

1 **Title:**

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

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5 **Running title:**

6 Joint effect of temperature and genotype on dormancy variation

7
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23 The maternal environment interacts with genetic
24 variation in regulating seed dormancy in *Arabidopsis*
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26 **Highlight**

27 The effect of low seed-maturation temperatures on seed dormancy is highly variable in
28 *Arabidopsis thaliana* accessions from Sweden, denoting strong genotype-environment
29 interactions, and a genome-wide association study identified compelling candidates that
30 could account for this plasticity.

31 **Abstract**

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

43 **Key-words**

44 *Arabidopsis thaliana*, genotype-environment interaction, germination, GWAS, maternal
45 environment, plasticity, temperature, seed dormancy

46 **Introduction**

47 Life-stage transitions, the timing of which is critical to plant fitness, are regulated by both
48 genes and the environment, usually in interaction (G x E) (El-Soda *et al.*, 2014; Koornneef *et*

49 *al.*, 1998; Chiang *et al.*, 2013). Plants have evolved ways to sense and integrate
50 environmental inputs in order to adjust their life cycle to seasonal environments, the best-
51 known example of which is vernalization and the perception of winter cold (Amasino, 2004).
52 In *A. thaliana*, vernalization results in the stable repression of the central regulator
53 *FLOWERING LOCUS C (FLC)*, a prerequisite for the vegetative-to-reproductive transition to
54 occur (Sheldon *et al.*, 2000).

55 While flowering time determines the reproductive environment, seed dormancy,
56 another major life-history trait crucial for local adaptation, regulates the timing of
57 germination and determines the post-germination environment (Donohue, 2002; Donohue *et*
58 *al.*, 2010; Chiang *et al.*, 2013). Dormancy is highly plastic and can be modulated both by pre-
59 and post-dispersal environmental cues such as temperature, light, and to a lesser extent, nitrate
60 (Fenner, 1991; Baskin and Baskin, 1998; Footitt *et al.*, 2013; Huang *et al.*, 2015; Penfield and
61 MacGregor, 2016; Finch-Savage and Footitt, 2017). In particular, low temperatures during
62 seed production dramatically increase dormancy in *A. thaliana* (Chiang *et al.*, 2011; Kendall
63 *et al.*, 2011; Huang *et al.*, 2014; He *et al.*, 2014) as well as in other species such as wheat
64 (Reddy *et al.*, 1985) and wild oat (Peters, 1982).

65 Central to this temperature-dependent process in *A. thaliana* is the upregulation of a
66 major genetic determinant of seed dormancy variation, *DELAY OF GERMINATION1*
67 (*DOG1*) (Bentsink *et al.*, 2006; Chiang *et al.*, 2011; Kendall *et al.*, 2011). The *DOG1* locus
68 exhibits genetic signatures suggestive of local adaptation, and field experiments have
69 emphasized its pivotal role in controlling the timing of germination in wild *A. thaliana*
70 populations (Huang *et al.*, 2010; Kronholm *et al.*, 2012; Postma and Ågren, 2016; Kerdaffrec
71 *et al.*, 2016). Independently of their action on *DOG1*, low seed maturation temperatures
72 induce deep dormancy by increasing the abscisic acid (ABA) / gibberellins (GA) ratio, two
73 antagonistic phytohormones repressing and activating germination, respectively (Chiang *et*
74 *al.*, 2011; Kendall *et al.*, 2011). Finally, low temperatures can promote coat-imposed
75 dormancy by altering seed coat permeability through the upregulation of the flavonoids
76 biosynthesis pathway, both during seed production (MacGregor *et al.*, 2015) and vegetative
77 phase (Chen *et al.*, 2014).

78 Thus, it is clear that the induction of primary dormancy is regulated by both genetic
79 and environmental factors, and the fact that distinct genotypes differ in their response to low
80 temperatures indicates that genotype-environment interactions partly control the trait
81 (Schmuths *et al.*, 2006; Penfield and Springthorpe, 2012; He *et al.*, 2014; Burghardt *et al.*,
82 2016a). A direct consequence of this plasticity is that similar germination trajectories,

83 defined as the evolution of the germination phenotype over time, can be promoted by
84 different combinations of genotypes and environments. For example, strong *DOG1* alleles
85 combined with a warm maternal environment and weak *DOG1* alleles combined with a cold
86 maternal environment can both lead to highly dormant phenotypes (Burghardt *et al.*, 2016a).

