression and fitness as well as natural selection on subsequent traits (Evans and Cabin 1995; Donohue 2001; Donohue et al. 2010; Huang et al. 2010).

Likewise, environmental conditions experienced in the previous generation can influence early developmental stages of progeny through maternal effects (Schmitt 1995; Andalo et al. 1999; Munir et al. 2001; Galloway 2002). In particular, the seasonal timing of flowering determines both the seed-maturation conditions and postdispersal seasons experienced by seeds, factors that influence dormancy and germination in *A. thaliana* (Donohue et al. 2007). In that manner, maternal traits, such as flowering time, can influence traits of progeny, such as germination. Here we examine these dynamics at the genetic level by testing how allelic variation of genes that regulate early developmental transitions, specifically germination, interact with the genes regulating late-stage phenotypes, specifically flowering.

We used the annual plant *A. thaliana*, whose life cycle is determined by the developmental transitions of germination and flowering, to examine allelic effects of naturally variable developmental genes in the wild, and to test the extent of the magnification of gene effects through environmentally induced pleiotropy. The genetic model *A. thaliana* is native to the Old World (Sharbel et al. 2000; Hoffman 2002), and exhibits life-history variation across its range (Ratcliffe 1965; Thompson 1994; Donohue 2009). A winter-annual life history is most common, with seeds germinating in autumn and flowering occurring in spring. A spring-annual life history also occurs, in which germination and flowering both occur during the same spring. Autumn flowering has also been documented, when seeds germinate and flower in autumn, potentially leading to a rapid-cycling life history.

Natural genetic variation has been documented for genes that regulate the developmental transitions of germination and flowering (Mitchell-Olds 1996; Alonso-Blanco et al. 1998; Alonso-Blanco et al. 2003; Clerkx et al. 2004; Meng et al. 2008; Alonso-Blanco et al. 2009; Atwell et al. 2010; Bentsink et al. 2010; Brachi et al. 2010; Fournier-Level et al. 2011; Salome et al. 2011). We focus on three of these genes that exhibit pronounced natural allelic variation: *DELAY OF GERMINATION 1* (*DOG1*), which regulates dormancy and germination timing, and two flowering-time genes—*FLOWERING LOCUS C* (*FLC*) and *CRYPTOCHROME 2* (*CRY2*).

DOG1 was identified by QTL studies (Alonso-Blanco et al. 2003; Bentsink et al. 2010), and is one of the major genes that causes the Cape Verde Island (Cvi) ecotype to be exceptionally dormant. It is also associated with germination timing in the field (Huang et al. 2010) and is the only cloned naturally variable dormancy gene (Bentsink et al. 2006). Natural variation in its expression is associated with geographic variation in dormancy and germination among *A. thaliana* populations (Bentsink et al. 2006; Chiang et al. 2011; Nakabayashi et al. 2012), and patterns of genetic differentiation among ecotypes indicate a role for *DOG1* in local adaptation (Kronholm et al. 2012). *DOG1* has also been implicated in maternal effects on germination. Maternal effects on dormancy are strong in *A. thaliana*. In particular, when the maternal plant matures its seeds under cool temperature, those seeds exhibit stronger dormancy and delayed germination compared to seeds that are matured under warmer temperatures. Recent studies have indicated that *DOG1* has a critical role in this temperature-dependent dormancy, because *DOG1* expression correlates with dormancy levels induced by varying seed-maturation temperature, and *DOG1* mutations significantly compromise the ability of the seeds to enter deep dormancy in response to low seed-maturation temperatures (Chiang et al. 2011; Kendall et al. 2011; Nakabayashi et al. 2012).

A complex network of converging pathways controls the flowering transition in *A. thaliana* (Ausin et al. 2005; Baurle and Dean 2006), and much of its natural genetic variation lies in the photoperiod and vernalization (chilling) pathways (Alonso-Blanco et al. 2009; Mendez-Vigo et al. 2011). *FLC* expression is mediated by the vernalization pathway and inhibits flowering. The expression of *FLC* can be upregulated by *FRIGIDA* (*FRI*) and further delay flowering (Koornneef et al. 1994; Lee et al. 1994). Variation in functionality among these two genes results in a wide range of flowering time under controlled environments (Gazzani et al. 2003; Michaels et al. 2003; Caicedo et al. 2004; Werner et al. 2005), but this variation decreases when the juvenile plants experience a long period of winter cold (vernalization) (Amasino 2005; Dennis and Peacock 2007). Moreover, *FLC* potentially regulates both flowering and germination, because it has been shown to mediate germination timing in response to temperatures experienced after dispersal, with high *FLC* expression promoting germination especially at low temperature (Chiang et al. 2009).

Within the photoperiod pathway, an extremely early flowering phenotype is induced by a gain of function *CRY2* allele isolated from the Cvi ecotype. This allele inhibits the sensing of daily photoperiod change by inducing a constitutive long-day acceleration of flowering (Alonso-Blanco et al. 1998; El-Assal et al. 2001; Botto et al. 2003).

