MOLECULAR ECOLOGY

Molecular Ecology (2011) **20**, 3336–3349 doi: 10.1111/j.1365-294X.2011.05181.x

DOG1 expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in *Arabidopsis thaliana*

GEORGE C. K. CHIANG,*†** MELANIE BARTSCH,† DEEPAK BARUA,‡ KAZUMI NAKABAYASHI,† MARILYNE DEBIEU,† ILKKA KRONHOLM,† MAARTEN KOORNNEEF,†§ WIM J. J. SOPPE,† KATHLEEN DONOHUE¶ and JULIETTE DE MEAUX†

*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA, †Department of Plant Breeding and Genetics, Max Planck Institute for Plant Breeding Research, Carl-von-Linne Weg 10, 50829 Cologne, Germany, ‡Indian Institute of Science Education and Research, Pune, Maharashtra 411021, India, §Laboratory of Genetics, Wageningen University, Arboretumlaan 4, 6703 BD, Wageningen, The Netherlands, ¶Department of Biology, Duke University, Box 90338, Durham, NC 27708, USA

Abstract

Seasonal germination timing of *Arabidopsis thaliana* strongly influences overall life history expression and is the target of intense natural selection. This seasonal germination timing depends strongly on the interaction between genetics and seasonal environments both before and after seed dispersal. *DELAY OF GERMINATION 1* (*DOG1*) is the first gene that has been identified to be associated with natural variation in primary dormancy in *A. thaliana*. Here, we report interaccession variation in *DOG1* expression and document that *DOG1* expression is associated with seed-maturation temperature effects on germination; *DOG1* expression increased when seeds were matured at low temperature, and this increased expression was associated with increased dormancy of those seeds. Variation in *DOG1* expression suggests a geographical structure such that southern accessions, which are more dormant, tend to initiate *DOG1* expression earlier during seed maturation and achieved higher expression levels at the end of silique development than did northern accessions. Although elimination of the synthesis of phytohormone abscisic acid (ABA) results in the elimination of maternal temperature effects on dormancy, *DOG1* expression predicted dormancy better than expression of genes involved in ABA metabolism.

Keywords: abscisic acid, dormancy, germination, natural variation, trans-generational plasticity

Received 30 January 2011; revision received 10 May 2011; accepted 1 June 2011

Introduction

As sessile organisms, plants cannot actively determine the location of their establishment, but they can choose the environment they experience by controlling their seasonal life history. In this regard, seasonal life history expression is crucial to the survival and fitness of the plants because they need to avoid harsh conditions and reproduce when the environment is conducive to establishment and growth. Two developmental decisions are critical determinants of overall life history in many annual plants: germination and flowering (Effmertova 1967). The mechanisms of these developmental switches are complex and environmental sensitive, and could be coordinately regulated, at least in part, through shared genetic pathways (Chiang *et al.* 2009). Seasonal germination timing is particularly essential for determining overall life history of the individual because it is capable of influencing subsequent seasonal life history expression (Evans & Cabin 1995; Andalo *et al.* 1999; Galloway 2002; Donohue *et al.* 2005c) and more specifically the timing of

Correspondence: Wim Soppe, Fax: 49-221-5062-470; Kathleen Donohue, Fax: 919-660-7293; Juliette de Meaux, Fax: 49-251-8324-668; E-mail: soppe@mpipz.mpg.de (W. Soppe), k.donohue@duke.edu (K. Donohue), juliette.de.meaux@uni-muenster.de (J. de Meaux)

**Present address: Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA 90024, USA

reproduction (Schmitt 1995; Donohue 2002; Korves *et al.* 2007; Wilczek *et al.* 2009). In *Arabidopsis thaliana*, germination timing has been shown to be under intense and geographically variable natural selection (Donohue *et al.* 2005b; Huang *et al.* 2010). Germination timing is also notoriously plastic in response to seasonal environmental factors that vary geographically (Biere 1991; Munir *et al.* 2001; Griffith *et al.* 2004; Donohue *et al.* 2005a,b).

One aspect of the seasonal life history of a plant that is often ignored and rarely examined is that the timing of reproduction, or flowering time, can influence germination timing because of the seasonal environments the plants experience during seed maturation (Donohue 2009). Environmental signals during seed-maturation stage, such as daily photoperiod and temperature, can manipulate the dormancy levels of the seeds at dispersal and precondition the environmental requirements needed to break that dormancy (Donohue *et al.* 2005c, 2007, 2008). How seasonal factors experienced during seed maturation influence dormancy and interact with postdispersal seasonal cues to determine germination timing is poorly characterized in any system, and the genetic basis of this germination plasticity is virtually unknown.

Seasonal germination timing is also strongly influenced by natural variation in the genetic basis of seed dormancy. The only cloned (Bentsink *et al.* 2006) naturally variable dormancy gene is *DELAY OF GERMINATION 1* (*DOG1*), which was first identified through QTL analysis as having the largest role in creating the dormancy difference in *A. thaliana* between the extremely dormant Cape Verde Island (Cvi) and weakly dormant Landsberg (L*er*) accessions (Alonso-Blanco *et al.* 2003). Further studies revealed *DOG1* as the major dormancy QTL among natural accessions (Bentsink *et al.* 2010). Another study revealed that a major QTL for dormancy and for field germination phenology itself co-located with *DOG1*, strongly suggesting that *DOG1*-mediated dormancy is a major determinant of germination phenology in the field (Huang *et al.* 2010). This QTL was under very strong natural selection, with the frequency of the marker allele associated with increased dormancy changing from below 50% to over 80% within a single generation, further suggesting that *DOG1* dormancy alleles were strong determinants of fitness in this experimental field population. *DOG1* encodes a protein with unknown molecular function. *DOG1* is specifically expressed in seeds, and it exhibits the highest level of expression during mid to late stages of silique development and seed maturation (Bentsink *et al.* 2006). In comparison with the *DOG1* expression in the Cvi accession, *DOG1* expression in the weakly dormant L*er* genotype is much lower. It would be of great interest to evaluate the ecological importance of genetic variation in *DOG1* and its interaction with seed-maturation environments to control the earliest life history transition.

To understand the importance of natural variation and environmental influence on dormancy, we must first identify the molecular framework of dormancy pathways. The physiological pathways of dormancy induction and breakage are somewhat characterized, even if their interactions with extrinsic environmental factors are not. Two plant hormones are of major importance: abscisic acid (ABA) induces and maintains dormancy, and gibberellins (GA) stimulate germination (Koornneef *et al.* 2002; Finkelstein *et al.* 2008; Holdsworth *et al.* 2008a,b). Endogenous levels of these two hormones largely determine whether a seed will germinate. Within the ABA signalling network, *NCED9* is an ABA biosynthesis gene with a seed-specific expression pattern during silique development, and its expression induces seed dormancy (Lefebvre *et al.* 2006). In contrast, *CYP707A1* encodes a catabolic enzyme important for reducing ABA accumulation during seed maturation and functions to properly control dormancy levels (Okamoto *et al.* 2006). It also has a seed-specific expression that peaks during seed maturation (Okamoto *et al.* 2006). The level of ABA in seeds, which can be regulated by both *NCED9* and *CYP707A1*, influences the degree of endogenous dormancy upon seed maturation (Koornneef *et al.* 1982). It has also been documented that *DOG1* requires ABA to induce dormancy (Bentsink *et al.* 2006), and *DOG1* is induced by the ABA-mediated sugar-signalling hormonal pathways (Teng *et al.* 2008).

To understand the potential adaptive importance of *DOG1*, we focused on *DOG1* expression plasticity in response to environmental variation during seed maturation and proceeded in four steps. First, we analysed a near-isogenic line differing in the region encoding *DOG1* to reveal the role played by this locus in the plastic response to seed-maturation environment. Second, we analysed *DOG1* expression variation under three distinct seed-maturation environments across a panel of 12 genotypes collected in 12 locations covering a broad environmental range of the species. We revealed extensive genetic variation. Third, we examined whether genetic variation for *DOG1* expression correlated with the actual germination behaviour of seeds matured in different environments. We found that *DOG1* expression level is a good indicator of the extent of dormancy imposed by seed-maturation environment. Eventually, we examined whether *DOG1* expression polymorphism has an improved predictive power compared with genes controlling ABA levels, a hormone known to be essential for delayed seed germination. Our work highlights the potential use of *DOG1* expression level as a diagnostic marker for environmental effects at the time of seed maturation.