87 Field studies have demonstrated that the maternal environment contributes greatly to
88 seed dormancy variation under natural conditions (Postma and Ågren, 2015). Thus, given the
89 existence of strong selection for timing of germination (Donohue *et al.*, 2005; Huang *et al.*,
90 2010; Postma and Ågren, 2016; Kerdaffrec *et al.*, 2016), it has been speculated that the
91 temperature-dependent regulation of primary dormancy is adaptive. For example, it may
92 provide the mother plant with information regarding the seasonal environment, information
93 that can be used to set dormancy appropriately (Galloway and Etterson, 2007). In addition,
94 this mechanism is expected to enable bet-hedging strategies, in which seeds from the same
95 population — or plant — express various dormancy phenotypes and germinate throughout
96 the year to maximize fitness in unpredictable environments (Venable and Brown, 1988;
97 Simons and Johnston, 2006; Wilczek *et al.*, 2010; Mitchell *et al.*, 2016).

98 Although genotype-environment interactions have previously been reported to
99 influence dormancy and germination plasticity in *A. thaliana* (Schmuths *et al.*, 2006; Penfield
100 and Springthorpe, 2012; He *et al.*, 2014; Burghardt *et al.*, 2016a), the extent of this
101 phenomenon and whether it is universal at the species level is unknown. Moreover, the
102 genetic basis of the differential response to seed maturation temperatures, and more
103 generally, of G x E variation, remains to be thoroughly investigated. Here, by growing a set
104 of *A. thaliana* lines from Sweden in two different environments, we assess the effect of
105 temperature on seed dormancy in a local sample, before performing a GWAS to identify the
106 genes responsible for the observed variation.

107 Material and methods

108 *Plant material and phenotyping*

109 The 92 Swedish lines used in this study (Table S1) were previously described by Kerdaffrec
110 *et al.* (2016). For each genotype, six biological replicates were vernalized for eight weeks
111 (4°C, standard long days, 90% humidity). Then, three randomly chosen replicates were
112 placed in a warm environment (21°C day, 16°C night), while the other three received a cold
113 treatment (15°C day, 10°C night). Both treatments were applied from rosette stage to ripening

114 and seed harvest. Seeds were harvested when about 50% of the siliques of a given plant had
115 come to maturity and were subsequently placed in dry environment for after-ripening (30%
116 relative humidity, 16°C, dark). The germination rate seeds after-ripened for 21, 63 and 105
117 days (GR21, GR63 and GR105) was estimated by scoring radicle emergence after a week of
118 incubation at 23°C under standard long days (Alonso-Blanco *et al.*, 2003; Kerdaffrec *et al.*,
119 2016).

120 *Broad sense heritability*

121 Broad sense heritabilities (H) were calculated as the genotypic variance divided by the total
122 variance. Both variances were estimated using a linear mixed model from the lme4 package
123 in the R framework (R Core Team, 2014). The model was as follows:

124

$$125 \quad Y \sim GEN + REP + e$$

126

127 where *GEN* is the genotype (line), *REP* is technical replicate, and *e* is the error. All
128 variables were fitted as random effects.

129 *Variance components analysis*

130 The variance-component analysis was described earlier by Sasaki *et al.* (2015). It was carried
131 out in LIMIX (Lippert *et al.*, 2014) using the following model:

132

$$133 \quad Y = [\mu_{warm}, \mu_{cool}] \otimes \mathbf{I}_{N,1} + U_{global} + \psi$$

134

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

137 *Genome-wide association mapping*

138 Genome-wide scans were performed on arcsine transformed mean phenotypic values using a
139 mixed-model accounting for population structure and SNPs derived from the 1001 genomes
140 project (Kang *et al.*, 2010; Zhang *et al.*, 2010; The 1001 Genomes Consortium, 2016). The
141 analysis was performed using the GWA-Portal (<https://gwas.gmi.oeaw.ac.at/>; Seren *et al.*,
142 2012), and both settings and results can be viewed interactively online: <http://bit.ly/2lkzAsp>.
143 In this manuscript, rare alleles (minor allele frequency < 14%) were filtered and a 5%
144 genome-wide significance threshold was determined using Bonferroni correction. A given

145 peak was considered to have a ‘specific’ effect when its highest score for any of the three
146 warm phenotypes did not exceed 2, or a ‘common’ effect when its score for at least one
147 phenotype in both environment was higher than 4. Peaks that did not meet any of these
148 arbitrary requirements were considered to have an ‘unclear’ effect.

149 *Gene enrichment analyses*

150 The seed dormancy *a priori* candidate gene list (91 genes; Table S2) was built regardless of
151 the GWAS results by searching the literature (Kendall *et al.*, 2011; Graeber *et al.*, 2012) and
152 the ARAPORT11 database. In a nutshell, a gene was considered significantly associated
153 when at least one SNP in the 40 kb surrounding its coding sequence had a *P* value lower than
154 10^{-4} . Lists of non *a priori* significantly associated TAIR11 genes were built in a similar
155 manner for each phenotype, and the overrepresentation of *a priori* genes in the resulting lists
156 was assessed using Fisher exact tests.

157 *Data availability*

158 Both raw and processed data used in this manuscript have been uploaded at GitHub:
159 <https://github.com/Gregor-Mendel-Institute/dormancy>.

160 **Results**

161 *Seed dormancy variation in the Swedish population*

162 We have previously shown that there is extensive natural variation for seed dormancy among
163 *A. thaliana* lines from Sweden, and that focusing on such a local sample increases one’s
164 ability to detect local adaptation (Kerdaffrec *et al.*, 2016). For these reasons, we used a set of
165 92 Swedish lines (Table S1) to assess the effect of the maternal environment on dormancy
166 variation. Several replicates of each line were vernalized for 8 weeks before being allowed to
167 self-fertilize and produce seed under either warm (21°C) or cold (15°C) temperatures. The
168 dormancy levels of the seeds were estimated by measuring their germination rate (GR) as a
169 function of seed age (21, 63 and 105 days), also known as after-ripening, resulting in six
170 different dormancy primary phenotypes (GR21, GR63 and GR105, for both warm and cold
171 treatments) (Fig. 1).

172 As expected, genotypes that experienced warm maternal temperature displayed great
173 variation for seed dormancy, although more than one third of the lines remained dormant
174 even after 105 days (Fig. 1). In contrast, the vast majority of seeds produced at cold maternal

175 temperature remained dormant throughout the experiment, in line with previous studies that
176 have shown that a decrease in seed maturation temperature generally induces a deeper
177 dormancy (Chiang *et al.*, 2011; Kendall *et al.*, 2011; Penfield and Springthorpe, 2012; Huang
178 *et al.*, 2014; He *et al.*, 2014; Burghardt *et al.*, 2016a).

179 All six phenotypes were correlated, especially within temperature treatments (Fig.
180 S1), and broad sense heritabilities were remarkably high (ranging from 0.85 to 0.94),
181 although they became moderate when considering lines with intermediate phenotypes only
182 (GR > 5% and < 95%; ranging from 0.67 to 0.75; Table 1). To assess the relative effects of
183 genes and the environment on dormancy variation, we performed a variance components
184 analysis, in which we modelled the effect of genotype (G; line), environment (E; maturation
185 temperature) and the interaction of both (G x E; line x maturation temperature) (Sasaki *et al.*,
186 2015). G and G x E effects contributed equally after 21 days (37 and 38 percent,
187 respectively), but the purely genetic effect increased over time. Environment effects were
188 responsible for 20% of the variance regardless of time point (Table 2). Although the accuracy
189 of this analysis is limited because of the relatively small sample size (due to many of the
190 tested lines being too dormant to be ‘informative’), these findings agree with previous studies
191 and indicate that the dormancy variation observed in the Swedish sample is, to a large extent,
192 explained by G x E effects (Schmuths *et al.*, 2006; Penfield and Springthorpe, 2012; He *et*
193 *al.*, 2014; Burghardt *et al.*, 2016a).

194 *Genetic variation in the response to low seed maturation temperatures*

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

202 To characterize the genetic variation in the response to low seed maturation
203 temperatures, we clustered lines based on their germination phenotypes across environments
204 and time. We identified six main clusters representing distinct germination behaviors (Fig. 2).
205 The largest cluster (cluster 1; n = 43) is not only deeply dormant but also insensitive to after-
206 ripening, making it ‘non-informative’ in the sense that it is not possible to assess its degree of
207 responsiveness to temperature. Two smaller clusters 2 (clusters 2; n = 12, and 3; n = 14)

208 display shallow to mild dormancy at 21°C that can be lifted with after-ripening, but the cold
209 treatment induces deep dormancy that cannot be broken. Two clusters (5 and 6, $n = 11$ and n
210 $= 8$, respectively) are both non-dormant at 21°C, but cold seed maturation temperatures
211 dramatically increased dormancy levels of one, but had very little effect on the latter. This
212 last observation clearly confirms that there is natural genetic variation in the response to low
213 temperatures and that genotype-environment interactions underlie dormancy variation in the
214 Swedish population. A spectacular example of this differential response can be found in the
215 opposite trajectories of Gro-3 (cluster 6) and Löv-1 (cluster 5) (Fig. 2 and S2). Finally, a
216 small cluster (cluster 4; $n = 4$) shows low dormancy regardless of the maternal environment,
217 demonstrating that the degree of responsiveness is independent of the dormancy level.

218 Burghardt *et al.* (2016a) have previously shown that similar germination phenotypes
219 and trajectories can be reached via different paths, and although we only assess the effect of
220 pre-dispersal temperatures, our results go in the same direction. For instance, cluster 5 and to
221 some extent cluster 3 are non-dormant when seeds are produced at 21C, but lower seed
222 maturation temperatures induced a deep dormancy, comparable to that of cluster 1.

223 *Geographic pattern of the response to low seed maturation temperatures*

224 It is well established that seed dormancy correlates with latitude and climate variables such as
225 temperature and precipitation, with northern lines generally being less dormant than southern
226 ones (Kronholm *et al.*, 2012; Debieu *et al.*, 2013; Kerdaffrec *et al.*, 2016). This geographic
227 pattern, thought to reflect local adaptation, was also observed in this study: both GR21 warm
228 (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
229 with latitude. However, we note that these relationships are not strict, possibly reflecting
230 adaptation to microenvironmental variation.

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

239 *GWAS for the response to low seed maturation temperatures*

240 To uncover the polymorphisms underlying the differential response to low seed maturation
241 temperatures, we assessed the significance of associations between the seed dormancy
242 phenotypes and genome-wide SNP markers from the 1001 genomes project using a mixed-
243 model accounting for population structure (Kang *et al.*, 2010; Zhang *et al.*, 2010; The 1001
244 Genomes Consortium, 2016). Four lines with missing genotype information were removed
245 from the dataset, bringing the number of lines to 88 (Table S1). GWAS results were very
246 comparable between time points, as expected given the strong correlations between traits
247 within treatments (Fig. S1), but they differed markedly between treatments, with no strong
248 association for the warm phenotypes while several peaks reached genome-wide significance
249 for the cold phenotypes (Fig. 4).

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

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

261 The only two associations with a ‘common’ effect, peaks 3 and 4, lie on
262 chromosomes 1 and 2, respectively, but no clear candidate could be spotted in the vicinity of
263 those peaks. The major ‘specific’ hit on chr. 5 (peak 9) falls directly in *SOS3-INTERACTING*
264 *PROTEIN 1 (SIP1)*, a gene encoding a SnRK3-type protein kinase likely to be involved in
265 stress and ABA signaling (Halfter *et al.*, 2000; Hrabak *et al.*, 2003). However, as the peak is
266 quite broad (more than 100 kb; see Fig. S3), we also examined other genes in the region.
267 Among those was *PHOTOTROPIN2 (PHOT2)*, a promising candidate not only because of its
268 role in the photoregulation of germination, but also because *PHOT1*, a gene with similar
269 function, was identified on chr. 3 below peak 5 (also ‘specific’). Both genes encode blue/UV-
270 A photoreceptors and are known to be involved in the transition from dormancy to
271 germination (Jedynak *et al.*, 2013). The third ‘specific’ association, peak 1, colocalizes with

272 *SnRK2-substrate 1 (SNS1)*, a gene required in ABA signaling (Umezawa *et al.*, 2013).
273 Among the four remaining peaks with an ‘unclear’ effect, we note the presence of *URGT2*, a
274 seed specific gene controlling mucilage formation (chr. 1, peak 2) (Rautengarten *et al.*,
275 2014). Finally, in contrast with our previous work (Kerdaffrec *et al.*, 2016), no strong
276 association was detected at the *DOG1* locus (peak 8, see Fig. S4), a point we discuss below.

277 Discussion

278 The regulation of seed dormancy by maternal environment temperature has been described in
279 numerous plant species and appears to be conserved among higher plants (Penfield and
280 MacGregor, 2016). In *A. thaliana*, the underlying mechanism has mainly been studied at the
281 molecular level, using very specific, often artificial backgrounds (He *et al.*, 2014; Burghardt
282 *et al.*, 2016a). Few studies have approached this temperature-dependent regulation from a
283 natural variation perspective, and both its extent and genetic basis remain unknown. Here, by
284 focusing on a set of Swedish lines, we aimed to characterize this phenomenon at the
285 population level and to identify its underlying genetic basis.

286 *The role of maternally-regulated dormancy in plant adaptation*

287 Despite the prevailing deep dormancy in the Swedish sample, several lines let us assess the
288 effect of maternal temperature on seed dormancy variation (Fig. 1). In agreement with
289 previous reports (Penfield and Springthorpe, 2012; He *et al.*, 2014; Burghardt *et al.*, 2016a),
290 we find that, although low seed production temperatures generally increase primary
291 dormancy, the effect differs between lines, indicating that the trait is influenced by genotype-
292 environment interactions. This observation is further supported by a variance component
293 analysis, which estimates that almost 40% of the dormancy variation in the Swedish sample
294 is due to G x E effects (Table 2; GR21). This, and the fact that high G x E variation was
295 previously observed in a set of world-wide lines (Penfield and Springthorpe, 2012), suggests
296 that the maternal regulation of seed dormancy by environmental cues is conserved not only at
297 the population, but also at the species level.

298 By combining different genotypes and seed maturation temperatures, Burghardt *et al.*
299 (2016a) have demonstrated that identical germination trajectories can be achieved by going
300 down different paths. Likewise, we found that cold seed maturation temperatures can produce
301 highly dormant phenotypes, similar in depth to those caused by genetic effects, showing that

302 environmental variation can have large repercussions on the expression of genetic variation
303 (Fig. 2).

304 Because the timing of germination is one of the major fitness components for *A.*
305 *thaliana* (Postma and Ågren, 2016; Kerdaffrec *et al.*, 2016), the idea that such maternal
306 regulation may be adaptive is attractive, although the rationale for its existence in this species
307 is yet to be established (Burghardt *et al.*, 2016a). In Sweden, where *A. thaliana* mainly
308 behaves as a winter annual, seed dispersal usually occurs in spring, and germination in fall.
309 Therefore, we hypothesize that Swedish populations use ambient temperatures to fine-tune
310 the depth of primary dormancy, should flowering happen earlier or later in the season. This is
311 especially true in northern Sweden, where plants vernalize before winter (Duncan *et al.*,
312 2015) and usually flower as soon as the snow melts (day length and temperature permitting),
313 the timing of which is likely to vary from year to year.

314 On the other hand, it is difficult to make sense of the great variability in the response
315 to low temperatures observed among northern lines (Fig. 2 and 3B), as one would expect low
316 dormancy levels to be necessary to make the most of an extremely short growing season
317 (which is the norm at these latitudes). This suggest that these lines, despite their common
318 geographical origin and high vernalization requirement (Shindo *et al.*, 2006; Duncan *et al.*,
319 2015), have different germination phenologies. Alternatively, although modelling approaches
320 predict that reproduction occurs under similar temperatures across the species range
321 (Springthorpe and Penfield, 2015), it is possible that populations from northern Sweden set
322 seeds in slightly warmer temperatures (Burghardt *et al.*, 2016b), which would diminish the
323 environmental effect and result in weaker dormancy.

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

332 *The genetic basis of the response to low seed maturation temperatures*

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

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

340 Among the candidates for the ‘specific’ genes, we identified *PHOT1* and *PHOT2*,
341 which both encode phototropins that mediate several light-dependent processes such as
342 hypocotyl phototropism (Zhao *et al.*, 2013), stomatal opening (Kinoshita *et al.*, 2001) and
343 germination (Jedynak *et al.*, 2013). Light, along with temperature, is one of the factors
344 regulating primary dormancy induction, and later in the soil seed bank dormancy release and
345 germination. Light-induced germination is mainly promoted by phytochromes, especially
346 *PHYB* and *PHYA* (Shinomura *et al.*, 1994; Heschel *et al.*, 2007; Jiang *et al.*, 2016), and
347 phototropins are assumed to act downstream of them, by modulating the germination
348 response via the integration of light and temperature signals (Jedynak *et al.*, 2013).
349 Interestingly, *TRANSPARENT TESTA 12 (TT12)*, a gene central to the induction of coat-
350 imposed dormancy in response to low seed maturation temperatures (MacGregor *et al.*,
351 2015), was identified earlier in a GWAS for germination traits under various light treatments
352 (Morrison and Linder, 2014). This stresses the point that light and temperature signaling
353 pathways may interact both during the induction and the release of dormancy. Therefore, it is
354 possible that *PHOT1* and *PHOT2* play a role in dormancy regulation, direct evidence of
355 which remains to be established. Finally, it should be mentioned that *TT12* and
356 *PHYTOCHROME-INTERACTING FACTOR-LIKE 6 (PIL6)*, a gene negatively regulating
357 *PHYB* (Fujimori *et al.*, 2004), are located 45 kb and 35 kb downstream of the strongest
358 association detected in our analysis (peak 6, chr.3), respectively.

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

366 As previously mentioned, both the power and the resolution of our GWAS are
367 undermined by the limited dormancy variation observed among Swedish lines.
368 Conspicuously, almost half of the tested lines are deeply dormant (‘noninformative’ lines). In

369 future experiments, it could be interesting to focus only on lines with mild- or non-dormant
370 phenotypes, or alternatively, to apply variable cold stratification treatments to gradually
371 alleviate dormancy and maximize the variation. On the other hand, classical quantitative trait
372 locus (QTL) mapping could be performed in segregating populations derived from contrasted
373 lines such as, for example, Gro-3 (insensitive) and Löv-1 (sensitive) (Fig. S2).

374 Finally, we have previously shown by performing a GWAS on 161 Swedish lines that
375 *DOG1* is the major regulator of seed dormancy in Sweden (Kerdaffrec *et al.*, 2016). The
376 *DOG1* region was also associated in the present study (Fig. S4), but to a lesser degree,
377 although similar phenotypes were used in both cases (GR21 warm). There are two likely
378 reasons for this discrepancy. First, the phenotypes are not perfectly correlated ($r = 0.84$) and
379 several lines were slightly more dormant in this study than in the previous (Fig. S6),
380 reflecting the plastic nature of seed dormancy. Secondly, even if the previously identified
381 *DOG1* alleles segregate among the lines used here, a different sample size is likely to give
382 different results because of the pitfalls intrinsic to GWAS (altered power, changes in allele
383 frequencies, epistasis, among others) (Korte and Farlow, 2013). As a demonstration, a
384 GWAS on both GR21 warm phenotypes (previous and present) using the exact same set of
385 lines (86, the overlap between both studies) gave very similar results at the *DOG1* locus (Fig.
386 S7).

387 *Towards a better understanding of environmentally-dependent life-history transitions*

388 In this study, we confirm that the maternal environment interacts with genotype in controlling
389 seed dormancy variation and characterize this interaction in a natural variation context. Our
390 GWAS results, in spite of their limitations, clearly support the fact the maternal environment
391 impacts the genetic basis of seed dormancy.

392 Because the gene networks and signaling pathways involved in the regulation of
393 environmentally-dependent transitions are starting to be well characterized, it will become
394 increasingly possible to integrate them into predictive models that can later be validated in
395 field experiments. The need for a better understanding of the molecular genetic basis of
396 genotype-environment interactions is real, as they are of importance not only to evolutionary
397 biology (Via and Lande, 1985; Fournier-Level *et al.*, 2011), but also to modern agriculture,
398 especially in the light of climate change (Saranga *et al.*, 2001; Li *et al.*, 2014; Fournier-Level
399 *et al.*, 2016).

400 Supplementary material

- 401 Table S1. List of Swedish lines used in this study.
402 Table S2. List of *a priori* seed dormancy genes used in this study.
403 Table S3. Top associated *a priori* seed dormancy genes.
404 Figure S1. Pairwise correlations between the dormancy traits and latitude.
405 Figure S2. The diverse germination trajectories in Sweden.
406 Figure S3. Enlarged view of the region surrounding peak 9 (1 Mb).
407 Figure S4. Enlarged view of the region surrounding peak 8 (1 Mb).
408 Figure S5. Quantile-quantile plots of GWAS *P* values.
409 Figure S6. The relationship between present and previously published GR21 phenotypes.
410 Figure S7. *DOG1* region association scans for GR21 warm phenotypes.

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Table 1. Heritability of seed dormancy traits. Broad sense heritabilities (H) were calculated using either the full sample or subsets of lines (whose number is indicated by n) with intermediate phenotypes.

	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 (GR \geq 5% and GR \leq 95%)				
GR21	0.75	38	0.65	20
GR63	0.75	42	0.65	27
GR105	0.71	38	0.67	32

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	35.06

Table 3: Summary of the GWAS for seed dormancy phenotypes. Are listed top SNPs for the nine GWAS peaks with a score $[-\log_{10}(P \text{ value})]$ greater than or equal to six for at least one phenotype. Genome-wide significant scores (≥ 7.33) are highlighted in bold. MAF stands for minor allele frequency.

Peak	Chr.	Pos.	MAF	Warm			Cold			Effect	Candidate genes
				GR21	GR63	GR105	GR21	GR63	GR105		
1	1	7,381,921	0.28	3.60	3.06	2.47	4.86	7.10	5.77	unclear	<i>URGT2</i>
2	1	9,148,998	0.14	0.33	0.47	0.46	7.36	5.21	4.86	specific	<i>SNS1</i>
3	2	1,882,558	0.26	4.23	3.69	3.89	5.52	6.16	6.67	common	-
4	3	10,504,938	0.22	5.32	3.79	2.57	5.80	7.35	6.26	common	-
5	3	16,820,806	0.14	1.97	1.83	1.39	6.82	4.89	4.11	specific	<i>PHOT1</i>
6	3	21,867,111	0.15	3.19	3.36	2.39	7.24	8.76	9.42	unclear	-
7	5	15,630,623	0.14	2.80	3.27	2.88	5.32	6.42	6.26	unclear	-
8	5	18,726,653	0.20	2.96	2.06	1.58	4.99	5.21	6.09	unclear	<i>DOG1</i>
9	5	23,596,831	0.14	1.22	1.80	1.75	9.07	6.48	7.76	specific	<i>PHOT2, SIP1</i>

Figure 1. The effect of low seed maturation temperatures 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 either for (A) 21, (B) 63 or (C) 105 days. Error bars represent the standard deviation within genotypes (n = 3, in few cases n = 2).

Figure 2. The effect of maternal environment on germination phenotypes and 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.

Figure 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 Fig. S1 for the correlations between latitude and the other phenotypes.

Figure 4. GWAS for seed dormancy phenotypes. 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 lower than 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 greater than or equal to 14%. The GWAS results can be viewed interactively online:

<http://bit.ly/2lkzAsp>

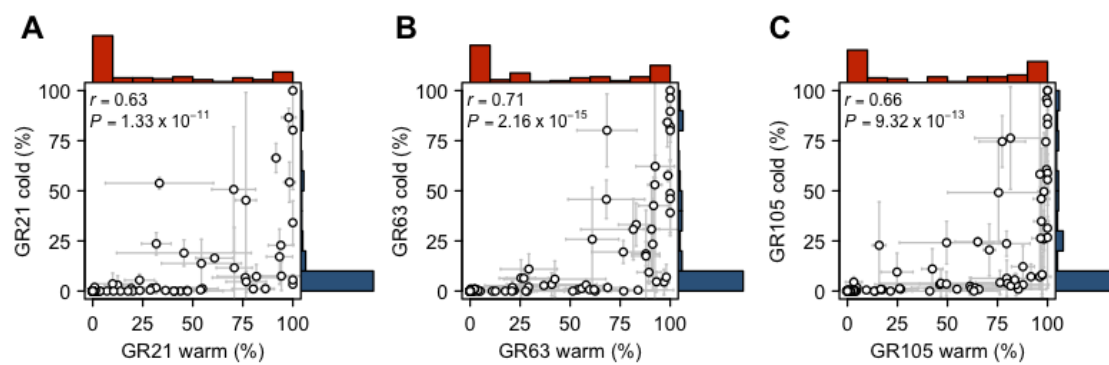


Figure 1.

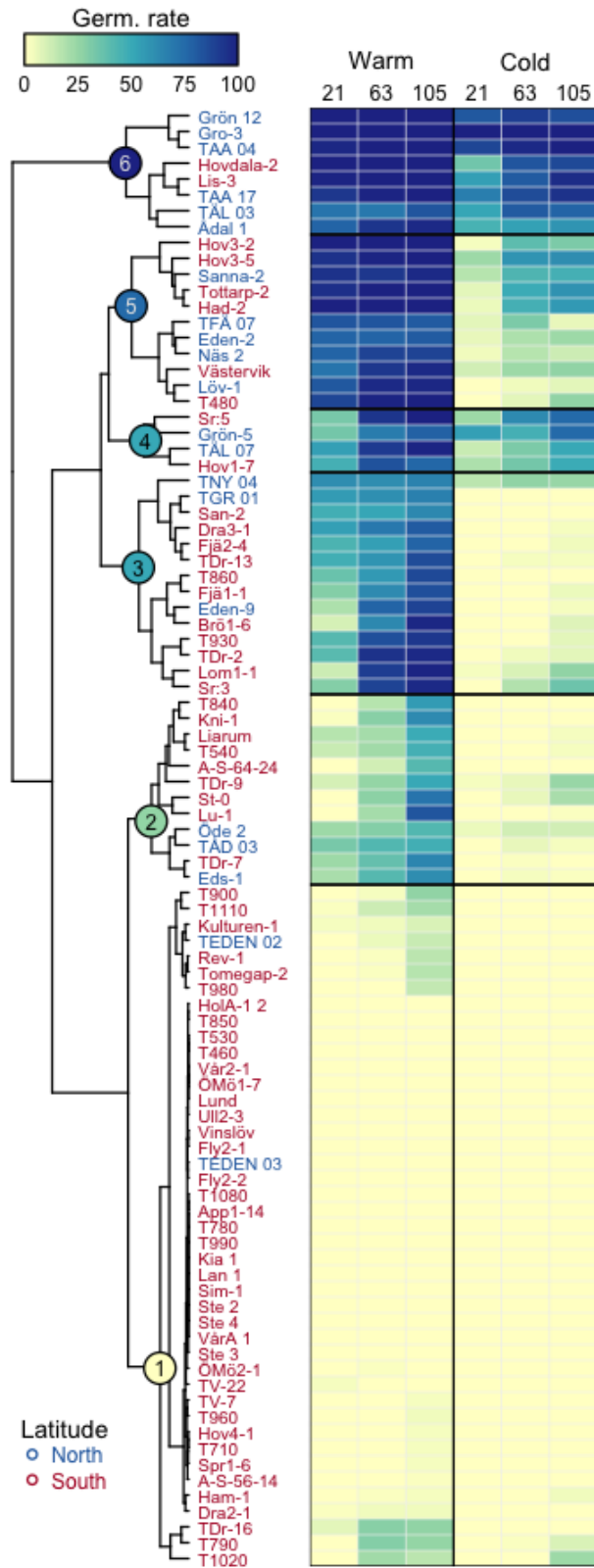


Figure 2.

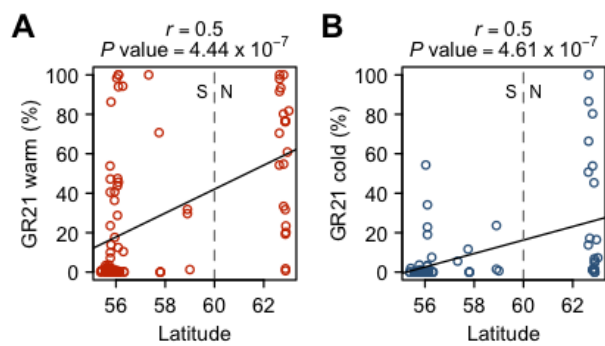


Figure 3.

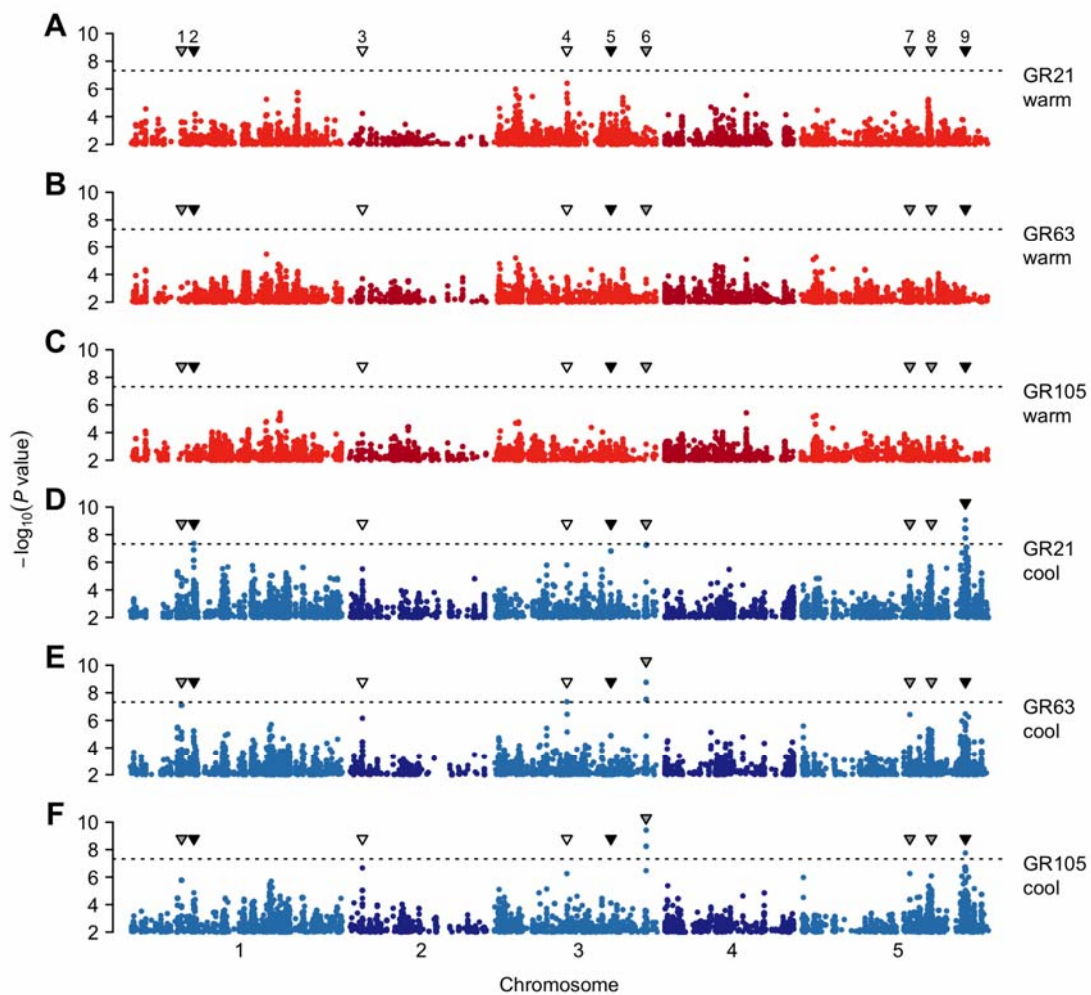


Figure 4.