Title: Environmental filtering of life-history trait diversity in urban populations of *Arabidopsis thaliana*

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Abstract (max 350)

1. The challenges to which plants are exposed in urban environments represent, in miniature, the challenges plants face as a result of global environmental change. Hence, urban habitats provide a unique opportunity to assess whether processes of local adaptation are taking place despite the short temporal and geographical scales that characterize the Anthropocene.
2. We quantified the ecological diversity of urban habitats hosting *A. thaliana* populations. Using plant community indicators, we show that these patches differ in their levels of soil nutrient content and disturbance. Accordingly, plants in each patch displayed a range of flowering time, size and fitness.
3. Using a deep sampling approach coupled with reduced genome-sequencing, we demonstrate that most individuals can be assigned to a limited set of clonal lineages; the genetic diversity of these lineages is a sample of the diversity observed in western European populations of the species, indicating that established urban populations originate from a broad regional pool of lineages.
4. We assessed the genetic and phenotypic diversity of these lineages in a set of common garden experiments. We report marked genetic differences in life-history traits, including time of primary and secondary dormancy as well as of flowering. These genetic differences in life-history traits are not randomly distributed but sorted out by ecological differences among sites of origin.

5. **Synthesis:** Our study shows that the genetically diverse phenology of a regional *A. thaliana* gene pool is not randomly distributed but filtered by heterogeneity in the urban environment. To out knowledge, this report is the first to show a pattern indicative of environmental filtering enhancing local genetic adaptation within urban environments. We conclude that environmental filtering helps maintain functional diversity within species.

Keywords: urban ecology, local adaptation, environmental filtering, life-history trait adaptation, ruderal species

1. Introduction

Urban environments provide untapped opportunities to link ecological and phenotypic diversity over the short temporal and spatial scales that define the Anthropocene (Johnson & Munshi-South, 2017; Szulkin et al., 2020). Understanding how plant functional variation is optimized within local urban environments can help understand the potential of species to cope with upcoming global changes.

Compared to that in neighboring rural areas, the climate in urban environments is generally characterized by increased temperature and reduced moisture, and thus a higher drought likelihood (Lambrecht et al. 2016). In addition, small-scale differences within urban microclimates are observable, depending on urban geometry and construction materials (Chatzidimitriou & Yannas, 2015). Urban microhabitats differ in edaphic characteristics: soils vary considerably in their developmental stage, depth, compaction, and surface transformation (Foti, 2017; Johnson & Munshi-South, 2017; Tresch et al., 2018). Shallow, sealed soils with a low capacity for water storage and low nutrient content typify the so-called urban grey spaces associated with built urban elements, such as sidewalks. They occur in close vicinity to nutrient-rich urban green spaces, often with eutrophic or even contaminated soils, which results in huge variation in soil-mediated plant resources over small spatial scales (Bonthoux et al., 2019; Gilbert, 2012; Vega & Küffer, 2021). Cities also host numerous ruderal sites, which are exposed to frequent and often severe disturbances, fragmentation, and high turnover rates among populations (Dubois & Cheptou, 2017; Guo et al., 2018). In addition, human-driven disturbances, such as weeding or mowing, may shorten the time windows for growth and reproduction, and thus modulate further the disturbance patterns.

Major life-history decisions, such as the timing of germination and the timing of flowering, shape the timing of plant phenological transitions and are critical for resource acquisition and reproductive success. They have been shown to contribute to the persistence of populations in heterogeneous urban settings (Liu et al., 2021; Nord & Lynch, 2009). Plant phenology is plastic. It can be adjusted locally in response to the environment because the life-history traits underlying it are modulated both by nutrient resources and by environmental cues, such as temperature and photoperiod (Vidal et al., 2014). For example, flowering is accelerated by winter temperature and resource stress and by high temperatures in spring, and delayed by the shortening days of the fall (Andrés & Coupland, 2012; Li et al., 2019). Plant phenology thus shifts in response to environmental cues that are modified by climate change (Chmielewski & Rötzer, 2001; Piao et al., 2019; Primack & Miller-Rushing, 2012).

The genetic composition of the population can also be tuned by local selective pressures acting on the genetic basis of phenological variation. Adaptation to local environments has been observed experimentally in many organisms (Kawecki & Ebert 2004; Leimu & Fischer 2008). Clearly,

adaptation via new genetic mutations is unlikely within urban settings because urban populations are small, fragmented, and prone to frequent extinction(Orr, 2005). However, if the pool of potential migrant genotypes is sufficiently diverse, then individual genotypes may establish or not depending on their genetic makeup, which results in local adaptation by environmental filtering. Indeed, patterns of local adaptation detected over small distances suggest that this kind of environmental filtering could happen in urban habitats (Halbritter et al., 2018; Lenssen et al., 2004; Robionek-Selosse et al., 2021).

In recent years, several studies have described phenotypic differences and genetic differentiation between individuals from urban and neighboring rural sites, consistently indicating that populations adapt rapidly to cities. These studies documented selection for novel optima in life-history traits, such as a late flowering time and decreased dispersal in holy hawksbeard (*Crepis sancta);* an early flowering time in common ragweed (*Ambrosia artemisiifolia*); and decreased cyanogenesis in white clover (*Trifolium repens*) (Cheptou et al., 2008; Gorton et al., 2018; Lambrecht et al., 2016; Santangelo et al., 2022; Thompson et al., 2016). Altogether, however, the amount of genetic variation in life-history traits, the distribution of this variation within the heterogeneous environments of cities, and its contribution to local adaptation in these environments remain unknown (Johnson & Munshi-South, 2017).

Urban environments in regions with temperate climates often host populations of short-lived herbaceous species, including the Brassicaceae species *Arabidopsis thaliana* (Bomblies et al., 2010; Herben et al., 2016; Huang et al., 2018). This species -- the initial model of plant molecular biology -- has become a powerful system for answering fundamental questions of ecology, evolution, and global environmental change (Exposito-Alonso et al., 2018; Fournier-Level et al., 2011; Takou et al., 2019; Vasseur et al., 2018). *A. thaliana*, which is native to large areas of Eurasia and Africa, has recently colonized many other parts of the world (Durvasula et al., 2017; Lee et al., 2017). The deep sampling of natural populations across various regions of its area of distribution has shown how environmental heterogeneity shapes the distribution of genetic diversity in *A. thaliana* (Castilla et al., 2020; DeLeo et al., 2020; Dubin et al., 2015, p. 20; Luo et al., 2015). Life-history trait adaptation appears to happen in concert across regional environments (reviewed in Takou et al. 2019). This includes adaptation in the timing of distinct phenological stages such as germination (Kronholm et al., 2012), or the onset of flowering (Ågren et al., 2017; Akiyama & Ågren, 2018; Le Corre, 2005), but also the tuning of other life-history traits such as the rate of rosette growth or leaf economic spectrum (Debieu et al., 2013; Sartori et al., 2019; Wieters et al., 2021).

In the present study, we determined the relative importance of phenotypic plasticity and genetic variation in shaping the phenology and fitness of eight spontaneously occurring urban populations of

A. thaliana. We asked i) Are *A. thaliana* urban populations occupying ecologically diverse habitats? ii) Are *A. thaliana* populations genetically diverse? iii) Is this genetic diversity randomly distributed across habitats? By combining plant-community and trait-based ecological measurements with analyses of genetic diversity at genomic and phenological levels, we show that genotypes growing in urban habitats exposed to high resource limitations and increased disturbance levels tend to have traits tailored to local conditions.

2. Materials and Methods

Study area

Our study focused on urban *A. thaliana* populations in the city of Cologne, situated in midwestern Germany (about 50.9°N, 7.0°E). Cologne is Germany's fourth most populous city, with more than one million inhabitants (Ansmann et al., 2021). One of Germany's warmest cities, it has a temperate oceanic climate with a mean annual temperature of 11.7 °C during the day and 6.3 °C at night, and a mean annual precipitation of 840 mm.

Field surveys, phenology monitoring, and functional trait measurements

We surveyed eight *A. thaliana* populations within a 4.3 x 3.7 km area in southwestern Cologne. Study populations were selected based on their accessibility and on their isolation from other populations, with all habitat patches located at least 0.6 km from one another. Urban habitat patches where viable *A. thaliana* populations were observable included green spaces such as disturbed lawns; various habitats within gray spaces, such as sidewalks, wall tops, and bases; and vegetated roadsides (Figure 1a; Figure S1; Table S1). Depending on habitat characteristics, patch size ranged from 4 m^2 for populations on wall tops to > 100 m^2 for populations in vegetated roadsides, and population size ranged from 15 to 1,000 individuals. All populations were studied throughout their growth period in 2017 (which extended between early March and the end of June), and revisited yearly during five subsequent growth periods (2018-2022) to assess population persistence. No field permissions were necessary for the collection of the plant samples in this study.

