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The maternal environment interacts with genetic variation in regulating seed dormancy in Swedish *Arabidopsis thaliana*

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Abstract

Seed dormancy is a complex adaptive trait that controls the timing of seed germination, one of the major fitness components in many plant species. Despite being highly heritable, seed dormancy is extremely plastic and influenced by a wide range of environmental cues. Here, using a set of 92 *Arabidopsis thaliana* lines from Sweden, we investigate the effect of seed maturation temperature on dormancy variation at the population level. The response to temperature differs dramatically between lines, demonstrating that genotype and the maternal environment interact in controlling the trait. By performing a genome-wide association study (GWAS), we identified several candidate genes that could presumably account for this plasticity, two of which are involved in the photoinduction of germination. Altogether, our results provide insight into both the molecular mechanisms and the evolution of dormancy plasticity, and can serve to improve our understanding of environmentally dependent life-history transitions.

Introduction

Life-stage transitions, the timing of which is critical to plant fitness, are regulated by both genes and the environment, usually in interaction (G x E) [1–3]. Plants have evolved ways to sense and integrate environmental inputs in order to adjust their life cycle to seasonal environments, the best-known example of which is vernalization and the perception of winter cold [4]. In *A. thaliana*, vernalization results in the stable repression of the central regulator *FLOWERING LOCUS C* (*FLC*), a prerequisite for the vegetative-to-reproductive transition to occur [5].

While flowering time determines the reproductive environment, seed dormancy, another major life-history trait crucial for local adaptation, regulates the timing of germination and determines the post-germination environment [1, 6, 7]. Dormancy is highly plastic and can be modulated both by pre- and post-dispersal environmental cues such as temperature, light, and to a lesser extent, nitrate [8–13]. In particular, low temperatures during seed production dramatically increase dormancy in *A. thaliana* [14–17] as well as in other species such as wheat [18] and wild oat [19].

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Central to this temperature-dependent process in *A. thaliana* is the upregulation of a major genetic determinant of seed dormancy variation, *DELAY OF GERMINATION1* (*DOG1*) [14, 15, 20]. The *DOG1* locus exhibits genetic signatures suggestive of local adaptation, and field experiments have emphasized its pivotal role in controlling the timing of germination in wild *A. thaliana* populations [21–24]. Independently of their action on *DOG1*, low seed maturation temperatures induce deep dormancy by increasing the abscisic acid (ABA) / gibberellins (GA) ratio, two antagonistic phytohormones repressing and activating germination, respectively [14, 15]. Finally, low temperatures can promote coat-imposed dormancy by altering seed coat permeability through the upregulation of the flavonoids biosynthesis pathway, both during seed production [25] and vegetative phase [26].

Thus, it is clear that the induction of primary dormancy is regulated by both genetic and environmental factors, and the fact that distinct genotypes differ in their response to low temperatures indicates that genotype-environment interactions partly control the trait [17, 17, 27–29]. A direct consequence of this plasticity is that similar germination trajectories, defined as the evolution of the germination phenotype over time, can be promoted by different combinations of genotypes and environments. For example, strong *DOG1* alleles combined with a warm maternal environment and weak *DOG1* alleles combined with a cold maternal environment can both lead to highly dormant phenotypes [27].

Field studies have demonstrated that the maternal environment contributes greatly to seed dormancy variation under natural conditions [30]. Thus, given the existence of strong selection for timing of germination [22–24, 31], it has been speculated that the temperature-dependent regulation of primary dormancy is adaptive. For example, it may provide the mother plant with information regarding the seasonal environment, information that can be used to set progeny dormancy appropriately [32]. In addition, this mechanism is expected to enable bet-hedging strategies, in which seeds from the same population—or plant—express various dormancy phenotypes and germinate throughout the year to maximize fitness in unpredictable environments [33–36].

Although genotype-environment interactions have previously been reported to influence dormancy and germination plasticity in *A. thaliana* [27–29], the extent of this phenomenon and whether it is universal at the species level is unknown. Moreover, the genetic basis of the differential response to seed maturation temperatures, and more generally, of G x E variation, remains to be thoroughly investigated. Here, by growing a set of *A. thaliana* lines from Sweden in two different environments, we assess the effect of maternal temperature on seed dormancy in a local sample, before performing a GWAS to identify the genes responsible for the observed variation.

Materials and methods

Plant material and phenotyping

The 92 Swedish lines used in this study (S1 Table) were kindly donated by Joy Bergelson (University of Chicago) and were previously described in [22, 37]. Upon reception, lines were bulk propagated for one generation under standard lab conditions to minimize potential maternal effects. To produce seeds used in germination assays, six biological replicates of each genotype were first vernalized for eight weeks (4˚C, 16 h day / 8 h night, 90% humidity). Then, three randomly chosen replicates were placed in a warm environment (21˚C day / 16˚C night), while the other three received a cold treatment (15˚C day / 10˚C night). Both treatments were applied from rosette stage to ripening and seed harvest. Seeds were harvested when about 50% of the siliques of a given plant had come to maturity and were subsequently placed in dry environment for after-ripening (30% relative humidity, 16˚C, dark). The germination rate of seeds

(percentage of germinated seeds) after-ripened for 21, 63 and 105 days (GR21, GR63 and GR105) was estimated for each genotype following standard methods [22, 38]. Briefly, about 75 seeds per genotype were spread on wet filter paper (Whatman, ref. 1001-047) in Petri dishes (Greiner Bio-One, ref. 628102) that were subsequently placed in moisture chambers to maintain high, constant humidity (close to 99%). Moisture chambers consisted of reasonably airtight, transparent plastic boxes (Ikea), whose bottom was covered by ten layers of paper towel imbibed with tap water. Germination was scored by observing radicle emergence after a week of incubation at 23˚C in standard long days (16 h day / 8 h night) and under fluorescent light (Osram L 58W 840 Lumilux, 40 μm/m^2/sec).

Broad sense heritability

Broad sense heritability (H) was calculated for each dormancy phenotype as the genotypic variance divided by the total variance. Both variances were estimated using a linear mixed-model from the *lme4* package in R environment [39]. The model was as follows:

$$Y = GEN + REP + e \quad (1)$$

where Y is phenotype (germination rate), GEN is genotype (line), REP is technical replicate, and e is error. All variables were fitted as random effects.

Variance component analysis

The variance component analysis was described earlier in [40]. It was carried out in LIMIX [41] using the following model:

$$Y = [\mu_{warm}, \mu_{cold}] \otimes 1_{N,1} + U_{global} + \psi \quad (2)$$

where μ_{warm} and μ_{cold} are environment specific mean values, U_{global} is a matrix of global relatedness fitted as random effect, and ψ is noise.

Genome-wide association mapping

Genome-wide scans were performed on arcsine transformed mean phenotypic values (germination rate) using a mixed-model accounting for population structure and 3,333,502 SNPs ($\approx$ 266 SNPs / kb) derived from the 1001 genomes project [42–44]. The analysis was carried out on 88 lines using the GWA-Portal (https://gwas.gmi.oeaw.ac.at/; [45]), and both the settings and the results are fully browsable online (https://goo.gl/dt53nc). In this manuscript, rare SNPs [minor allele frequency (MAF) lower than 14%] were filtered out to minimize the risk of spurious associations (clearly amplified by the small population size) and to identify common variants that are more likely to explain the global pattern of variation observed across Sweden. We note that interactive plots displaying *P* values for rare SNPs (MAF lower than 14%) are available online (https://goo.gl/dt53nc). A 5% genome-wide significance threshold was determined using Bonferroni correction. However, because this correction is conservative when used in the context of GWAS, all marginally significant markers with a *P* value lower than 10^{-6} were considered of interest and grouped into peaks. A given peak had a 'specific' effect when its highest score [-$\log_{10}$(*P* value)] for any of the three warm phenotypes did not exceed 2, or a 'common' effect when its score for at least one phenotype in both environment was higher than 4. Peaks that did not meet any of these arbitrary criteria were regarded as having an 'unclear' effect. Finally, we considered all genes located between peaks borders plus-minus 20 kb when looking for candidate genes.