Although pathways of developmental transitions have been mostly examined individually under controlled conditions, how they interact under seasonally variable environments across generations is essential to life-history expression and local adaptation (Penfield and Springthorpe 2012). In the current study, we analyzed life-cycle behavior in different naturally occurring genotypes that were exposed to different experimental flowering times with respect to the environmental conditions during seed maturation and postdispersal seasons. This allowed us to test (1) whether interactions between dormancy and flowering-time alleles can create the various life histories observed in natural populations, (2) whether allelic effects of these life-history loci were modified by seasonal conditions or by the genetic background at other loci, (3) whether natural selection on life-history alleles was modified by seasonal conditions or by the genetic background at other loci. In this manner, we demonstrated that the seasonal context of life-history expression can fundamentally modify allelic effects, pleiotropic relationships, and natural selection on key life-history loci.

Materials and Methods

We used seeds of near isogenic lines (NILs) containing natural allelic variants of germination and flowering-time loci (Table S1). NILs remain the best tool to mimic naturally occurring mutations and to study the evolutionary consequences of individual allelic substitutions (Bradshaw and Schemske 2003). In the laboratory, these NILs differ in generation time as a result of introgression of a dormancy-inducing allele of *DOG1* from the Cvi ecotype into the L*er* background ($DOG1_{\mathrm{Cvi}}$) (Alonso-Blanco et al. 2003), combined with other early-flowering ($CRY2_{\mathrm{Cvi}}$) or late-flowering ($FLC_{\mathrm{Sf-2}} + FRI_{\mathrm{Sf-2}}$) alleles at flowering loci. NILs $DOG1_{\mathrm{Cvi}}$, FLC_{Cvi}, and $CRY2_{\mathrm{Cvi}}$ that contain introgressions of alleles from the Cvi ecotype were constructed and supplied by C. Alonso-Blanco, L. Bentsink, and M. Koornneef. $DOG1_{\mathrm{Cvi}}$ was constructed by selecting for linked genetic markers DFR, MBK, nga129, and g2368. *DOG1* is the only known gene regulating germination contained within the introgressed region of the $DOG1_{\mathrm{Cvi}}$ genotype. FLC_{Cvi} was selected by using linked genetic markers nga158 and nga151, and the $CRY2_{\mathrm{Cvi}}$ was selected with linked genetic markers PVV4, AXR1, PhyA, and g2395. The construction and characterization processes were detailed in previous publications (Alonso-Blanco et al. 1998; El-Assal et al. 2001; Alonso-Blanco et al. 2003). ($FLC_{\mathrm{Sf-2}} + FRI_{\mathrm{Sf-2}}$) contains the *FRI* and *FLC* alleles from San Feliu (Sf-2) introgressed into the L*er* background and was validated by linked markers g3843 (*FRI*), nga158 (*FLC*), nga 249 (*FLC*). These NILs were constructed and supplied by I. Lee, S. D. Michaels, and R. M. Amasino as previously described (Lee et al. 1994). Line [$DOG1_{\mathrm{Cvi}} + (FLC_{\mathrm{Sf-2}} + FRI_{\mathrm{Sf-2}})$] was constructed by crossing $DOG1_{\mathrm{Cvi}}$ and ($FLC_{\mathrm{Sf-2}} + FRI_{\mathrm{Sf-2}}$), and line ($DOG1_{\mathrm{Cvi}} + CRY2_{\mathrm{Cvi}}$) was constructed by crossing line $DOG1_{\mathrm{Cvi}}$ and $CRY2_{\mathrm{Cvi}}$. All lines were homozygous and were monitored for at least two generations to validate their stable phenotypes.

We simulated variation in seasonal flowering time by growing maternal plants under different conditions resembling different seasons, and then dispersing their seeds during different seasons. In this way, we decoupled maternal effects operating through seed-maturation conditions from those operating through postdispersal conditions determined by dispersal time. Specifically, we grew replicates of all genotypes under long-warm days ("LW" = 22°C, 14 h light/24 h) resembling late spring, short-warm days ("SW" = 22°C, 10 h), and short-cool days ("SC" = 10°C, 10 h) resembling early spring/late autumn. "LW" versus "SW" reveals effects of seed-maturation photoperiod; "SW" versus "SC" reveals effects of seed-maturation temperature.

All plants were vernalized for four weeks to synchronize flowering. Seeds from all treatments and genotypes matured synchronously within each of three dispersal cohorts: May 15, July 17, October 18 in 2007. These dates correspond to natural dispersal periods in New England populations. For each dispersal cohort, four chambers were used for each condition, and four plants per genotype were grown in each chamber. Cohorts of fresh seeds, harvested in May, July, and October, were pooled across chambers and dispersed into an old-field site within one week of harvest at the Harvard Concord Field Station, Bedford, MA, USA. Field methods followed standard protocols detailed elsewhere (Donohue et al. 2005b). Fifteen seeds of a given genotype x maternal treatment were placed in a 2.25-inch diameter peat pot (Jiffy Poly-Pak, Jiffy Products) filled with Metromix 360 (Gro Horticulture, Bellevue, WA). There were 15 blocks for each dispersal cohort, with genotype and maternal treatment randomized within each block.