We recorded phenological stages and fitness-related plant functional traits of *A. thaliana* individuals distributed over the eight habitat patches. At each patch, we established 10 to 14 observation quadrats (10 x 10 cm), each at least 50-60 cm from one another. We marked from 1 to 3 *A. thaliana* individuals per quadrat (15-24 per habitat patch; 174 in total) and visited them twice a week during the study period to score their development. For all marked individuals, we recorded the day of bolting (i.e., when the inflorescence became visible) and, subsequently, the number of open flowers and the number of closed fruits. Rosette diameter and the number of rosette leaves were measured at the time of bolting. We used these observation data to derive five phenological stages and five fitness-related plant functional traits ("fitness proxies"; see Table S2). The selection of plant

145 functional traits follows recommendations for the assessment of plant fitness (Gibson, 2014), while
146 phenological stages were selected to represent life-history stages of the entire growth period, as
147 recommended for herbaceous species (Huang et al., 2018; Nordt et al., 2021). Germination was
148 generally not possible to determine *in situ*, except in populations growing on wall tops (SGY, KAD,
149 HLU).

150 *Assessment of environmental conditions of habitat patches*

151 Abiotic environmental conditions and characteristics of disturbance regime were recorded at the
152 level of habitat patches, making use of the indicator function of plant communities (Kollmann &
153 Fischer, 2003). We used species' Ellenberg indicator values, EIVs (Ellenberg & Leuschner, 2010), as a
154 proxy for abiotic conditions. EIVs, which express the optimal positions of Central European plant
155 species along gradients of abiotic factors, provide information about species' niche and habitat
156 requirements (Diekmann 2003), in both rural and urban habitats (Fanelli et al. 2006). EIVs are
157 expressed on an ordinal scale ranging from 1 to 9 and refer to light regime (L), temperature (T), and
158 continentality of climate (K). Edaphic conditions are captured as soil moisture (F), pH (R), and
159 nutrient availability (N). Likewise, species' disturbance indicator values (DIVs) (Herben et al., 2016),
160 were used to characterize the disturbance regimes of urban habitat patches. To this end, we
161 extracted species-level indicators for disturbance severity (DS), disturbance frequency (DF), and
162 disturbance effects on vegetation structure (DV) from the data compiled in Herben et al., 2016. DIVs
163 are based on vegetation-plot records of the Czech flora, with a species' DS defined as the mean
164 disturbance severity of all vegetation classes weighted by its occurrence frequencies in these classes;
165 DF calculated as the mean of logarithmic disturbance frequency of all vegetation classes weighted by
166 a species' occurrence frequencies in these classes; and DV calculated from vegetation structural
167 parameters by summing covers and community-weighted means of all species' growth height per
168 vegetation class. We determined the composition of plant communities in autumn (late August and
169 early September) 2017 by recording the presence of all vascular plant species in the herbaceous layer
170 of the habitat patches. Based on species' specific indicator values (see Table S4) and excluding *A.*
171 *thaliana*, weighted average EIVs and DIVs were calculated for the plant community of each habitat
172 patch.

173 *Assessment of genomic variation*

174 At the end of the 2017 growth period, we harvested seeds from 5 to 17 individuals per population
175 and used single seeds from each individual to produce progenies. For four of the eight habitat
176 patches (BER, BIS, KAD, and MIL), we also included seeds from individuals sampled in the year prior
177 to field data collection, referring to these as population samples (Table S3). In order to broaden our
178 view of the genomic diversity of *A. thaliana* in Cologne, we also produced progenies from seeds

179 collected at 30 additional habitat patches distributed throughout the sampling area but not included
180 in the *in situ* monitoring (hereafter called scattered samples). Field collected seeds were grown in a
181 randomized setting, under conditions simulating winter, i.e., in growth chambers with 10 h light at
182 18 °C / 14 h dark at 16 °C, 60 % humidity, for 6 weeks; followed by 8 h light/16 h dark at 4°C, 80 %
183 humidity, for 6 weeks; and ultimately ripened in the greenhouse in spring-like conditions with 16 h
184 light, at about 20-24 °C / 8 h dark at about 18-20 °C until harvest. Seeds for progeny were harvested
185 using Aracons (Betatech, Gent, Belgium).

186 Three leaves per plant were sampled for DNA extraction, deep frozen, and homogenized using a
187 Precellys Evolution homogenizer (Thermo Fisher Scientific, Waltham, MA, USA). DNA was isolated
188 using the NucleoSpin® Plant II kit (Macherey-Nagel, Düren, Germany). Genomic DNA was prepared
189 for RAD-Seq as described by (Dittberner et al., 2019) using 10 pools with 20 individually barcoded
190 plant samples each, and sequencing was performed at the Max Planck Institute of Plant Breeding
191 Research. The bioinformatics pipelines used for read mapping, and identification of each of the 12
192 genotypes present in the studied habitat patches, population structure and admixture are described
193 in the supplementary information.

194 *Common garden experiments:*

195 For each identified genotype, two individuals were randomly selected and amplified in the
196 greenhouse. Since they were raised in the same maternal environment, variance among genotypes
197 can be assigned to genetic effects and variance among replicate lines of each genotype allows testing
198 whether all maternal effects were effectively removed when growing parents in common garden
199 conditions. We then took eight individuals from each of these two lines and grew them in growth
200 chambers (Johnson Controls, Milwaukee, WI, USA) in three trials under different growth conditions.
201 Growth conditions were chosen to identify genetic variation in the major pathways regulating
202 flowering time: constant long days (16 h light at 20°C/8h dark at 18°C); constant short days (8 h light
203 at 18°C day/16 dark at 16°C night); and short days/vernalization/long days (8 h day light at 18°C /16 h
204 dark at 16°C for 5 weeks; 8h day light/16h dark at 4 °C for 4 weeks, 16 h day light at 20°C/8h dark at
205 18°C until ripening). The *A. thaliana* genotypes Col-0 was included as a reference in all experiments.
206 Because we did not detect differences between replicated lines, we considered the number of
207 replicates to be 2x8= 16 replicates/genotype. Replicates were sown in individual 6-cm-diameter pots
208 that were watered regularly by flooding the trays. Flowering time was determined as the time span
209 between sowing and the first flower to open petals.

210 Ripe seeds from the short-day/vernalization/long-day experiment were harvested and stored at
211 room temperature for the germination assay. Germination rates were determined after three
212 months to quantify primary dormancy (Baskin & Baskin, 2004). Seeds were sown on wet filter paper

213 and incubated in closed microtiter plates. Before incubation under long-day conditions (16 h light at
214 20°C/8h dark at 18°C), seeds were incubated in the dark either at 4 °C for 7 days to quantify their
215 capacity to germinate when dormancy is released, or at -21 °C for 4 d and at 35 °C for 4 d to quantify
216 variation in secondary dormancy. Germination was scored after 10 days.

217 To quantify genetic variation under outdoor field conditions, we conducted a set of common garden
218 experiments. For this, seeds from 4 independent replicate lines per genotype were sown directly at a
219 density of 8 seeds per pot. In total, we planted 32 pots per genotype and randomized 9-cm- diameter
220 pots at a field site in the botanical garden of Cologne University. Again, because we found no
221 significant differences between replicate lines, we determined that the experiment was complete
222 with 32 replicates per genotype. Seeds were sown in late summer (August 23) and in early autumn
223 (September 20), mid-autumn (November 8), and late winter (February 26). Rather than being
224 watered artificially, pots were put on a fabric that retained water after rainfall. The site was
225 protected against birds and rabbits by a net. Plants were inspected for germination twice a week.
226 Flowering time was scored when the first flower showed open petals. For fertility measurement,
227 siliques were counted at the end of each plant's life cycle.

228 Phenotypic data was analyzed using the glm function in R version 4.1.0 (R Core Team, 2022). We
229 used one-way ANOVA with habitat patch as the fixed factor to analyze differences in environmental
230 conditions (EIVs and DIVs of plant species co-occurring with *A. thaliana*); and in the phenological
231 stages and fitness proxies of *A. thaliana* plants observed *in situ*. Measurements of flowering time in
232 common garden experiments were analyzed with two-way ANOVA, using genotype and cohort or
233 growth condition as fixed factors.

234 For germination assays under controlled indoor conditions, the number of germinants per Petri dish
235 was analyzed with the R function zeroinfl (Package pscl; Zeileis et al., 2008) to correct for zero
236 inflation. For germination rates in the outdoor common garden experiment, we proceeded as under
237 controlled conditions, except that we used a quasipoisson distribution of error. Because the two
238 replicate lines we used for each genotype never showed significant effects, the line effect was not
239 included in any of the models. We used the function ANOVA with a Chi-squared test to determine
240 the overall significance of effects. In all cases, the significance of pairwise differences was tested with
241 a Tukey post-hoc test, using the function glht from the multcomp package in R (Hothorn et al., 2008).
242 We used the Pearson coefficient of the cor.test function in R to quantify the correlation between
243 phenotypes.

244

245 **Results**

246 **Urban habitat patches of *A. thaliana* are ecologically diverse**

247 The eight urban habitat patches differed significantly in environmental conditions related to climate,
248 soil, and severity of disturbance. Indicator values for light (L), and edaphic conditions -- humidity (F),
249 and soil nutrients (N) -- differed between sites (Figure 1b, supplemental Figure 1a, b; humidity (F):
250 $F_{7,113}$=2.855, p=0.00883; soil nutrients (N): $F_{7,93}$=2.777, p=0.0114, light (L): $F_{7,130}$=2.329, p=0.0285).
251 Indicator values of the accompanying flora for herb-layer disturbance frequency (DF) and herb-layer
252 structure-based disturbance (DV) differed between sites (Figure 1c, supplemental Figure 1c; DF:
253 $F_{7,84}$=2.897, p=0.0092; DV: $F_{7,84}$=3.108, p=0.00577), whereas differences in herb-layer disturbance
254 severity (DS) were not significant. The three values for disturbance were strongly and positively
255 correlated with each other (range of r: 0.802 – 0.958; p<0.00172) and with the temperature indicator
256 (range of r: 0.736 – 0.803; p<0.00632; Figure 1d; Figure S3, Table S4) .