Materials and methods

The use of a near-isogenic line to examine germination effects of natural DOG1 allele substitutions

To test whether different natural alleles of *DOG1* exhibit different germination responses to seed-maturation conditions, a near isogenic line (NIL) of *Arabidopsis thaliana* containing a naturally highly expressed *DOG1* allele from the Cape Verde Island (Cvi) accession introgressed in the Landsberg *erecta* (L*er*) background (NIL-$DOG1_{Cvi}$) was grown with its two parental lines under three seed-maturation environments that differed in daily photoperiod and temperature. This NIL was constructed by multiple backcrosses to L*er* and contains approximately 10 cM of Cvi introgression fragment centring around the *DOG1* locus. It is described in detail in Alonso-Blanco *et al.* 2003. The first seed-maturation treatment, 'long-day, warm' (LW), imposed a 14-h light/10-h dark cycle of full-spectrum light at 22 °C and resembles seasonal conditions during a typical seed-maturation season in late spring and early summer. The second treatment, 'short-day, warm' (SW), imposed a 10-h light/14-h dark cycle at 22 °C. The third treatment, 'short-day, cold' (SC), imposed a 10-h light/14-h dark cycle at 10 °C and resembles the seasonal conditions during seed maturation when plants mature seed in the autumn or very early spring. The LW and SC conditions allow us to examine the seasonal environments most likely will be experienced by *A. thaliana* populations during the reproductive stages for the two dominant life history groups – winter annuals and summer annuals. Comparing the LW with SW treatment reveals the effect of the photoperiod during seed maturation. Comparing the SW with SC reveals the effect of seed-maturation temperature. In each treatment, plants were grown in four different chambers (Conviron E7/2 growth chambers; Controlled Environment Ltd, Winnipeg, Manitoba, Canada) as four biologically independent replicates, and in each replicate compartment, there were four plants of each genotype.

The seeds freshly harvested from those maternal plants were used to assess germination frequency. Germination assays were conducted using 20 seeds of a given genotype in a single Petri plate (50 × 9 mm) containing 0.5% agar dissolved in distilled water. Sixteen Petri plates, equally distributed over four biological replicates, were used for each genotype in each seed-maturation treatment (320 seeds per genotype). Seeds imbibed in the agar plates were first placed in the complete dark at 10 °C for 5 days before moving to light. During germination, seeds were given a 12-h photoperiod of white florescent light with a photon flux density of approximately 90 μmol/m^2/s in Percival germination incubators (Percival Scientific Inc, Perry, IA, USA). Plates were scored for germination at 10 days after being transferred to light.

For germination experiments, we estimated germination proportion in each Petri plate as the total number of germinants after 10 days in the light for each treatment, divided by the total number of viable seeds. We conducted a series of ANOVAs using both SAS (SAS Institute Inc, Cary, NC, USA) and STATISTICA (StatSoft Inc, Tulsa, OK, USA) programs to test for the effects of genotype, seed-maturation condition and the interaction.

Variation of germination in natural accessions

To examine the germination response to seed-maturation conditions of natural accessions originating from different locations, 12 accessions of *A. thaliana* (L.) Heynh (Brassicaceae) collected from Eurasia and Africa, *A. thaliana*'s native range (Table 1), were grown under the three seed-maturation conditions, and their *DOG1* expression was measured in developing siliques at two time points. Experimental procedures followed those of the NIL experiment unless otherwise noted.

The 12 accessions were divided into northern and southern groups based on their latitudinal origins. All the southern accessions experienced much warmer winters (and drier climate Fig. S1, Supporting information) with an average January temperature above 4 °C in their native habitats (Fig. 1), while all northern accessions had an average January temperature below 4 °C. Seeds of the accessions were obtained through The Arabidopsis Information Resource. Planting was staggered to facilitate synchronous seed maturation across all accessions. All lines were grown at 22 °C and 12 h of light in the beginning of the rosette stages before they were moved to 5 °C for a 3-week vernalization period. After vernalization, all plants were moved to three seed-maturation environmental treatments in Conviron E7/2 growth chambers. Within each seed-maturation treatment, two plants of each accession were grown in one chamber, and three individual chambers were used to represent three biological replicates.

Germination assays were conducted using 12 seeds of a given accession in a single Petri plate. And 12 Petri plates, equally distributed over three biological replicates, were used for each accession in each seed-maturation and germination treatment (144 seeds per genotype per treatment). Fresh seeds of the 12 accessions from the three seed-maturation conditions were exposed to four germination conditions that roughly approximate natural seasonal changes in germination temperature. A constant 10 °C (10–10 °C: 7-days dark, 14-days 12-h light) germination temperature resembles

Table 1 Geographical distribution of 12 accessions (or ecotypes), divided into two geographical groups based on their latitudes of origin

Geography	Ecotype	Code	TAIR No	Region	Country
North	Lov-5	Lv	N22575	Lovvik	Sweden
North	Bil-7	Bi	N22579	Billaberget	Sweden
North	Edi-0	Ed	N1122	Edinburgh	UK
North	Ull2-3	Ul	N22587	Ullstorp	Sweden
North	Tamm-27	Tm	N22605	Tammisari	Finland
North	Ge-0	Ge	N1186	Geneva	Switzerland
South	Fei-0	Fe	N22645	St. Maria D. Feiria	Portugal
South	Sf-2	SF	N1516	San Feliu	Spain
South	Pro-0	Pr	N22649	Proaza, Asturios	Spain
South	Mir-0	Mi	N1378	Miramare/Trieste	Italy
South	Cvi-0	Cv	N902	Cape Verdi Islands	Cape Verde
South	Ct-1	Ct	N1094	Catania	Italy

TAIR, The Arabidopsis Information Resource.

Fig. 1 Geographical and climatic basis for the divide between the northern and southern groups. Accessions of *Arabidopsis thaliana* were divided into northern and southern groups based on the latitudes of their original collection sites. The green dotted line represents the January average temperature of 4 °C (<4 °C for north and east of green dotted line), while the solid black line indicates the latitudinal divide.

the average temperature of the early spring and autumn, while a constant 22 °C (22–22 °C: 7-days dark, 14-days 12-h light) germination temperature resembles that of the late spring and early summer. Changes in germination conditions from 10 to 22 °C (10–22 °C: 7-days dark at 10 °C, 14-days 12-h light at 22 °C) and from 10 to 28 °C (10–28 °C: 7-days dark at 10 °C, 14-days 12-h light at 28 °C) mimic the temperature transitions from late winter or early spring to late spring and mid summer, respectively.

Examination of DOG1 expression variation

To examine interaccession and geographical variation in *DOG1* expression and the influence of seed-maturation conditions, quantitative gene expression experiments were conducted. About 8–14 siliques were collected for each biological replicate of each accession × seed-maturation treatment. We examined two silique developmental stages for *DOG1* expression. Because of the difference in the speed of silique growth among accessions and among seed-maturation environments, the siliques were harvested according to the developmental physiological state of the siliques instead of according to the arbitrary number of days after flowering. The first time point (early stage) was the mid seed-maturation stage when the siliques were first fully extended (Fig. 2). The second time point (late stage) was the late seed-maturation period when the siliques were beginning to turn yellow prior to drying out. Each sample was preserved in Ambion RNAlater solution frozen at −30 °C and was taken out of RNAlater immediately before the start of the RNA extraction process. RNA extractions were performed according to Vicient & Delseny (1999), with the small modification that the phenol, phenol/chloroform and chloroform extractions were performed only once. The dissolved RNA subsequently went through at least one run of high salt and LiCl purification and then treated with DNAse I. cDNA was synthesized using Invitrogen SuperScript® II First Strand Synthesis kit with 3000 ng of total RNA from each sample. Quantitative PCR was performed using the Eppendorf SYBR Green PCR kit and run on the Mastercycler ep realplex (Eppendorf) according to the manufacturer's instructions. At least eight commonly used reference genes were examined for their stability and suitability for this study (Schwab 2008). Ultimately, *ACTIN8* (*ACT8*) was used as an internal standard to

Fig. 2 Illustration of the two silique collection stages. The plants from which these siliques were taken were grown in an Elbanton growth chamber, 22 °C, 16 h light, 70% RH. Under this constant condition, L*er* siliques completed seed maturation around 19 days after pollination (DAP) or flower opening. Because of the difference in the speed of silique growth among accessions and among seed-maturation environments, the experimental siliques were harvested according to the developmental stage of the siliques instead of according to the arbitrary number of days after flowering. The first time point of collection (early stage) was the mid seed-maturation stage when the siliques were just fully extended, similar to the stage of 10–11 DAP shown previously. The second time point (late stage) was the late seed-maturation period when the siliques were beginning to turn yellow prior to drying out, similar to the stage of 17 DAP shown previously.

normalize the data, and a second reference gene *APC2* was used to re-confirm the results (Fig. S2, Supporting information). These two reference genes were among the most stable and accurate ones during seed maturation (Schwab 2008).

We used the following primers: *ACTIN8*, forward 5′-CTCAGGTATTGCAGACCGTATGAG-3′ and reverse 5′-CTGGACCTGCTTCATCATACTCTG-3′; *APC2*, forward 5′-GAAACATCAATTGCCTCTGTGGAAGA-3′ and reverse 5′-AAGGATCAGCCACACAAAACATCTTG-3′; *DOG1*-overall-expression, forward 5′-TAGGCTCGTTTATGCTTTGTGTGG-3′ and reverse 5′-CGCACTTAAGTCGCTAAGTGATGC-3′. The *DOG1*-overall-expression primers amplified all of the alternative splicing types (Bentsink *et al*. 2006). The cDNA samples of each accession from the same growth chambers (constituting one biological replicate) of the same developmental stage were randomized in one 96-well plate with two technical replicates, and the mean expression of that one biological replicate was derived from averaging the two technical replicates. All of the samples of the same biological replicate (overall three biological replicates) were standardized against the same 4-point serial dilution standard curve amplified with the same reaction mix in the 96-well plate. Overall, there were 12 accessions × 3 maternal treatments × 3 biological replicates × 2 technical replicates.

To assess natural variation in gene expression, expression ratios of *DOG1* over *ACT8* were used. The three normalized biological replicates were averaged to give an overall expression value of the accession for the specific treatment and developmental stage. The plasticity of the gene expression to the seed-maturation environment was defined as the proportional directional change of the germination or expression value with either an increase of 4 h of daily photoperiod (plasticity to photoperiod (LW-SW)/SW) or a decrease of 10 °C (plasticity to temperature (SC-SW)/SW) of the seed-maturation environment. When comparing specifically among the accessions under the same treatment and developmental stages, biological replicates of each accession were used as the statistical units. Otherwise, the overall values averaged from three biological replicates were used as the statistical units. Series of ANOVAs were used to test for the interactions among expression, germination, developmental stages and the environments. Correlation matrices and multiple regression analysis were used to test for associations between gene expression and germination.

Comparative analysis between DOG1 and dormancy-controlling ABA genes

To test whether ABA is essential for the dormancy plasticity imposed by seed-maturation environments, ABA-deficient *aba1*-1 mutants in both the L*er* and Col background, acquired from ABRC, were gown in SW and SC together with their wild-type lines. In each seed-maturation treatment, two plants of each genotype were grown in a chamber, and three individual chambers were used as three biologically independent replicates. Fresh seeds of each genotype × seed-maturation treatment were germinated on nine Petri plates, with each one containing 15 seeds (135 seeds per genotype × seed maturation). ANOVAs were used to test for effects of genotype, seed-maturation condition and the interaction. To further analyse ABA genes' comparative ability to predict dormancy induced by seed-maturation conditions, *NCED9* and *CYP707A1* expressions were measured from the same samples used for detecting *DOG1*. Experimental procedures and statistical analyses followed those of the *DOG1*'s. The used primers were the following: *NCED9*, forward 5′-ATCGACCGGAGAG ATTCGAAAG-3′ and reverse 5′-TCACCTTCTCCTCGT CGTGAAC-3′; *CYP707A1*, forward 5′-TCCATCGCTC AAGACTCTCTCC-3′ and reverse 5′-ACCTCGTCTTTT CCGAAGATCG-3′.

Analysis of quantitative gene expression and normalization

Inter-run control was performed prior to the experiments to ensure the precision of the qRT-PCR machine and that the results from different plates are comparable. No template controls were included in every plate

and were consistently found to have no amplification. RNA quality control with NanoDrop measurement was performed, and the A260/A280 ratios of all our samples were better than 1.8. The specificity of the amplifications was verified by melting curve analysis, which showed single amplification on every sample, and the efficiency was evaluated by standard curve analysis. *DOG1* standard curve template was constructed by using the serial dilution set of the cloned *DOG1* vector with the absolute quantity known. *NCED9*, *CYP707A1* and *ACT8* standard curve templates were constructed by using the serial dilution set of the PCR product of each gene. All amplifications had R^2 above 0.95 and efficiency above 0.91. We used standard curves on every plate for every gene investigated and the PCR efficiency information from these standard curves to correct for the gene expression of the respective genes individually. The standard curves used in every qPCR plates for measuring relative expression varied very little (Ct SYBR No. for one shared standard curve point: *DOG1*-Mean 16.84, SE 0.20; *ACT8*-Mean 22.02, SE 0.05; *NCED9*-Mean 23.07, SE 0.05; *CYP707A1*-Mean 21.46, SE 0.04), and this little variation also should not alter the conclusion, because the samples from the complete set of the same biological replicates were amplified in the same plate standardized against the same standard curve. There was also no significant difference between the early- and late-stage plates for their standard curve point comparison (*DOG1*: $P = 0.29$; *ACT8*: $P = 0.54$; *NCED9*: $P = 0.61$; *CYP707A1*: $P = 1.00$).

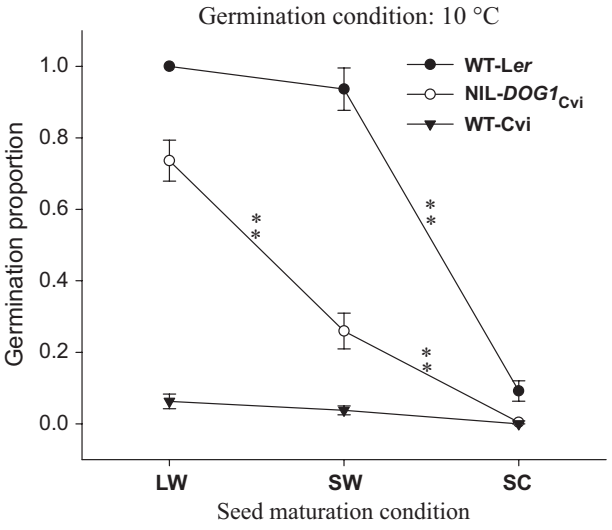

Fig. 3 Germination response reaction norm of *DELAY OF GERMINATION 1 (DOG1)* alleles to seed-maturation environments. The mean proportion of fresh offspring seed germination (±1 SE) at 10 °C. The seeds from three lines (L*er*, Cvi and near isogenic line (NIL)-*DOG1*$_{Cvi}$) were matured under three conditions (long-day, warm = 14-h daily photoperiod, 22 °C; short-day, warm = 10-h daily photoperiod, 22 °C; and short-day, cold = 10-h daily photoperiod, 10 °C). The connecting lines across the seed-maturation conditions indicate norms of reaction. Germination proportion is defined as the average of the number of germinated seeds with radicle protrusion divided by the total number of viable seeds. Significance levels indicate comparisons between seed-maturation environments within the same genotypes. The NIL-*DOG1*$_{Cvi}$ genotype was significantly more dormant than the L*er* genotype in all treatments.*$P < 0.05$; **$P < 0.01$.

Results

To dissect the ecological relevance of *DOG1* expression, we conducted four experiments to successively address (i) its role in plasticity to seed-maturation environment, (ii) the natural variation in plastic responses, (iii) its association with the phenotype under selection and (iv) its precedence over ABA genes to predict phenotypic plasticity in response to seed-maturation environments. In the sections that follow, we outline for each set of experiments the details of the expression and germination responses, along with their statistical significance.

DOG1 influences seed dormancy level in response to seed-maturation environments

When matured under SW condition (short daily photoperiod, warm temperature), seeds with *DOG1*$_{Cvi}$ introgression had significantly greater dormancy (Fig. 3). However, an increase of 4 h of daily photoperiod during seed maturation (LW – long daily photoperiod, warm temperature) significantly released the dormancy of the NIL-*DOG1*$_{Cvi}$ seeds, although they were still more dormant than the line with the low-expressing *DOG1*$_{Ler}$ allele. An analysis of *DOG1* relative expression in L*er* × Cvi-0 F1 individuals in warm conditions had demonstrated that the L*er*-0 allele of the *DOG1* promoter is weaker and *DOG1* is expressed at lower level (Bentsink *et al.* 2006). Our experiment thus indicates that seeds with a stronger *DOG1* allele might be more responsive to the photoperiod during seed maturation. The Cvi-0 parental line remained dormant under both photoperiods. Therefore, the comparative analysis of the action of the Cvi-0 allele in the L*er*-background (NIL) vs. its native background (e.g. Cvi-0) shows the existence of interactions between the *DOG1*$_{Cvi}$ allele and L*er* allele at other loci is required for the environmental plasticity of *DOG1*-induced dormancy.

The *DOG1* NIL also differed in the magnitude of response to seed-maturation temperature. Seeds of all genotypes were dormant when matured under cool temperature (SC, short daily photoperiod, cool temperature). The L*er* genotype exhibited less dormancy at the warmer temperature, so it had a larger response to

cool temperature. Consequently, as for responses to photoperiod, the difference among the three genotypes in dormancy under SW conditions accounted for differences in the response of these genotypes to seed-maturation temperature. L*er* seeds exhibited dormancy when matured at low temperature; this response reflects the increase in *DOG1* expression at low temperature, even of this comparatively low-expressing allele (Fig. S3, Supporting information). Thus, increased dormancy is associated with increased *DOG1* expression. We could not exclude that the dormancy plasticity in response to seed-maturation condition was also caused by other factors than the plasticity of the *DOG1* allele expression because we did not fully quantify *DOG1* expression in the NIL experiment. In addition, there is always a possibility that other genes on the chromosomal introgression of the NIL caused the phenomena we observed. Nevertheless, Bentsink *et al.* (2006) have demonstrated that many accessions including L*er* and Cvi exhibit large differences in *DOG1* cis-regulation under some conditions, while these accessions largely lack polymorphisms in the coding regions. Together with previous knowledge, the NIL results provide us with a logical motivation to examine natural variation in *DOG1* expression among selected accessions.

Interaccession variation in DOG1 expression and plasticity to seed-maturation environment

In agreement with previously published results (Bentsink *et al.* 2006), *DOG1* expression was significantly higher during the second time point of the later stage of seed maturation (Fig. 4), and there was no exception for all 12 accessions examined.

DOG1 expression differed among accessions in response to seed-maturation temperature but not seed-maturation photoperiod. A 4-h difference in the seed-maturation photoperiod did not significantly influence *DOG1* expression when pooled over all 12 accessions (Fig. 4a) (LW vs. SW early stage: $F = 0.03$, $P = 0.85$; LW vs. SW late stage: $F = 0.26$, $P = 0.61$). Early in development, there was no significant difference in *DOG1* expression between the warm and the cool temperatures (SW vs. SC early: $F = 1.44$, $P = 0.24$). However, at the later developmental stage, when seeds are induced into dormancy just before desiccation (Bentsink *et al.* 2006), *DOG1* expression was significantly lower at the warmer temperature than at the cool temperature (SW vs. SC late: $F = 6.23$, $P = 0.02$).

Significant geographical variation in *DOG1* expression was detected. *DOG1* expression both early and late in seed development was significantly higher in southern accessions than in northern accessions in all seed-maturation conditions except the late-stage seeds under LW (Fig. 4a).

Significant interaccession variation in early-stage *DOG1* expression was detected in all treatments, in large part because of unusually high levels of *DOG1* expression by the Sf-2 accession from Spain (Fig. 4b). When that accession was dropped from the analysis, the accessions still differed significantly in the early-stage *DOG1* expression (LW: $F = 9.81$, $P < 0.01$; SW: $F = 8.63$, $P < 0.01$; SC: $F = 16.28$, $P < 0.01$). In contrast, the accessions differed significantly in late-stage *DOG1* expression only when seeds were matured under SW. This result is in agreement with the result from the NILs (Fig. 3), in which differences in dormancy between genotypes were most pronounced under the SW treatment.

Collectively, accessions did not exhibit plasticity of *DOG1* expression in response to seed-maturation photoperiod [F-photoperiod (LW vs. SW) -early = 0.03, $P = 0.85$] or temperature for the early developmental stage [F-temperature (SW vs. SC) early = 1.44, $P = 0.24$]. During the late developmental stage, all accessions were similarly nonresponsive to photoperiod (F-accession nested in region × photoperiod = 1.25, $P > 0.05$), and the two regions also did not differ in their response to photoperiod (F-region × photoperiod = 2.74, $P > 0.05$; Fig. 4c). Most accessions increased *DOG1* expression at cool temperature (Fig. 4c), and accessions differed significantly in response to temperature (F-accession nested in region × temperature = 3.93, $P < 0.01$), indicating genetic variation in *DOG1* expression plasticity to temperature.

Correlation between DOG1 expression and germination

Seeds matured in cooler temperatures were much more dormant than seeds matured in warmer temperatures under all germination conditions (Fig. 5: *F*-temperature = 135.6, $P < 0.01$). This result is in agreement with the higher *DOG1* expression under the cooler seed-maturation temperature. In contrast, we detected no main effect of seed-maturation photoperiod on germination, but we detected significant variation among accessions in their germination response to seed-maturation photoperiod (F-photoperiod = 1.84, $P = 0.17$; F-accession × photoperiod = 1.88, $P = 0.03$; F-region × photoperiod = 3.7, $P = 0.054$).

Dark imbibition at various temperatures prior to light germination significantly influenced germination behaviours. Cold, dark imbibition stimulated germination (*F*: 22–22 vs. 10–22 = 281.3, $P < 0.01$), and seeds that experienced cold dark imbibition germinated more if transferred to warm temperature than if kept at cool

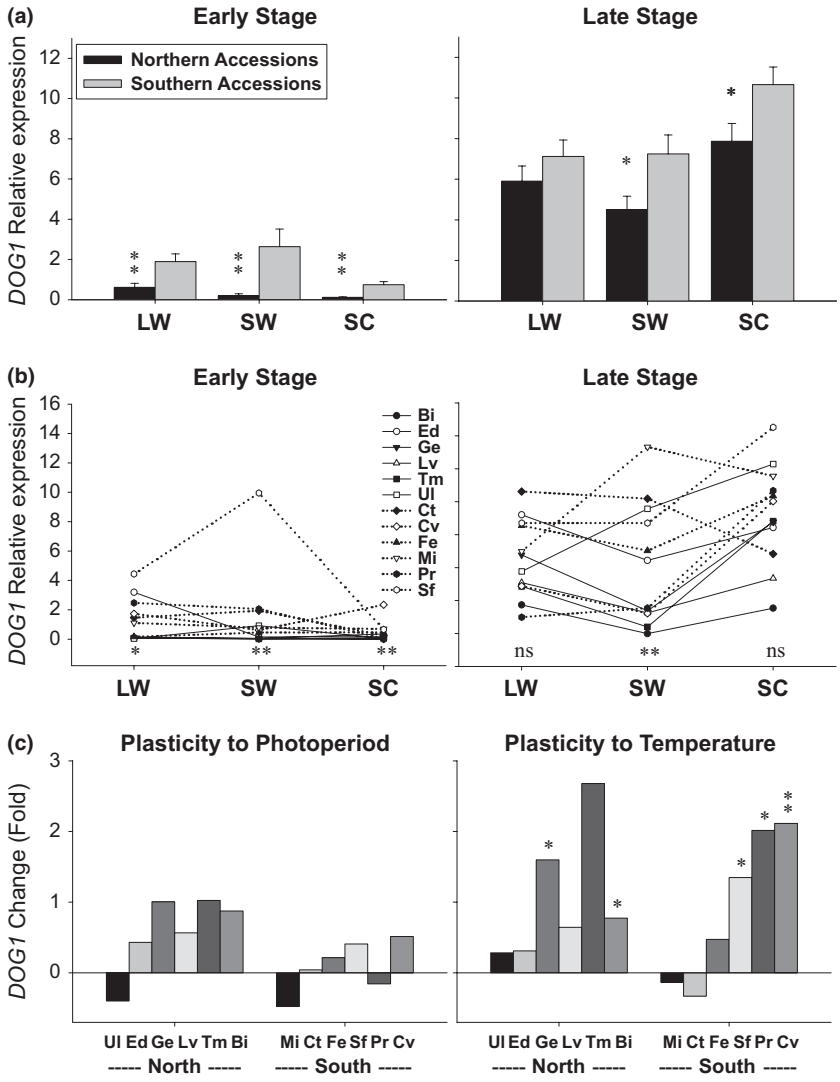

Fig. 4 Natural variation among accessions in *DELAY OF GERMINATION 1 (DOG1)* expression and its plasticity to seed-maturation conditions. (a) Comparison of *DOG1* relative expression (±1 SE) between early (left) and late (right) seed-maturation stages for each of three seed-maturation environments, for northern and southern accessions. Asterisks indicate the significance level for expression differences between the northern and southern groups. (b) *DOG1* expression reaction norms of individual accessions under three seed-maturation conditions [long-day, warm (LW), short-day, warm (SW), short-day, cold (SC)] for early (left) and late (right) seed-maturation stages. Lines (norms of reaction) connect the accession means across the three seed-maturation treatments. Solid lines represent northern accessions, while dotted lines represent southern accessions. Asterisks indicate the significance level for accession differences within each seed-maturation treatment. (c) Relative *DOG1* expression change (fold) in each accession in response to an increase of 4 h of daily photoperiod (left, LW vs. SW, all ns) or a decrease in temperature (right, SC vs. SW) during the late stage of seed maturation. All data were normalized by *ACT8*. Asterisks indicate the significance level of the response to seed-maturation temperature. $*P < 0.05$; $**P < 0.01$. Plasticity to photoperiod = (LW-SW)/SW; plasticity to temperature = (SC-SW)/SW. Three biological replicates were used for each expression data point.

temperature (*F*: 10–10 vs. 10–22 = 63.83, $P < 0.01$; *F*: 10–10 vs. 10–28 = 10.47, $P < 0.01$).

Significant interaccession variation was detected for germination under all seed-maturation conditions when the germinating seeds experienced any period of cool temperature during imbibition (10–10, 10–22, 10–28 °C), except for seeds matured under cool conditions that did not experience any warm temperature during germination (10–10 °C) (Fig. 5 & Table S1, Supporting information). Accession differences were highly diminished in all seeds matured at cool temperature because all seeds were highly dormant in that treatment. Significant interaccession variation in response to seed-maturation temperature was also detected in all germination conditions

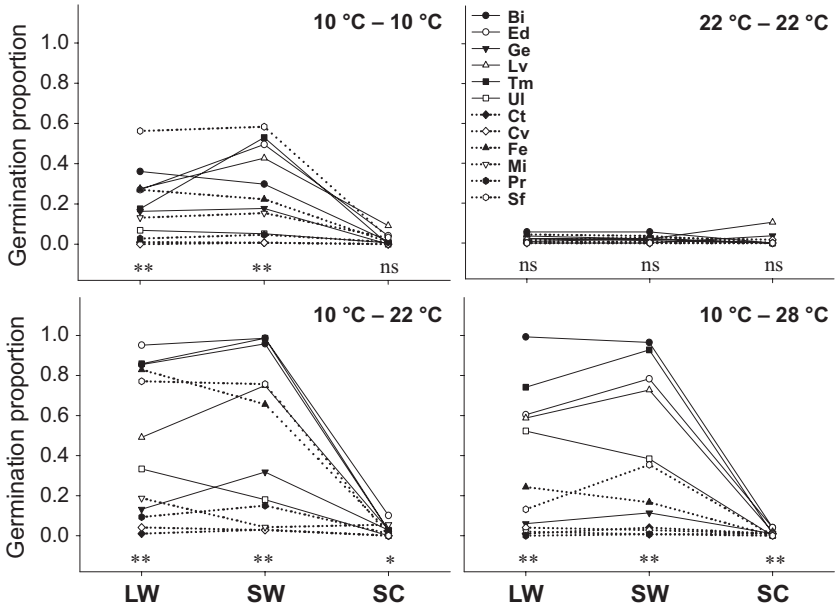

Fig. 5 Variation in germination among accessions under different germination conditions. Lines connect the accession means across three seed-maturation treatments. Solid lines represent northern accessions, while dotted lines represent southern accessions. Asterisks indicate the significance level for accession differences within each seed-maturation treatment. *$P < 0.05$; **$P < 0.01$.

Germination condition	*DOG1*	*NCED9*	*CYP707A1*
Early stage			
10–10 °C	***r* = 0.43, *P* = 0.01**	$r = 0.23$, $P = 0.19$	***r* = 0.41, *P* = 0.01**
22–22 °C	$r = 0.20$, $P = 0.25$	$r = 0.22$, $P = 0.20$	$r = 0.20$, $P = 0.26$
10–22 °C	$r = 0.19$, $P = 0.27$	$r = 0.13$, $P = 0.46$	$r = 0.16$, $P = 0.37$
10–28 °C	$r = 0.09$, $P = 0.60$	$r = 0.09$, $P = 0.60$	$r = 0.11$, $P = 0.54$
Late stage			
10–10 °C	$r = 0.28$, $P = 0.10$	$r = 0.02$, $P = 0.91$	$r = 0.10$, $P = 0.57$
22–22 °C	***r* = 0.33, *P* = 0.04**	***r* = 0.34, *P* = 0.04**	$r = 0.30$, $P = 0.08$
10–22 °C	***r* = 0.35, *P* = 0.04**	$r = 0.06$, $P = 0.73$	$r = 0.13$, $P = 0.44$
10–28 °C	***r* = 0.52, *P* = 0.00**	$r = 0.17$, $P = 0.32$	$r = 0.07$, $P = 0.70$

Table 2 Regression analysis indicates significant association between *DOG1* expression and germination. Correlations ($N = 12$) between germination proportions and the expression of *DOG1*, *NCED9* and *CYP707A1* at early and late developmental stages and in the four germination conditions, pooled over all seed-maturation treatments

DOG1, *DELAY OF GERMINATION 1*.
Letters in bold indicate significance.

that experienced a period of cold during imbibition (Table S1, Supporting information).

Significant variation in germination between the northern and southern accession was detected, which suggests the existence of geographical structure in germination among *Arabidopsis thaliana* populations. Northern accessions had a significantly higher germination proportion than southern accessions, especially when higher temperatures were experienced during seed germination (10–10 °C: F-region = 1.54, $P = 0.22$; 22–22 °C: $F = 3.67$, $P = 0.06$; 10–22 °C: $F = 3.78$, $P = 0.06$; 10–28 °C: $F = 14.75$, $P < 0.01$). The decreased germination of southern accessions is in agreement with their higher *DOG1* expression. The geographical difference in germination was significant only under warmer seed-maturation conditions (SW & 10–28 °C, F-region = 14.63, $P < 0.01$; LW & 10–28 °C, F-region = 14.01, $P < 0.01$). No differences in germination between northern and southern accessions were detected for seeds matured at cool temperatures. Therefore, although *DOG1* expression was less in northern accessions even at cool seed-maturation temperature, all accessions attained high enough *DOG1* levels to be induced into dormancy when matured under cool conditions.

Early *DOG1* expression was not significantly associated with dormancy, except when pooled over seed-maturation treatments at the 10–10 °C germination condition (Table 2, Tables S2 and S3, Supporting information). A significant, strong inverse correlation was detected between late *DOG1* expression and germination in all germination conditions except the lowest temperature, when pooled over all seed-maturation treatments (Table 2 and Fig. 6). Within a given seed-maturation environment, however, the association

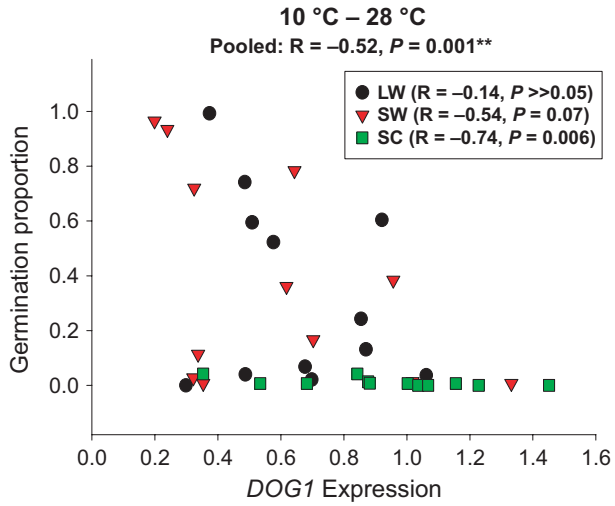

Fig. 6 Correlation between *DELAY OF GERMINATION 1 (DOG1)* expression and germination. Correlation between the means of late-stage *DOG1* expression of accessions and their seed germination proportion. The graph shows the correlation pooled across three seed-maturation environments, and the box shows the correlation coefficient for each seed-maturation treatment separately.

between late *DOG1* expression and germination was weaker and almost always nonsignificant (Table S3, Supporting information). The correlations between *DOG1* expression and germination under specific treatments are not significant if corrected for multiple comparisons. With the mild caveat that the statistical power was weaker for tests conducted within seed-maturation treatments, these results suggest that late-stage *DOG1* expression predicts the germination responses to seed-maturation temperature better than it predicts germination within a given seed-maturation treatment.

Genotypic plasticity of *DOG1* expression and of germination in response to seed-maturation temperature was not significantly correlated ($P > 0.05$ for both early and late *DOG1* expression) because of the nearly complete dormancy of all accessions when they were matured at low temperature. It appears that the greatly increased expression of *DOG1* at low temperature obscured accession differences in dormancy response.

DOG1 expression predicts germination better than the expression of two other dormancy-regulating ABA genes NCED9 and CYP707A1

While both the L*er* and Col wild types showed a dramatic induction into strong dormancy by SC, their *aba1* mutants completely eliminated this dormancy induced by cold temperature during seed maturation (Fig. 7). This effect occurred without regard of the imbibition/germination treatment. The results suggest that,

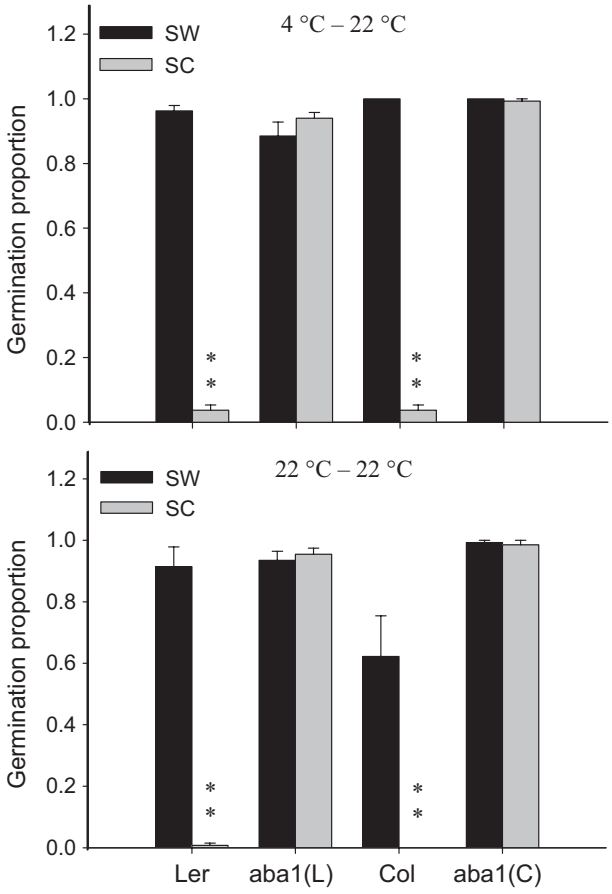

Fig. 7 Requirement of endogenous abscisic acid synthesis in dormancy induced by seed-maturation environments. L*er* and Col represent the two wild types, while L-aba1 and C-aba1 indicate *aba1* mutants in L*er* and Col background, respectively. Asterisks indicate the significance difference between the wild type and its *aba1* mutant. *$P < 0.05$; **$P < 0.01$.

while *DOG1* predicts germination in response to seed-maturation conditions, it occurs under the framework of ABA pathways. This motivates us to investigate whether ABA regulatory genes can also predict the germination behaviours in response to seed-maturation environments. We chose *NCED9* and *CYP707A1* because they have a specific function in regulating dormancy levels, and their expression profiles are very similar to those of *DOG1* during silique development.

Even though *DOG1*, *NCED9* and *CYP707A1* expressions are mostly coordinated, they have significant differences in their ability to respond to seed-maturation environments among accessions (Figs S4–S6, Supporting information). Unlike *DOG1* expression, *NCED9* and *CYP707A1* expression patterns were not good predictors of seed germination under most conditions within any seed-maturation treatment or when pooled over seed-maturation treatments (Table 2 and Table S3, Supporting information). Late-stage *NCED9* expression was

significantly correlated with germination only when the seeds did not experience any cold imbibition. In contrast, early *CYP707A1* expression was correlated with germination only when the germinating seeds were constantly in the lower temperature, suggesting that ABA synthesis could be important for regulating dormancy under warm temperatures, whereas ABA degradation may be important for regulating dormancy at low temperature. Furthermore, under the germination conditions and expression stages when the two ABA genes were correlated with germination, *DOG1* expression also correlated with germination equally well. Nevertheless, during the early stage and only when the germinating seeds did not experience any period of cold, the plastic responses of *NCED9* expression to seed-maturation photoperiod and the plastic responses of *CYP707A1* expression to both photoperiod and temperature were correlated with germination (Table S2, Supporting information). Because *DOG1* expression better predicted germination than did expression of the ABA biosynthetic and degradation genes, *DOG1*-mediated dormancy might involve more than just the ABA pathways. Alternatively, multiple genes in the ABA network may redundantly regulate dormancy or *DOG1* via ABA levels. Both *NCED9* and *CYP707A1* have a paralogous gene, *NCED6* and *CYP707A2*, respectively, which have largely over-lapping function in seed dormancy regulation (Lefebvre *et al.* 2006; Okamoto *et al.* 2006). While their highly coordinated expressions during development suggest that *NCED6* and *CYP707A2* most likely will have similar expression patterns as *NCED9* and *CYP707A1* in response to seed-maturation conditions, there remains a possibility that either of the two not tested might be more involved in linking the seed-maturation environments to dormancy.

Discussion

Four lines of experimentation were carried out to examine the role of *DOG1* in responding to seed-maturation environments and its natural variation. First, to establish that *DOG1* is involved in the response to seed-maturation environments, seeds of NIL-$DOG1_{Cvi}$ (near-isogenic line) were matured in three different conditions (varying in daily photoperiod and temperature). Second, variation in *DOG1* expression induced by the environment was confirmed with 12 genotypes grown in the same conditions. This experiment revealed that Southern genotypes tend to have higher *DOG1* expression. Third, seed germination was examined to estimate whether changes in *DOG1* expression induced by seed-maturation conditions correlate with changes in seed germination. A significant correlation showed that *DOG1* expression is a good predictor of the action of seed-maturation environments on dormancy. This experiment also revealed significant genetic variation in the response to seed-maturation environments. Fourth, to evaluate whether *DOG1* is a better predictor of this response than genes controlling ABA levels, another essential component for germination, the expression of two ABA genes was quantified and compared with seed germination. Taken together, these complementary experiments highlight the potential of *DOG1* for understanding the ecological realm of seed dormancy evolution.

Germination behaviour is known to be under the decisive influence of environmental conditions, and *DOG1* is a major determinant of seed dormancy variation (Bentsink *et al.* 2006, 2010; Huang *et al.* 2010). Therefore, the study of natural variation in *DOG1* expression among various environmental conditions provides an excellent opportunity to evaluate the ecological importance of molecular variation for the evolution of seasonal plasticity in *Arabidopsis thaliana*. While many other hormonal, resource mobilization and seed-coat genes are known to be involved in the physiological processes of germination and dormancy, based on studies of mutants, none of these has been shown to be associated with natural variation in dormancy (Finch-Savage & Leubner-Metzger 2006; Finkelstein *et al.* 2008; Holdsworth *et al.* 2008a,b). In this study, we documented *A. thaliana* interaccession variation in *DOG1* expression and showed the association between *DOG1* and germination responses to seed-maturation environments, a factor that is highly likely to influence seasonal dormancy induction in the field. In addition, *DOG1* expression varied geographically, with southern accessions exhibiting higher *DOG1* expression and higher dormancy.

Genetic variation in dormancy may be due in a large part to genetic variation in *DOG1* expression. Although *DOG1* expression levels did not predict germination as well within a given seed-maturation treatment, expression predicted the broader patterns of parental environmental effects and geographical variation. The lack of significant correlations when each treatment was analysed separately could be in part because of the reduced statistical power, as the direction of the correlation was negative in all treatments. However, it is also likely that cold-induced *DOG1* expression strongly induces dormancy in general, but natural variation in *DOG1* expression is not as strongly related to natural variation in dormancy under a given set of conditions. This could be caused by several reasons. The proteins encoded by the *DOG1* alleles in various accessions might have differences in functionality. In addition, fine-tuning of the overall dormancy levels could be due to other minor QTLs. As an example, *DOG1* expression of Cvi-0 is

relatively low under warm seed-maturation conditions even though its dormancy is among the strongest, indicating improved functionality of the protein or dormancy contribution by other loci. Another possibility is that the predominant ecological role of *DOG1* in imposing dormancy is environmental sensing. For instance, the *DOG1* allele of Fei-0 was recently found to be relatively less dormant under the warmer seed-maturation condition (Bentsink *et al.* 2010), and this also is accompanied by its higher germination proportion among southern accessions. However, cooler seed-maturation condition induces significant increases in both the *DOG1* expression and dormancy in Fei-0, suggesting *DOG1*'s effects on dormancy depends crucially on the environment. Nevertheless, overall latitudinal differences in *DOG1* expression reflected latitudinal differences in dormancy, suggesting some functionally significant differences among accessions in *DOG1* expression. It should be noted that our laboratory studies of germination might not have included the most appropriate environmental conditions for assessing the relationship between *DOG1* expression and germination under complex field conditions.

Another important finding is that genetic variation for *DOG1* expression and for dormancy itself was not expressed in all seed-maturation conditions. This is shown both by the NILs and by the analysis of various genotypes. Despite the geographical differences in *DOG1* expression under cool seed-maturation temperatures, extremely little interaccession variation for dormancy was expressed when seeds were matured at low temperature because nearly all freshly harvested seeds of any accession were dormant. One possibility for the lack of interaccession variation in dormancy in the low-temperature treatment is that, as *DOG1* levels increase, all accessions attained a high enough level of *DOG1* that is capable of inducing dormancy. Based on that hypothesis, it could be argued that genetic variation in *DOG1* expression is not as important when seeds are matured at low temperature and that *DOG1* might not be under selection in the early spring- or autumn-flowering *A. thaliana* cohorts for that reason. More generally, when expression levels determine threshold phenotypes, environmental conditions that alter or elevate expression may mask variation in the phenotype and shield genotypes from selection.

Alternatively, rather than masking genetic variation completely, cool seed-maturation conditions might increase the intensity of an environmental factor required for breaking dormancy. Genetic variation for *DOG1* expression might still influence dormancy under conditions not assessed in this study. For example, the fresh seeds studied here were all uniformly dormant, but their response to after-ripening has not been studied. Accessions with higher *DOG1* expression might have seeds that maintain their dormancy for a longer period of time, thereby contributing to dormancy differences after longer periods of after-ripening.

In this study, we first showed that the hormone ABA is required for the plastic response in germination. But despite the fact that the expression of the ABA biosynthetic and catabolic genes studied here were correlated with *DOG1* expression within a given treatment, the responses of the ABA genes to seed-maturation treatments did not reflect the responses of *DOG1* to these treatments. It therefore appears that the changes in *DOG1* expression in response to seed-maturation conditions, and temperature in particular, are not accounted for by changes in expression of the ABA biosynthetic and catabolic genes studied here.

The significant correlation between the dormancy levels and overall *DOG1* expression measured in this study indicates a quantitative relationship that is shaped by seed-maturation environments. Both cis- and trans-regulatory differences may influence the expression level of *DOG1*, and it is obvious from our data that other naturally variable genes interact with *DOG1* to influence germination. However, it should be noted that the phenotypic difference caused by various *DOG1* genotypes could have been the effect of variation in expression, coding sequence or a combination of the two. Further functional experimentation is needed to distinguish the effects of expression level and coding sequence polymorphism on the environmentally induced dormancy variation. In addition, multiple transcript variants are transcribed from the *DOG1* gene by alternative splicing (Bentsink *et al.* 2006). While the functionality and molecular role of any of the splicing types has not been determined, the existence of complex transcript profiles may confer *DOG1* extra plasticity in response to environmental conditions, both before and after dispersal.

Exploring the link between natural variation in seed germination, individual fitness and life history evolution has been a major focus because of the intensity of natural selection on germination timing (Simons & Jognston 2003, 2006; Donohue *et al.* 2005b; Evans & Dennehy 2005). Our study strongly suggests that the role of *DOG1* expression in adaptive evolution will be greatly influenced by the seed-maturation environment and its seasonal or geographical variability. There is, however, no definitive data indicating that the allelic differences between L*er* and Cvi are because of expression differences alone. But, because the function of *DOG1* remains unknown, it is difficult to assess variation in its molecular action. Instead, an analysis of expression levels may provide a first view on the importance of *DOG1* variation in germination ecology.

For future investigation, detailed life history of *A. thaliana* wild populations across diverse geographical ranges should be compared with their natural *DOG1* expression levels during seasonal silique development, to infer the degree of adaptive significance between *DOG1* expression and seed-maturation environment. A recent genome-wide association mapping study has linked the *DOG1* locus with flowering time determination in *A. thaliana* (Atwell *et al.* 2010), even though the exact physiological or molecular connection, if any, is still unknown (Brachi *et al.* 2010 provided an alternative suggestion that it might be the nearby gene *SAG12* influencing flowering time). It does not come as a surprise that there are extensive cross-talks between germination and flowering time pathways, as it has also been documented that the naturally variable flowering time gene *FLC* regulates seed germination through its downstream targets (Chiang *et al.* 2009). This suggests that germination and flowering, the two most significant developmental transitions for local adaptation, could be partially determined for an individual even before dispersal through the interaction of *DOG1* and seed-maturation environments. Our results investigating geographical patterns of variation in *DOG1* expression and correlations between *DOG1* expression and germination behaviour, conditioned by the seed-maturation environments, provide the first look into the possible role of local adaptation of this major dormancy QTL in *A. thaliana*.

Acknowledgements

We thank ABRC for supplying seed stocks. Four anonymous reviewers made very helpful suggestions that improved the manuscript. This work was supported by the Max Planck Society, the Deutsche Forschungsgemeinschaft grant within the Collaborative Research Center SFB680 to JM and SFB 572 to WJJS and the National Science Foundation (NSF) grants DEB 0807973 to GCKC and KD, ISO 0544053 to KD.

References

Alonso-Blanco C, Bentsink L, Hanhart CJ, Vries HB, Koornneef M (2003) Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. *Genetics*, **164**, 711–729.

Andalo C, Mazer SJ, Godelle B, Machon N (1999) Parental environmental effects on life history traits in *Arabidopsis thaliana*. *New Phytologist*, **142**, 173–184.

Atwell S, Huang YS, Vilhjalmsson BJ *et al.* (2010) Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*, **465**, 627–631.

Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of DOG1, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA*, **103**, 17042–17047.

Bentsink L, Hanson J, Hanhart CJ *et al.* (2010) Natural variation for seed dormancy in Arabidopsis is regulated by additive genetic and molecular pathways. *Proceedings of the National Academy of Sciences of the USA*, **107**, 4264–4269.

Biere A (1991) Parental effects in *Lychnis flos cuculi*. II. Selection on time of emergence and seedling performance in the field. *Journal of Evolutionary Biology*, **3**, 467–486.

Brachi B, Faure N, Horton M *et al.* (2010) Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. *PLoS Genetics*, **6**, e1000940.

Chiang GCK, Barua D, Kramer EM, Amasino RM, Donohue K (2009) Major flowering time gene, *FLOWERING LOCUS C*, regulates seed germination in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the USA*, **106**, 11661–11666.

Donohue K (2002) Germination timing influences natural selection on life-history characters in *Arabidopsis thaliana*. *Ecology*, **83**, 1006–1016.

Donohue K (2009) Completing the cycle: maternal effects as the missing link in plant life cycles. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **364**, 1059–1074.

Donohue K, Polisetty CR, Wender NJ (2005a) Genetic basis and consequences of niche construction: plasticity-induced genetic construction on the evolution of seed dispersal in *Arabidopsis thaliana*. *The American Naturalist*, **165**, 537–550.

Donohue K, Dorn L, Griffith C *et al.* (2005b) The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. *Evolution*, **59**, 758–770.

Donohue K, Dorn L, Griffith C *et al.* (2005c) Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. *Evolution*, **59**, 740–757.

Donohue K, Heschel MS, Chiang GCK, Butler CM, Barua D (2007) Phytochrome mediates germination responses to multiple seasonal cues. *Plant, Cell & Environment*, **30**, 202–212.

Donohue K, Heschel MS, Butler CM *et al.* (2008) Diversification of phytochrome contributions to germination as a function of maternal environment. *New Phytologist*, **177**, 367–379.

Effmertova E (1967) The behaviour of 'summer annual', 'mixed', and 'winter annual' natural populations as compared with early and late races in field conditions. *Arabidopsis Information Service*, 4.

Evans AS, Cabin RJ (1995) Can dormancy affect the evolution of post-germination traits-the case of *Lesquerella-fendleri*. *Ecology*, **76**, 344–356.

Evans MEK, Dennehy JJ (2005) Germ banking: bet-hedging and variable release from egg and seed dormancy. *Quarterly Reviews of Biology*, **80**, 431–451.

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. *New Phytologist*, **171**, 501–523.

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. *Annual Review of Plant Biology*, **59**, 387–415.

Galloway LF (2002) The effect of maternal phenology on offspring characters in the herbaceous plant *Campanula americana*. *Ecology*, **90**, 851–858.

Griffith C, Kim E, Donohue K (2004) Life-history variation and adaptation in the historically mobile plant *Arabidopsis thaliana* (Brassicaceae) in North America. *American Journal of Botany*, **91**, 837–849.

Holdsworth MJ, Bentsink L, Soppe WJJ (2008a) Molecular networks regulating *Arabidopsis* seed maturation, after-ripening, dormancy and germination. *New Phytologist*, **179**, 33–54.

Holdsworth MJ, Finch-Savage WE, Grappin P, Job D (2008b) Post-genomic dissection of seed dormancy and germination. *Trends in Plant Science*, **13**, 7–13.

Huang XQ, Schmitt J, Dorn L *et al.* (2010) The earliest stages of adaptation in an experimental plant population: strong selection on QTLs for seed dormancy. *Molecular Ecology*, **19**, 1335–1351.

Koornneef M, Jorna ML, Brinkhorst-van der Swan DLC, Karssen CM (1982) The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in nongerminating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Heynh. *Theoretical and Applied Genetics*, **61**, 385–393.

Koornneef M, Bentsink L, Hilhorst H (2002) Seed dormancy and germination. *Current Opinion in Plant Biology*, **5**, 33–36.

Korves TM, Schmid KJ, Caicedo AL *et al.* (2007) Fitness effects associated with the major flowering time gene *FRIGIDA* in *Arabidopsis thaliana* in the field. *The American Naturalist*, **169**, E141–E157.

Lefebvre V, North H, Frey A *et al.* (2006) Functional analysis of Arabidopsis *NCED6* and *NCED9* genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. *The Plant Journal*, **45**, 309–319.

Munir J, Dorn LA, Donohue K, Schmitt J (2001) The effect of maternal photoperiod on seasonal dormancy in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany*, **88**, 1240–1249.

Okamoto M, Kuwahara A, Seo M *et al.* (2006) *CYP707A1* and *CYP707A2*, which encodes abscisic acid 8′-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. *Plant Physiology*, **141**, 97–107.

Schmitt J (1995) Genotype-environment interaction, parental effects, and the evolution of plant reproductive traits. In:*Experimental and Molecular Approaches to Plant Biosystematic* (ed. Hoch P). pp. 1–16, Missouri Botanical Garden, St. Louis, MO, USA.

Schwab M (2008) *Identification of Novel Seed Dormancy Mutants in* Arabidopsis thaliana *and Molecular and Biochemical Characterization of the Seed Dormancy Gene DOG1*. PhD thesis, Universität zu Köln, University of Cologne, Cologne, Germany.

Simons AM, Jognston MO (2003) Suboptimal timing of reproduction in Lobelia inflata may be a conservative bet-hedging strategy. *Journal of Evolutionary Biology*, **16**, 233–243.

Simons AM, Jognston MO (2006) Environmental and genetic sources of diversification in the timing of seed germination: implications for the evolution of bet hedging. *Evolution*, **60**, 2280–2292.

Teng S, Rognoni S, Bentsink L, Smeekens S (2008) The Arabidopsis GSQ5/DOG1 Cvi allele is induced by the ABA-mediated sugar signalling pathway, and enhances sugar sensitivity by stimulating ABI4 expression. *The Plant Journal*, **55**, 372–381.

Vicient CM, Delseny M (1999) Isolation of total RNA from *Arabidopsis thaliana* seeds. *Analytical Biochemistry*, **268**, 412–413.

Wilczek AM, Roe JL, Knapp MC *et al.* (2009) Effects of genetic perturbation on seasonal life history plasticity. *Science*, **323**, 930–934.

G.C.K.C., D.B. and K.D. are interested in gene–environment interactions and evolutionary ecology of plant adaptation. M.B., K.N. and W.S. focus on seed dormancy in *Arabidopsis thaliana* and aim to identify its molecular mechanisms. M.D., I.K. and J.de-M. address the molecular consequences of life-history evolution and local adaptation in *A. thaliana* and its relatives. M.K. strives to advance knowledge on important processes that determine plant growth and development by making use of genetic and genomic tools available and being developed for both crop and model plants.

Data accessibility

Gene Expression and Germination Data: DRYAD entry doi: 10.5061/dryad.18pn5.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Geographic and climatic basis for the divide between the northern and southern groups.

Fig. S2 Comparison between two reference gene expression patterns.

Fig. S3 Elevated *DOG1* expression under cold seed-maturation conditions.

Fig. S4 Natural variation of *NCED9* expression across accessions.

Fig. S5 Natural variation of *CYP707A1* expression across accessions.

Fig. S6 Coordinated expression of *DOG1* and ABA genes.

Table S1 The accessions showed highly significant differences in most of their germination responses.

Table S2 Upper: Correlations between plasticity of *DOG1*, *NCED9*, and *CYP707A1* in response to seed-maturation photoperiod (left) and seed-maturation temperature (right) during the two developmental stages. Correlations are based on accession means. Lower: Correlations between plasticity to photoperiod or temperature and germination proportions under four germination treatments. Correlations are based on mean expression and mean germination of accessions.

Table S3 Correlation between the expression of *DOG1*, *NCED9*, and *CYP707A1* at early and late developmental stages in each seed-maturation treatment (LW, SW, SC) with germination proportions in each of four germination conditions.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.