

1 **Title:**

2 **Assortment of flowering time and immunity alleles in natural *Arabidopsis thaliana***  
3 **populations suggests immunity and vegetative lifespan strategies coevolve**

4  
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14 Running title: Assortment of immunity and flowering time alleles

15

16 **ABSTRACT:**

17

18 The selective impact of pathogen epidemics on host defenses can be strong but remains  
19 transient. By contrast, life-history shifts can durably and continuously modify the balance  
20 between costs and benefits of immunity, which arbitrates the evolution of host defenses. Their  
21 impact on the evolutionary dynamics of host immunity, however, has seldom been documented.  
22 Optimal investment into immunity is expected to decrease with shortening lifespan, because a  
23 shorter life decreases the probability to encounter pathogens or enemies. Here, we document that  
24 in natural populations of *Arabidopsis thaliana*, the expression levels of immunity genes correlate  
25 positively with flowering time, which in annual species is a proxy for lifespan. Using a novel  
26 genetic strategy based on bulk-segregants, we partitioned flowering time-dependent from –  
27 independent immunity genes and could demonstrate that this positive co-variation can be  
28 genetically separated. It is therefore not explained by the pleiotropic action of some major  
29 regulatory genes controlling both immunity and lifespan. Moreover, we find that immunity genes  
30 containing variants reported to impact fitness in natural field conditions are among the genes  
31 whose expression co-varies most strongly with flowering time. Taken together, these analyses  
32 reveal that natural selection has likely assorted alleles promoting lower expression of immunity  
33 genes with alleles that decrease the duration of vegetative lifespan in *A. thaliana* and *vice versa*. This  
34 is the first study documenting a pattern of variation consistent with the impact that selection on  
35 flowering time is predicted to have on diversity in host immunity.

36

37 Keywords: Immunity, Flowering time, Lifespan, *Arabidopsis thaliana*, Transcriptomics, Trade-off,  
38 Pleiotropy, bulk-segregant sequencing

39

40 **INTRODUCTION**

41 The ability of organisms to defend against pathogens is a major determinant of survival in natural  
42 populations (Parker & Gilbert, 2004; Chisholm *et al.*, 2006; Lee & Mazmanian, 2010). Pathogens  
43 have long been suspected to impose a fast evolution of the host immune system and the “Red  
44 Queen” Hypothesis is nowadays a keystone of evolutionary biology (Van Valen, 1973; Liow *et al.*,  
45 2011). Evidence that pathogens drive the molecular evolution of host defense systems has been  
46 accumulating in an array of plant and animal systems (Bergelson *et al.*, 2001; de Meaux &

47 Mitchell-Olds, 2003; Moeller & Tiffin, 2005; Ravensdale *et al.*, 2010; Laine *et al.*, 2010; Maekawa *et al.*, 2011; Dybdahl *et al.*, 2014; Karasov *et al.*, 2014; Siddle & Quintana-Murci, 2014; Parker *et al.*, 2015; Metzger *et al.*, 2016).

50 Yet, the possible impact of changes in ecology on the evolution of defense systems should also  
51 be considered as they may durably change the exposure of hosts to pathogens. Invasive species,  
52 for example, owe much of their success to the release from pathogen and pest pressures (Mitchell  
53 & Power, 2003; Mitchell *et al.*, 2010). Similarly, shifts in life history can alter the balance between  
54 costs and benefits of host defense systems (Herms & Mattson, 1992). Shifting from perennial to  
55 annual life cycles, or evolving from a winter-annual to summer-annual cycling occurs frequently  
56 across plant phylogenies (Garnier, 1992; Michaels *et al.*, 2003; Franks *et al.*, 2007; Tank &  
57 Olmstead, 2008; Matthew Ogburn & Edwards, 2015; Kiefer *et al.*, 2017). The reduction in  
58 lifespan that follows such life history changes concomitantly reduces the probability to encounter  
59 enemies (Jokela *et al.*, 2000). As a matter of fact, woody plant species with longer lifespan often  
60 display stronger herbivore defenses (Endara & Coley, 2010). As a consequence, immunity and  
61 lifespan are expected to coevolve.

62 *Arabidopsis thaliana* populations offer an optimal model for catching the co-evolution of life  
63 history and immunity in the act. *A. thaliana* has become over the last decade a powerful model  
64 system to address ecological questions at the genetic level (Mitchell-Olds & Schmitt, 2006;  
65 Bergelson & Roux, 2010; Roux & Bergelson, 2016). Experiments in common gardens have been  
66 performed to describe the architecture of natural variation in fitness and to infer geographic  
67 distributions of locally adaptive mutations (Fournier-Level *et al.*, 2011; Hancock *et al.*, 2011;  
68 Fournier-Level *et al.*, 2016). Analyses of mutants and recombinant inbred lines (RIL) have  
69 allowed reconstructing the contribution of phenotypes to fitness (Wilczek *et al.*, 2009; Chiang *et al.*,  
70 2013; Fournier-Level *et al.*, 2013). Secondary chemical compounds were shown to have  
71 evolved to deter predominant herbivores in natural populations (Brachi *et al.*, 2013; Kerwin *et al.*,  
72 2015). Clinal variation along the latitudinal range of the species reveals how phenotypes  
73 expressed along the life cycle are jointly shaped by natural selection (Lasky, 2012; Debieu *et al.*,  
74 2013; Vidigal *et al.*, 2016).

75 *A. thaliana* is arguably one of the species for which we have the largest amount of genetic and  
76 phenotypic information on both immune reactions against pathogens and variation in the  
77 duration of the vegetative lifespan. As such, it is an optimal model system for assessing the  
78 impact of life history changes, which modify plant vegetative lifespan, on the evolution of the  
79 immunity system. Indeed, in annual (monocarpic) species, which grow and reproduce only once,  
80 flowering time marks the end of the vegetative growth phase. Seed production in monocarpic  
81 species is terminated by senescence and death, so that flowering time provides a good proxy for  
82 lifespan. In *A. thaliana*, it has been scored in a number of conditions (Brachi *et al.*, 2010; Sasaki  
83 *et al.*, 2015; Roux & Bergelson, 2016) and flowering time changes are often locally adaptive (Le  
84 Corre, 2005; Toomajian *et al.*, 2006; Montesinos-Navarro *et al.*, 2011; Debieu *et al.*, 2013; Li *et al.*,  
85 2014; Hu *et al.*, 2017). Natural variation in flowering time can thus be used to investigate the  
86 impact of lifespan changes on host defenses.

87 The immune system has also been intensively studied in this species, revealing multiple layers of  
88 defenses, ranging from basal immunity, which is sufficient to control most microbes, to severe  
89 reactions that actively defeat virulent pathogens (Jones & Dangl, 2006). Strain-specific immunity  
90 components are likely to be linked in their evolution to the virulence specificity of co-occurring  
91 pathogens (de Meaux & Mitchell-Olds, 2003; Moeller & Tiffin, 2005; Roux & Bergelson, 2016).  
92 Recent fluctuations in the composition of the pathogen population may therefore affect the

93 specific components of immunity targeted by these epidemics and thereby mask or blur the long-  
94 term impact of lifespan modifications. To minimize this effect and to highlight the impact of  
95 lifespan variation, we took a genomics approach and examined how flowering time co-varies with  
96 expression levels of genes with an experimentally-supported function in immunity. These  
97 approximately 700 genes jointly reflect a broad spectrum of traits, which, when their expression  
98 increases have a positive effect on immunity (Eulgem, 2005; Vetter *et al.*, 2012; Boccara *et al.*,  
99 2014). We test below whether their expression level, a proxy for their effectiveness, co-varies  
100 with flowering time, a proxy for lifespan in the field and further examine the roles played by  
101 demographic history and pleiotropy in shaping patterns of co-variation.

102

## 103 RESULTS

### 104 Positive co-variation between expression levels of immunity genes and the timing of 105 flowering in Swedish *A. thaliana* populations

106

107 We first focused on a set of 138 genotypes originating from Sweden because high quality data  
108 were available for both genome-wide expression profiles and flowering time estimates (Dubin *et al.*,  
109 2015; Sasaki *et al.*, 2015). These two studies were part of a single experiment in which  
110 flowering time and gene expression were characterized at both 16°C and 10°C under long day  
111 conditions in growth chambers. We focused on the data collected at 16°C and computed  
112 Spearman correlation coefficients between the expression level of each gene and flowering time.  
113 Of 22,686 genes, for which expression levels could be quantified, 1,374 (6%) were significantly  
114 correlated with flowering time under a 5% false discovery rate (FDR). We first verified that genes  
115 annotated for their function in flowering time were among the genes whose expression correlates  
116 with the phenotype. Overall, genes with an experimentally validated function in flowering time in  
117 the genome were not enriched among those genes (6.9% of 630 genes at FDR 0.05,  
118 Hypergeometric test,  $p=0.19$ ), yet the two well-known regulators of flowering time,  
119 FLOWERING LOCUS C and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS-1  
120 (FLC and SOC1, Spearman correlation  $\rho=0.50$  and  $-0.62$ , FDR-corrected  $p=2.87e-6$  and  $p=7e-$   
121  $12$ , respectively) were the two most strongly correlated genes. In addition, using the R-package  
122 TopGO, we examined patterns of functional enrichment among genes that tended to be more  
123 expressed in early flowering genotypes. Many functional gene ontology (GO) categories related to  
124 cell differentiation and growth were enriched (Suppl. Table S1) and the GO category “regulation  
125 of flower development” was among the most over-represented ( $p=8.00e-14$ , Suppl. Table 1).  
126 This observation confirmed the biological relevance of the data set examined.