257 **Plants differ across habitat patches in their phenology and fitness**

258 Across the eight urban *A. thaliana* populations, all phenological stages and plant functional traits
259 measured *in situ* showed significant differences (p<2.43e-06; F=6.094, Table S6; Figure S4). These are
260 exemplified in data on flowering time and rosette size (Figure S1a,b). The diverse traits describing
261 phenological time points, the temporal spacing between phenological states, and the size of the
262 plants also covaried positively (Figure S5).

263 **Community-level indicators of environmental variables explain part of the phenotypic differences**
264 **between field sites**

265 *In situ* observations of phenotypic differences between sampling sites were partly correlated with
266 the differences in environmental characteristics: as expected, plants from sites with high mean
267 values for N- and F-indicators, which reflect differences in nutrient and soil moisture levels,
268 respectively, were on average larger than those from sites with lower N- and F-indicator values (p-
269 values smaller than 0.05 for F: inflorescence length, maximal number of open flowers, rosette
270 diameter; for N: rosette diameter at bolting, number of rosette leaves, and number of fruits; Figure
271 S1c). Plants from sites with higher disturbance values bolted later than plants from other sites, but
272 formed larger inflorescences bearing more fruits.

273 **Urban populations in Cologne consist of clones of diverse western European genotypes**

274 In order to determine the extent of genomic variation and the history of population establishment,
275 we genotyped 5-17 individuals per population as well as plants sampled from additional sites
276 scattered across the study area. In total, we genotyped about 1% of the genome, or 13 Mbp, of 149
277 individuals using RAD-sequencing (Table S5). Overall, we uncovered 11,489 single nucleotide
278 polymorphisms (SNPs) in the 8 focal populations, and 17,288 SNPs if we included the scattered
279 sample. Individuals were overall highly homozygous (>99%), indicating that self-fertilization
280 dominates in Cologne populations. Populations showed a strong genetic structure, as can be seen

281 from the genetic distance matrix (Figure 2a): Populations BER, GHU, RKO, and SHY consisted
282 exclusively of genetically identical individuals, with the exception of a single plant with 99%
283 heterozygosity from the GHU site. Populations BIS, HLU, KAS, and MIL each consisted of two
284 genotypes. Overall Fst reached 0.72. Genetic similarity allowed clustering genotypes into three
285 groups: Group 1 comprised RKO and BER, group 2 KAS-1, KAS-2, and BIS-2, group 3 consisted of HLU-
286 1, HLU-2, and SGY. The genotype of plants collected in 2016 was the same as that of individuals
287 collected in 2017 in the same location, confirming that local populations are most often formed from
288 one (maximum two) single genotypes that persist over years.

289 In order to understand the origin of the genotypes that established populations in the city, we
290 compared the Cologne samples to the European genotypes of the 1001-genome collection (Alonso-
291 Blanco et al., 2016) . We took a subset of representative genotypes from both datasets and first ran a
292 principal component analysis (PCA) (see Materials and Methods). If habitats had been colonized via a
293 stepping stone model, we would expect that the genotypes of the Cologne sample form a cluster of
294 genotypes that stands out of the 1001 genomes. Cologne samples, however, co-localized with
295 western and Central European accessions in the PCA, especially with accessions from Germany,
296 France, the Netherlands, and the UK. They did not form a defined subgroup separate from the other
297 genotypes (Figure 2b, c, Figure S6). Hierarchical clustering using admixture analysis confirmed that
298 individual lineages share ancestry with individuals collected in Germany, the Netherlands or France in
299 variable proportions (Figure 3d). In fact, the average number of pairwise differences between
300 Cologne lineages was comparable to the number of differences between samples of the 1001
301 genomes (Figure S6). In conclusion, at each site, *A. thaliana* individuals formed either one or two
302 clonal lineages, and collectively they do not form a local endemic group of similar genotypes. In
303 addition, populations displayed no signal of isolation by geographic distance (Figure S6, Mantel test
304 $p>0.05$) nor isolation by environmental distance (Mantel test $p>0.05$ for all environmental factors).
305 We conclude that new populations are often established by long range migration and that the
306 stepping stone model, by which new patches would be colonized by propagules of neighbouring
307 patches, is inconsistent with the pattern of genetic diversity we observed (Duforet-Frebourg &
308 Slatkin, 2016).

309 **Cologne urban populations show genetic variation in flowering time and dormancy regulation**

310 Variation in genetic pathways regulating flowering time and germination may contribute to life-
311 history differences recorded on-site. To link the germination and flowering behavior of the urban
312 genotypes to knowledge about these well-studied pathways, we analyzed flowering under standard
313 conditions in a randomized block design and included the well-studied genotype Col-0 as a reference.
314 In total, three controlled environmental conditions were set: long days (LD), short days (SD), and a

315 combination of short days/vernalization/long days (SDV). This setup thus allowed us to thoroughly
316 examine genetic variation in the regulation of flowering time. The first two conditions allowed us to
317 derive photoperiod-dependent differences in the regulation of flowering; the third mimicked the
318 succession of environmental cues experienced in the fall, winter, and spring (Andrés & Coupland,
319 2012). Differences between LD and SDV conditions revealed variation in vernalization requirement,
320 because it reveals the extent to which flowering time differences in LD tend to decrease in SDV as a
321 result of exposure to the cold temperatures.

322 Flowering times differed between genotypes under all conditions ($F_{12,134to158}$>39.53, p<2 e^{-16}) and the
323 interaction between genotype and experimental condition was signicant ($F_{24,461}$= 36.72, p<2 e^{-16}).
324 Under LD conditions, BER and RKO plants flowered as early as the early flowering reference genotype
325 Col-0, whereas BIS2 plants had not flowered after 105 days, indicating that the former do not require
326 vernalization to flower (Figure 3a, Table S7). Under SD conditions, several genotypes flowered
327 significantly earlier than Col-0, indicating that natural Cologne genotypes might flower in the short
328 days of the fall or the late winter: RKO plants flowered earliest; BER, KAD1, KAD2, and MIL1 flowered
329 only slightly earlier than Col-0. Both genotypes from the BIS site were unable to flower under SD
330 conditions and were thus unlikely to flower in the fall (Figure 3b). When plants were grown under
331 conditions simulating winter (SDV), most genotypes flowered within a narrow time period, with only
332 BIS1 and BIS2 displaying significantly later flowering (Figure 3c). This experiment demonstrated that
333 there is considerable genetic variation among the 12 Cologne genotypes for the regulation of
334 flowering time. The magnitude of these differences depended on photoperiod and vernalization.

335 The genotypes also differed in how they regulated germination (Table S8). After three months of
336 seed storage at room temperature, germination assays revealed that the genotypes differed in
337 primary and secondary dormancy. When seeds were untreated, their germination rates indicated
338 levels of primary dormancy (Baskin & Baskin, 2004; Finkelstein et al., 2008). Untreated seeds of BER,
339 BIS2, MIL1, and MIL2 plants had high germination rates, whereas seeds of plants from the other
340 genotypes had a less than 50% germination rate (χ^2, df=11, N=48, p<2.2e^{-16}; Figure 4a). Incubating
341 non-dormant seeds at very low (-21°C) or high (35 °C) temperatures induced variable levels of
342 secondary dormancy in Cologne genotypes, as indicated by a significant interaction between
343 genotypes and germination conditions (likelihood ratio test, χ^2= 83.2, df=24, p=1.81e^{-8}, Figure 4c and
344 supplemental Figure S7).

345

346 **Outdoor common garden experiments confirm differences in germination and flowering regulation**
347 **under field conditions**

348 The 12 genotypes were further assayed in an outdoor common garden located in Cologne, and the
349 genetic variation underlying their differences in survival and fertility was quantified. Here again, we
350 included the widely used genotype Col-0 in the experiment as a reference (Table S9a-b). Since
351 phenological variation is known to depend on the timing of germination, seeds were sown directly
352 into pots at a field site in late August, mid-September, early November (6 weeks, 9 weeks, and 3
353 months after harvest), and the end of February. As pots were not watered artificially, germination
354 and further development depended on natural rainfall and temperature. Germination rates among
355 genotypes strongly differed in the August and September sowing cohorts. In the August cohort, 43
356 days after seeds were sown, only Col-0 plants had germinated to 100%; BER and MIL1 showed 43 and
357 28 % germination, respectively, 10 % or fewer of the seeds of the other genotypes had germinated
358 (differences between the genotypes: $F_{12,195}$=16.97, p<2e-16, Figure 4c). In the September cohort, 15
359 days after seeds were sown, most genotypes had germinated at 80% or more, and only HLU1, GHU,
360 and SGY had germinated at less than 60% (after 22 d: $F_{12,195}$=15.84, p<2e-16; Figure 4d). Eventually,
361 most seed germinated, but the most delayed was the August cohort (Figure S8a-d). Later
362 germination in this cohort can be explained by heat-induced secondary dormancy. In contrast,
363 differences in germination for the November and February cohorts were minor (Figure S8e,f).