Pots were monitored weekly for germination, bolting, and silique number. A random germinant was designated the focal plant for observations of life history and fitness, and all other germinants were removed. "Days to germination" was the number of days from dispersal into the field until germination. "Days to bolting" (flowering) was the number of days from germination to bolting. The number of siliques was recorded after natural senescence. If a plant died before reproducing, it had a value of zero siliques. ANOVA (SAS GLM) of normalized data tested for effects of genotype, maternal treatment, dispersal season, and their interactions, on germination day, bolting day, and silique number, with all factors as fixed effects. Single introgressions were compared to the L*er* background, and multiple introgressions were compared to L*er* and to each single introgression with Fisher's LSD. Submodels tested for interactions between genotype and

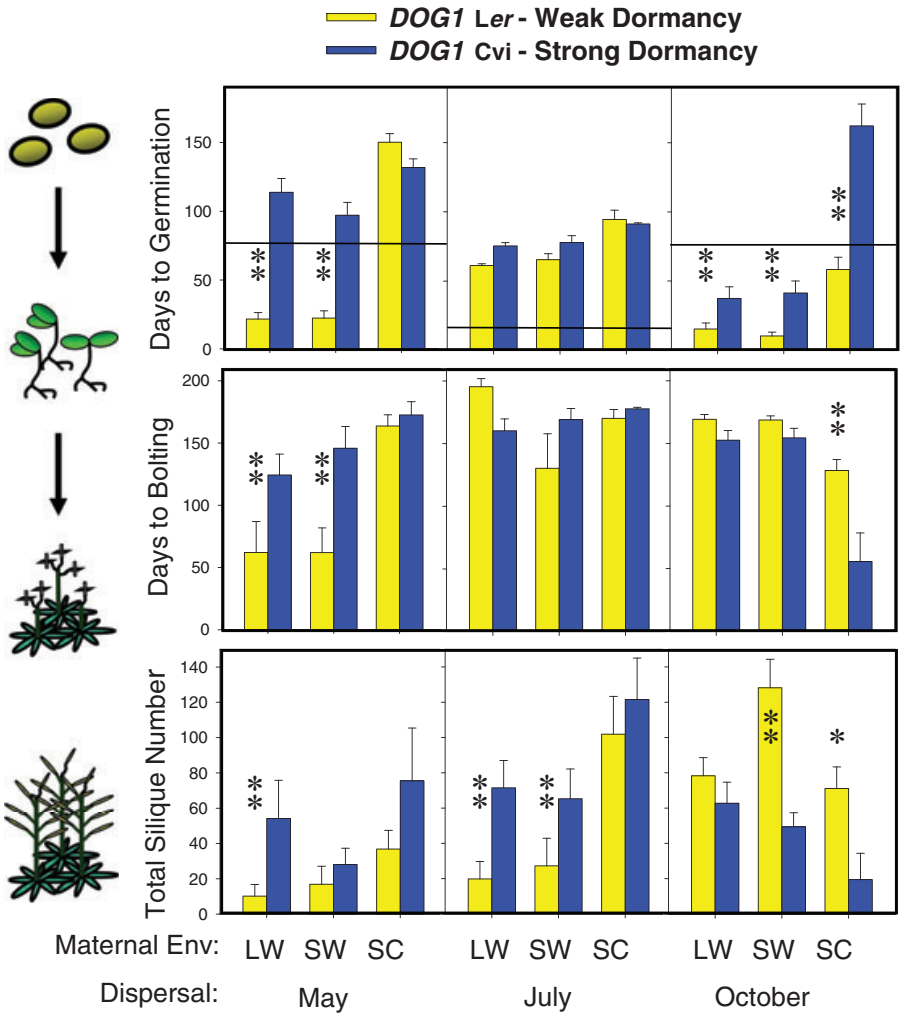

Figure 1. Effects of *DOG1* allelic differences on germination, bolting, and fitness. This illustration compares genetic lines containing $DOG1_{Cvi}$ alleles to those containing $DOG1_{Ler}$ alleles. "Days to germination" = days between dispersal and germination. "Days to bolting" = days between germination and bolting. LW = long warm, SW = short-warm, SC = short-cool. Dispersal cohorts = May, July, October. Horizontal black lines in the upper panels correspond to a date of August 1 for the May and July dispersal cohorts, and January 1 for the October dispersal cohort. $^{*}P < 0.05$, $^{**}P < 0.01$ for the $DOG1_{Cvi}$ effect based on Fisher's LSD test.

maternal treatment within each dispersal cohort and for interactions between genotype and dispersal season within each maternal treatment.

Results and Discussion

THE EARLY DEVELOPMENTAL GENE *DOG1* INFLUENCED MAJOR LIFE-CYCLE TRANSITIONS AND FITNESS

Based on laboratory phenotypes, we predicted that dormancy and flowering-time loci combined would determine life history. Weak dormancy ($DOG1_{Ler}$) plus early flowering ($CRY2_{Cvi}$) alleles were predicted to have the shortest life cycle, whereas strongly dormant ($DOG1_{Cvi}$) plus late flowering ($FLC_{Sf-2} + FRI_{Sf-2}$) alleles should have the longest. In contrast to predictions, only *DOG1* significantly influenced the life cycle in the wild (Fig. 1, Table 1). Genotypes with the $DOG1_{Cvi}$ allele had delayed germination under some, but not all conditions (Fig. 1, Tables 1, S2), verifying that this naturally variable dormancy gene indeed affects germination time under natural seasonal conditions. $DOG1_{Cvi}$ had delayed germination because of its strong dormancy.