Gene enrichment analyses

The seed dormancy *a priori* candidate gene list (91 genes; S2 Table) was built regardless of the GWAS results by searching the literature (mainly [15, 46]) and by querying the ARAPORT11 database using the following GO terms: GO:0048838 'release of seed from dormancy', GO:1902039 'negative regulation of seed dormancy process', and GO:0010162 'seed dormancy process'. This list is non exhaustive and we only included major dormancy regulators as well as seed specific genes affected by temperature during seed maturation. A gene was considered significantly associated when at least one SNP located 20 kb upstream or 20 kb downstream its coding sequence had a *P* value lower than 10^{-4}. Lists of non *a priori* significantly associated TAIR11 genes were built in a similar manner for each phenotype, and the overrepresentation of *a priori* genes in the resulting lists was assessed using one-sided Fisher's exact test as described in [47]. More specifically, we used a 2 x 2 contingency table in which row entries consisted of significant and non significant genes while column entries consisted of *a priori* and non *a priori* genes, which gave for categories: significant *a priori* genes, non significant *a priori* genes, significant non *a priori* genes and non significant non *a priori* genes. This analysis was performed in R environment [39] using the built-in 'fisher.test()' function, with the alternative parameter set to 'greater' as we tested for overrepresentation.

Results

Seed dormancy variation in the Swedish population

As expected, genotypes that experienced warm maternal temperature displayed great variation for seed dormancy, although more than one third of the lines remained dormant even after 105 days (Fig 1). In contrast, the vast majority of seeds produced at cold maternal temperature remained dormant throughout the experiment, in line with previous studies that have shown that a decrease in seed maturation temperature generally induces a deeper dormancy [14–17, 27, 28].

All six phenotypes were correlated, especially within temperature treatments (S1 Fig), and broad sense heritabilities were remarkably high (ranging from 0.85 to 0.94; Table 1), although they became moderate when considering lines with intermediate phenotypes only (GR $\geq$ 5% and $\leq$ 95%; ranging from 0.67 to 0.75; Table 1). To assess the relative effects of genes and the environment on dormancy variation, we performed a variance components analysis, in which

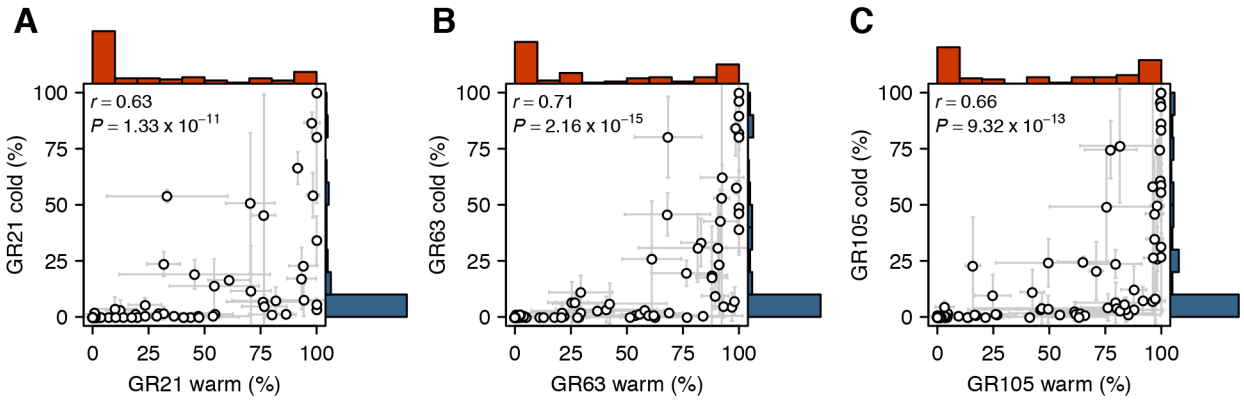

Fig 1. The effect of seed maturation temperature on seed dormancy variation. Scatter plots and histograms showing the relationship between dormancy traits as well as their phenotypic distribution. Seeds were produced either under warm (21˚C; red) or cold (15˚C; blue) conditions and after-ripened for either (A) 21, (B) 63 or (C) 105 days. Error bars represent the standard deviation within genotypes (n = 3, in few cases n = 2).

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Table 1. Heritability of seed dormancy traits.

	H_{warm}	n_{warm}	H_{cold}	n_{cold}
Full sample				
GR21	0.93	92	0.85	92
GR63	0.93	92	0.87	92
GR105	0.94	92	0.87	92
Intermediate lines				
GR21	0.75	38	0.65	20
GR63	0.75	42	0.65	27
GR105	0.71	38	0.67	32

Broad sense heritabilities (H) were calculated using either the full sample or subsets of lines (whose number is indicated by 'n') with intermediate phenotypes (GR $\geq$ 5% and $\leq$ 95%).

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we modelled the effect of genotype (G; line), environment (E; maturation temperature) and the interaction of both (G x E; line x maturation temperature) [40]. G and G x E effects contributed equally after 21 days (37% and 38%, respectively), but the purely genetic effect increased over time. Environment effects were responsible for 20% of the variance regardless of time point (Table 2). Although the accuracy of this analysis is limited because of the relatively small sample size (due to many of the tested lines being too dormant to be 'informative'), these findings agree with previous studies and indicate that the dormancy variation observed in the Swedish sample is, to a large extent, explained by G x E effects [17, 27–29].

Genetic variation in the response to low seed maturation temperatures

It is thus clear that the effect of the maternal environment differs between Swedish genotypes. About one third of the mild- and non-dormant lines appeared to be relatively insensitive to the maternal environment after 21 days, and one line, Gro-3, reached 100% of germination in both conditions. In sharp contrast, other non-dormant lines such as Löv-1 and T480 were heavily affected by the maternal environment and displayed very low germination rates when seeds were produced at low temperatures, even after 105 days of after-ripening (S2 Fig).

To characterize the genetic variation in the response to low seed maturation temperatures, we clustered lines based on their germination phenotypes across environments and time. We identified six main clusters representing distinct germination behaviors (Fig 2). The largest cluster (cluster 1; n = 43) is not only deeply dormant but also insensitive to after-ripening, making it 'non-informative' in the sense that it is not possible to assess its degree of responsiveness to temperature. Two smaller clusters (clusters 2; n = 12, and 3; n = 14) display shallow to mild dormancy at 21˚C that can be lifted with after-ripening, but the cold treatment induces deep dormancy that can not be broken. Two clusters (5 and 6, n = 11 and n = 8, respectively) are both non-dormant at 21˚C, but cold seed maturation temperatures dramatically increased dormancy levels of the former, but had very little effect on the latter. This last observation clearly confirms that there is natural genetic variation in the response to low temperatures and

Table 2. Genetic and environmental effects on seed dormancy variation.