364 More than 95 % of the germinated seedlings survived until seed set. Most flowered in spring with the
365 exception of the Col-0 and BER plants from the August cohort, which germinated almost immediately
366 and flowered already in late autumn/winter. Differences in flowering time, which were observed for
367 each of the four cohorts ($F_{12,195}$>13.33, p<2 e^{-16}), revealed a significant interaction between genotype
368 and sowing date ($F_{34,755}$= 20.89, p<2e^{-16}, Table S3, Figure 3d, Figure S9). Flowering times observed for
369 the 12 genotypes were generally not correlated across the various common garden cohorts grown
370 outdoor, with the exception of the February and September cohorts (Figure S10). Fertility among the
371 genotypes, determined by the number of fruits per pot, differed between the genotypes for the
372 August, September, and November cohorts ($F_{12,195}$=3.072, p=0.00054; $F_{12,194}$=7.721, p=1.15e^{-12}; and
373 $F_{12,195}$=4.702, p=1.01e^{-06}). Again, a significant interaction between genotype and seed-sowing date
374 was observed ($F_{24,584}$=3.534, p= 4.75e^{-08}). For the August cohort, only two genotypes (RKO and SGY)
375 were less fertile than most of the genotypes (Table S3, Figure S11). Fitness differences for the plants
376 grown from seeds sown in February, all of which germinated within a week in early March and
377 flowered 23-39 days later, were not significant. With 52% of the plants dying after an unusually warm
378 dry spell in May, the average fitness of the spring cohort was low .

379 In summary, plants from the 12 Cologne genotypes displayed remarkable genetic differences in their
380 ability to control germination and flowering in common garden experiments. Flowering times
381 measured in controlled growth chamber and in the common garden were significantly correlated,

382 but correlation depended on the various experimental indoor conditions and/or the seed dispersal
383 times characterizing the different common garden cohorts (Figure S10).

384 **Environmental variation among sites of origin correlates with genetic variation observed in**
385 **common gardens and controlled environments**

386 The phenotypes of urban *A. thaliana* individuals in situ correlate with the ecological characteristics of
387 the sites in which they are monitored. We further tested whether the distribution of genetic
388 differences revealed in common gardens and under lab conditions is independent of the ecological
389 characteristics at the sites of origin. We found that genetic variation in the rate of germination under
390 late summer conditions in the August cohort or after cold treatment correlates positively with
391 variation in the severity of disturbance (r=0.763, p=0.0039 and r=0.816, p=0.0012, respectively).
392 Furthermore, in the absence of vernalization, variation in the time to flowering correlates negatively
393 with variation in disturbance severity and temperatures (e.g., indicator value T (flowering date in LD
394 or SD): r=-0.814 and -0.806; p=0.0013 and 0.0016; Figure 5, Figure S12). Genetic variation in
395 phenology is thus not randomly distributed across habitats. Its distribution matches the disturbance
396 gradient along which the *A. thaliana* plant populations monitored in this study are distributed.

397 Both genetic variation for the regulation of flowering time in LD conditions and phenotypic variation
398 for flowering time *in situ* were correlated with disturbance frequency, but the slope of the
399 relationship changed signed ($F_{1,20}$=14.6984, p=0.001, Figure 5, Figure S13). We conclude that genetic
400 variants accelerating flowering in the absence of vernalization are found in the most disturbed sites,
401 where plants are observed to flower at a later date.

402

403 **Discussion**

404 Urban habitats form a mosaic of diverse environments that challenge plant growth in different ways.
405 However, the impact of this heterogeneity on the distribution of adaptive genetic variation is not
406 known (Rifkin, 2018). While the influence of urban heterogeneity on gene flow and population
407 differentiation has been reported in animal and plant species, its role in shaping the distribution of
408 genetic variation for adaptive traits remains unexplored (Beninde et al., 2015; Gorton et al., 2018;
409 Rivkin et al., 2019). By combining plant community and trait-based ecological measurements with an
410 in-depth exploration of genetic diversity at the genomic and phenotypic levels, this study bridges this
411 knowledge gap. The results provide compelling evidence that the genetic variation in *A. thaliana* is
412 not randomly distributed across environmentally diverse urban habitats. These findings enhance our
413 understanding of the actual ecology of a species that serves as a model in plant molecular biology
414 (Takou et al., 2019), and further provide valuable insights into adaptive genetic traits in urban
415 environments (Rivkin et al., 2019).

416 *A. thaliana* populations sampled here confirm previous reports about urban populations (Bomblies et
417 al., 2010): Each habitat patch is colonized by one, sometimes two, clonal lineages. An analysis of
418 genomic variation showed that these lineages belong to the broadly distributed gene pool of
419 northwestern European *A. thaliana* genotypes, supporting the notion that urban city patches were
420 established from a pool of regionally diverse migrants. Furthermore, our study shows that the urban
421 habitats hosting spontaneously occurring *A. thaliana* stands are heterogeneous. The advantage of
422 ecological characteristics based on community indicators, which leverage the particularly extensive
423 information available for the German flora, is that they provide robust measures of environmental
424 variation (Diekmann, 2003; Fanelli et al., 2006). Here, these characteristics reveal that *A. thaliana*
425 habitats form a continuum of varying levels of humidity and nutrients, as well as varying frequencies
426 of disturbance. The ecological differences we quantified were clearly associated with the phenotypic
427 differences displayed by plants *in situ,* as plants growing under better edaphic conditions (more
428 nutrients, more water) were larger, and plants growing in disturbed habitats bolted later and bore
429 more fruits. *In situ* observations of plant diversity, however, do not allow disentangling the effects of
430 plant plasticity to the environment from the effect of genetic differences between populations
431 (Aguilar-Trigueros et al., 2017; Cadotte & Tucker, 2017; Thakur & Wright, 2017). Unraveling whether
432 the successful occupation of diverse urban habitat patches is solely facilitated by plant plasticity
433 represents a critical step towards understanding the ecological dynamics of biodiversity in urban
434 environments (Brans et al., 2017; Rivkin et al., 2019).

435 Focusing on the 12 clonal lineages identified by genomic analysis, we thus explored whether genetic
436 variation contributes to the phenological variation observed *in situ*. By comparing the lineages
437 forming each population patch under common growth conditions, we found strong genetic
438 differences in how they regulate seed germination and flowering. In total, we relied on at least seven
439 indoor and outdoor common garden conditions to show that the 12 clonal lineages varied in their
440 requirements for vernalization and their ability to flower during shortening daylength, as well as in
441 the ability of their seeds to delay germination after exposure to cold and heat. We confirm previous
442 findings that the timing of germination plays a major role on life-history traits manifested later in the
443 life cycle (Donohue, 2009; Wilczek et al., 2009). By contrasting variation in plant traits expressed *in*
444 *situ* with *ex situ* observations made in common garden settings, we showed that genetic variation in
445 phenology tends to buffer the effect that environmental heterogeneity has on trait variation. Indeed,
446 we found that genotypes that flowered early under LD or SD conditions (*ex situ*) were found in sites
447 that were more disturbed. Whereas, those from the less disturbed sites flowered later under LD or
448 SD conditions but flowered early *in situ* (Figure 5). Indeed, the timing of flowering in LD conditions
449 and *in situ* showed opposite patterns of covariation with disturbance frequency (Figure 5).
450 Therefore, genotypes growing in habitats with relatively harsher environmental conditions

(characterized by resource stress, frequent disturbance, and a delay in development) tend to have traits genetically tailored to a life cycle that is shorter in the absence of vernalization. We thereby reveal a remarkable pattern of counter-gradient adaptation (Conover & Schultz, 1995), wherein genetic variation for flowering time in LD reduces the extent of phenological variation that would otherwise typify the heterogeneous environments hosting *A. thaliana* in the city. Therefore, variable genetic traits may not always be directly observable across environmental gradients. Counter-gradient variation is indeed a well-known confounding experimental error for *in-situ* studies, that can only be resolved with genetic studies in common gardens (Conover & Schultz, 1995).

We believe that, in this study, the distribution of genetic differences in the regulation of flowering reflects the outcome of a process driven by local selective pressures. Indeed, we found no correlation between genetic distance and geographic or ecological distance, so the non-random pattern of co-variation between disturbance level and genetic variation in life-history cannot be explained by local seed dispersal. We identify two other potential explanations: i) specialized genotypes have adapted via the selection of new mutations affecting life history in an otherwise non-adapted lineage (Orr, 2005), or ii) local environments filtered mis-adapted genotypes out, in the same way that they exclude mis-adapted species (Guo et al., 2018; Kraft et al., 2015; Laliberté et al., 2014; Yang et al., 2022). Since many alleles altering the genetics of flowering time have already been characterized in the western European region from which the populations originate (Le Corre, 2005; Lopez-Arboleda et al., 2021), it is highly likely that genotypes with diverse regulatory alleles were among the pool of migrants that populated habitat patches in the city of Cologne. Environmental filtering thus appears to be the most parsimonious explanation for the non-random distribution of genetic variation in flowering time documented in this study. Considering that the assessment of variation *in situ* and at the genetic level constrained us to the study of only 8 populations, our findings suggest that urban environmental heterogeneity has a strong filtering power on the genetic basis of intraspecific trait variation. Environmental filters imposed by habitat heterogenetiy in cities have been shown to diversify plant communities (Aronson et al., 2016). Indeed, cities can preserve species diversity, depending, for example, on the size and connectivity of habitats within cities (Beninde et al., 2015; Hahs et al., 2009). To the best of our knowledge, this study is the first to show that environmental filtering also has the potential to maintain functionally diverse and locally adapted genetic lineages. Such lineages form *bona fide* ecotypes, a term often used in the literature but seldom scrutinized.