$DOG1_{Cvi}$ also altered flowering, because its increased dormancy altered the season of postgermination development, which in turn altered flowering time (Fig. 1, Tables 1, 2). Germination time was strongly associated with flowering time, with the strength and direction of the correlation depending on dispersal season (Fig. S1). Moreover, when germination timing was included in an analysis of covariance that included *DOG1*

Table 1. ANOVA of (A) germination, (B) days to bolting (duration between germination and bolting), (C) total silique production. *F*-ratios for the effect of genotype are given for specific contrasts. "May," "July," and "October" refer to the timing of seed dispersal. "LW," "SW," and "SC" refer to the seed-maturation treatments, as described in the text. All comparisons to L*er* test for significant effects of the introgressed alleles. Comparisons between single and double NILs tested for the effect of the second introgression while controlling for the effects of the first introgression. $N = 15$ for each genotype x treatment combination for "Germination" and "Total siliques." Sample sizes for flowering time ranged from 3 to 9 for May dispersal, 3 to 12 for July dispersal, and 3 to 14 for October dispersal; sample sizes for flowering time ranged from 3 to 14 for "LW", 3 to 14 for "SW", and 3 to 14 for "SC."

		May			July			October		
Comparison pairs		LW	SW	SC	LW	SW	SC	LW	SW	SC
(A) Germination										
WT-L*er*	NIL-FLC_{Cvi}	1.18	0.72	1.45	3.03	0.81	6.24*	0.87	1.50	0.15
WT-L*er*	NIL-FRI_{Sf2}+FLC_{Sf2}	0.02	1.24	1.63	2.91	1.14	0.76	4.27*	1.00	0.12
WT-L*er*	NIL-$CRY2_{Cvi}$	3.16	0.73	0.07	0.14	1.83	0.27	2.44	2.15	0.16
WT-L*er*	NIL-$DOG1_{Cvi}$	40.23**	28.74**	1.96	5.88*	4.22	0.40	10.07**	22.31**	4.62
WT-L*er*	NIL-$DOG1_{Cvi}$+FRI_{Sf2}+FLC_{Sf2}	15.31**	143.30**	3.56	2.14	9.92**	0.84	4.06	30.65**	9.26**
WT-L*er*	NIL-$DOG1_{Cvi}$+$CRY2_{Cvi}$	7.15*	4.27*	0.21	4.99*	21.16**	0.35	11.01**	14.09**	9.26**
NIL-$DOG1_{Cvi}$	NIL-$DOG1_{Cvi}$+FRI_{Sf2}+FLC_{Sf2}	2.10	3.03	0.34	0.58	0.23	1.05	2.14	0.14	0.67
NIL-FRI_{Sf2}+FLC_{Sf2}	NIL-$DOG1_{Cvi}$+FRI_{Sf2}+FLC_{Sf2}	18.99**	41.21**	0.53	8.81**	1.02	0.06	11.42**	30.65**	7.47*
NIL-$DOG1_{Cvi}$	NIL-$DOG1_{Cvi}$+$CRY2_{Cvi}$	5.92*	5.79*	1.83	0.00	0.18	0.43	0.12	2.17	0.67
NIL-$CRY2_{Cvi}$	NIL-$DOG1_{Cvi}$+$CRY2_{Cvi}$	25.22**	7.67*	0.70	7.19*	8.94**	0.00	18.36**	9.57**	9.95**
(B) Days to bolting										
WT-L*er*	NIL-FLC_{Cvi}	0.01	0.60	1.39	0.02	∼∼∼	1.71	1.07	1.28	1.33
WT-L*er*	NIL-FRI_{Sf2}+FLC_{Sf2}	0.60	1.47	1.44	∼∼∼∼	∼∼∼∼	0.04	1.13	1.33	0.27
WT-L*er*	NIL-$CRY2_{Cvi}$	1.80	0.44	0.14	3.22	2.31	0.99	1.60	3.15	0.11
WT-L*er*	NIL-$DOG1_{Cvi}$	0.78	0.45	0.03	1.78	4.48	1.44	3.20	2.39	2.22
WT-L*er*	NIL-$DOG1_{Cvi}$+FRI_{Sf2}+FLC_{Sf2}	0.19	604.20**	2.41	10.20*	98.79**	1.86	0.83	3.54	1.38
WT-L*er*	NIL-$DOG1_{Cvi}$+$CRY2_{Cvi}$	0.06	1.33	1.03	2.42	9.49*	1.06	2.39	0.45	7.76*
NIL-$DOG1_{Cvi}$	NIL-$DOG1_{Cvi}$+FRI_{Sf2}+FLC_{Sf2}	0.52	1.60	1.44	0.76	3.09	1.89	0.98	0.06	0.03
NIL-FRI_{Sf2}+FLC_{Sf2}	NIL-$DOG1_{Cvi}$+FRI_{Sf2}+FLC_{Sf2}	2.17	5.51*	5.58*	∼∼∼∼	∼∼∼∼	1.57	1.04	3.26	0.73
NIL-$DOG1_{Cvi}$	NIL-$DOG1_{Cvi}$+$CRY2_{Cvi}$	1.36	0.71	0.50	0.27	0.20	0.31	0.15	1.84	1.24
NIL-$CRY2_{Cvi}$	NIL-$DOG1_{Cvi}$+$CRY2_{Cvi}$	1.73	2.78	0.61	0.50	0.36	0.03	1.71	0.69	5.43*
(C) Total siliques produced										
WT-L*er*	NIL-FLC_{Cvi}	0.11	1.03	2.24	0.33	0.02	0.10	0.00	0.48	3.99
WT-L*er*	NIL-FRI_{Sf2}+FLC_{Sf2}	0.56	2.38	1.81	2.72	0.89	0.30	1.76	3.17	2.99
WT-L*er*	NIL-$CRY2_{Cvi}$	0.11	2.21	2.41	0.69	0.07	0.06	1.31	3.69	0.29
WT-L*er*	NIL-$DOG1_{Cvi}$	1.61	5.65*	2.66	2.97	1.05	1.64	3.60	14.14**	0.05
WT-L*er*	NIL-$DOG1_{Cvi}$+FRI_{Sf2}+FLC_{Sf2}	5.08*	4.83*	2.53	0.99	2.14	0.52	0.02	18.00**	4.50*
WT-L*er*	NIL-$DOG1_{Cvi}$+$CRY2_{Cvi}$	0.36	1.72	0.77	1.87	1.96	0.05	3.79	10.52**	1.73
NIL-$DOG1_{Cvi}$	NIL-$DOG1_{Cvi}$+FRI_{Sf2}+FLC_{Sf2}	3.28*	0.06	0.10	0.60	0.04	0.25	3.44	0.33	1.61
NIL-FRI_{Sf2}+FLC_{Sf2}	NIL-$DOG1_{Cvi}$+FRI_{Sf2}+FLC_{Sf2}	11.74**	0.22	0.02	6.73*	4.72*	3.72	2.70	0.46	16.08**
NIL-$DOG1_{Cvi}$	NIL-$DOG1_{Cvi}$+$CRY2_{Cvi}$	0.74	2.34	1.06	0.45	0.00	1.02	0.09	0.02	0.51
NIL-$CRY2_{Cvi}$	NIL-$DOG1_{Cvi}$+$CRY2_{Cvi}$	1.42	0.00	0.39	10.26**	4.04	0.18	0.29	1.32	1.73