	G	E	G x E	noise
GR21	37.27	20.48	38.13	4.13
GR63	50.06	19.46	19.18	11.30
GR105	47.75	22.33	24.86	5.06

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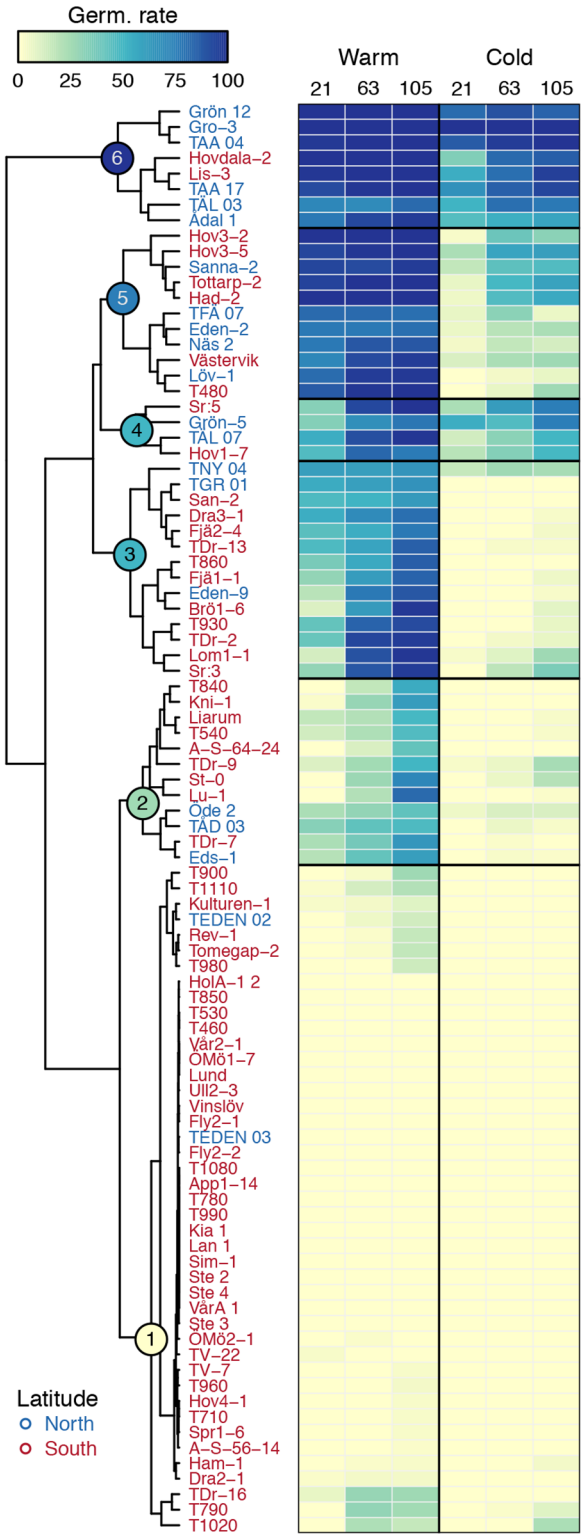

Fig 2. The effect of seed maturation temperature on germination trajectories. Clustering dendrogram reporting the high disparity in germination trajectories across maternal environments (warm or cold) and time (21, 63 or 105 days of after-ripening). The six major clusters are numbered from 1 to 6 and are indicated with colored circles on the nodes of the dendrogram. Lines names are colored according to latitude of origin: south Sweden (red) is defined as the region below 60˚N and north Sweden (blue) as the region above 60˚N. Heatmap colors represent germination phenotypes, with darker shades indicating higher germination rates.

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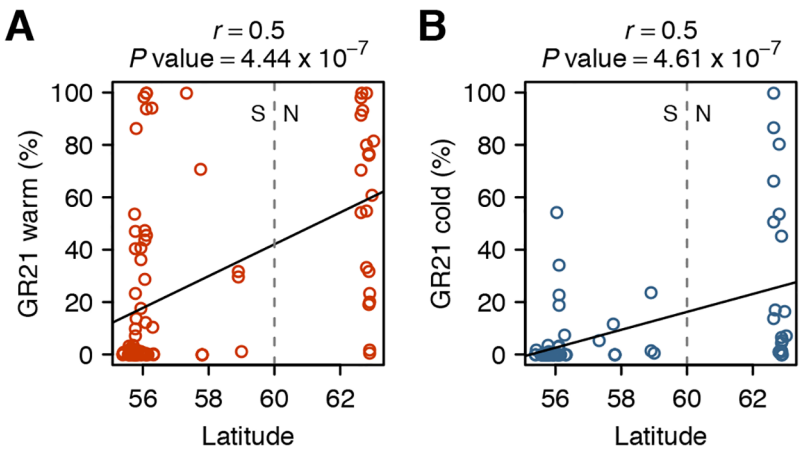

Fig 3. The geographic pattern of the dormancy variation. Correlation between latitude and either (A) GR21 warm or (B) GR21 cold. As in Fig 2, we define south Sweden (S) as the region below 60˚N and north Sweden (N) as the region above 60˚N. See S1 Fig for the correlations between latitude and the other dormancy phenotypes.

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that genotype-environment interactions underlie dormancy variation in the Swedish population. A spectacular example of this differential response can be found in the opposite trajectories of Gro-3 (cluster 6) and Lövv-1 (cluster 5) (Fig 2 and S2 Fig). Finally, a small cluster (cluster 4; n = 4) shows low dormancy regardless of the maternal environment, demonstrating that the degree of responsiveness is independent of the dormancy level. [27] have previously shown that similar germination phenotypes and trajectories can be reached via different paths, and although we only assess the effect of pre-dispersal temperatures, our results go in the same direction. For instance, cluster 5 and to some extent cluster 3 are non-dormant when seeds are produced at 21˚C, but lower seed maturation temperatures induced a deep dormancy, comparable to that of cluster 1.

Geographic pattern of the response to low maturation temperatures

It is well established that seed dormancy in *A. thaliana* correlates with latitude and climate variables such as temperature and precipitation, with northern lines generally being less dormant than southern ones [21, 22, 48]. This geographic pattern, thought to reflect local adaptation, was also observed in this study: both GR21 warm (Fig 3A; $r = 0.5$, $P = 4.44 \times 10^{-7}$) and cold (Fig 3B; $r = 0.5$, $P = 4.61 \times 10^{-7}$) are correlated with latitude. However, we note that these relationships are not strict, possibly reflecting adaptation to microenvironmental variation.

When grown under cold maternal conditions, almost all non-dormant lines from the south appear to be severely affected, and exhibit strongly reduced germination rates. Northern lines, however, display a greater variation in their response to low seed maturation temperature, with some genotypes being insensitive (Fig 3B). This suggests that the response not only varies along a latitudinal gradient but also at a very local scale. These findings are nicely captured by the above-mentioned clustering approach, in which most lines from northern Sweden are binned in the sensitive and insensitive clusters 5 and 6, respectively (Fig 2).

GWAS for the response to low seed maturation temperatures

To uncover the polymorphisms underlying the differential response to low seed maturation temperatures, we assessed the significance of associations between the seed dormancy phenotypes and genome-wide SNP markers from the 1001 genomes project using a mixed-model

accounting for population structure [42–44]. Four lines with missing genotype information were removed from the dataset, bringing the number of lines to 88 (S1 Table). GWAS results were very comparable between time points, as expected given the strong correlations between traits within treatments (S1 Fig), but they differed markedly between treatments, with no strong association for the warm phenotypes while several peaks reached genome-wide significance for the cold phenotypes (Fig 4).