Our assessment of *in situ* and *ex situ* variation in phenology highlights the importance of interdisciplinary approaches in ecology, quantitative genetics, and genomics. Indeed, our study identifies a pattern of adaptive genetic trait variation that is not directly observable in situ. With our sample of a small number of highly structured populations composed of one or two clonal lineages, we do not yet have the power to identify the loci responsible for adaptive life history variation

(Hancock et al., 2011; Herden et al., 2019). The number of variants that contribute to the genetic variation of plant phenology in Cologne *A. thaliana* populations will have to be determined in future studies.

Finally, one can speculate that disturbances may be the driving force behind the environmental filtering of genetic variation in life history within the city. However, in urban plant communities, environmental filters are shaped by a complex interplay of various factors, including urban habitat transformation, fragmentation, typical urban alterations such as heat islands or pollution, and human preferences(Williams et al., 2009). It is highly likely that each of these factors co-varies with the degree of disturbance. Consequently, future studies focusing on genetic variation within cities should intend to disentangle the specific factors filtering genetic variation in life history traits within urban plant populations and communities.

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Authors’ Contributions

AL, JM, GS, AS conceived the ideas and designed the methodology; JM, GS and AL wrote the manuscript; AF, GS, HD, JT, GC collected and/or analyzed the data; all authors contributed to the draft and gave final approval for publication. Sequencing data was deposited as submission numbers ERS5063293 to ERS5063441, project PRJEB40091 in the ENA repository. We acknowledge funding from the Deutsche Forschungsgemeinschaft within the framework of TRR341.

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767 **Figure Legends**

768 **Fig. 1**: Characteristics of eight urban *Arabidopsis thaliana* populations and their habitat conditions in
769 the city of Cologne, with (a) differences in size and phenological stage between *A. thaliana* from two
770 adjacent habitats (both photographs from 2017-04-03); (b) differences between habitat patches
771 based on plant species' Ellenberg indicator values for soil nutrients and (c) the same based on plant
772 species' Herben indicator for disturbance frequency. Letters indicate significant differences at
773 $p<0.05$. (d) Similarity of habitat patches based on a PCA with plant communities' average indicator
774 values for abiotic environmental conditions and disturbances. For indicator names, see Materials and
775 Methods.

776 **Fig. 2:** a) Analysis of genetic distance between progeny of 85 individual plants from 8 sampling sites
777 in Cologne determined by RAD-sequencing. Individual plants are colour coded according to their
778 origin. b) Principal component analysis of genetic differences between Cologne plants from the 8
779 sampling sites (blue), plants from additional sites of the sampling area (red) and 357 selected
780 genotypes of the Arabidopsis 1001 genome set (grey). Origin of 1001 genome genotypes is indicated
781 for countries represented by higher number of accessions (AUT-Austria, CZE-Czech Republic, ESP-
782 Spain, FRA-France, GER-Germany, ITA-Italy, NED-The Netherlands, SUI-Switzerland, SWE-Sweden,
783 UK-United Kingdom). c) Magnification of the yellow marked part of the plot b). d) Proportions of
784 ancestry shared between Cologne genomes (CLN) and genomes collected in Europe was estimated by
785 ADMIXTURE. On the basis of minimum cross-validation error, K=8 clusters were identified in the
786 sample of 357 previously published European genomes . From top to bottom are shown genomes
787 from Cologne, then from European dataset. The latter are grouped by country of origin and sorted by
788 ancestry within groups.

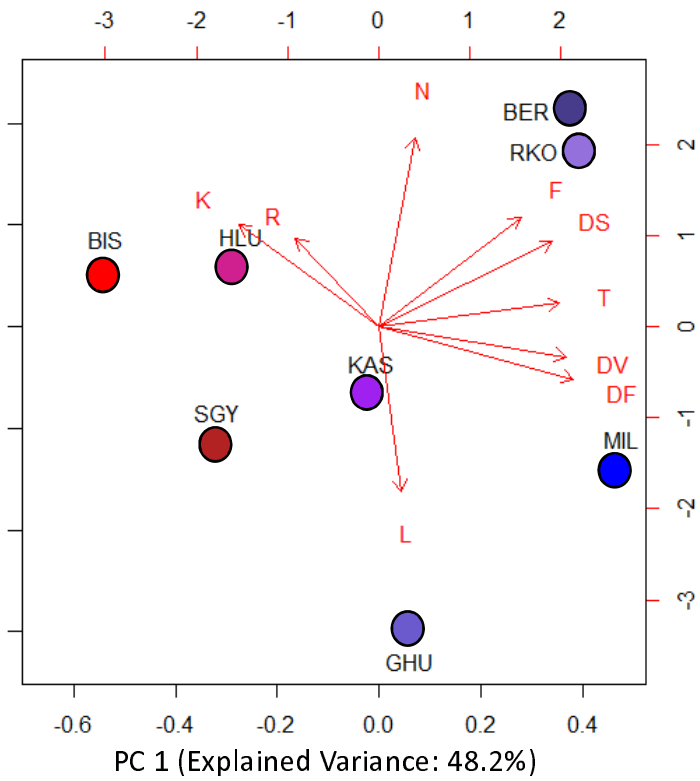
789 **Fig. 3:** Flowering time (days after sowing) of plants grown in controlled conditions: long days (16 h
790 light/20 °C; 8 h dark/18°C) (a), short days (10 h light/18 °C; 14 h dark/14 °C) (b), or short days
791 followed by vernalisation (8 h light/4 °C; 16 h dark/4 °C) and shift to long days at day 64 (c). Flowering
792 time (days of following year) of plants sown on dry soil on September 20th and grown without
793 artificial watering in the botanical garden (d). Letters indicate significant differences at a level $p<0,05$.

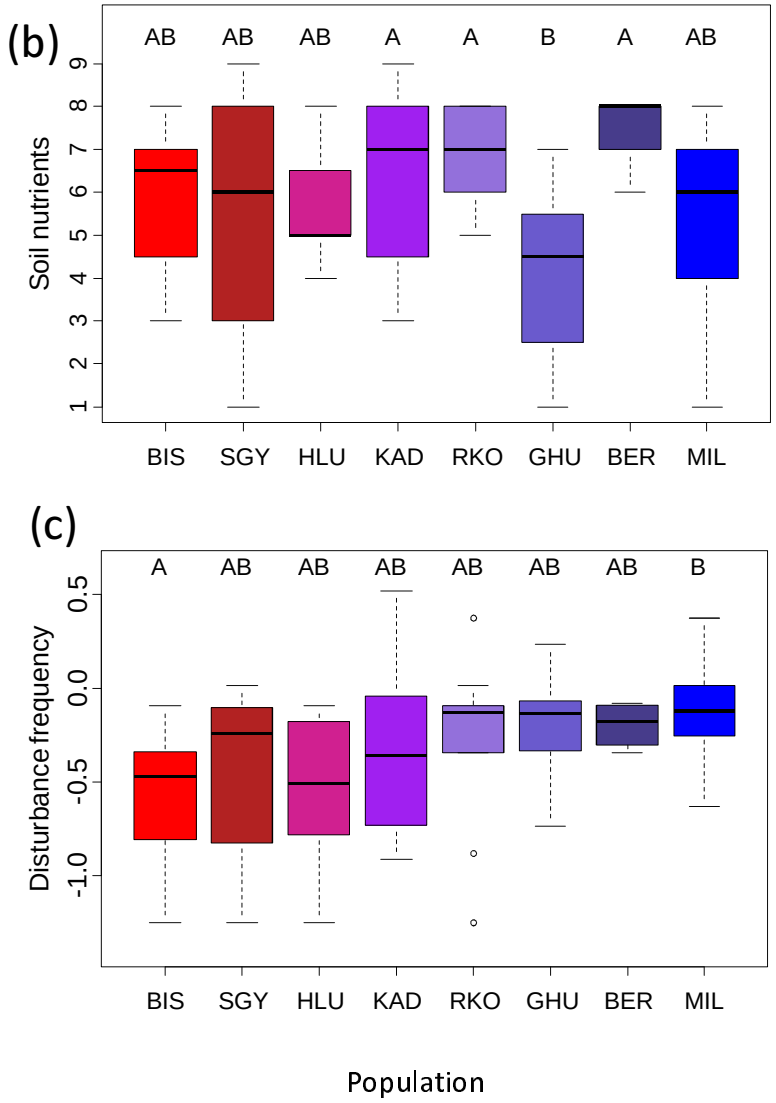
794 **Fig. 4:** Germination frequency of seeds from the 12 different Cologne genotypes and Col-0 controls.
795 Seeds were germinated on filter paper at 20 °C in petri dishes after 3-4 moths of afterripening to test
796 for primary dormancy (a) or incubated at 35 °C in the dark for 4 days before incubation at 20 °C to
797 observe heat induced secondary dormancy (b) (4 replicates per genotype). Germination frequency of
798 seeds counted on October 5th, that were sown on dry soil in a common garden experiment without
799 artificial watering on August 23rd (c) or September 20th (d) demonstrating different reaction to heat
800 induced secondary dormancy under natural field conditions. Letters indicate significant differences at
801 a level $p<0,05$.