127 Next, we tested whether immunity genes were enriched among genes whose expression  
128 correlated with the timing of flowering. Among genes with significant correlation with the  
129 phenotype, we observed a significant excess of immunity genes (8.6% of 691 genes at 5% FDR,  
130 hypergeometric test,  $p=0.002$ ). The distribution of correlation coefficients was also significantly  
131 skewed towards higher correlation coefficients for immunity genes (Fig. 2A, Kolmogorov-  
132 Smirnov test,  $p<2.2e-16$ ). GO enrichment analysis showed that genes involved in GO  
133 “oxidation-reduction process” and “response to wounding” were among the most strongly  
134 enriched ( $p<1e-30$ ,  $p=1.1e-19$ , respectively, Suppl. Table 1). This first analysis revealed a  
135 pronounced pattern of positive co-variation between flowering time and the expression of  
136 immunity genes.

137 In laboratory conditions, genotypes with a strong requirement for vernalization tend to show a  
138 strong delay in flowering that often does not translate into late flowering in the field (Brachi *et al.*,

139 2010; Li *et al.*, 2014). Indeed, in the field plants often experience sufficient levels of cold to fulfill  
140 their vernalization requirement. In fact, only the 51 genotypes that advanced their flowering time  
141 at 16°C compared to 10°C (e.g. those that did not need low temperatures to induce flowering),  
142 showed a correlation in their flowering across temperatures (Sasaki *et al.*, 2015). Flowering time  
143 variation across the latter sub-sample of genotypes may therefore allow a more accurate  
144 classification of genotypes with increasing vegetative lifespan. Among the 507 out of 22,686  
145 (2.2%) genes that displayed a significant positive correlation with flowering time at 10% FDR  
146 across this restricted sample of genotypes, 16/630 genes were annotated for their function in  
147 flowering. As in the above, several known flowering time regulators were among the genes  
148 associated with flowering time, such as FLOWERING LOCUS C (FLC), GIGANTEA,  
149 FLOWERING PROMOTING FACTOR 1-LIKE PROTEIN 2 (FLP2) or even the genes  
150 PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and PHYTOCHROME  
151 INTERACTING FACTOR 5 (PIF5), which had been associated with accelerated flowering  
152 (Andrés & Coupland, 2012; Thines *et al.*, 2014). Although the whole set of flowering time genes  
153 was not significantly enriched among genes correlating positively with the timing of flowering  
154 (2.5%, hypergeometric test,  $p=0.24$ ), they tended to be more highly expressed in early flowering  
155 genotypes (excess of negative correlations, Kolmogorov-Smirnov test,  $p=1.16e-13$ , Fig. 2B). The  
156 GO category “regulation of flower development” was even more over-represented in this dataset  
157 ( $p<1e-30$ , Suppl. Table 1). Higher expression of genes associated with the positive regulation of  
158 flowering was observed among early-flowering genotypes. This further confirms that expression  
159 variation was correctly quantified.

160 We also observed that variation in immunity gene expression tended to correlate positively with  
161 variation in flowering time, after excluding vernalization-sensitive genotypes. In total expression  
162 of 28 of the 691 genes belonging to the immunity gene category correlated significantly with  
163 flowering at 10% FDR. They were significantly enriched (4%, 1.8-fold enrichment,  
164 hypergeometric test,  $p=0.0009$ ). Compared to the ensemble of expressed genes in the genome,  
165 they generally tended to be more highly expressed in late flowering genotypes (marked excess of  
166 positive correlations, Kolmogorov-Smirnov test,  $p<2.2e-16$ , Fig. 2B). GO enrichment analysis  
167 showed that genes involved in the GO categories “response to chitin” and “regulation of plant-  
168 type hypersensitive response” were the two most strongly enriched (both  $p<1e-30$ , Suppl. Table  
169 1). We thus conclude that the correlation between expression of immunity genes and the timing  
170 of flowering is independent of allelic variation in vernalization requirements.

171  
172 **Positive co-variation of immunity gene expression with flowering time is independent of**  
173 **population structure and is also detected in a second sample of broader geographic**  
174 **origin**

175  
176 Relatedness among individuals in the sample may drive the correlation between expression of  
177 immunity genes and the timing of flowering. In fact, flowering time in the Swedish lines is  
178 strongly associated with the demographic history of these populations and thus with their  
179 population structure (Dubin *et al.*, 2015; Sasaki *et al.*, 2015). We therefore also computed for each  
180 gene, the correlation between gene expression and flowering time with a mixed-model that  
181 included a kinship matrix for the 51 genotypes that lacked vernalization requirement (see  
182 methods; Yu *et al.*, 2006; Stich *et al.*, 2008). This analysis revealed that, for immunity genes, the  
183 distribution of correlation coefficient estimates remained strongly skewed towards positive  
184 values, after population structure was accounted for (Kolmogorov-Smirnov test,  $p=2.2e-16$ ,



185 Suppl. Fig. 1). However, the whole set of immunity genes was no longer enriched among genes  
186 with a significant co-variation with flowering time (5.2% vs. 5%, hypergeometric test,  $p=0.6$ ).  
187 We note that accounting for population structure also did not change the pattern of co-variation  
188 between gene expression of flowering genes and timing of flowering itself. They showed a  
189 coefficient distribution that was strongly skewed towards negative values (Kolmogorov-Smirnov  
190 test,  $p=2.2e-16$ ) and were significantly over-represented among genes with expression  
191 significantly associated with flowering time (8% vs 5% at 5% FDR, hypergeometric test,  
192  $p=0.0005$ ).  
193 We further investigated whether the skew towards positive co-variation between immunity gene  
194 expression and flowering time is limited to the regional subset of genotypes growing in Sweden  
195 or whether it is a feature of diversity that segregates across the whole range of the species. For  
196 this, we turned to a species-wide dataset of gene expression variation collected in young seedlings  
197 (Schmitz *et al.*, 2013). For 52 of these genotypes, the duration of vegetative growth had been  
198 determined under natural conditions in the field (Brachi *et al.*, 2010). Although a skew towards  
199 negative correlation for flowering time genes was observed (Kolmogorov Smirnov test,  $p=5.3e-5$ ,  
200 Fig. 2C), the seedling of these earlier flowering genotypes did not yet express genes important for  
201 the formation of flower (Suppl. Table 1).  
202 Nevertheless, we again observed a strong skew towards positive correlation between immunity  
203 gene expression and flowering time, indicating that genotypes that will flower later expressed  
204 them at a higher level (Kolmogorov Smirnov test,  $p<2.2e-16$ , Fig. 2C). Immunity genes were not  
205 particularly enriched among genes with significantly correlated expression and flowering time at  
206 5% FDR (5% for both, hypergeometric test,  $p=0.24$ ). Yet, GO categories such as “response to  
207 chitin”, “respiratory burst involved in immunity response”, “response to wounding” and  
208 immunity response to fungus” were the four most strongly enriched functions among genes with  
209 highest Spearman correlation coefficients (all  $p<1e-30$ , Suppl. Table 1).  
210 Contrasting genotypes of diverse flowering time (e.g. lifespan) revealed that, in natural  
211 populations, immunity genes tend to co-vary positively with this trait. The latter two analyses  
212 showed that this effect remained when population structure was accounted for and was also  
213 detectable in another gene expression dataset and with a different set of genotypes.

## 214 215 **A bulk-segregant analysis demonstrates that co-variation is not due to pleiotropic effect** 216 **of flowering time control** 217

218 The tendency of immunity genes to show expression levels correlating positively with flowering  
219 may be due to the pleiotropic action of regulatory genes that co-regulate flowering time and  
220 immunity. In plants, the impact of development and growth regulators on defense systems is  
221 being increasingly recognized (Alcázar *et al.*, 2011). There is evidence that flowering time and  
222 defense control each other (Korves & Bergelson, 2003; Pagán *et al.*, 2008; Fan *et al.*, 2014;  
223 Lozano-Durán & Zipfel, 2015; Jiménez-Góngora *et al.*, 2015; Davila Olivas *et al.*, 2017; Develey-  
224 Riviere & Galiana, 2007; Pajeroska-Mukhtar *et al.*, 2009; Martinez *et al.*, 2004; Whalen, 2005;  
225 Kerwin *et al.*, 2015; Lyons *et al.* 2015). If so, the pattern we observed would not reflect the joint  
226 optimization of immunity and life history strategy but only the pleiotropic action of their  
227 regulators. We therefore asked to which extent flowering time and the expression of immune-  
228 related genes could be genetically separated and thus evolve independently.  
229 We therefore designed an experiment to describe the level of pleiotropy of flowering time  
230 regulators on the expression of immunity genes. If such regulators control the pattern of

231 covariation reported in Fig. 2, it should not be possible to separate variation in immunity gene  
232 expression from variation in flowering time in a segregating recombinant inbred population. We  
233 used the two genotypes Col-0 and Bur-0, which differ in flowering time (Simon *et al.*, 2008) and  
234 were also reported to exhibit markedly distinct sensitivities to flagellin, with the later flowering  
235 genotype Bur-0 displaying stronger basal immunity (Vetter *et al.*, 2012). We analyzed the  
236 transcriptomes of these two lines at 14 and 28 days after germination (see methods) and found  
237 that the skews shown in Fig. 2 remain when the dataset was reduced to the genes that differed in  
238 expression between these two lines (Suppl. Fig. 2). This confirmed that these two genotypes  
239 could help identify immunity genes that share genetic regulators with flowering time.

240 We designed a cost-effective approach to identify the genes whose expression variation cannot be  
241 separated from flowering time. We used 244 recombinant inbred lines (RILs) derived from a  
242 cross between the parents Bur-0 and Col-0, followed by >8 generations of selfing (Simon *et al.*,  
243 2008). We bulked RILs by their flowering time and characterized their transcriptomes at 14 and  
244 28 days after germinations using RNA sequencing (see methods). In RILs, the genomes of the  
245 parental genotypes are randomly shuffled by recombination. Because of this genetic property,  
246 RILs are commonly used to identify Quantitative Trait Loci (QTL), which are genomic regions  
247 underlying the genetic control of phenotypic variation. In our approach, this means that  
248 differences in gene expression between early- and late-flowering RILs reflect differences that are  
249 genetically associated with flowering time. The experimental strategy is described in Suppl. Fig. 3-  
250 4. This strategy does not allow characterizing the exact genetic architecture of gene expression  
251 variation, but it allows the identification of genes whose expression variation is controlled either  
252 by flowering-time regulators or by genes located in the genomic vicinity of these regulators.  
253 Thereafter, we named these genes Flowering-Time (FT)-dependent genes.

254 Of a total of 20,553 genes expressed in both the parental genotypes and RIL pools, 6,097 (29%)  
255 were differentially expressed between early and late flowering RIL pools, i.e. FT-dependent. As  
256 expected, there was a strong excess of genes annotated as having a function in flowering time  
257 among FT-dependent genes (223/630 – 36%, hypergeometric test,  $p=3.7e-5$ ). This demonstrated  
258 that this strategy effectively highlighted genes whose expression is under the genetic control of  
259 flowering time regulators. By contrast, immunity genes were not over-represented among FT-  
260 dependent genes. More so, they were clearly under-represented among FT-dependent genes at  
261 the second time point of sampling (1.15 fold less abundant than expected by chance at day 28,  
262 hypergeometric test,  $p=0.01$ ). Only 19% of all immunity genes were FT-dependent. These genes,  
263 however, did not explain the skew towards positive co-variation with flowering time reported in  
264 Fig. 2. Immunity genes, whose expression was not differently expressed between RIL pools (i.e.  
265 genes whose expression is not dependent on the regulators of flowering time), in fact, tended to  
266 be more skewed towards positive correlation coefficients than FT-dependent immunity genes  
267 (Kolmogorov-Smirnov test,  $p=0.01$ , Fig. 2A). We observed that FT-dependent flowering time  
268 genes did not shift significantly from the distribution of correlation in the rest of the genome  
269 (Kolmogorov-Smirnov test,  $p=0.15$ , Fig. 2A). Therefore, the excess of positive expression co-  
270 variation with flowering time observed among immunity genes is most strongly driven by genes  
271 whose expression level was easily separated from variation in flowering by recombination.

272

### 273 **Age-regulated immunity genes often show positive co-variation with flowering time**

274

275 Immunity genes are often observed to change their activity with age and development (Barton &  
276 Boege, 2017). Because we had sampled material at day 14 and day 28 after germination, we could

277 also separate genes whose expression changed with age (here after named age-regulated genes)  
278 from genes with similar expression levels in 14- and 28-day-old plants (see methods). Age-  
279 regulated genes were markedly more frequent among annotated immunity genes than among  
280 annotated flowering time genes (243/630 – 38% vs 334/691 - 48%, for flowering-time and  
281 immunity genes, respectively, Chi Square test,  $p= 7.2e-11$ ). In *A. thaliana*, a so-called age-related  
282 resistance is activated in older *A. thaliana* plants, providing them with a immunity barrier against a  
283 broad spectrum of pathogens (Rusterucci *et al.*, 2005). In agreement with our findings, the timing  
284 of age-related resistance had been reported not to stand under the direct control of flowering  
285 time (Wilson *et al.*, 2013).

286 The subset of genes, whose expression variation in natural populations correlated with flowering  
287 time, were also enriched among age-regulated genes (hypergeometric test,  $p= 7.2e-11$ ).  
288 Altogether, 4% (348/8565) and 6% (498/7935) of age-independent and age-regulated genes,  
289 respectively, were correlated with flowering time at 5% FDR. Immunity genes contributed  
290 significantly to this excess, because the expression levels of immunity genes that were age-  
291 regulated tended to show a strong skew towards positive correlation with flowering time in  
292 natural populations (Fig. 2B, Kolmogorov-Smirnov test,  $p=0.0009$ ). Our analysis thus indicates  
293 that the tendency of immunity genes to co-vary positively with flowering time in natural  
294 population is i) not explained by the genetic control of flowering time and ii) increased among  
295 genes whose expression is regulated by plant age.

### 296 297 **Genes activated by elicitors of basal immunity also show an excess of positive** 298 **correlations with flowering time**

299  
300 In the above analyses, immunity levels were represented by a set of 731 genes annotated for  
301 functions related to immunity. To test whether this trend towards positive covariation between  
302 immunity gene expression and flowering time was limited to the set of genes defined by Gene  
303 Ontology categories, we analyzed an independent set of immunity-related genes: the 245 genes  
304 whose expression is activated in *Arabidopsis* seedlings upon perception of flagellin by the PAMP  
305 receptor kinase FLAGELLIN SENSING 2 (FLS2), hereafter named FlaRe genes (Navarro *et al.*,  
306 2004). FlaRe genes coordinate cellular and developmental responses to exposure of molecular  
307 signatures of bacteria. Only 10 FlaRe genes overlapped with the immunity-annotated genes used  
308 above. We observed that FlaRe genes were enriched among genes showing positive co-variation  
309 with flowering time (Fig. 2A-C, Kolmogorov-Smirnov test,  $p< 2.2e-16$ ). This observation  
310 remained when accounting for population structure (Suppl. Fig. 1, Kolmogorov-Smirnov test,  
311  $p<2.2e-16$ ) and was also seen for flowering time measured in the field in a species-wide sample  
312 of genotypes (Fig. 2C, Kolmogorov-Smirnov test,  $p<2.2e-16$ ). When partitioning genes according  
313 to whether or not they were FT-dependent or age-regulated, we observed that FT-dependence  
314 did not significantly change the distribution of correlation coefficients between FlaRe gene  
315 expression and flowering time across natural genotypes (Fig. 2A-B, Kolmogorov-Smirnov test,  
316  $p=0.15$  and  $p=0.32$ , for FT-controlled and age-regulated genes, respectively). Nevertheless, FlaRe  
317 genes were significantly under-represented among FT-dependent genes, especially at the second  
318 sampling time point (1.8-fold less frequent among flowering time controlled genes,  
319 hypergeometric test,  $p= 2.24e-05$ ). By contrast, they were over-represented among age-regulated  
320 genes (2.1-fold more frequent among age-regulated genes, hypergeometric test,  $p= 2.6e-08$ ).  
321 Thus, the positive co-variation reported in Fig. 1A-C is unlikely to result from the pleiotropic  
322 action of flowering time regulators on FlaRe genes. This suggests that, like for annotated

323 immunity genes, alleles attenuating the expression of FlaRe genes were assorted with early-  
324 flowering alleles in natural populations and vice versa.

325

### 326 **Fitness-associated immunity genes show higher correlation coefficients with flowering** 327 **time**

328

329 We further asked whether genes with fitness-relevant variation have expression levels that are  
330 more strongly assorted with variation in the timing of flowering. A reciprocal transplant  
331 experiment performed in 4 locations throughout Europe identified 866 nucleotide variants in the  
332 genome of *A. thaliana* that significantly associated with fitness differences manifested in natural  
333 conditions (Fournier-Level *et al.* 2011). Of these variants, 15 mapped to immunity genes and 17  
334 to flowering genes. Association with fitness coincided with a skew towards higher correlation  
335 coefficients for immunity genes only (Fig. 3, Kolmogorov-Smirnov test,  $D=0.46$ ,  $p=0.014$  and  
336  $p>0.05$  for immunity and flowering time genes, respectively). One of the immunity genes  
337 (AT3G16720), which is activated upon exposure to the fungal PAMP chitin, was FT-dependent  
338 but it did not explain this pattern (Kolmogorov-Smirnov test,  $p=0.028$  without AT3G16720).  
339 Five of the immunity genes with FT-independent immune functions were age-regulated  
340 (AT1G18150, AT1G80840, AT4G01700, AT5G19510, AT5G57220) but this did not explain the  
341 pattern either (Kolmogorov-Smirnov test,  $p=0.009$  without these genes). Of the 245 FlaRe genes,  
342 3 contained fitness-associated SNPs. These three genes were among the genes with highest  
343 correlation coefficients (AT1G19670:  $\rho=0.397$ , AT3G16720:  $\rho=0.282$ , AT4G38860:  $\rho=0.487$ ).  
344 We thus observe that immunity genes that can be most relevant for fitness in natural populations  
345 of *A. thaliana* are also genes whose expression levels were most strongly assorted with alleles  
346 determining flowering time.

347

## 348 **DISCUSSION**

349

### 350 **Evidence for concerted evolution of immunity and flowering time in *A. thaliana***

351

352 Our analyses reveal that, in *A. thaliana*, individuals with a shorter vegetative lifespan tend to  
353 express immunity genes at a lower level. The bulk analysis of early- and late-flowering RILs  
354 shows that this pattern of co-variation results from the combination of independent alleles  
355 controlling immunity gene expression and flowering time in natural populations, because these  
356 alleles could be separated in the segregating recombinant offspring of an early- and a late-  
357 flowering genotype. Because co-variation is also i) robust to the demographic history of the  
358 populations and ii) particularly pronounced for immunity-gene variants that associate with  
359 fitness, our analyses suggest that this allelic combination is assembled by natural selection. This  
360 pattern is confirmed by the examination of genes annotated with a function in immunity and  
361 genes observed to respond to elicitation by the common bacterial elicitor flagellin. Our data  
362 further suggests that much of the positive co-variation between immunity gene expression and  
363 flowering depends on plant age. This factor is of recognized importance in plant immunity  
364 (Alcázar *et al.*, 2011; Lozano-Durán & Zipfel, 2015; Carella *et al.*, 2015) and also very well  
365 documented in ecological studies (Barton & Boege, 2017). Based on our findings, it is tempting  
366 to speculate that variation in age-dependent regulation of immunity may mediate the co-variation  
367 we report.

368



369 **Co-variation between immunity and flowering time is not explained by variation in**  
370 **vernalization requirements**

371 Flowering time variation depends on seasonal fluctuations, on the timing of germination and on  
372 the genetics of its control (Lempe *et al.*, 2005; Balasubramanian *et al.*, 2006; Korves *et al.*, 2007;  
373 Burghardt *et al.*, 2015; Hu *et al.*, 2017). Genotypes with a strong vernalization requirement, which  
374 are thought to have an obligate winter annual strategy, contribute strongly to the variation  
375 reported in the literature because they display much delayed flowering in the laboratory (Lempe *et al.*  
376 *et al.*, 2005; Li *et al.*, 2014; Sasaki *et al.*, 2015). The pattern we report, however, is not due to the  
377 assortment of immunity gene expression variants with alleles imposing a strong vernalization  
378 requirement. Indeed, since we observed that the pattern of co-variation between immunity gene  
379 expression and flowering time is magnified in plants whose flowering is not accelerated by cold  
380 exposure (Fig. 2A-B), this pattern is not driven by the genotypes requiring vernalization. In  
381 addition, the same pattern of co-variation is observed in a global sample of ecotypes, whose  
382 flowering time was scored in an outdoor common garden experiment, where plants were  
383 naturally vernalized (Fig. 2C). Therefore, we believe that the flowering time measures we used  
384 here do capture some of the natural lifespan variation. Future studies will have to confirm that  
385 flowering time variation scales with average differences in the lifespan expressed at the location  
386 of origin of each genotype.

387

388 **Positive co-variation between lifespan and immunity suggests cascading effect of**  
389 **flowering time adaptation on immunity evolution**

390 Two alternative scenarios may lead to concerted evolution of flowering time and immunity. First,  
391 in conditions where disease pressure is high, both shorter lifespan and stronger immunity can be  
392 expected to be advantageous, in order to simultaneously minimize the probability of attack, and  
393 maximize the probability of survival in case of attack. Under such scenario, negative co-variation  
394 between immunity and lifespan is expected. Alternatively, if lifespan is evolving under  
395 evolutionary forces independent of disease pressure, a reduced probability to encounter  
396 pathogens will favor mutations transferring energy allocated to immunity into energy allocated to  
397 growth. Indeed, defensive functions are known to be costly for the organism (Lochmiller &  
398 Deerenberg, 2000; Purrington, 2000). As a consequence the allocation into immunity is predicted  
399 to decrease where shorter lifespan evolves. Under this second scenario, a pattern of positive co-  
400 variation is expected between immunity and lifespan.

401 The pattern of co-variation we report here for immunity vs. flowering time is indeed positive and  
402 thus lends support to the second scenario. Local adaptation of flowering time is well documented  
403 in *A. thaliana* (Le Corre, 2005; Toomajian *et al.*, 2006; Méndez-Vigo *et al.*, 2011; Brachi *et al.*, 2013;  
404 Li *et al.*, 2014; Debieu *et al.*, 2013; Burghardt *et al.*, 2015; Vidigal *et al.*, 2016). In addition, several  
405 studies support the idea that increased basal level in immunity components improves immunity  
406 (Vetter *et al.*, 2012; Boccara *et al.*, 2014). At the same time, variants involved in the surveillance  
407 systems directed against pathogenic virulence factors were shown to incur substantial fitness  
408 costs (Tian *et al.*, 2003; but see also MacQueen *et al.*, 2016) and variation in basal immunity was  
409 negatively correlated with plant growth (Vetter *et al.*, 2012). Our results are thus compatible with  
410 an evolutionary scenario in which local adaptation of flowering time has cascading effect on  
411 immunity, possibly because a reduction of the plant's lifespan increases the cost/benefit ratio of  
412 immunity. This may also explain why genes involved in local adaptation in China are enriched  
413 among both flowering time and immunity genes (Zou *et al.* 2017).

414 However, a positive pattern of covariation could also arise even if the two traits evolve  
415 independently. Indeed, it is possible that populations where early flowering is advantageous  
416 coincide with populations where disease pressure is lower and *vice versa*. We cannot formally  
417 exclude that this scenario does not apply, because too little is known about variation in disease  
418 pressure in *A. thaliana* natural populations. Several elements, however, indicate it is unlikely.  
419 First, the rapid cycling genotypes are more frequent at intermediate latitudes, where summers are  
420 mild and wet (Lempe *et al.*, 2005; Debieu *et al.*, 2013). Since these conditions are also favorable to  
421 diseases, it is unlikely that higher disease pressure is found in areas where delayed flowering is  
422 more adaptive. Second, it is unlikely that this pattern may be due to herbivore enemies. Indeed,  
423 more severe herbivory damage has been observed on early-flowering *A. thaliana* individuals  
424 grown in the field (Weinig *et al.*, 2003). This seems to be common in plant species and should  
425 select for higher defense among early-flowering genotypes (Carmona *et al.*, 2010). Third, such  
426 scenario would assume that variation in disease pressure does not alter the trade-off between  
427 survival and reproductive output. This trade-off, however, is central in many models explaining  
428 the evolution of the timing of flowering in monocarpic plant species (Mitchell-Olds, 1996;  
429 Metcalf & Mitchell-Olds, 2009; Ashworth *et al.*, 2016).

430 Our results are therefore compatible with a scenario, in which adaptation of life history traits has  
431 a cascading effect on the evolution of immunity in *A. thaliana*. These findings do not contradict  
432 evidence that a tug of war characterizes the evolution of pathogen-specific components of  
433 immunity (Tellier & Brown, 2007; Roux & Bergelson, 2016). Indeed, by examining the basal  
434 expression level of a large set of genes involved in the immune reaction, the impact of durable  
435 selective forces on general immunity levels can be detected. This approach circumvents the  
436 potentially confounding signature left by a recent epidemics on strain-specific R-genes. Indeed,  
437 testing phenotypic variation in disease resistance across genotypes with different life-history  
438 alleles would probably reveal variation in gene-for-gene resistance, but the pervasive impact of  
439 selection fine-tuning energetic costs associated with immunity strategies would remained masked.

440  
441 Interspecific differences in the investment in defence against herbivory has been often associated  
442 with differences in lifespan and growth rate (Endara & Coley, 2010; Kooyers *et al.*, 2017). Future  
443 studies will also have to examine whether a similar evolutionary trend has emerged in species that  
444 have reshaped their life history to decrease overall vegetative lifespan. Early flowering is actually  
445 often favored when the favorable season is shortened (Franks *et al.*, 2007; Kenney *et al.*, 2014).  
446 Ongoing selection for early flowering is clearly widespread at temperate latitudes (Munguía-Rosas  
447 *et al.*, 2011) and transitions from perenniality to annuality occur frequently within phylogenies  
448 (Kiefer *et al.*, 2017). Testing whether life span reduction associates with an attenuation of  
449 immunity gene expression should therefore be possible in many taxa.

450

451

## 452 **The impact of life history evolution on defense systems is expected across all kingdoms**

453

454 In animals, the idea that the optimal investment in immunity depends on the life history of a  
455 species was also incorporated in evolutionary models (Jokela *et al.*, 2000). For plants and animals  
456 alike, resources available to the organism are limited. Energetic demands on growth may compete  
457 with those required for mounting immunity or counteracting the negative effects of parasites and  
458 pathogens (van Boven & Weissing, 2004; Lazzaro & Little, 2009; Dowling & Simmons, 2009;  
459 Seppälä, 2015). Several evolutionary models show that a prolonged lifespan is predicted to favor

460 resource investment into immunity (Jokela *et al.*, 2000; Medzhitov & Janeway, 2000; van Boven &  
461 Weissing, 2004; Miller *et al.*, 2007). As a consequence, changes in life history can mold the  
462 evolution of immune systems in animals as well (Van Valen, 1973; Sheldon & Verhulst, 1996;  
463 Schulenburg *et al.*, 2009). This theoretical prediction is supported by analyses of sexual  
464 dimorphism in the duration of effective breeding: females with increased reproductive longevity  
465 show stronger immune-competence but also by a meta-analysis of selection experiments (Rolf, 2007;  
466 Nunn *et al.*, 2009, van der Most *et al.* 2011). In frogs, fast developing species were also  
467 shown to be more susceptible to infection by trematodes (Johnson *et al.*, 2012). Yet, such studies  
468 cannot exclude that longevity and immunity are constrained in their evolution by common  
469 regulatory factors or causal inter-dependence. To the best of our knowledge, this study is the first  
470 to provide evidence that natural variation in the activity of genes that are important for defeating  
471 pathogens is assorted with alleles controlling variation in a life history trait of considerable  
472 importance for adaptation. Local adaptation for lifespan should therefore be considered as a  
473 potentially important contributor to the maintenance of genetic diversity in immune systems.  
474  
475  
476

## 477 MATERIAL AND METHODS

478

### 479 Flowering and immunity candidate genes

480 Gene Ontology (GO) categories were used to identify functionally related genes whose  
481 annotation was inferred from experiments, direct assays, physical interaction, mutant phenotype,  
482 genetic interactions or from expression patterns. Based on the keyword “flowering” in the TAIR  
483 database, 659 flowering time genes were selected. For immunity genes, we united 17 GO  
484 categories yielding 731 genes (Suppl. Table S2). For flagellin responsive (FlaRe) genes, we took  
485 the set of 245 genes that were activated in seedlings described in (Navarro *et al.*, 2004) (Suppl.  
486 Table S2). Subsets of flowering, immunity and FlaRe genes containing fitness-associated single  
487 nucleotide polymorphisms (SNPs) were retrieved from Fournier-Level *et al.* 2011.

488

### 489 Correlation between gene expression and flowering time in a natural population

490 We analyzed two published sets of natural ecotypes for which both genome-wide expression  
491 profiles and flowering time estimates were available. The first dataset comprised 138 lines from  
492 Sweden scored for both flowering time (for plants grown at 16h light-8hour dark at constant  
493 16°C) and gene expression in whole rosette collected at the 9-true-leaf stage (Dubin *et al.*, 2015;  
494 Sasaki *et al.*, 2015). For this first dataset, gene expression and flowering were determined in the  
495 same experiment. The second dataset combined data from two sources. RNA extracted from 7-  
496 day old seedlings of 144 genotypes grown on agar plate in long days had been sequenced  
497 (Schmitz *et al.*, 2013) and expression levels quantified as quantile normalized fragment numbers  
498 per kilobases and million reads (FPKM). For 52 of these genotypes, flowering time, measured in  
499 cumulative photothermal units, had been scored in the field (Brachi *et al.*, 2010). Photo-thermal  
500 units sum up the combination of temperature and day length and thus provide an estimate of the  
501 duration of the favorable season.

502

503 Expression counts were  $\log_2 + 1$ -transformed to include null values of expression and a Spearman  
504 correlation coefficient between flowering time and expression level was computed for each gene.  
505 P-values were adjusted for false discovery rate using the `p.adjust` function in R (Benjamini &  
506 Hochberg, 1995; Yekutieli & Benjamini, 1999). A Kolmogorov-Smirnov test was used to  
507 compare the distribution of Spearman correlation coefficients  $\rho$  of flowering time and immunity  
508 genes with the distribution of  $\rho$  for 22,686 genes for which gene expression was quantified. Gene  
509 enrichments were tested using hypergeometric tests in R. The GO enrichment analysis was  
510 performed with the Gene Set Enrichment Analysis (GSEA) test akin to non-parametric  
511 Kolmogorov-Smirnov tests, first described by Subramanian *et al.*, 2005, and implemented in the  
512 “topGO” R package (Alexa and Rahnenfuhrer, 2010). We further applied the *elim* procedure,  
513 available in this package, which calculates enrichment significance of parent nodes after  
514 eliminating genes of significant children nodes. This controls for the dependency among nested  
515 parent-child GO categories so that the significance of each enrichment can be interpreted  
516 without over-conservative p-value corrections for multiple-testing (Alexa *et al.* 2006). To test the  
517 impact of population structure on the correlation, we ran a mixed model with the help of the R  
518 package *lme4*. For each gene, we used gene expression level as a dependent variable. Flowering  
519 time was used as independent variable and a kinship matrix, generated with a matrix of SNPs  
520 segregating among Swedish genotypes (Dubin *et al.*, 2015), was included as random effect. The  
521 estimate of the flowering time effect was extracted. This allowed compared the distribution of



522 estimates observed for the whole genome, the subset of flowering time genes, or the subsets of  
523 defense genes.

524

### 525 **Analysis of gene expression in segregant pools bulked by flowering time**

526

527 Seeds of Bur-0, Col-0 and 278 Bur-0xCol-0 Recombinant Inbred Lines (RIL) obtained after 8  
528 generations of selfing were provided by the Arabidopsis Stock Center at INRA Versailles  
529 (France, (Simon *et al.*, 2008). Each line was grown individually in six replicates, each in 6cm  
530 diameter pots randomly allocated to 24 trays, each containing 35 pots. Seeds were stratified at  
531 5°C for 3 days and grown in growth chambers (Elbanton BV, Holland, equipped with Sylvania  
532 Gro-Lux F36W /Gro (T8) fluorescent tubes and Osram 25 W 220 Lumen light bulbs) under  
533 long-day conditions (21°C, 16h light, 18°C, 8h dark). Trays were rotated within the chamber  
534 every other day. Flowering time was scored as the day to the first open flower. Genotypes of  
535 individuals lines were retrieved from Simon *et al.* (2008) and mapping of flowering time recovered  
536 the same QTL (not shown).

537 We selected the 40 RIL in the 15% and 85% quantiles of flowering time for RNA sequencing.  
538 Each RIL and the two parental lines were planted in 20 replicates in the conditions described  
539 above. At days 14 and 28, the oldest true leaf was flash-frozen in liquid nitrogen. Three pools,  
540 each combining 13 RIL, were produced at each time point for early and late lines, for a total of 3  
541 biological replicates, 2 pool types (early and late RIL) and 2 time points (14 and 28 days). For  
542 each of the two parental lines, leaves of 12 replicates were pooled for each time point.

543 RNA was isolated using the TRIzol extraction protocol (ThermoFisher Scientific, USA). DNA  
544 traces were removed with the Ambion DNA-free kit (ThermoFisher Scientific, USA) and  
545 purified RNA was stored in TE buffer at -80°C. RNA quality and integrity was confirmed with  
546 the 2100 Expert Software on a Bioanalyzer (Agilent Technologies, Inc. Waldbronn, Germany).  
547 All samples had RNA integrity index (RIN) larger than 8. Single-read libraries were prepared with  
548 1µg of total RNA per sample using the Illumina TruSeq RNA Sample Preparation Kit v2  
549 (Illumina Inc. San Diego, USA) based on poly-A RNA purification. Sequencing of 75bp single  
550 reads was performed on the Illumina HighScan SQ system of the Core Facility of the  
551 Department of Genetic Epidemiology, Institute of Human Genetics, University of Münster,  
552 Germany. Raw data has been deposited in NCBI's Gene Expression Omnibus (Edgar *et al.*, 2002)  
553 and are accessible through GEO Series accession number GSE97664.

554

### 555 **Data analysis of RNA-seq from bulk segregant pools**

556 In total, 24 RNA libraries were sequenced. Raw sequences were demultiplexed and read quality  
557 validated with FastQC. Bad quality base calls were trimmed using the fastx-toolkit (Version  
558 0.013, Li *et al.* 2009). Trimmed reads (FastQ, quality score 33, quality threshold 20 and minimum  
559 length 30 base pair) were mapped to the *A. thaliana* TAIR10 annotated transcriptome using  
560 Bowtie 2 (version 2-2.0.0-beta6, (Langmead & Salzberg, 2012). Tophat (version-  
561 2.0.5.Linux\_x86\_64) was used to discover splice sites and Cufflinks for assembling the  
562 transcriptome (Trapnell *et al.*, 2010). In total 411,5 M sequence reads were obtained, with a mean  
563 read count per sample of 17,1 M reads. After trimming, 96.5% of the reads were mapped  
564 uniquely with a final average coverage of 66 reads per base pair.

565 We used a custom R script to verify that coverage was uniform across transcripts and confirmed  
566 that the RNA sequenced was not degraded. Read counts were calculated by counting the number  
567 of reads that mapped uniquely to the corresponding gene (isoforms were not considered). Lowly  
568 expressed genes with less than 20 reads over all samples were excluded from the analysis. The

569 samples clustered by time point of sampling (Fig. 2), with the exception of RNA samples from  
570 the Col-0 at 28 days, which resembled more expression levels measured at 14 days, probably  
571 because of its early shift to flowering. Differentially expressed (DE) genes were identified by  
572 running a nested analysis of sampling time effects within parental genotype (and/or early- and  
573 late-flowering leaf pools) with DESeq2 version 1.2.5 (Anders *et al.*, 2013; Love *et al.*, 2014). P-  
574 values were corrected for false discovery rate (Benjamini-Hochberg correction; (Benjamini &  
575 Hochberg, 1995). DE genes were defined as having an adjusted p-value<0.05. This analysis  
576 allowed the identification of genes showing differential expression between the parents (Suppl.  
577 Table S3) and genes showing flowering time dependent expression (differential expression  
578 between early and late flowering RIL pools, i.e. FT-regulated genes Suppl. Table S4) both at day  
579 14 and at day 28. We performed further analyses to disentangle significant sources of gene  
580 expression variation. To test whether gene expression was significantly modified at each time  
581 point, separate tests were performed for each parental genotype and RIL pool type. Genes  
582 differentially regulated at 14- and 28-days in Bur-0 (adjusted p-value<0.05) were defined as age-  
583 regulated genes (Suppl. Table S5). To determine whether one or both sampling time points drove  
584 significant differential expression, separate tests were performed for each time point (not shown).

585

### 586 **Confirmation with qRT PCR**

587 We confirmed gene expression levels for 11 selected immunity genes with differential expression  
588 between Bur-0 and Col-0 or early vs. late flowering pools (log<sub>2</sub>-fold change > 1.5) using RT-  
589 PCR. We followed standard protocols and used RNA Helicase (AT1G58060), Protein  
590 Phosphatase 2A Subunit A3 (PP2AA3) and transcript AT5G12240 as control genes. Gene  
591 expression based on RNA sequencing and RT-PCR were strongly correlated (Pearson  
592 correlation, 0.58<R<0.96, max p <0.01).

593

594

### 595 **Figure legends**

596

597

598

599 **Figure 1:** Distribution of Spearman correlation coefficients between expression levels of each  
600 expressed *A. thaliana* gene and flowering time. Grey: All expressed genes; Blue: Genes annotated  
601 as flowering time genes (FT genes); Red: Genes annotated as immunity genes; Pink: Flagellin-  
602 responsive (FlaRe) genes (Navarro *et al.* 2004). **A.** For 138 Swedish genotypes; **B.** Analysis  
603 restricted to 51 Swedish genotypes showing correlated flowering time at 10°C and 16°C; **C.**  
604 Species-wide sample of 52 genotypes. Distribution for each group of genes was compared to the  
605 genome-wide distribution (black double-head arrow) with a Kolmogorov-Smirnov test. P-values  
606 are given in the color corresponding to the gene class. Spearman correlation coefficients were  
607 computed between expression levels of each of 23,511 expressed *A. thaliana* genes, reported in  
608 Durbin *et al.* 2015 for 9th leaf seedlings, and flowering time measured in the same condition for  
609 51 genotypes originating from natural populations in Sweden (Sasaki *et al.* 2015). \*\*\* p < 0.001.

610

611

612 **Figure 2:** Distribution of Spearman correlation coefficients between gene expression level and  
613 flowering time. **A.** Partition of genes controlled by flowering time (hatched boxes with blue  
614 border) vs independent from flowering time (uniform boxes with black border); **B.** Partition of

615 genes controlled by development (hatched boxes with orange border) vs independent from  
616 development (uniform boxes with black border). Inserts in the top of the figure illustrates how  
617 these gene classes were defined. Immunity genes that are not controlled by flowering time but  
618 controlled by development tend to have higher correlation coefficients of natural variation for  
619 expression with natural variation for flowering time. Grey: All expressed genes; Blue: Genes  
620 annotated as flowering time genes (FT genes); Red: Genes annotated as immunity genes; Pink:  
621 Flagellin-responsive (FlaRe) genes (Navarro *et al.* 2004). P-values for Kolmogorov-Smirnov test  
622 comparing the distribution of genes within each category that are independent of or regulated by  
623 **A.** flowering time or **B.** age are shown when significant. Note that only 12 FlaRe genes are  
624 controlled by flowering time in our experiment. Spearman correlation coefficients were  
625 computed between expression levels of each of 23,511 expressed *A. thaliana* genes, reported in  
626 Durbin *et al.* 2015 for 9th leaf seedlings, and flowering time measured in the same condition for  
627 51 genotypes originating from natural populations in Sweden (Sasaki *et al.* 2015). \*  $p < 0.05$ , \*\*\*  $p$   
628  $< 0.001$ .

629  
630 **Figure 3:** Distribution of Spearman correlation coefficients between gene expression level and  
631 flowering time. All expressed genes –uniform boxes with black border- vs. genes with fitness-  
632 associated SNPs in Fournier-Level *et al.* (2011), - hatched boxes with purple border-. Grey: All  
633 expressed genes; Blue: Genes annotated as flowering time genes (FT genes); Red: Genes  
634 annotated as immunity genes. Immunity genes that carry SNPs associating with fitness tend to  
635 have higher correlation coefficients of natural variation for expression with natural variation for  
636 flowering time. P-values for Kolmogorov-Smirnov test comparing the distribution for genes  
637 within each category are shown when significant. Spearman correlation coefficients were  
638 computed between expression levels of each of 23,511 expressed *A. thaliana* genes, reported in  
639 Durbin *et al.* 2015 for 9th leaf seedlings, and flowering time measured in the same condition for  
640 51 genotypes originating from natural populations in Sweden (Sasaki *et al.* 2015). \*  $p < 0.05$ .

641

642

### 643 **Supplementary Tables**

644

645 **Suppl. Table 1:** GO categories enriched among genes correlating either positively or negatively  
646 with flowering time.

647

648 **Suppl. Table 2:** List of immunity genes and GO categories (only gene annotations based on  
649 experimentally validated open reading frames were considered). FlaRe genes and flowering time  
650 genes used in the study.

651

652 **Suppl. Table 3:** FT-dependent genes (DE between early and late flowering RIL pools). Output  
653 of gene expression analysis includes mean read count (FPKM), log<sub>2</sub> fold-change, Standard error  
654 of the log<sub>2</sub> fold-change; p-value and FDR adjusted p-value. FT-dependent genes have FDR  
655 adjusted p-values  $< 0.05$ .

656

657 **Suppl. Table 4:** Differentially expressed genes between Col-0 and Bur-0. Output of gene  
658 expression analysis includes mean read count (FPKM), log<sub>2</sub> fold-change, Standard error of the  
659 log<sub>2</sub> fold-change; p-value and FDR adjusted p-value. Genes differently regulated between Col-0  
660 and Bur-0 have FDR adjusted p-values  $< 0.05$ .

661

662 **Suppl. Table 5:** Age-regulated genes defined as differential gene expression changes in Bur-0  
663 between 14- and 28-days. Output of gene expression analysis includes mean read count (FPKM),  
664 log<sub>2</sub> fold-change, Standard error of the log<sub>2</sub> fold-change; p-value and FDR adjusted p-value. FT-  
665 dependent genes have FDR adjusted p-values <0.05.

666

667

668

## 669 **Supplementary Figures**

670

671 **Suppl. Figure 1:** Distribution of estimated effect of flowering time as explanatory factor of gene  
672 expression variation, taking into account population structure between Swedish genotypes of  
673 group A (Group A genotypes advance their flowering at 16°C compared to 10°C and show  
674 correlated flowering at 10°C and 16°C, Sasaki *et al.* 2015). The trend shown in Figure 1 is  
675 maintained after accounting for population structure. P-values for Kolmogorov-Smirnov test  
676 comparing estimate distribution for the gene subset compared to the genome-wide distribution  
677 are given. **A.** Density distribution, **B.** Boxplots.

678

679 **Suppl. Figure 2:** Distribution of correlation coefficients between gene expression and flowering  
680 time restricted to the genes showing differential expression in Col-0 vs. Bur-0. Expression  
681 differences between the genotypes Col-0 and Bur-0 recapitulate the pattern reported within  
682 natural populations in Figure 1. Distribution of Spearman correlation coefficients between gene  
683 expression level and flowering time for the set of genotypes showing consistent differences in  
684 flowering at 10°C and 16°C (Sasaki *et al.* 2015). This analysis is restricted to the 6980 genes  
685 showing differential expression between Col-0 and Bur-0. Immunity genes have a stronger skew  
686 in correlation coefficients with flowering time. Spearman correlation coefficients were computed  
687 between expression level of each of 6980 expressed *A. thaliana* genes, reported in Durbin *et al.*  
688 2015 for 9th leaf seedlings, and flowering time measured in the same condition. Genotypes  
689 originate from natural populations in Sweden (Sasaki *et al.* 2015). Black line: All expressed genes,  
690 Blue lines: Gene annotated as flowering time genes (FT genes), Red lines: Genes annotated as  
691 immunity genes, Pink line: Flagellin-responsive (FlaRe) genes (Navarro *et al.* 2004).

692

693 **Suppl. Figure 3:** Bulk-sequencing strategy used to identify FT-dependent genes, i.e. genes whose  
694 expression is genetically controlled by flowering time regulators or by genes located closely to  
695 and therefore co-segregating with flowering time regulators.

696

697 **Suppl. Figure 4:** Heatmap of the correlation in gene expression variation for the 1000 genes  
698 showing highest expression variation between samples. Clustering of gene expression levels  
699 shows that samples partition by genotype (Col-0, Bur-0), sampling time point (14d, 28d), and  
700 flowering time (Early Fl.: Early flowering RIL pool, Late Fl.: Late flowering RIL pool).

701

702

703 **Supplementary datasets:** For each expressed genes, Spearman correlation coefficients and their  
704 FDR significance presented separately for each dataset (whole Swedish populations, only  
705 vernalization-independent Swedish line, species-wide dataset).

706



707

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712 *al.* 2002) and are accessible through GEO Series accession number GSE97664.

713

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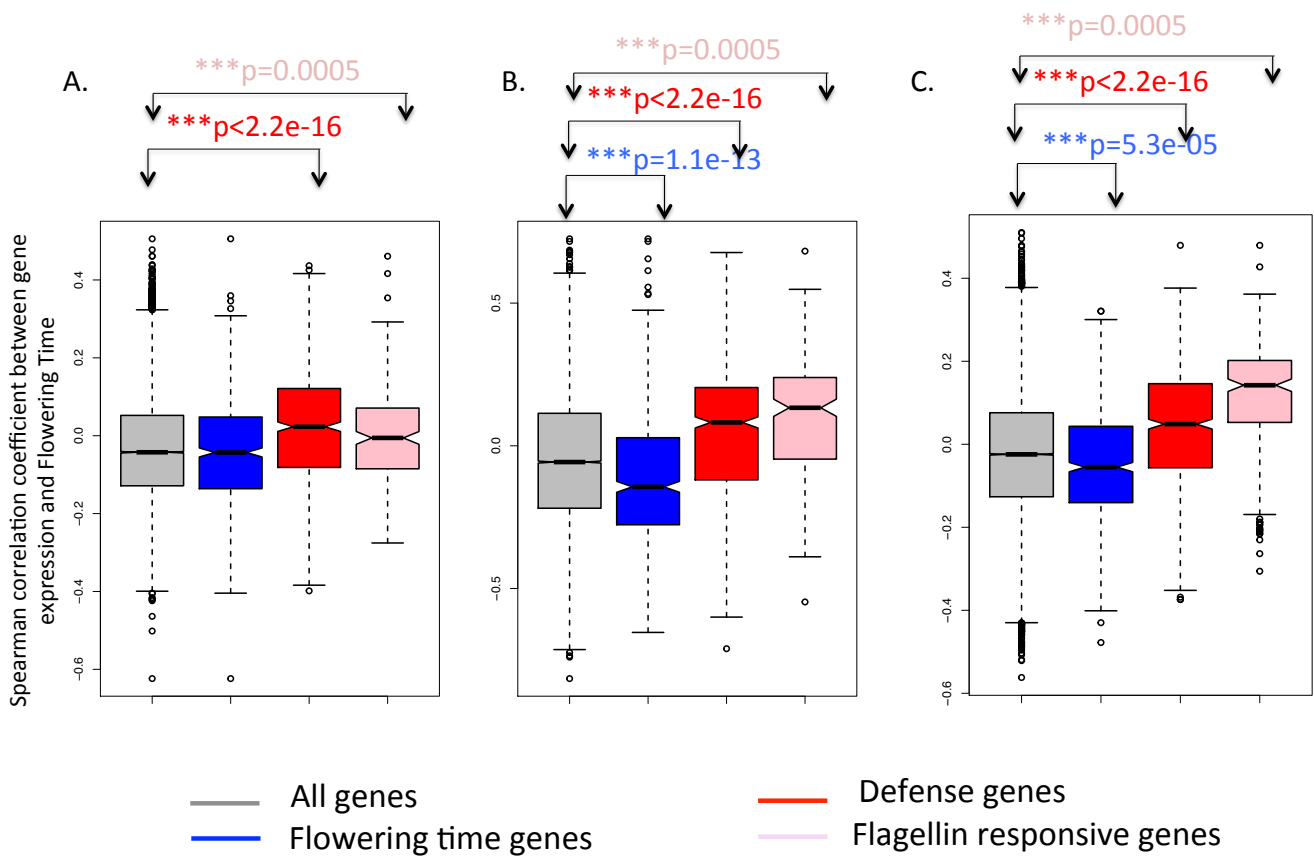
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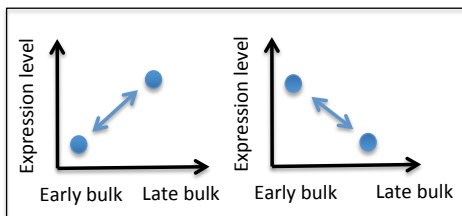
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Figure 1



A- FT-dependent regulation



B- Age-dependent regulation

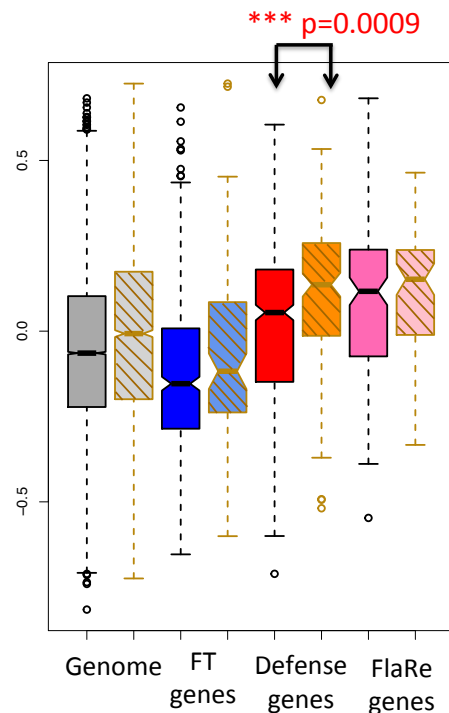
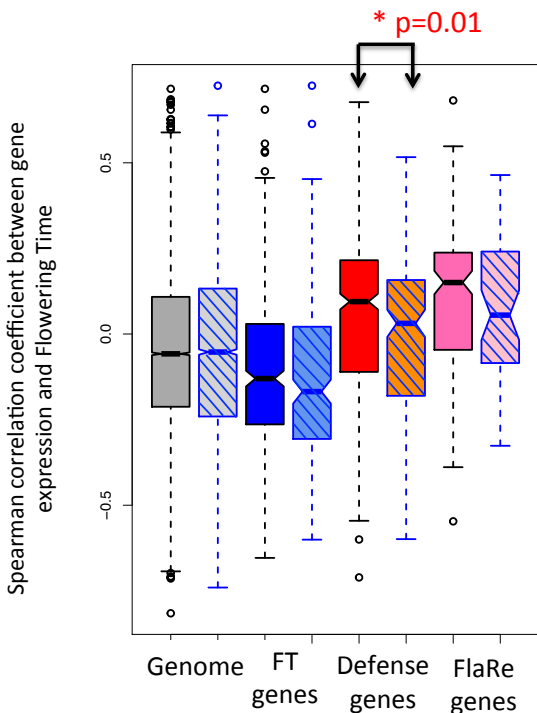
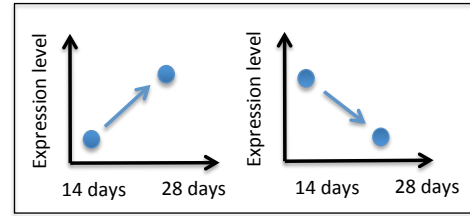
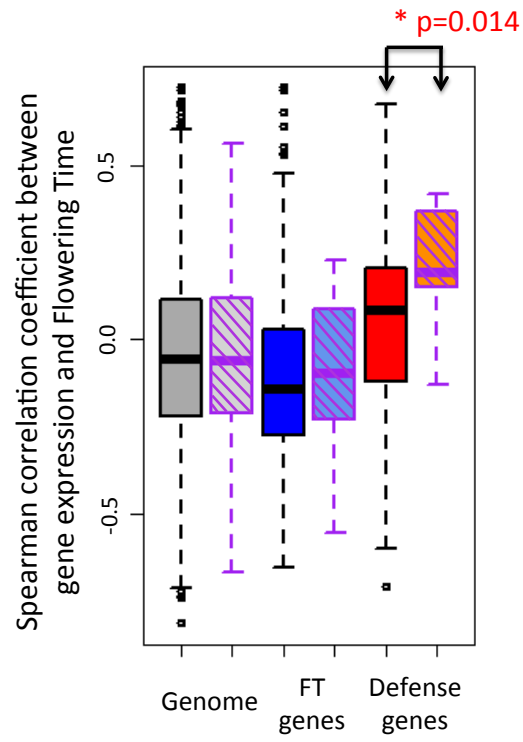


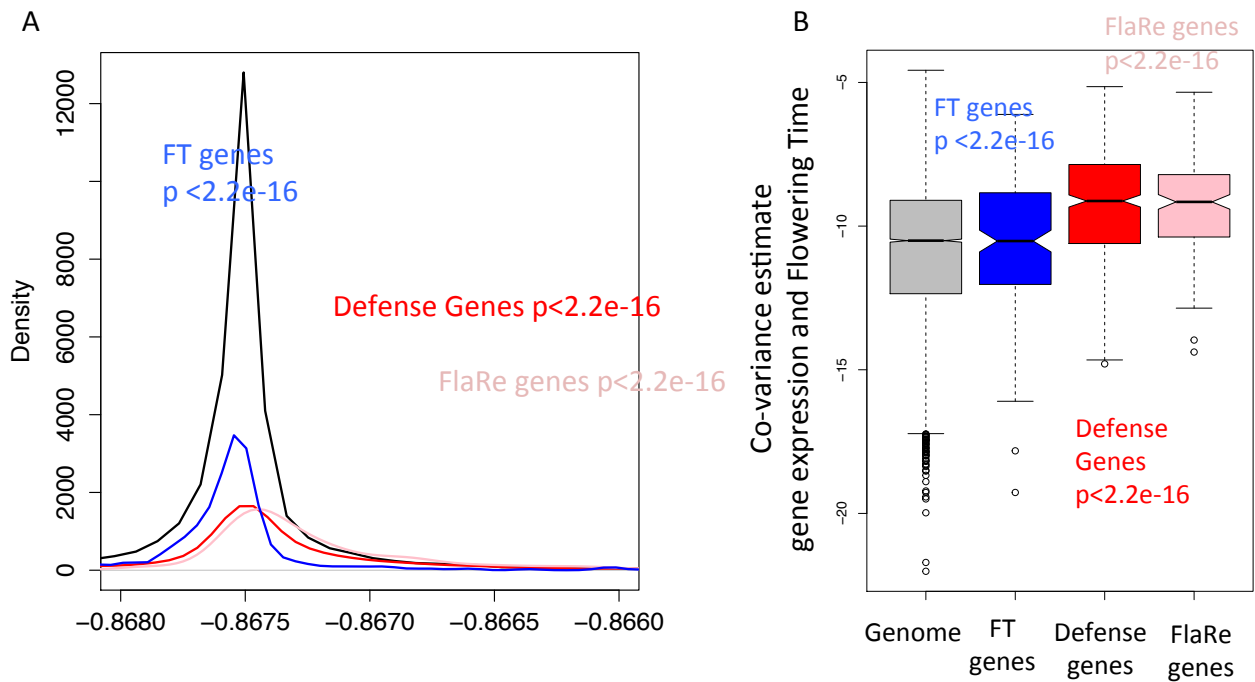
Figure 2



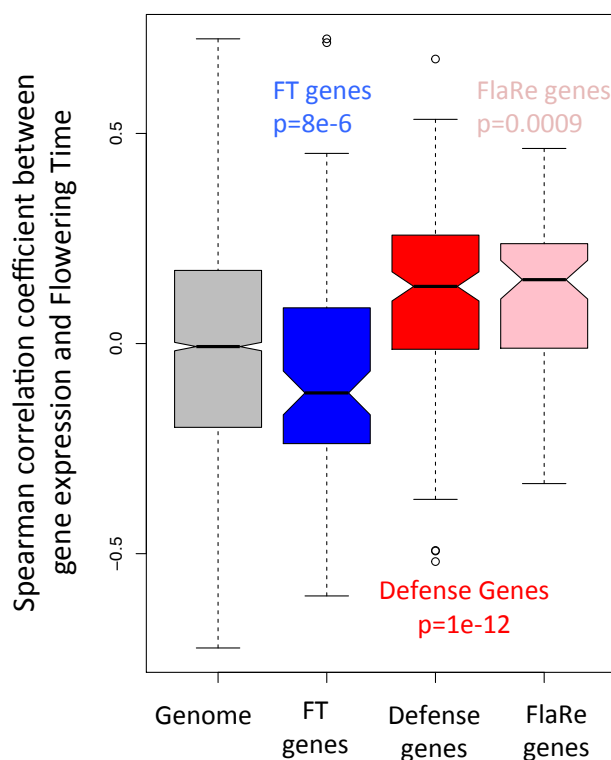
Figure 3



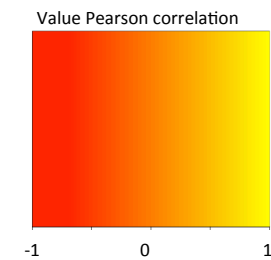
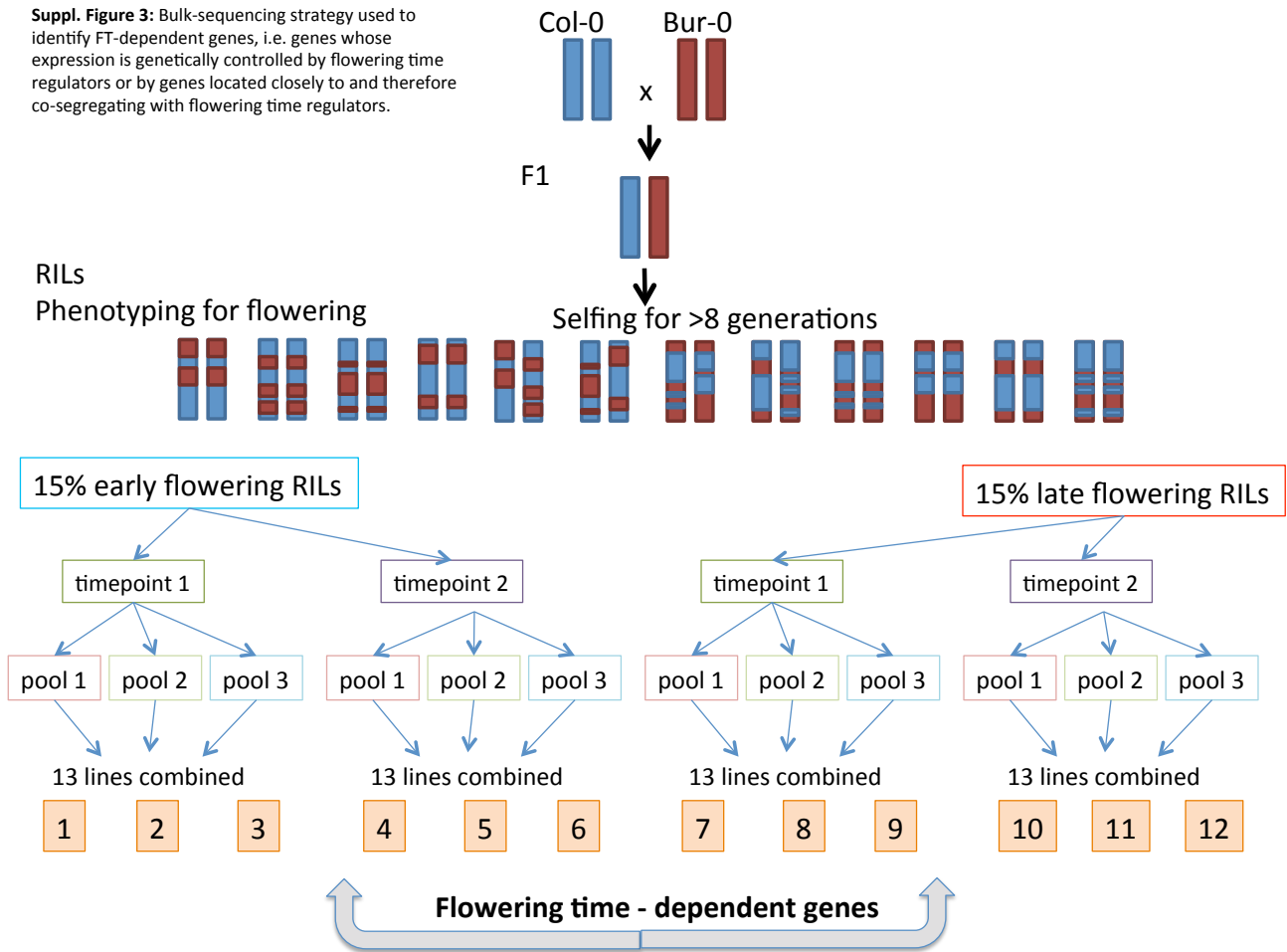
**Suppl. Figure 1:** Distribution of estimated effect of flowering time as explanatory factor of gene expression variation, taking into account population structure between vernalization-independent genotypes from Sweden. The trend shown in Figure 1 is maintained after accounting for population structure. P-values for Kolmogorov-Smirnov test comparing estimate distribution for the gene subset compared to the genome-wide distribution are given. **A.** Density distribution, **B.** Boxplots.



**Suppl. Figure 2:** Distribution of correlation coefficients between gene expression and flowering time restricted to the genes showing differential expression in Col-0 vs. Bur-0. Expression differences between the genotypes Col-0 and Bur-0 recapitulate the pattern reported within natural populations in Figure 1. Distribution of Spearman correlation coefficients between gene expression level and flowering time for a set of genotypes showing consistent differences in flowering at 10°C and 16°C (Group A genotypes, Sasaki et al. 2015). This analysis is restricted to the 6980 genes showing differential expression between Col-0 and Bur-0. Defense genes have a stronger skew in correlation coefficients with flowering time. Spearman correlation coefficients were computed between expression level of each of 6980 expressed *A. thaliana* genes, reported in Durbin et al. 2015 for 9th leaf seedlings, and flowering time measured in the same condition. Genotypes originate from natural populations in Sweden (Sasaki et al. 2015). Black line: All expressed genes, Blue lines: Gene annotated as flowering time genes (FT genes), Red lines: Genes annotated as defense genes, Pink line: Flagellin-responsive (FlaRe) genes (Navarro et al. 2004).



**Suppl. Figure 3:** Bulk-sequencing strategy used to identify FT-dependent genes, i.e. genes whose expression is genetically controlled by flowering time regulators or by genes located closely to and therefore co-segregating with flowering time regulators.



**Suppl. Figure 4:** Heatmap of the correlation in gene expression variation for the 1000 genes showing highest expression variation between samples. Clustering of gene expression levels shows that samples partition by genotype (Col-0, Bur-0), sampling time point (14d, 28d), and flowering time (Early Fl.: Early flowering RIL pool, Late Fl.: Late flowering RIL pool).