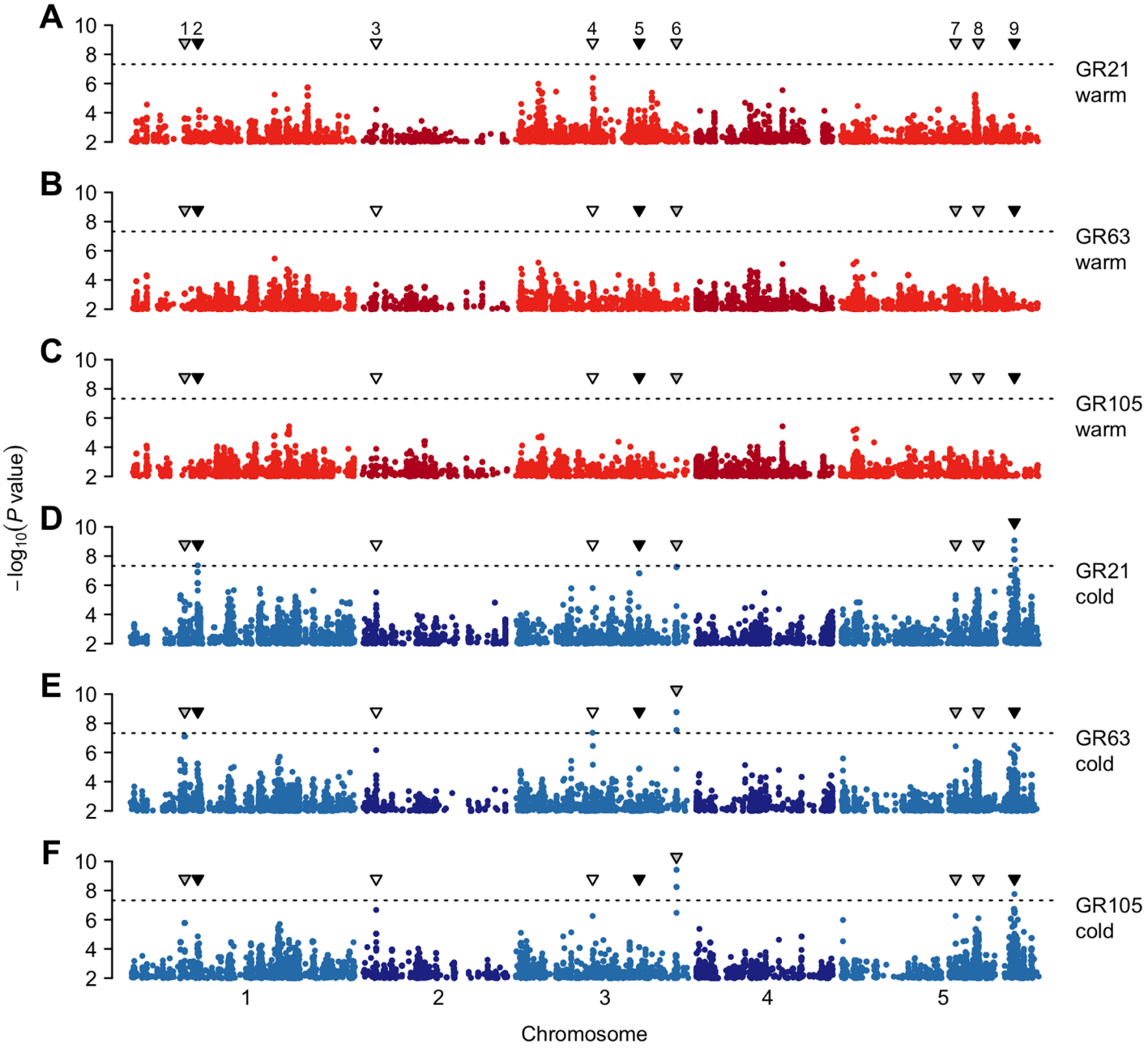

Fig 4. GWAS for seed dormancy traits. Manhattan plots of genome-wide association results for germination rate of seeds set either in (A-C) warm or (D-F) cold environments and after-ripened for (A and D) 21, (B and E) 63 or (C and F) 105 days. The dotted horizontal line indicates a significance level of 0.05 after Bonferroni correction for multiple testing. Triangles show the position of the nine peaks with *P* values < 10^{-6} for at least one phenotype. Triangle color indicates the type of effect: white, 'common'; black, 'specific'; grey, 'unclear'. Are only displayed SNPs with a minor allele frequency ≥ 14%. The GWAS results are fully browsable online: https://goo.gl/dt53nc.

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Table 3. GWAS for seed dormancy traits summary.

Peak	Chr.	Pos.	MAF	Warm			Cold			Effect	Number of genes	Candidate genes
				GR21	GR63	GR105	GR21	GR63	GR105			
1	1	7381921	0.28	3.60	3.06	2.47	4.86	7.10	5.77	unclear	10	*URGT2*
2	1	9148998	0.14	0.33	0.47	0.46	**7.36**	5.21	4.86	specific	25	*SNS1*
3	2	1882558	0.26	4.23	3.69	3.89	5.52	6.16	6.67	common	12	–
4	3	10504938	0.22	5.32	3.79	2.57	5.80	**7.35**	6.26	common	17	–
5	3	16820806	0.14	1.97	1.83	1.39	6.82	4.89	4.11	specific	8	*PHOT1*
6	3	21867111	0.15	3.19	3.36	2.39	7.24	**8.76**	**9.42**	unclear	10	–
7	5	15630623	0.14	2.80	3.27	2.88	5.32	6.42	6.26	unclear	10	–
8	5	18726653	0.20	2.96	2.06	1.58	4.99	5.21	6.09	unclear	11	–
9	5	23596831	0.14	1.22	1.80	1.75	**9.07**	6.48	**7.76**	specific	34	*SIP1, PHOT2*

Are listed top SNPs for the nine GWAS peaks with a score [$-\log_{10}(P \text{ value})$] ≥ 6 for at least one phenotype. Genome-wide significant scores (≥ 7.33; Bonferroni correction) are highlighted in bold. The number of genes encompassed by each peak is also indicated.

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To explore the GWAS results, we first performed an *a priori* gene enrichment analysis using a set of 91 genes with known or predicted function in seed dormancy regulation (S2 Table). No enrichment was detected for any of the six traits, but the fact that *DOG1* is the most strongly associated *a priori* candidate suggests that some of the associations are true signals rather than noise (S3 Table).

Next, we looked at the associations in greater detail, limiting ourselves to an arbitrarily chosen *P* value cutoff of 10^{-6}. This yielded a total of nine regions across the six phenotypes, regions that we classified into three categories (see Material and methods): those with a 'common' effect (they tend to have a similar effect on both warm and cold phenotypes), those with a 'specific' effect (they tend to influence only the cold phenotypes), and last, those with an 'unclear' effect (Fig 4 and Table 3).

The only two associations with a 'common' effect, peaks 3 and 4, lie on chromosomes 1 and 2, respectively, but no clear candidate could be identified among the genes tagged by those peaks (S4 Table). The major 'specific' hit on chr. 5 (peak 9) falls directly in *SOS3-INTERACTING PROTEIN 1* (*SIP1*), a gene encoding a SnRK3-type protein kinase likely to be involved in stress and ABA signalling [49, 50]. However, as the peak is quite broad (more than 100 kb; see S3 Fig), we also examined the 33 other genes present in the associated region (S4 Table). Among those was *PHOTOTROPIN2* (*PHOT2*), a promising candidate not only because of its role in the photoregulation of germination [51], but also because *PHOT1*, a gene with similar function, was identified on chr. 3 below peak 5 (also 'specific'). The third 'specific' association, peak 1, colocalizes with *SnRK2-substrate 1* (*SNS1*), a gene required in ABA signalling [52]. Among the four remaining peaks with an 'unclear' effect, we note the presence of *URGT2*, a seed specific gene controlling mucilage formation (chr. 1, peak 2) [53]. Finally, in contrast with our previous work [22], no strong association was detected at the *DOG1* locus (peak 8, see S4 Fig), a point we discuss below.

Discussion

The regulation of seed dormancy by maternal environment temperature has been described in numerous plant species and appears to be conserved among higher plants [12]. In *A. thaliana*, the underlying mechanism has mainly been studied at the molecular level, using very specific, often artificial backgrounds [17, 27]. Few studies have approached this temperature-dependent regulation from a natural variation perspective, and both its extent and genetic basis remain

unknown. Here, by focusing on a set of Swedish lines, we aimed to characterize this phenomenon at the population level and to identify its underlying genetic basis.

The role of maternally-regulated dormancy in plant adaptation

Despite the prevailing deep dormancy in the Swedish sample, several lines let us assess the effect of maternal temperature on seed dormancy variation (Fig 1). In agreement with previous reports [17, 27, 28], we find that, although low seed production temperatures generally increase primary dormancy, the effect differs between lines, indicating that the trait is influenced by genotype-environment interactions. This observation is further supported by a variance component analysis, which estimates that almost 40% of the dormancy variation in the Swedish sample is due to G x E effects (Table 2; GR21). This, and the fact that high G x E variation was previously observed in a set of world-wide lines [28], suggests that the maternal regulation of seed dormancy by environmental cues is conserved not only at the population, but also at the species level.

By combining different genotypes and seed maturation temperatures, [27] have demonstrated that identical germination trajectories can be achieved by going down different paths. Likewise, we found that cold seed maturation temperatures can produce highly dormant phenotypes, similar in depth to those caused by genetic effects, showing that environmental variation can have large repercussions on the expression of genetic variation (Fig 2).

Because the timing of germination is one of the major fitness components in *A. thaliana* [22, 23], the idea that such maternal regulation may be adaptive is attractive, although the rationale for its existence in this species is yet to be established [27]. In Sweden, where *A. thaliana* mainly behaves as a winter annual, seed dispersal usually occurs in spring, and germination in fall. Therefore, we hypothesize that Swedish populations use ambient temperatures to fine-tune the depth of primary dormancy, should flowering happen earlier or later in the season. This is especially true in northern Sweden, where plants vernalize before winter [54] and usually flower as soon as the snow melts (daylength and temperature permitting), the timing of which is likely to vary from year to year.

On the other hand, it is difficult to make sense of the great variability in the response to low temperatures observed among northern lines (Figs 2 and 3B), as one would expect low dormancy levels to be necessary to make the most of an extremely short growing season (which is the norm at these latitudes). This suggest that these lines, despite their common geographical origin and high vernalization requirement [54, 55], have different germination phenologies. Alternatively, although modelling approaches predict that reproduction occurs under similar temperatures across the species range [56], it is possible that populations from northern Sweden set seeds in slightly warmer temperatures [57], which would diminish the environmental effect and result in weaker dormancy.

Germination in Sweden also happens—to a much lesser extent—in spring and/or summer, as it is not uncommon to observe flowering plants at different times of the year in some southern Swedish populations (Kerdaffrec and Nordborg, personal observations). This could be evidence of bet-hedging, and it is clear that, in this case, the ability to adjust dormancy levels through maternal regulation according to seasonal environment would be advantageous. However, a constant monitoring of these populations across several years would be necessary to rule out the possibility that distinct genotypes expressing different life cycles segregate at these locations.

The genetic basis of the response to low seed maturation temperatures

Although the molecular mechanisms involved in the temperature-dependent maternal regulation of seed dormancy are being revealed, its underlying genetic basis has not been studied yet.

Here, by performing a GWAS on six dormancy traits, we identify a total of nine distinct associations, three of which have a 'specific' (i.e., interaction) effect (Table 3). SNPs within these three peaks are associated with high germination rates in response to cold seed maturation temperatures, which suggests that they tag genes involved in the temperature-dependent regulation of dormancy.

Among the candidates for the 'specific' genes, we identified *PHOT1* and *PHOT2*, which both encode phototropins that mediate several light-dependent processes such as hypocotyl phototropism [58], stomatal opening [59] and germination [51]. Light, along with temperature, is one of the factors regulating primary dormancy induction, and later in the soil seed bank dormancy release and germination. Light-induced germination is mainly promoted by phytochromes, especially *PHYB* and *PHYA* [60–62], and phototropins are assumed to act downstream of them, by modulating the germination response via the integration of light and temperature signals [51]. Interestingly, *TRANSPARENT TESTA 12* (*TT12*), a gene central to the induction of coat-imposed dormancy in response to low seed maturation temperatures [25], was identified earlier in a GWAS for germination traits under various light treatments [63]. This stresses the point that light and temperature signalling pathways may interact both during the induction and the release of dormancy. Therefore, it is possible that *PHOT1* and *PHOT2* play a role in dormancy regulation, direct evidence of which remains to be established. Finally, it should be mentioned that *TT12* and *PHYTOCHROME-INTERACTING FACTOR-LIKE 6* (*PIL6*), a gene negatively regulating *PHYB* [64], are located 45 kb and 35 kb downstream of the strongest association detected in our analysis (peak 6, chr.3), respectively.

Although our GWAS identified compelling candidates, we emphasize that most signals are driven by the same few lines, a consequence of the small sample size and the limited phenotypic variation. Indeed, most of the associated SNPs, and especially those with a 'specific' effect, are often private to a small subset of non-dormant, cold-temperature-insensitive northern lines (Table 3). Some of these associations may also be false positives due to confounding by population structure, although quantile-quantile plots do not show extremely inflated *P* values (S5 Fig).

As previously mentioned, both the power and the resolution of our GWAS are undermined by the limited dormancy variation observed among Swedish lines. Conspicuously, almost half of the tested lines are deeply dormant ('non-informative' lines). In future experiments, it could be interesting to focus only on lines with mild- or non-dormant phenotypes, or alternatively, to apply variable cold stratification treatments to gradually alleviate dormancy and maximize the variation. On the other hand, classical quantitative trait locus (QTL) mapping could be performed in segregating populations derived from contrasted lines such as, for example, Gro-3 (insensitive) and Löv-1 (sensitive) (S2 Fig).

Finally, we have previously shown by performing a GWAS on 161 Swedish lines that *DOG1* is the major regulator of seed dormancy in Sweden [22]. The *DOG1* region was also associated in the present study (S4 Fig), but to a lesser degree, although similar phenotypes were used in both cases (GR21 warm). There are two likely reasons for this discrepancy. First, the phenotypes are not perfectly correlated (Pearson's $r = 0.84$) and several lines were slightly more dormant in this study than in the previous (S6 Fig), reflecting the plastic nature of seed dormancy. Secondly, even if the previously identified *DOG1* alleles segregate among the lines used here, a different sample size is likely to give different results because of the pitfalls intrinsic to GWAS (altered power, changes in allele frequencies, epistasis, among others) [65]. As a demonstration, a GWAS on both GR21 warm phenotypes (previous and present) using the exact same set of lines (86, the overlap between both studies) gave very similar results at the *DOG1* locus (S7 Fig).

Conclusion

In this study, we confirm that the maternal environment interacts with genotype in controlling seed dormancy variation and characterize this interaction in a natural variation context, at the population level. Our GWAS results, in spite of their limitations, agree with the fact that the maternal environment impacts the genetic basis of seed dormancy, although functional evidence is required to validate these findings and confirm the role of the identified candidates.

Because the genes and pathways involved in the regulation of environmentally-dependent transitions are starting to be well characterized, it will become increasingly possible to integrate them into predictive models that, ultimately, could be validated in the field. This should lead towards a better understanding of the molecular and genetic basis of genotype-environment interactions, which is not only important to evolutionary biology [66, 67] but also to modern agriculture, especially in the light of climate change [68–70].

Supporting information

S1 Fig. Pairwise correlations between seed dormancy traits and latitude. Lower panel: pairwise scatter plots showing the relationships between variables; Upper panel: pairwise Pearson's correlation coefficients between variables. All correlations presented here are significant ($P < 0.05$).
(TIF)

S2 Fig. The diverse germination trajectories in Sweden. Replicates are represented using different types of dashed lines while plain lines indicate mean values. Red and blue lines correspond to warm (21˚C) and cold (15˚C) maternal environments, respectively.
(TIF)

S3 Fig. Enlarged view of the region surrounding peak 9 (1 Mb). Local manhattan plots for (A-C) GR21-GR105 warm and (D-F) GR21-GR105 cold. Triangles in (A) denote the *PHOT2* and *SIP1* locus and SNP color reflects the extent of linkage disequilibrium (LD) starting from the most strongly associated SNP in the region at position 23,596,831 on chromosome 5.
(TIF)

S4 Fig. Enlarged view of the region surrounding peak 8 (1 Mb). Local manhattan plots for (A-C) GR21-GR105 warm and (D-F) GR21-GR105 cold. Triangle in (A) denotes the *DOG1* locus. SNP color reflects the extent of LD starting from the most strongly associated SNP in the vicinity of the *DOG1* locus at position 18,580,359 on chromosome 5.
(TIF)

S5 Fig. Quantile-quantile plots of GWAS *P* values. Comparisons of the expected and observed -$\log_{10}$(*P* value) from GWAS for GR21-GR105 warm (A-C, red) and GR21-GR105 cold (D-F, blue). Are only displayed common SNPs (minor allele frequency $\geq$ 14%).
(TIF)