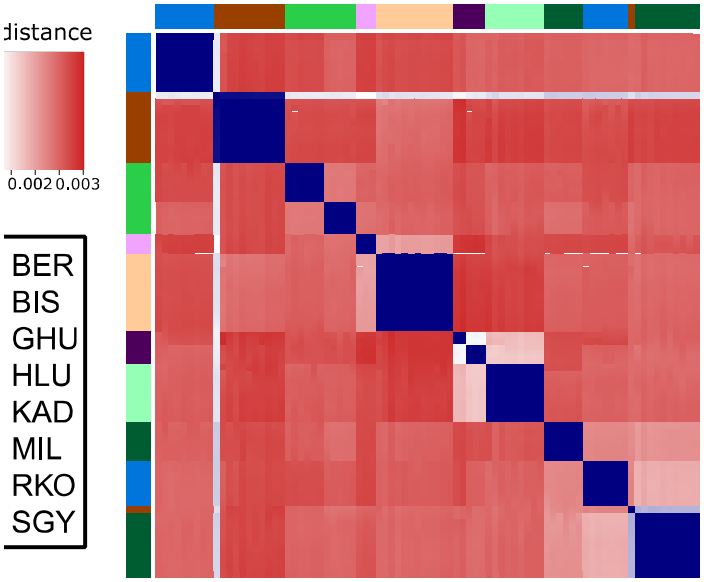
802 **Fig. 5:** Variation of phenology in urban *A. thaliana* genotypes, as observed within urban habitat
803 patches in the City of Cologne (in-situ; blue), and in a common-garden experiment in the same city
804 (ex-situ; red) as a function of variation in the index of Disturbance frequency at the site of origin. The
805 timing of flowering in-situ (blue) increases with disturbance frequency, whereas the opposite trend is
806 observable for the timing of flowering in indoor common garden conditions (red).

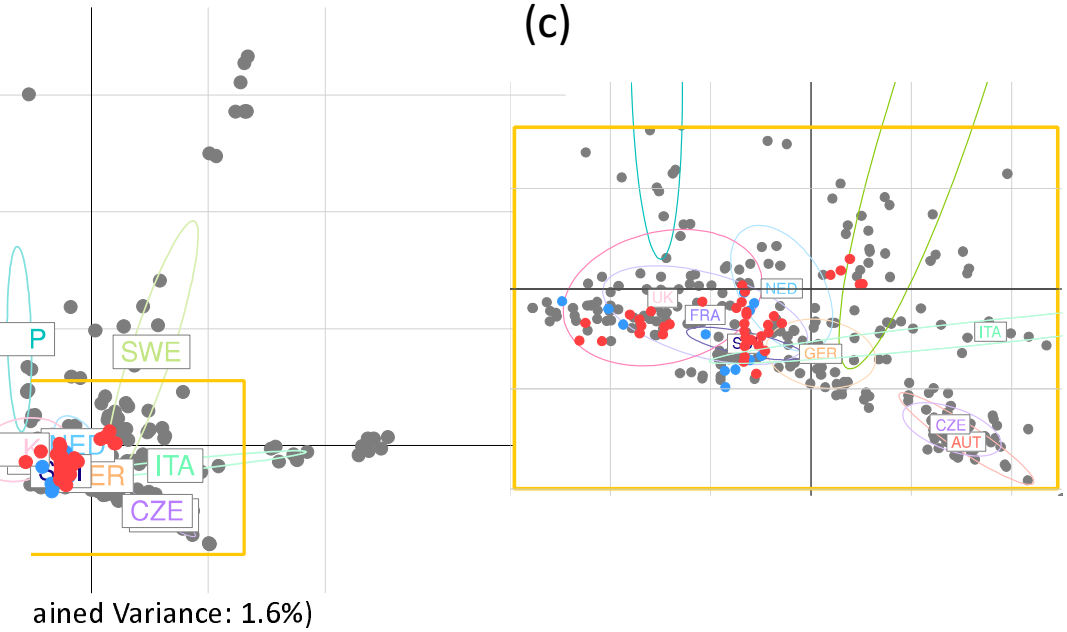
807

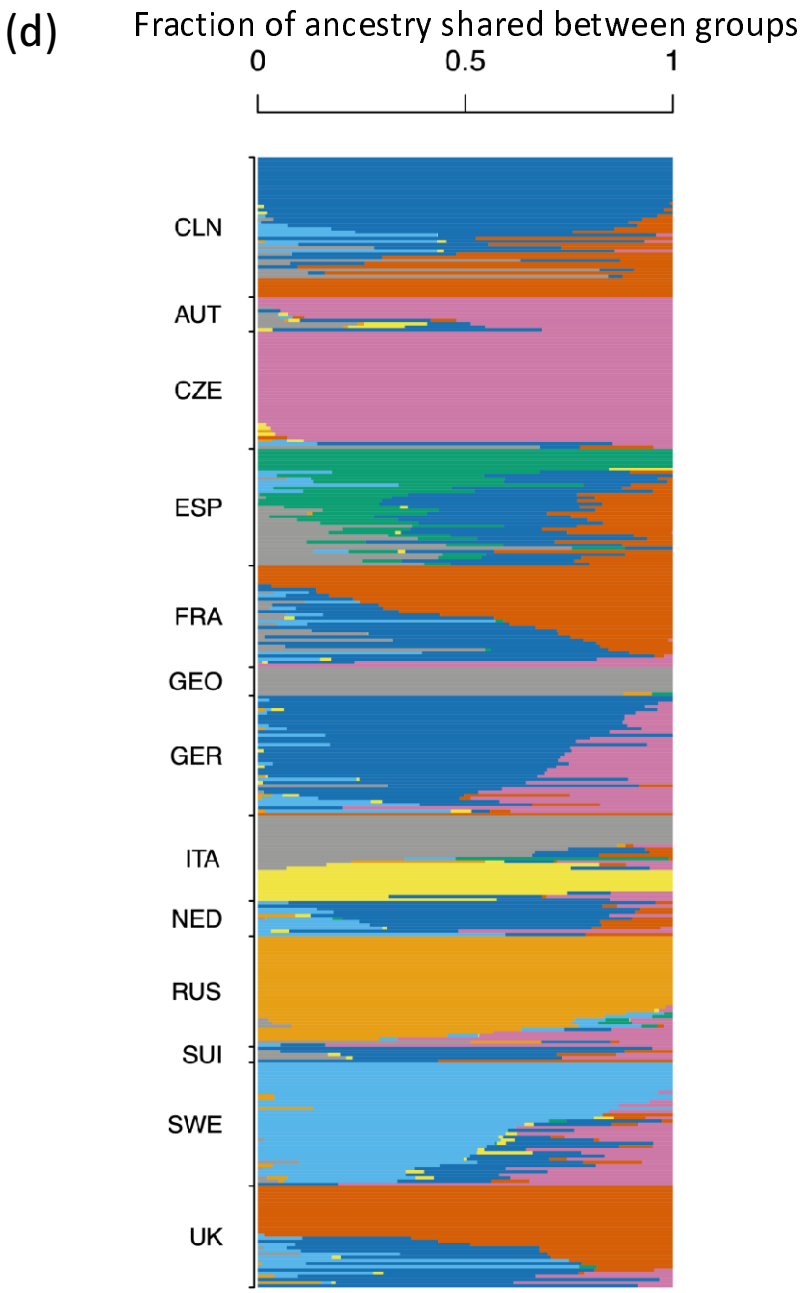
RICHARTZ

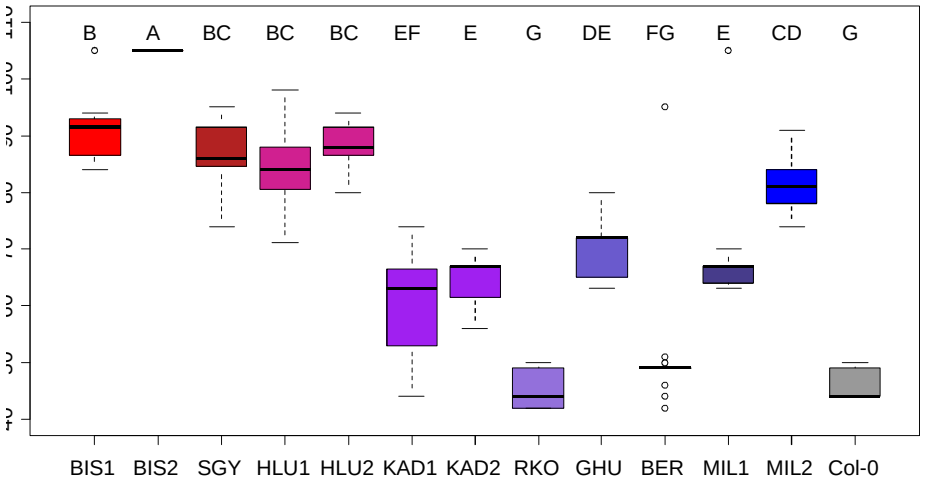

-3
-2
-1
0
1
2
N
BER
RKO
F
DS
K
R
BIS
HLU
T
KAS
DV
DF
SGY
MIL
L
GHU
-0.6
-0.4
-0.2
0.0
0.2
0.4
PC 1 (Explained Variance: 48.2%)