*$P < 0.05$; **$P < 0.01$, ∼∼∼∼ = insufficient replicates. $P < 0.01$ is also significant after Bonferroni correction for analysis of multiple traits ($P = 0.017$ for three traits).

alleles, the effect of the *DOG1* allele was either no longer significant or the effect was largely reduced (Table 2), indicating that the effect of *DOG1* on flowering time was largely accounted for by its effect on germination. Therefore under natural conditions, *DOG1* is also a major flowering-time gene. By impacting both life stages, *DOG1* controlled the entire life cycle and consequently was the only locus to significantly influence fitness (Fig. 1, Tables 1, 2).

The only evidence for epistasis between the dormancy and flowering-time alleles for phenology was the interaction between *DOG1* and *CRY2* QTLs for effects on germination time. Under the warm seed-maturation conditions (LW and SW), the genetic line containing both $DOG1_{Cvi}$ and $CRY2_{Cvi}$ alleles had an intermediate germination timing that was significantly different from both the dormant $DOG1_{Cvi}$ line and the nondormant $CRY2_{Cvi}$ line (F (*DOG1* x *CRY2* vs. *DOG1*) = 5.923, $P = 0.022$; F (*DOG1* x *CRY2*

Table 2. **(A) Effect of *DOG1* alleles on flowering time (duration from germination to bolting). (B) Effect of *DOG1* alleles on fitness (silique number). The effect of *DOG1* alleles on flowering time and fitness is mediated largely through germination timing. *F*-ratios are given for the effect of *DOG1* alleles in ANOVA without germination as a covariate (*DOG1*), when germination time is used as a covariate in the ANOVA model (+Germ), or when flowering time is used as a covariate in the ANOVA model ("+Flower" in the lower table). If the effect of *DOG1* on flowering or fitness were no longer significant when germination was included in the model, this was evidence that *DOG1* influences flowering or fitness through germination.**

(A)

	May		July		October	
	DOG1	+Germ	*DOG1*	+Germ	*DOG1*	+Germ
LW	3.682	0.220	2.788	12.390**	3.197	19.500**
SW	10.23**	6.200*	3.446	0.550	3.041	62.400**
SC	0.213	1.691	1.488	1.400	5.715*	3.500

(B)

	May			July			October		
	DOG1	+Germ	+Flower	*DOG1*	+Germ	+Flower	*DOG1*	+Germ	+Flower
LW	9.004**	0.091	0.400	14.600**	0.300	0.840	0.749	0.015	3.626
SW	2.696	1.940	0.697	7.962**	4.249*	2.760	14.200**	5.346*	24.630**
SC	0.791	1.083	1.094	1.986	1.769	2.256	7.261**	1.746	1.272

***$P < 0.05$; **$P < 0.01$.**

vs. $CRY2 = 25.22$, $P < 0.001$), such that effects of $CRY2_{Cvi}$ were only apparent on the $DOG1_{Cvi}$ background. However, the *CRY2* introgression from Cvi is very close to a putative *DOG2* locus that was indicated to have an opposite effect of *DOG1* (Alonso-Blanco et al. 2003), therefore introducing the possibility that this epistatic germination behavior was caused by *DOG2*.

MAJOR FLOWERING-TIME GENES HAD NONSIGNIFICANT EFFECTS ON LIFE HISTORY IN THE FIELD

Natural genetic variation in flowering time in *A. thaliana* is hypothesized to contribute to locally adaptive life histories across its natural range. However, little is known about how this genetic variation is manifested in association with variation in other developmental pathways under natural conditions. Under our experimental scenarios that manipulated seasonal environments and genetic background, major natural flowering-time alleles surprisingly did not significantly influence flowering time (Table 1), although sample sizes for flowering time were usually smaller than for germination timing (but they were comparable in some treatments and genotypes). In fact, the most significant effect that our natural flowering-time alleles had on life-history traits was on germination. The high *FLC*-expressing line promoted by functional *FRI* alleles, ($FLC_{Sf-2} + FRI_{Sf-2}$), germinated earlier than the L*er* background in the autumn dispersal cohort under the LW maternal condition [mean days to germination (SD): $FLC_{Sf-2} + FRI_{Sf-2} = 8.0$ (0.73); L*er* = 10.7 (1.07); Table 1], indicating a possible role of this flowering-time gene on seasonal germination timing. The observation that high *FLC* expression influenced germination when seeds were dispersed under cool conditions of late autumn is consistent with a previous study documenting that high *FLC* expression promoted germination particularly at low temperature, as in autumn (Chiang et al. 2009). In addition, we detected no significant main-effect influence of any of the naturally variable flowering-time genes on silique number under any conditions. This lack of effect of flowering-time alleles on silique number cannot be attributed to reduced sample sizes because plants with zero fitness were also used in the analysis. However for the May dispersal cohort LW seeds, high *FLC* expression of the ($FLC_{Sf-2} + FRI_{Sf-2}$) genotype increased fitness when in the $DOG1_{Cvi}$ background but not the L*er* background (Table 1 and Fig. S2). Thus epistasis for fitness was apparent, such that selection on *FLC* or *FRI* depended on the allele at the *DOG1* locus, although the mechanism for this is unknown. In summary, the effect of a single early-developmental dormancy gene was magnified in the field to impact the subsequent transition to flowering, while masking the effect of major flowering-time genes that have pronounced effects under laboratory conditions. This dormancy locus also altered natural selection on flowering-time alleles.

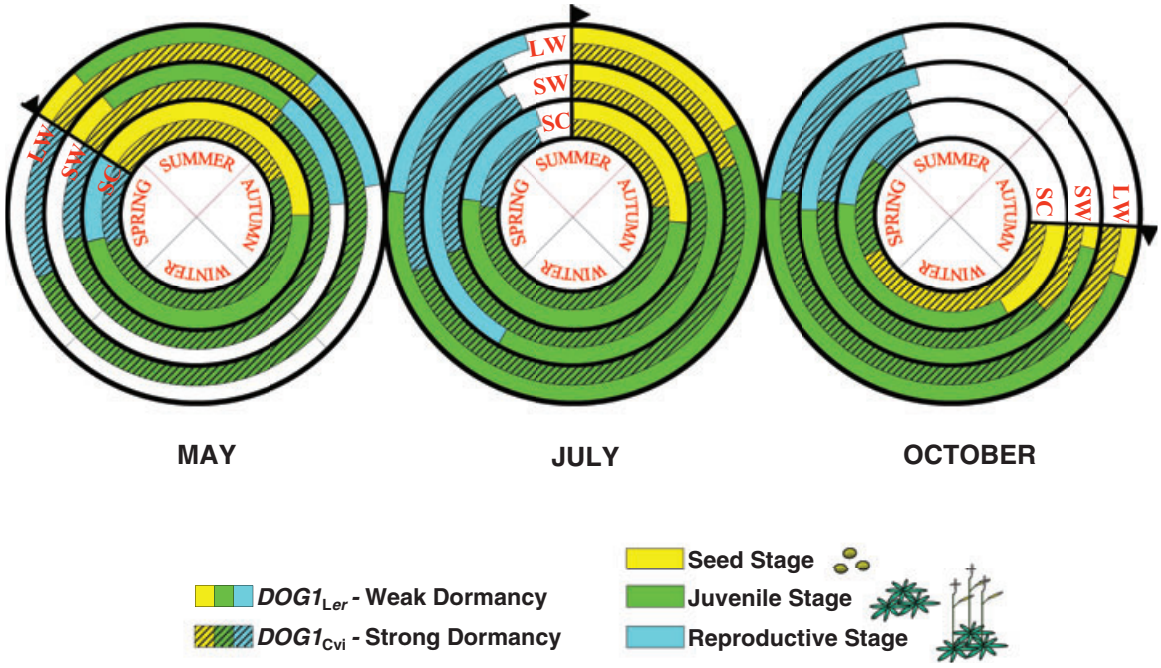

Figure 2. Summary of the expression of life-cycle stages of *DOG1* genotypes throughout the seasons, based on Julian days. The year was divided into Spring (March, April, May), Summer (June, July, August), Autumn (September, October, November), and Winter (December, January, February). Maternal environments: LW = long day-warm, SW = short day-warm, SC = short day-cool. Dispersal cohorts = May, July, October. The life cycle begins with dispersal and ends with death. For May dispersal, those with spring reproduction continued until early summer. The winter-annual life history is prevalent in *Arabidopsis thaliana*, with the life cycle starting with seeds germinating in the autumn and rosettes overwintering before completing reproduction in the early spring (Baskin and Baskin 1983). A spring-annual germinates in the spring and completes its life cycle in the early summer. Autumn annuals have also been observed, and the entire life cycle is completed in the autumn. Genotypes that can express both spring-annual and autumn- or winter-annual life cycles are considered rapid cycling. $DOG1_{Cvi}$ maintained winter-annual life histories in the May dispersal cohort under LW and SW, but it induced spring annuals in the October dispersal cohort under SC maturation conditions.

DOG1 EFFECTS ON LIFE HISTORY DEPENDED ON FLOWERING TIME

Although *DOG1* was the only gene we examined that influenced life cycles in the wild, our experimental manipulation of maternal flowering season altered *DOG1* effects on germination, life history, and natural selection on *DOG1* alleles (Table S2). It did so through effects of seed-maturation conditions and dispersal season combined. For example, hot conditions during the July dispersal inhibited germination even of nondormant seeds until after summer, but it also permitted after-ripening and relieved dormancy, thereby minimizing *DOG1* effects on germination and the life cycle in that dispersal cohort. In contrast, seeds dispersed in May required strong dormancy from *DOG1* to maintain the predominant winter-annual life history in which individuals germinate in the autumn and flower the next spring (Figs. 1 and 2), and *DOG1* allelic effects were pronounced in that dispersal cohort.

Moreover, while seed-maturation photoperiod had no effect, cool seed-maturation temperature delayed germination. This cool-induced dormancy resulted in complete masking of the $DOG1_{Cvi}$ effect on germination and life cycles in the May dispersal cohort. In contrast, in the October cohort, delayed germination of $DOG1_{Cvi}$ was so pronounced under cool seed-maturation conditions that it caused a shift from a winter-annual to spring-annual life history (Fig. 2). *DOG1* therefore induced a basic life-history change in plants growing in the wild.

MATERNAL EFFECTS IMPOSED BALANCING SELECTION ON *DOG1* ALLELES

DOG1 was the only locus examined that significantly altered total lifetime fitness. However, manipulation of flowering season altered natural selection on *DOG1* alleles, as the strength and even direction of natural selection on *DOG1* alleles depended on seed-maturation temperature and the season of seed dispersal (Table S2). In spring dispersal cohorts, the delayed germination of $DOG1_{Cvi}$ induced higher fitness (in warm-matured seeds), but in the October dispersal cohort $DOG1_{Cvi}$ had lower fitness. Therefore, variation in flowering time imposed balancing selection on *DOG1*.

This variable selection on *DOG1* alleles can be explained by the observation that spring germination was associated with

low fitness compared to autumn germination. If seeds are dispersed in spring, the induction of dormancy by $DOG1_{Cvi}$ or by cool seed-maturation temperature would be adaptive, because it would delay germination until autumn (Figs. 1 and 2). In October-dispersed seeds, in contrast, dormancy induction by $DOG1_{Cvi}$ or by cool seed-maturation temperature would prevent germination in the autumn and delay germination until spring, causing lower fitness. Thus, the adaptive value of *DOG1* alleles and of maternal temperature effects on dormancy both depend on the season of seed dispersal.

$DOG1_{Cvi}$ did not significantly influence germination or flowering in the July dispersal cohort (Table 1, Figs. 1 and 2). Hot temperatures immediately after dispersal apparently masked *DOG1* allelic differences. However, $DOG1_{Cvi}$ had somewhat (but not significantly) delayed germination and also had higher fitness. It is possible that this two-week germination delay allowed the germinants to avoid the warm, dry period of early autumn (Fig. S3), leading to a higher juvenile survival rate (Survival%: with $DOG1_{Cvi}$: 51%, without $DOG1_{Cvi}$: 13%; $P < 0.01$. No significant difference in silique number was detected for those that survived to bolting between the groups with or without $DOG1_{Cvi}$).

In summary, certain seed-maturation and postdispersal conditions masked allelic differences at *DOG1*. Other combinations of environmental conditions caused delayed germination to increase fitness, favoring $DOG1_{Cvi}$, while still other combinations caused delayed germination to lower fitness, disfavoring $DOG1_{Cvi}$. Therefore, variation in flowering time imposed balancing selection on *DOG1*.

Summary and Conclusions

In natural seasonal environments, the effect of the dormancy gene *DOG1* was magnified to the point that it dominated the entire life cycle as well as altered fitness. The pronounced effect of *DOG1* on overall life history is caused by the ramifying effects of early life stages on subsequent life stages through their interaction with seasonal environmental conditions. Genes that regulate the timing of prior developmental transitions determine seasonal conditions experienced subsequently, which in turn alter the expression of subsequent life stages. In this case, $DOG1_{Cvi}$ delayed the germination of spring-dispersed seeds in the autumn, which slowed growth and delayed flowering. In autumn-dispersed seeds, the delay of germination until springtime caused greatly accelerated flowering of spring germinants, as they were induced to flower by the long days of spring, soon after germination, very early in development (Figs. 1 and 2), resulting in a total change of life history from winter annual to spring annual. In this manner, genes acting early in development can have pleiotropic effects on later life stages under natural seasonal variation.

Likewise, flowering time of maternal plants influenced germination in the next generation and influenced the expression of genetic variation for germination. Specifically, hot postdispersal temperatures experienced by the July dispersal cohort delayed germination of all seeds until autumn (and broke dormancy of cool-matured seeds, promoting autumn germination). Cool seed-maturation temperature, moreover, induced dormancy and thereby delayed germination, thereby having effects similar to those of the $DOG1_{Cvi}$ allele. Interestingly, *A. thaliana* from warmer, southern climates tend to be more dormant and to have higher *DOG1* expression during seed development than those from colder, northern climates (Chiang et al. 2011). However, those northern ecotypes can obtain higher *DOG1* expression and be induced into dormancy when seeds are matured under cooler temperatures (Chiang et al. 2011; Kendall et al. 2011). This suggests that cool spring temperatures during seed maturation in northern locations could eliminate the requirement for strong *DOG1* alleles. Evidence for geographically variable natural selection on *DOG1* was recently reported based on *F*st/*Q*st comparisons, implicating *DOG1* in local adaptation (Kronholm et al. 2012). Results of the study presented here indicate that geographically variable natural selection on *DOG1* can occur because of this maternal temperature effect on dormancy, mediated by flowering time. It is therefore possible that genetically based variation in flowering time could also influence germination and natural selection on germination loci, even imposing balancing selection on them.

For flowering loci to pleiotropically influence germination in this manner, however, flowering-time genes must influence flowering time under natural conditions. In this study, they did not. The inability of flowering loci to significantly influence flowering time, as observed here, is not unprecedented. A study by Wilczek et al. (2009) showed that the expression of allelic differences between major flowering loci, such as *FRI*, depended acutely on the season of germination. Their simulation indicated that genotypic differences were detectable only when seeds were sown (analogous to germinating) in a very narrow window during the autumn. Under certain conditions allelic differences may be manifest in the field, but multigenerational experiments would be necessary to test this. The only effect of a flowering-time gene that we detected in the present study was on germination. Indeed flowering-time genes, such as *FLC*, pleiotropically regulate environment-dependent seed germination (Chiang et al. 2009). Furthermore, *FLC* and *DOG1* expression exhibits cycling patterns that are linked to dormancy cycling in buried seeds, suggesting a role for *FLC* in regulating germination (Footitt et al. 2011). Despite the large effect of *FLC* on flowering under laboratory conditions, selection on *FLC* variation in nature might be more dependent on its influence on germination than on flowering.

Pleiotropy—the effect of one gene on multiple traits—is among the most important factors influencing evolutionary rates and outcomes (Wagner et al. 2008), but it is likely to be greatly underestimated in the laboratory. In the wild, developmental-timing genes have cascading pleiotropic effects on other phenotypes because they determine the seasonal conditions of subsequent development, which in turn influences the expression of plastic phenotypes. This general mechanism of pleiotropy would apply to any organism developing in the wild. In this case, just one gene acting early in development altered the entire life cycle of this annual plant. This dynamic also operates across generations, such that maternal developmental timing has cascading effects on their progeny, even inducing balancing selection. The temporal sequence of development under natural conditions can fundamentally alter allelic effects of developmental genes and patterns of natural selection on life-history loci.

ACKNOWLEDGMENTS

We thank M. Koornneef, L Bentsink, and R. M. Amasino for supplying seeds, S. Gunner for assistance, F. M. Rosin for comments, and J. Schmitt for discussion. This work was supported by National Science Foundation grants DEB-0807973 to GCKC and KD, IOS-0844280 to KD.

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Associate Editor: K. Bomblies

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Genotypes used, with the constitution of natural variants of germination and flowering-time alleles and their corresponding phenotypes under laboratory conditions.

Table S2. Results of analysis of variance that test for interactions between alleles and seed-maturation conditions (maternal) or dispersal seasons (dispersal).

Figure S1. Relationship between germination timing and flowering time (days between germination and flowering) for the three dispersal cohorts.

Figure S2. Epistasis for fitness.

Figure S3. Recorded temperature and precipitation by NOAA (www.noaa.gov) from the Bedford, MA Station spanning the whole duration of the experiment.