S6 Fig. The relationship between present and previously published GR21 phenotypes.
(TIF)

S7 Fig. *DOG1* region association scans for GR21 warm phenotypes. Local scans for (A) the GR21 warm phenotype from the present study and (B) the previously published GR21 warm phenotype [22]. The exact same set of lines (86, the overlap between both studies) was used for the local scans.
(TIF)

S1 Table. List of the 92 Swedish accessions used in this study. Accessions that were excluded from the GWAS are marked with '0' in the 'GWAS panel' column.
(CSV)

S2 Table. List of *a priori* seed dormancy genes used in this study.
(CSV)

S3 Table. Top associated *a priori* seed dormancy genes. Seed dormancy genes with a score $[-\log_{10}(P \text{ value})] \geq 4$ for at least one phenotype.
(CSV)

S4 Table. GWAS for seed dormancy traits full summary and associated genes.
(CSV)

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References

1. Chiang George C K, Barua Deepak, Dittmar Emily, Kramer Elena M, de Casas Rafael Rubio, and Donohue Kathleen. Pleiotropy in the wild: the dormancy gene DOG1 exerts cascading control on life cycles. *Evolution*, 67(3):883–893, March 2013. https://doi.org/10.1111/j.1558-5646.2012.01828.x PMID: 23461337
2. Koornneef Maarten, Alonso-Blanco Carlos, Peeters Anton J M, and Soppe Wim. GENETIC CONTROL OF FLOWERING TIME IN ARABIDOPSIS. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 49:345–370, June 1998. https://doi.org/10.1146/annurev.arplant.49.1.345 PMID: 15012238
3. El-Soda Mohamed, Malosetti Marcos, Zwaan Bas J, Koornneef Maarten, and Aarts Mark G M. Genotype × environment interaction QTL mapping in plants: lessons from arabidopsis. *Trends Plant Sci.*, 19 (6):390–398, 2014. https://doi.org/10.1016/j.tplants.2014.01.001 PMID: 24491827
4. Amasino Richard. Vernalization, competence, and the epigenetic memory of winter. *Plant Cell*, 16 (10):2553–2559, October 2004. https://doi.org/10.1105/tpc.104.161070 PMID: 15466409
5. Sheldon C C, Rouse D T, Finnegan E J, Peacock W J, and Dennis E S. The molecular basis of vernalization: the central role of FLOWERING LOCUS C (FLC). *Proc. Natl. Acad. Sci. U. S. A.*, 97(7):3753–3758, 28 March 2000. https://doi.org/10.1073/pnas.060023597 PMID: 10716723
6. Donohue Kathleen. Germination timing influences natural selection on life-history characters in arabidopsis thaliana. *Ecology*, 83(4):1006–1016, 2002. https://doi.org/10.1890/0012-9658(2002)083%5B1006:GTINSO%5D2.0.CO;2
7. Donohue Kathleen, de Casas Rafael Rubio, Burghardt Liana, Kovach Katherine, and Willis Charles G. Germination, postgermination adaptation, and species ecological ranges. *Annu. Rev. Ecol. Evol. Syst.*, 41(1):293–319, 2010. https://doi.org/10.1146/annurev-ecolsys-102209-144715

8. Fenner M. The effects of the parent environment on seed germinability. *Seed Sci. Res.*, 1991. https://doi.org/10.1017/S0960258500000696
9. Baskin Carol C and Baskin Jerry M. *Seeds: Ecology, Biogeography, and, Evolution of Dormancy and Germination*. Academic Press, SanDiego, California, USA, 1998.
10. Huang Ziyue, Ölçer-Footitt Hulya, Footitt Steven, and Finch-Savage William E. Seed dormancy is a dynamic state: variable responses to pre- and post-shedding environmental signals in seeds of contrasting arabidopsis ecotypes. *Seed Sci. Res.*, 25(02):159–169, 12 June 2015. https://doi.org/10.1017/S096025851500001X
11. Footitt Steven, Huang Ziyue, Clay Heather A, Mead Andrew, and Finch-Savage William E. Temperature, light and nitrate sensing coordinate arabidopsis seed dormancy cycling, resulting in winter and summer annual phenotypes. *Plant J.*, 74(6):1003–1015, June 2013. https://doi.org/10.1111/tpj.12186 PMID: 23590427
12. Penfield Steven and MacGregor Dana R. Effects of environmental variation during seed production on seed dormancy and germination. *J. Exp. Bot.*, 10 December 2016.
13. Finch-Savage William E and Footitt Steven. Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. *J. Exp. Bot.*, 24 January 2017. https://doi.org/10.1093/jxb/erw477 PMID: 28391330
14. Chiang George C K, Bartsch Melanie, Barua Deepak, Nakabayashi Kazumi, Debieu Marilyne, Kronholm Ilkka, Koornneef Maarten, Soppe Wim J J, Donohue Kathleen, and de Meaux Juliette. DOG1 expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in arabidopsis thaliana. *Mol. Ecol.*, 20(16):3336–3349, 2011. https://doi.org/10.1111/j.1365-294X.2011.05181.x PMID: 21740475
15. Kendall Sarah L, Hellwege Anja, Marriot Poppy, Whalley Celina, Graham Ian A, and Penfield Steven. Induction of dormancy in arabidopsis summer annuals requires parallel regulation of DOG1 and hormone metabolism by low temperature and CBF transcription factors. *Plant Cell*, 23(7):2568–2580, July 2011. https://doi.org/10.1105/tpc.111.087643 PMID: 21803937
16. Huang Ziyue, Footitt Steven, and Finch-Savage William E. The effect of temperature on reproduction in the summer and winter annual arabidopsis thaliana ecotypes bur and cvi. *Ann. Bot.*, 113(6):921–929, May 2014. https://doi.org/10.1093/aob/mcu014 PMID: 24573642
17. He Hanzi, de Souza Vidigal Deborah, Snoek L Basten, Schnabel Sabine, Nijveen Harm, Hilhorst Henk, and Bentsink Leónie. Interaction between parental environment and genotype affects plant and seed performance in arabidopsis. *J. Exp. Bot.*, 65(22):6603–6615, December 2014. https://doi.org/10.1093/jxb/eru378 PMID: 25240065
18. Reddy L V, Metzger R J, and Ching T M. Effect of temperature on seed dormancy of wheat. *Crop Sci.*, 25:455–458, 1985. https://doi.org/10.2135/cropsci1985.0011183X002500030007x
19. Peters N C B. The dormancy of wild oat seed (avena fatua l.) from plants grown under various temperature and soil moisture conditions. *Weed Res.*, 22(4):205–212, 1 August 1982. https://doi.org/10.1111/j.1365-3180.1982.tb00165.x
20. Bentsink Leónie, Jowett Jemma, Hanhart Corrie J, and Koornneef Maarten. Cloning of DOG1, a quantitative trait locus controlling seed dormancy in arabidopsis. *Proc. Natl. Acad. Sci. U. S. A.*, 103(45):17042–17047, 7 November 2006. https://doi.org/10.1073/pnas.0607877103 PMID: 17065317
21. Kronholm Ilkka, Xavier Picó F, Alonso-Blanco Carlos, Goudet Jérôme, and de Meaux Juliette. Genetic basis of adaptation in arabidopsis thaliana: local adaptation at the seed dormancy QTL DOG1. *Evolution*, 66(7):2287–2302, July 2012. https://doi.org/10.1111/j.1558-5646.2012.01590.x PMID: 22759302
22. Kerdaffrec Envel, Filiault Danièle L, Korte Arthur, Sasaki Eriko, Nizhynska Viktoria, Seren Ümit, and Nordborg Magnus. Multiple alleles at a single locus control seed dormancy in swedish arabidopsis. *Elife*, 5:e22502, 14 December 2016. https://doi.org/10.7554/eLife.22502 PMID: 27966430
23. Postma Froukje M and Ågren Jon. Early life stages contribute strongly to local adaptation in arabidopsis thaliana. *Proc. Natl. Acad. Sci. U. S. A.*, 21 June 2016. https://doi.org/10.1073/pnas.1606303113 PMID: 27330113
24. Huang Xueqing, Schmitt Johanna, Dorn Lisa, Griffith Converse, Effgen Sigi, Takao Shaun, Koornneef Maarten, and Donohue Kathleen. The earliest stages of adaptation in an experimental plant population: strong selection on QTLS for seed dormancy. *Mol. Ecol.*, 19(7):1335–1351, April 2010. https://doi.org/10.1111/j.1365-294X.2010.04557.x PMID: 20149097
25. MacGregor Dana R, Kendall Sarah L, Florance Hannah, Fedi Fabio, Moore Karen, Paszkiewicz Konrad, Smirnoff Nicholas, andPenfield Steven. Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism. *New Phytol.*, 205(2):642–652, January 2015. https://doi.org/10.1111/nph.13090 PMID: 25412428
26. Chen Min, MacGregor Dana R, Dave Anuja, Florance Hannah, Moore Karen, Paszkiewicz Konrad, Smirnoff Nicholas, Graham Ian A, and Penfield Steven. Maternal temperature history activates

flowering locus T in fruits to control progeny dormancy according to time of year. *Proc. Natl. Acad. Sci. U. S. A.*, 111(52):18787–18792, 30 December 2014. https://doi.org/10.1073/pnas.1412274111 PMID: 25516986

27. Burghardt Liana T, Edwards Brianne R, and Donohue Kathleen. Multiple paths to similar germination behavior in arabidopsis thaliana. *New Phytol.*, 209(3):1301–1312, February 2016. https://doi.org/10.1111/nph.13685 PMID: 26452074

28. Penfield Steven and Springthorpe Victoria. Understanding chilling responses in arabidopsis seeds and their contribution to life history. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 367(1586):291–297, 19 January 2012. https://doi.org/10.1098/rstb.2011.0186 PMID: 22144391

29. Schmuths Heike, Bachmann Konrad, Weber W Eberhard, Horres Ralf, and Hoffmann Matthias H. Effects of preconditioning and temperature during germination of 73 natural accessions of arabidopsis thaliana. *Ann. Bot.*, 97(4):623–634, April 2006. https://doi.org/10.1093/aob/mcl012 PMID: 16464878

30. Postma Froukje M and Ågren Jon. Maternal environment affects the genetic basis of seed dormancy in arabidopsis thaliana. *Mol. Ecol.*, 24(4):785–797, February 2015. https://doi.org/10.1111/mec.13061 PMID: 25640699

31. Donohue Kathleen, Dorn Lisa, Griffith Converse, Kim Eunsuk, Aguilera Anna, Polisetty Chandra R, and Schmitt Johanna. The evolutionary ecology of seed germination of arabidopsis thaliana: variable natural selection on germination timing. *Evolution*, 59(4):758–770, April 2005. https://doi.org/10.1111/j.0014-3820.2005.tb01751.x PMID: 15926687

32. Galloway Laura F and Etterson Julie R. Transgenerational plasticity is adaptive in the wild. *Science*, 318(5853):1134–1136, 16 November 2007. https://doi.org/10.1126/science.1148766 PMID: 18006745

33. Venable D Lawrence and Brown Joel S. The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *Am. Nat.*, 131(3):360–384, 1988. https://doi.org/10.1086/284795

34. Simons Andrew M and Johnston Mark O. Environmental and genetic sources of diversification in the timing of seed germination: implications for the evolution of bet hedging. *Evolution*, 60(11):2280–2292, November 2006. PMID: 17236421

35. Wilczek A M, Burghardt L T, Cobb A R, Cooper M D, Welch S M, and Schmitt J. Genetic and physiological bases for phenological responses to current and predicted climates. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 365(1555):3129–3147, 12 October 2010. https://doi.org/10.1098/rstb.2010.0128 PMID: 20819808

36. Mitchell Jack, Johnston Iain G, and Bassel George W. Variability in seeds: biological, ecological, and agricultural implications. *J. Exp. Bot.*, 26 October 2016. https://doi.org/10.1093/jxb/erw397

37. Long Quan, Rabanal Fernando A, Meng Dazhe, Huber Christian D, Farlow Ashley, Platzer Alexander, Zhang Qingrun, Vilhjálmsson Bjarni J, Korte Arthur, Nizhynska Viktoria, Voronin Viktor, Korte Pamela, Sedman Laura, Mandáková Terezie, Lysak Martin A, Seren Ümit, Hellmann Ines, and Nordborg Magnus. Massive genomic variation and strong selection in arabidopsis thaliana lines from sweden. *Nat. Genet.*, 45(8):884–890, August 2013. https://doi.org/10.1038/ng.2678 PMID: 23793030

38. Alonso-Blanco Carlos, Bentsink Leónie, Hanhart Corrie J, Blankestijn-de Vries Hetty, and Koornneef Maarten. Analysis of natural allelic variation at seed dormancy loci of arabidopsis thaliana. *Genetics*, 164(2):711–729, June 2003. PMID: 12807791

39. R Core Team. R: A language and environment for statistical computing. R foundation for statistical computing, vienna, austria. 2013, 2014.

40. Sasaki Eriko, Zhang Pei, Atwell Susanna, Meng Dazhe, and Nordborg Magnus. "missing" G x E variation controls flowering time in arabidopsis thaliana. *PLoS Genet.*, 11(10):e1005597, 2015. https://doi.org/10.1371/journal.pgen.1005597 PMID: 26473359

41. Lippert Christoph, Casale Francesco Paolo, Rakitsch Barbara, and Stegle Oliver. LIMIX: genetic analysis of multiple traits. 22 May 2014.

42. Kang Hyun Min, Sul Jae Hoon, Service Susan K, Zaitlen Noah A, Kong Sit-Yee, Freimer Nelson B, Sabatti Chiara, and Eskin Eleazar. Variance component model to account for sample structure in genome-wide association studies. *Nat. Genet.*, 42(4):348–354, April 2010. https://doi.org/10.1038/ng.548 PMID: 20208533

43. Zhang Zhiwu, Ersoz Elhan, Lai Chao-Qiang, Todhunter Rory J, Tiwari Hemant K, Gore Michael A, Bradbury Peter J, Yu Jianming, Arnett Donna K, Ordovas Jose M, and Buckler Edward S. Mixed linear model approach adapted for genome-wide association studies. *Nat. Genet.*, 42(4):355–360, April 2010. https://doi.org/10.1038/ng.546 PMID: 20208535

44. The 1001 Genomes Consortium. 1,135 genomes reveal the global pattern of polymorphism in arabidopsis thaliana. *Cell*, 166(2):481–491, 14 July 2016. https://doi.org/10.1016/j.cell.2016.05.063 PMID: 27293186

45. Seren Ümit, Vilhjálmsson Bjarni J, Horton Matthew W, Meng Dazhe, Forai Petar, Huang Yu S, Long Quan, Segura Vincent, and Nordborg Magnus. GWAPP: a web application for genome-wide association mapping in arabidopsis. *Plant Cell*, 24(12):4793–4805, December 2012. https://doi.org/10.1105/tpc.112.108068 PMID: 23277364

46. Graeber Kai, Nakabayashi Kazumi, Miatton Emma, Leubner-Metzger Gerhard, and Soppe Wim J J. Molecular mechanisms of seed dormancy. *Plant Cell Environ.*, 35(10):1769–1786, October 2012. https://doi.org/10.1111/j.1365-3040.2012.02542.x PMID: 22620982

47. Filiault Daniele L and Maloof Julin N. A genome-wide association study identifies variants underlying the arabidopsis thaliana shade avoidance response. *PLoS Genet.*, 8(3):e1002589, 15 March 2012. https://doi.org/10.1371/journal.pgen.1002589 PMID: 22438834

48. Debieu Marilyne, Tang Chunlao, Stich Benjamin, Sikosek Tobias, Effgen Sigi, Josephs Emily, Schmitt Johanna, Nordborg Magnus, Koornneef Maarten, and de Meaux Juliette. Co-variation between seed dormancy, growth rate and flowering time changes with latitude in arabidopsis thaliana. *PLoS One*, 8 (5):e61075, 23 May 2013. https://doi.org/10.1371/journal.pone.0061075 PMID: 23717385

49. Halfter U, Ishitani M, and Zhu J K. The arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. *Proc. Natl. Acad. Sci. U. S. A.*, 97(7):3735–3740, 28 March 2000. https://doi.org/10.1073/pnas.040577697 PMID: 10725350

50. Hrabak Estelle M, Chan Catherine W M, Gribskov Michael, Harper Jeffrey F, Choi Jung H, Halford Nigel, Kudla Jorg, Luan Sheng, Nimmo Hugh G, Sussman Michael R, Thomas Martine, Walker-Simmons Kay, Zhu Jian-Kang, and Harmon Alice C. The arabidopsis CDPK-SnRK superfamily of protein kinases. *Plant Physiol.*, 132(2):666–680, June 2003. https://doi.org/10.1104/pp.102.011999 PMID: 12805596

51. Jedynak Paweł, Myśliwa-Kurdziel Beata, Turek Elżbieta, and Malec Przemysław. Photoinduction of seed germination in arabidopsis thaliana is modulated by phototropins. *Acta Biol. Crac. Ser. Bot.*, 55 (1), 2013.

52. Umezawa Taishi, Sugiyama Naoyuki, Takahashi Fuminori, Anderson Jeffrey C, Ishihama Yasushi, Peck Scott C, and Shinozaki Kazuo. Genetics and phosphoproteomics reveal a protein phosphorylation network in the abscisic acid signaling pathway in arabidopsis thaliana. *Sci. Signal.*, 6(270):rs8, 9 April 2013. https://doi.org/10.1126/scisignal.2003509 PMID: 23572148

53. Rautengarten Carsten, Ebert Berit, Moreno Ignacio, Temple Henry, Herter Thomas, Link Bruce, Doñas-Cofré Daniela, Moreno Adrián, Saéz-Aguayo Susana, Blanco Francisca, Mortimer Jennifer C, Schultink Alex, Reiter Wolf-Dieter, Dupree Paul, Pauly Markus, Heazlewood Joshua L, Scheller Henrik V, and Orellana Ariel. The golgi localized bifunctional UDP-rhamnose/UDP-galactose transporter family of arabidopsis. *Proc. Natl. Acad. Sci. U. S. A.*, 111(31):11563–11568, 5 August 2014. https://doi.org/10.1073/pnas.1406073111 PMID: 25053812

54. Duncan Susan, Holm Svante, Questa Julia, Irwin Judith, Grant Alastair, and Dean Caroline. Seasonal shift in timing of vernalization as an adaptation to extreme winter. *Elife*, 4, 23 July 2015. https://doi.org/10.7554/eLife.06620

55. Shindo Chikako, Lister Clare, Crevillen Pedro, Nordborg Magnus, and Dean Caroline. Variation in the epigenetic silencing of FLC contributes to natural variation in arabidopsis vernalization response. *Genes Dev.*, 20(22):3079–3083, 15 November 2006. https://doi.org/10.1101/gad.405306 PMID: 17114581

56. Springthorpe Vicki and Penfield Steven. Flowering time and seed dormancy control use external coincidence to generate life history strategy. *Elife*, 4, 31 March 2015. https://doi.org/10.7554/eLife.05557 PMID: 25824056

57. Burghardt Liana T, Metcalf C Jessica E, and Donohue Kathleen. A cline in seed dormancy helps conserve the environment experienced during reproduction across the range of arabidopsis thaliana. *Am. J. Bot.*, 103(1):47–59, January 2016. https://doi.org/10.3732/ajb.1500286 PMID: 26744481

58. Zhao Xiang, Wang Yan-Liang, Qiao Xin-Rong, Wang Jin, Wang Lin-Dan, Xu Chang-Shui, and Zhang Xiao. Phototropins function in high-intensity blue light-induced hypocotyl phototropism in arabidopsis by altering cytosolic calcium. *Plant Physiol.*, 162(3):1539–1551, July 2013. https://doi.org/10.1104/pp.113.216556 PMID: 23674105

59. Kinoshita T, Doi M, Suetsugu N, Kagawa T, Wada M, and Shimazaki K. Phot1 and phot2 mediate blue light regulation of stomatal opening. *Nature*, 414(6864):656–660, 6 December 2001. https://doi.org/10.1038/414656a PMID: 11740564

60. Shinomura T, Nagatani A, Chory J, and Furuya M. The induction of seed germination in arabidopsis thaliana is regulated principally by phytochrome B and secondarily by phytochrome a. *Plant Physiol.*, 104(2):363–371, February 1994. https://doi.org/10.1104/pp.104.2.363 PMID: 12232088

61. Heschel M Shane, Selby Jessica, Butler Colleen, Whitelam Garry C, Sharrock Robert A, and Donohue Kathleen. A new role for phytochromes in temperature-dependent germination. *New Phytol.*, 174 (4):735–741, 2007. https://doi.org/10.1111/j.1469-8137.2007.02044.x PMID: 17504457

62. Jiang Zhimin, Xu Gang, Jing Yanjun, Tang Weijiang, and Lin Rongcheng. Phytochrome B and REVEILLE1/2-mediated signalling controls seed dormancy and germination in arabidopsis. *Nat. Commun.*, 7:12377, 10 August 2016. https://doi.org/10.1038/ncomms12377 PMID: 27506149

63. Morrison Ginnie D and Linder C Randal. Association mapping of germination traits in arabidopsis thaliana under light and nutrient treatments: searching for G×E effects. *G3*, 4(8):1465–1478, August 2014. https://doi.org/10.1534/g3.114.012427 PMID: 24902604

64. Fujimori Toru, Yamashino Takafumi, Kato Takahiko, and Mizuno Takeshi. Circadian-controlled basic/helix-loop-helix factor, PIL6, implicated in light-signal transduction in arabidopsis thaliana. *Plant Cell Physiol.*, 45(8):1078–1086, August 2004. https://doi.org/10.1093/pcp/pch124 PMID: 15356333

65. Korte Arthur and Farlow Ashley. The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods*, 9(1):1–9, 2013. https://doi.org/10.1186/1746-4811-9-29

66. Via Sara and Lande Russell. Genotype-Environment interaction and the evolution of phenotypic plasticity. *Evolution*, 39(3):505–522, 1985. https://doi.org/10.1111/j.1558-5646.1985.tb00391.x PMID: 28561964

67. Fournier-Level A, Korte A, Cooper M D, Nordborg M, Schmitt J, and Wilczek A M. A map of local adaptation in arabidopsis thaliana. *Science*, 334(6052):86–89, 7 October 2011. https://doi.org/10.1126/science.1209271 PMID: 21980109

68. Li Yan, Cheng Riyan, Spokas Kurt A, Palmer Abraham A, and Borevitz Justin O. Genetic variation for life history sensitivity to seasonal warming in arabidopsis thaliana. *Genetics*, 196(2):569–577, February 2014. https://doi.org/10.1534/genetics.113.157628 PMID: 24281156

69. Fournier-Level Alexandre, Perry Emily O, Wang Jonathan A, Braun Peter T, Migneault Andrew, Cooper Martha D, Metcalf C Jessica E, and Schmitt Johanna. Predicting the evolutionary dynamics of seasonal adaptation to novel climates in arabidopsis thaliana. *Proc. Natl. Acad. Sci. U. S. A.*, 2 May 2016. https://doi.org/10.1073/pnas.1517456113 PMID: 27140640

70. Saranga Yehoshua, Menz Mónica, Jiang Chun-Xiao, Wright Robert J, Yakir Dan, and Paterson Andrew H. Genomic dissection of genotype× environment interactions conferring adaptation of cotton to arid conditions. *Genome Res.*, 11(12):1988–1995, 2001. https://doi.org/10.1101/gr.157201 PMID: 11731488