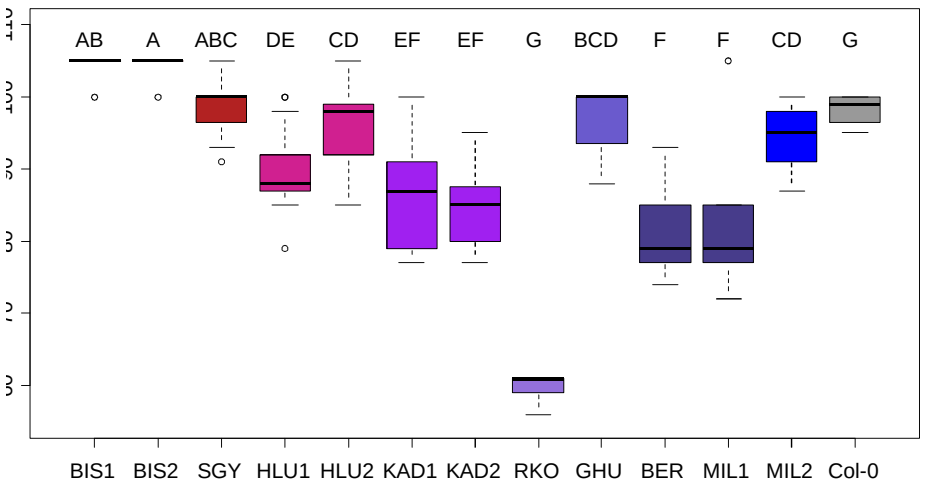

(b)
AB
AB
AB
A
A
B
A
AB
Soil nutrients
1
2
3
4
5
6
7
8
9
BIS
SGY
HLU
KAD
RKO
GHU
BER
MIL
(c)
A
AB
AB
AB
AB
AB
AB
B
Disturbance frequency
-1.0
-0.5
0.0
0.5
BIS
SGY
HLU
KAD
RKO
GHU
BER
MIL
Population


distance
0.002 0.003
BER
BIS
GHU
HLU
KAD
MIL
RKO
SGY

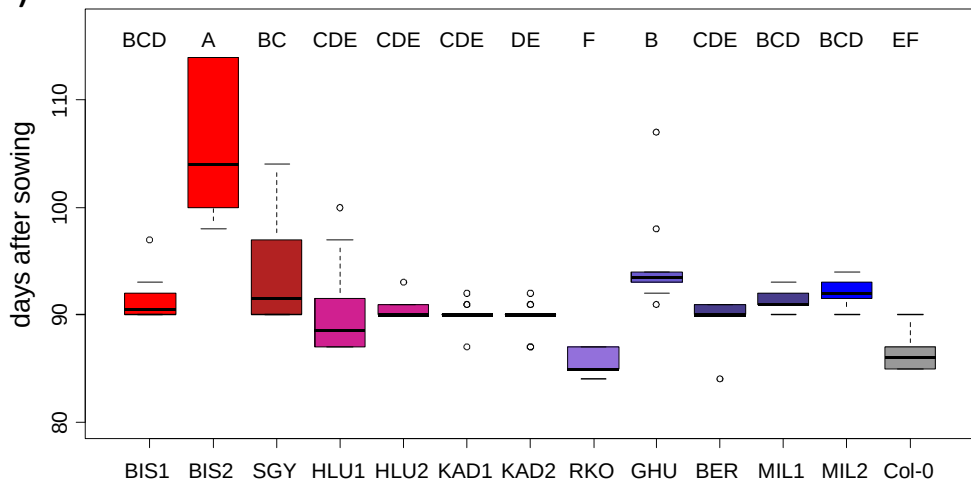

(c)
SWE
ITA
CZE
ained Variance: 1.6%)
NED
UK
FRA
GER
ITA
CZE
AUT


(d)
Fraction of ancestry shared between groups
0
0.5
1
CLN
AUT
CZE
ESP
FRA
GEO
GER
ITA
NED
RUS
SUI
SWE
UK

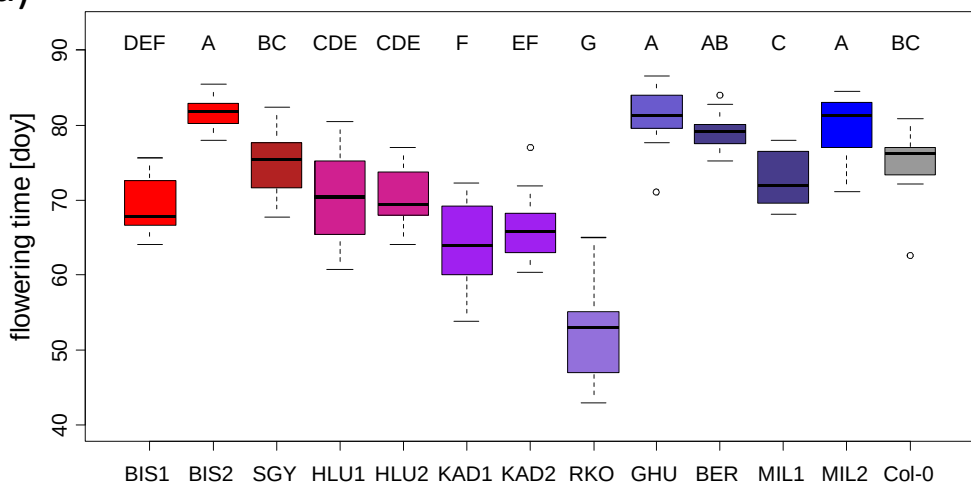

B A BC BC BC EF E G DE FG E CD G
BIS1 BIS2 SGY HLU1 HLU2 KAD1 KAD2 RKO GHU BER MIL1 MIL2 Col-0


AB A ABC DE CD EF EF G BCD F F CD G
BIS1 BIS2 SGY HLU1 HLU2 KAD1 KAD2 RKO GHU BER MIL1 MIL2 Col-0

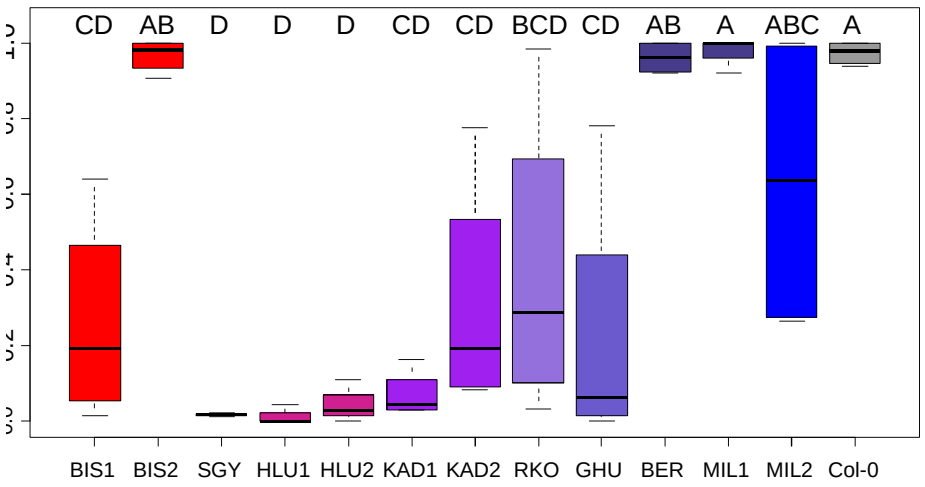
(c)


days after sowing
80
90
100
110
BCD A BC CDE CDE CDE DE F B CDE BCD BCD EF
BIS1 BIS2 SGY HLU1 HLU2 KAD1 KAD2 RKO GHU BER MIL1 MIL2 Col-0

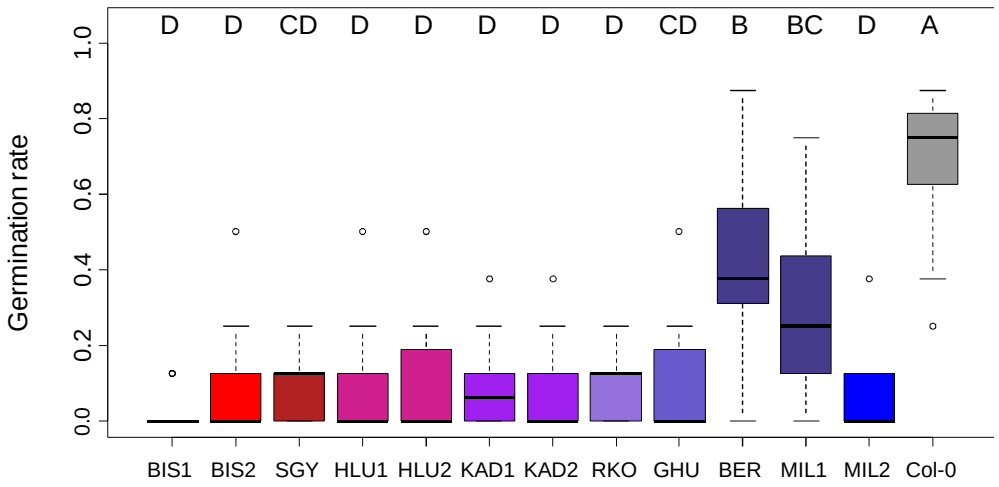
(d)

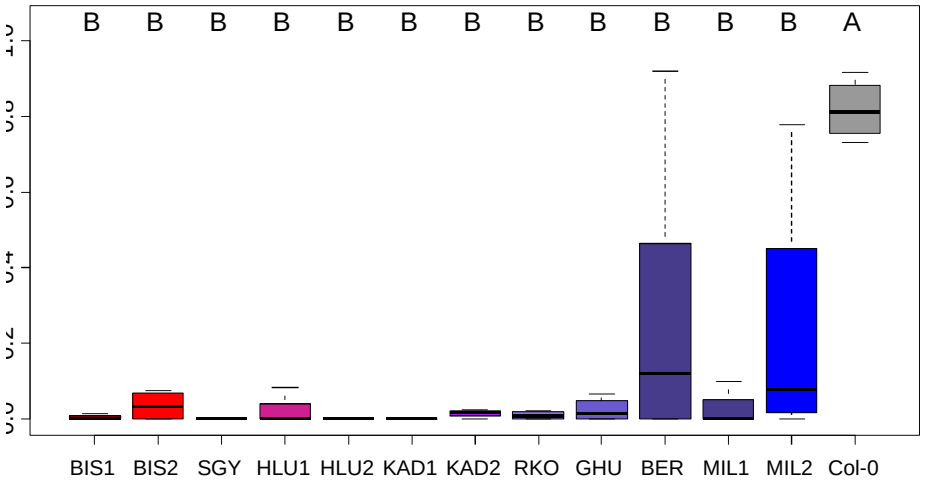

flowering time [doy]
40
50
60
70
80
90
DEF A BC CDE CDE F EF G A AB C A BC
BIS1 BIS2 SGY HLU1 HLU2 KAD1 KAD2 RKO GHU BER MIL1 MIL2 Col-0

g. 3:

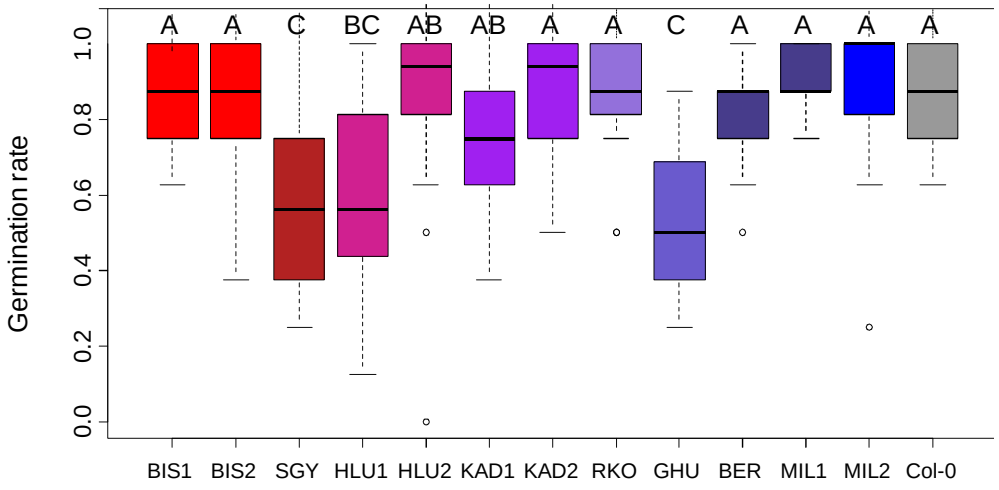

CD AB D D D CD CD BCD CD AB A ABC A
BIS1 BIS2 SGY HLU1 HLU2 KAD1 KAD2 RKO GHU BER MIL1 MIL2 Col-0

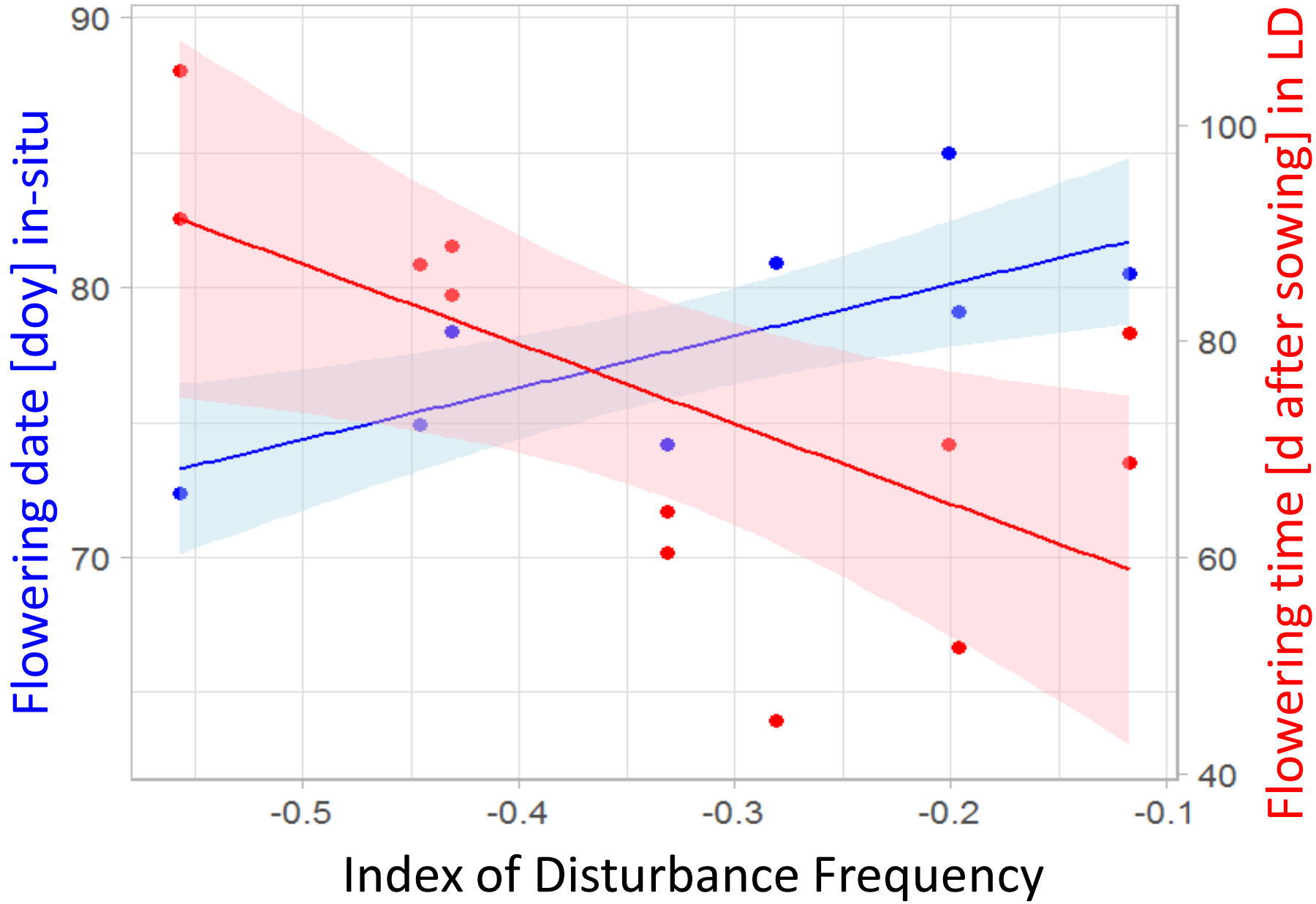
(c)


D D CD D D D D D CD B BC D A
Germination rate
0.0 0.2 0.4 0.6 0.8 1.0
BIS1 BIS2 SGY HLU1 HLU2 KAD1 KAD2 RKO GHU BER MIL1 MIL2 Col-0


B B B B B B B B B B B B A
BIS1 BIS2 SGY HLU1 HLU2 KAD1 KAD2 RKO GHU BER MIL1 MIL2 Col-0

(d)


A A C BC AB AB A A C A A A A
Germination rate
0.0 0.2 0.4 0.6 0.8 1.0
BIS1 BIS2 SGY HLU1 HLU2 KAD1 KAD2 RKO GHU BER MIL1 MIL2 Col-0


Flowering date [doy] in-situ
90
80
70
Flowering time [d after sowing] in LD
100
80
60
40
-0.5
-0.4
-0.3
-0.2
-0.1
Index of Disturbance Frequency

5: