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1 **Title: Local adaptation of life-history traits within urban populations of *Arabidopsis thaliana***

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12 **Abstract (max 350)**

13 1. The challenges to which plants are exposed in urban environments represent, in miniature,
14 the challenges plants face as a result of global environmental change. Hence, urban habitats
15 provide a unique opportunity to assess whether processes of local adaptation are taking
16 place despite the short temporal and geographical scales that characterize the anthropocene
17 .
18 2. We quantified the ecological diversity of spontaneously occurring urban habitat patches of *A.*
19 *thaliana*. Using plant community indicators, we show that these patches differ in their levels
20 of soil nutrient content and disturbance. Accordingly, plants in each patch displayed
21 significantly different flowering time, size, and fitness.
22 3. Using a deep sampling approach coupled with reduced genome-sequencing, we demonstrate
23 that most individuals can be assigned to a limited set of clonal lineages; the genetic diversity
24 of these lineages represents the diversity observed in western European populations of the
25 species, indicating that established urban populations originate from a broad regional pool of
26 lineages.
27 4. We assessed the genetic and phenotypic diversity of these lineages in a set of common
28 garden experiments. We report marked genetic differences in life-history traits, including
29 time of primary and secondary dormancy as well as of flowering. These genetic differences in
30 life-history traits are not randomly distributed but sorted out by ecological differences
31 among sites of origin.
32 5. **Synthesis:** Our study shows that the genetically diverse phenology of a regional *A. thaliana*
33 gene pool is not randomly distributed but filtered by urban environmental heterogeneity.

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34 This report is the first to show a pattern of local genetic adaptation within urban
35 environments. We conclude that environmental filtering helps maintain functional diversity
36 within species.

37

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

40

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41 **1. Introduction**

42 Predicting how plant and animal populations will cope with the challenges imposed by global
43 environmental change remains a pressing goal in ecology. Urban environments provide untapped
44 opportunities to link ecological and phenotypic diversity over the short temporal and spatial scales
45 that define the anthropocene (Johnson & Munshi-South, 2017; Szulkin et al., 2020). Understanding
46 how plant functional variation is optimized within local urban environments can provide crucial
47 information on species' potential to cope with upcoming global changes.

48 Compared to that in neighboring more rural areas, the climate in urban environments is generally
49 characterized by increased temperature and reduced moisture, and thus a higher drought likelihood
50 (Lambrecht et al. 2016). In addition, small-scale differences within urban microclimate are
51 observable, depending on urban geometry and construction materials (Chatzidimitriou & Yannas,
52 2015). Urban microhabitats differ in edaphic characteristics: soils vary considerably in their
53 developmental stage, depth, compaction, and surface transformation (Foti, 2017; Johnson & Munshi-
54 South, 2017; Tresch et al., 2018). Shallow, sealed soils with a low capacity for water storage and low
55 nutrient content typify the so-called urban gray spaces associated with built urban elements, such as
56 sidewalks. They occur in close vicinity to nutrient-rich urban green spaces, often with eutrophic or
57 even contaminated soils, which results in huge variation in soil-mediated plant resources over small
58 spatial scales (Bonthoux et al., 2019; Gilbert, 2012; Vega & Küffer, 2021). Cities also host numerous
59 ruderal sites, which are exposed to frequent and often severe disturbances, fragmentation, and high
60 turnover rates among populations (Dubois & Cheptou, 2017; Guo et al., 2018). In addition, human-
61 driven disturbances, such as weeding or mowing, may shorten the time windows for growth and
62 reproduction, and thus modulate further the disturbance patterns.

63 Major life-history decisions, such as the timing of germination and the timing of flowering, are critical
64 for resource acquisition and reproductive success, because of their impact on plant phenology. They
65 therefore contribute to the persistence of populations in heterogeneous urban settings (Liu et al.,
66 2021; Nord & Lynch, 2009). Plant phenology can be optimized locally by the environment because
67 the life-history traits underlying it are modulated both by nutrient resources and by environmental
68 cues, such as temperature and photoperiod (Vidal et al., 2014). For example, flowering time is
69 accelerated by winter temperature and resource stress and by high temperatures in spring, and
70 delayed by the shortening days of the fall (Andrés & Coupland, 2012; Li et al., 2019). Plant phenology
71 thus shifts in response to environmental cues that are modified by climate change (Chmielewski &
72 Rötzer, 2001; Piao et al., 2019; Primack & Miller-Rushing, 2012). The genetic composition of the
73 population can also be tuned by local selective pressures acting on phenology. Adaptation to local
74 environments has been observed experimentally in many organisms (Kawecki & Ebert 2004; Leimu &

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75 Fischer 2008). Clearly, adaptation via new genetic mutations is unlikely within urban settings
76 because urban populations are small, fragmented, and prone to frequent extinction (Orr, 2005).
77 However, if the pool of migrant genotypes is sufficiently diverse, and if each local environment
78 selects its fittest genotype from these pools, then individual genotypes may be locally adapted.
79 Indeed, patterns of local adaptation detected over small distances suggest that this kind of
80 environmental filtering could happen in urban habitats (Halbritter et al., 2018; Lenssen et al., 2004;
81 Robionek-Selosse et al., 2021). In recent years, several studies have described phenotypic differences
82 and genetic differentiation between individuals from urban and neighboring rural sites, consistently
83 indicating that populations adapt rapidly to urban environments. These studies documented direct
84 selection for novel optima in life-history traits, such as a late flowering time and decreased dispersal
85 in holy hawksbeard (*Crepis sancta);* an early flowering time in common ragweed (*Ambrosia*
86 *artemisiifolia*); and decreased cyanogenesis in white clover (*Trifolium repens*) (Cheptou et al., 2008;
87 Gorton et al., 2018; Lambrecht et al., 2016; Santangelo et al., 2022; Thompson et al., 2016).
88 Altogether, however, the amount of genetic variation in life-history traits, the distribution of this
89 variation across diverse urban sites, and its contribution to local adaptation remain unknown
90 (Johnson & Munshi-South, 2017).

91 Urban environments in regions with temperate climates often host populations of short-lived
92 herbaceous species, including the Brassicaceae species *Arabidopsis thaliana* (Bomblies et al., 2010;
93 Herben et al., 2016; Huang et al., 2018). This species -- the initial model of plant molecular biology --
94 has become a powerful system for answering fundamental questions of ecology, evolution, and
95 global environmental change (Exposito-Alonso et al., 2018; Fournier-Level et al., 2011; Takou et al.,
96 2019). *A. thaliana*, which is native to large areas of Eurasia and Africa, has recently colonized many
97 other parts of the world (Durvasula et al., 2017; Svardal et al., 2017). The deep sampling of natural
98 populations across various regions of its area of distribution has shown how environmental
99 heterogeneity shapes the distribution of genetic diversity in *A. thaliana* (Castilla et al., 2020; DeLeo et
100 al., 2020; Dubin et al., 2015, p. 20; Luo et al., 2015). Life-history trait adaptation appears to happen in
101 concert across regional environments (reviewed in Takou et al. 2019). This includes adaptation in the
102 timing of distinct phenological stages such as germination (Kronholm et al., 2012), or the onset of
103 flowering (Ågren et al., 2017; Akiyama & Ågren, 2018; Le Corre, 2005), but also the tuning of other
104 life-history traits such as the rate of rosette growth (Debieu et al., 2013; Wieters et al., 2021).

105 In the present study, we determined the relative importance of environmental plasticity and genetic
106 variation in shaping the phenology and fitness of eight spontaneously occurring urban populations of
107 *A. thaliana*. We asked i) Are *A. thaliana* urban populations occupying ecologically diverse habitats? ii)
108 Are *A. thaliana* populations genetically diverse? iii) Is this genetic diversity randomly distributed
109 across habitats? By combining plant-community and trait-based ecological measurements with

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110 analyses of genetic diversity at genomic and phenological levels, we show that genotypes growing in
111 urban habitats exposed to high resource limitations and increased disturbance levels tend to have
112 traits tailored to local conditions.

113 **2. Materials and Methods**

114 *Study area*

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

120 *Field surveys, phenology monitoring, and functional trait measurements*

121 We surveyed eight *A. thaliana* populations within a 4.3 x 3.7 km area in southwestern Cologne. Study
122 populations were selected based on their accessibility and on their isolation from other populations,
123 with all habitat patches located at least 0.6 km from one another. Urban habitat patches where
124 viable *A. thaliana* populations were observable included green spaces such as disturbed lawns;
125 various habitats within gray spaces, such as sidewalks, wall tops, and bases; and vegetated roadsides
126 (Figure 1a; Table S1). Depending on habitat characteristics, patch size ranged from 4 m^2 for
127 populations on wall tops to > 100 m^2 for populations in vegetated roadsides, and population size
128 ranged from 15 to 1,000 individuals. All populations were studied throughout their growth period in
129 2017 (which extended between early March and the end of June), and revisited yearly during five
130 subsequent growth periods (2018-2022) to assess population persistence. No field permissions were
131 necessary for the collection of the plant samples in this study.

132 We recorded phenological stages and fitness-related plant functional traits of *A. thaliana* individuals
133 distributed over the eight habitat patches. At each patch, we established 10 to 14 observation
134 quadrats (10 x 10 cm), each at least 50-60 cm from one another. We marked from 1 to 3 *A. thaliana*
135 individuals per quadrat (15-24 per habitat patch; 174 in total) and visited them twice a week during
136 the study period to score their development. For all marked individuals, we recorded the day of
137 bolting (i.e., when the inflorescence became visible) and, subsequently, the number of open flowers
138 and the number of closed fruits. Rosette diameter and the number of rosette leaves were measured
139 at the time of bolting. We used these observation data to derive five phenological stages and five
140 fitness-related plant functional traits ("fitness proxies"; see Table 1). The selection of plant functional
141 traits follows recommendations for the assessment of plant fitness (Gibson, 2014), while
142 phenological stages were selected to represent life-history stages of the entire growth period, as
143 recommended for herbaceous species (Huang et al., 2018; Nordt et al., 2021). Germination was

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144 generally not possible to determine *in situ*, except in populations growing on wall tops (SGY, KAD,
145 HLU).

146 **Table 1.** Phenological stages and fitness proxies measured *in situ* for urban populations of *A.*
147 *thaliana*; with units of measurement; minimum, maximum (Min/Max) and median trait values for the
148 whole data collected across the study sites. doy: days of year, mm: millimeter.

Variable set	Stage or Trait [Unit]	Min/Max	Median
Phenological stages	Bolting (inflorescence visible) [doy]	69/90	79.0
	Onset of flowering [doy]	75/131	89.0
	Onset of fruiting [doy]	86/144	94
	Flowering duration [days]	1/78	18
	Fruiting duration [days]	1/75	26
Fitness proxies	Rosette diameter at bolting [mm]	4/45	15
	Rosette leaf no. at bolting	3/24	10
	Generative height [mm]	9/330	83
	Max. number of open flowers	1/35	3
	Max. number of closed fruits	1/190	18

149

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). We determined the composition of plant
163 communities in autumn (late August and early September) 2017 by recording the presence of all
164 vascular plant species in the herbaceous layer of the habitat patches. Based on species' specific
165 indicator values (see Table S2), average EIVs and DIVs were calculated for each habitat patch.

166 *Assessment of genomic variation*

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167 At the end of the 2017 growth period, we harvested seeds from 5 to 19 individuals per population
168 and used single seeds from each individual to produce progenies. For four of the eight habitat
169 patches (BER, BIS, KAD, and MIL), we also included seeds from individuals sampled in the year prior
170 to field data collection, referring to these as population samples (Table S3). In order to broaden our
171 view of the genomic diversity of *A. thaliana* in Cologne, we also produced progenies from seeds
172 collected at 30 additional habitat patches distributed throughout the sampling area but not included
173 in the *in situ* monitoring (so-called scattered samples). Field collected seeds were grown in a
174 randomized setting, under conditions simulating winter, i.e., in growth chambers with 10 h light at
175 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 %
176 humidity, for 6 weeks; and ultimately ripened in the greenhouse in spring-like conditions with 16 h
177 light, at about 20-24 °C / 8 h dark at about 18-20 °C until harvest. Seeds for progeny were harvested
178 using Aracons (Betatech, Gent, Belgium).

179 Three leaves per plant were sampled for DNA extraction, deep frozen, and homogenized using a
180 Precellys Evolution homogenizer (Thermo Fisher Scientific, Waltham, MA, USA). DNA was isolated
181 using the NucleoSpin® Plant II kit (Macherey-Nagel, Düren, Germany). Genomic DNA was prepared
182 for RAD-Seq as described by Dittberner et al. (2019) using 10 pools with 20 plants each, and
183 sequencing was performed at the Max Planck Institute of Plant Breeding Research. The
184 bioinformatics pipelines used for read mapping, and identification of each of the 12 genotypes
185 present in the studied habitat patches are described in the supplementary information.

186 *Common garden experiments:*

187 For each identified genotype, two individuals were randomly selected and amplified in the
188 greenhouse. Since they were raised in the same maternal environment, variance among genotypes
189 can be assigned to genetic effects and variance among replicate lines of each genotype allows testing
190 whether all maternal effects were effectively removed. We then took eight individuals from each of
191 these two lines and grew them in growth chambers (Johnson Controls, Milwaukee, WI, USA) in three
192 trials under different growth conditions. Growth conditions were chosen to identify genetic variation
193 in the major pathways regulating flowering time: constant long days (16 h light at 20°C/8h dark at
194 18°C); constant short days (8 h light at 18°C day/16 dark at 16°C night); and short
195 days/vernalization/long days (8 h day light at 18°C /16 h dark at 16°C for 5 weeks; 8h day light/16h
196 dark at 4 °C for 4 weeks, 16 h day light at 20°C/8h dark at 18°C until ripening). The *A. thaliana* line
197 Col-0 was included as a reference in all experiments. Because we did not detect differences between
198 replicated lines, we determined that we had 16 replicates/genotype for this experiment. Replicates
199 were chosen randomly and sown in individual 6-cm-diameter pots that were watered regularly by

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200 flooding the trays. Flowering time was determined as the time span between sowing and the first
201 flower to open petals.

202 Ripe seeds from the short-day/vernalization/long-day experiment were harvested and stored at
203 room temperature for the germination assay. Germination rates were determined after three
204 months to quantify primary dormancy (Baskin & Baskin, 2004). Seeds were sown on wet filter paper
205 and incubated in closed microtiter plates. Before incubation under long-day conditions (16 h light at
206 20°C/8h dark at 18°C), seeds were incubated in the dark either at 4 °C for 7 days to demonstrate
207 their capacity to germinate at 100%, or at -21 °C for 4 d and at 35 °C for 4 d to quantify variation in
208 secondary dormancy. Germination was scored after 10 days.

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

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

225 For germination assays under controlled indoor conditions, the number of germinants per Petri dish
226 was analyzed with the R function zeroinfl to correct for zero inflation. For germination rates in the
227 outdoor common garden experiment, we proceeded as under controlled conditions, except that we
228 used a quasipoisson distribution of error. Because the two replicate lines we used for each genotype
229 never showed significant effects, the line effect was not included in any of the models. We used the
230 function ANOVA with a Chi-squared test to determine the overall significance of effects. In all cases,
231 the significance of pairwise differences was tested with a Tukey post-hoc test, using the function glht
232 from the multcomp package in R. We used the Pearson coefficient of the cor.test function in R to
233 quantify the correlation between phenotypes.

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234

235 **Results**

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

237 The eight urban habitat patches differed significantly in environmental conditions related to climate,
238 soil, and severity of disturbance. Indicator values for light (L), and edaphic conditions -- humidity (F),
239 and soil nutrients (N) -- differed between sites (Figure 1b, supplemental Figure 1a, b; humidity (F):
240 $F_{7,113}$=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).
241 Indicator values of the accompanying flora for herb-layer disturbance frequency (DF) and herb-layer
242 structure-based disturbance (DV) differed between sites (Figure 1c, supplemental Figure 1c; DF:
243 $F_{7,84}$=2.897, p=0.0092; DV: $F_{7,84}$=3.108, p=0.00577), whereas differences in herb-layer disturbance
244 severity (DS) were not significant. The three values for disturbance were strongly and positively
245 correlated with each other (range of r: 0.802 – 0.958; p<0.00172) and with the temperature indicator
246 (range of r: 0.736 – 0.803; p<0.00632; Figure 1d; supplemental Figure 2, supplemental Table 2) .

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

248 Across the eight urban *A. thaliana* populations, all phenological stages and plant functional traits
249 measured *in situ* showed significant differences (p<2.43e-06; F=6.094, Table S4; supplemental Figure
250 3). These are exemplified in data on flowering time and rosette size (Figure 2a,b). The diverse traits
251 describing phenological time points, the temporal spacing between phenological states, and the size
252 of the plants also covaried (supplemental Figure 4).

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

255 *In situ* observations of phenotypic differences between sampling sites were partly correlated with
256 the differences in environmental characteristics: as expected, plants from sites with high mean
257 values for N- and F-indicators, which reflect differences in nutrient and soil moisture levels,
258 respectively, were on average larger than those from sites with lower N- and F-indicator values (p-
259 values smaller than 0.05 for F: inflorescence length, maximal number of open flowers, rosette
260 diameter; for N: rosette diameter at bolting, number of rosette leaves, and number of fruits; Figure
261 2c). Plants from sites with higher disturbance values bolted later than plants from other sites, but
262 formed larger inflorescences bearing more fruits.

263 **Urban populations in Cologne consist of clones of diverse western European lines**

264 In order to determine the extent of genomic variation and the history of population establishment,
265 we genotyped 5-17 individuals per population as well as plants sampled from additional sites
266 scattered across the study area. In total, we genotyped about 1% of the genome of 149 individuals

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267 using RAD-sequencing (Table S3). Individuals were overall highly homozygous (>99%), indicating that
268 self-fertilization dominates in Cologne populations. Populations showed a strong genetic structure,
269 as can be seen from the genetic distance matrix (Figure 3a): Populations BER, GHU, RKO, and SHY
270 consisted exclusively of genetically identical individuals, with the exception of a single plant with high
271 heterozygosity from the GHU site. Populations BIS, HLU, KAS, and MIL each consisted of two
272 genotypes. The genotype of plants collected in 2016 was the same as that of individuals collected in
273 2017 in the same location, confirming that local populations are most often formed from one
274 (maximum two) single genotypes that persist over years.

275 Genotypes of the Cologne samples were compared to the European genotypes of the 1001-genome
276 collection (Alonso-Blanco et al., 2016) by running a principle component analysis (PCA) on a subset of
277 representative genotypes from both datasets (see Materials and Methods). Cologne samples co-
278 localize with western and Central European accessions, especially with accessions from Germany,
279 France, the Netherlands, and the UK, but they did not form a defined subgroup separate from the
280 other genotypes (Figure 3b, c, supplemental Figure 5). In conclusion, at each site, collected *A.*
281 *thaliana* individuals formed either one or two clonal lineages. The level of nucleotide diversity of
282 these lineages reflects region-wide levels of diversity.

283

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

285 Variation in genetic pathways regulating flowering time and germination may contribute to life-
286 history differences recorded on-site. To link the germination and flowering behavior of the urban
287 genotypes to knowledge about these well-studied pathways, we analyzed flowering under standard
288 conditions in a randomized block design and included the well-studied lab strain Col-0 as a reference.
289 In total, three controlled environmental conditions were set: long days (LD), short days (SD), and a
290 combination of short days/vernalization/long days (SDV). This setup thus allowed us to thoroughly
291 examine genetic variation in the regulation of flowering time. The first two conditions allowed us to
292 derive photoperiod-dependent differences in the regulation of flowering; the third mimicked the
293 succession of environmental cues experienced in the fall, winter, and spring (Andrés & Coupland,
294 2012). Differences between LD and SDV conditions revealed variation in vernalization requirements,
295 because it reveals the extent to which flowering time differences in LD tend to decrease in SDV as a
296 result of exposure to the cold temperatures.

297 Flowering times differed between genotypes under all conditions ($F_{12,134to158}>39.53$, $p<2\ e^{-16}$) and the
298 interaction between genotype and experimental condition was signicant ($F_{24,461}= 36.72$, $p<2\ e^{-16}$).
299 Under LD conditions, BER and RKO plants flowered as early as the early flowering reference genotype
300 Col-0, whereas BIS2 plants had not flowered after 105 days, indicating that the former do not require

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301 vernalization to flower (Figure 4a, Table S5). Under SD conditions, several genotypes flowered
302 significantly earlier than Col-0, indicating that natural Cologne genotypes were prone to flower either
303 in the short days of the fall or the late winter: RKO plants flowered earliest; BER, KAD1, KAD2, and
304 MIL1 flowered only slightly earlier than Col-0. Both genotypes from the BIS site were unable to
305 flower under SD conditions and were thus unlikely to flower in the fall (Figure 4b). When plants were
306 grown under conditions simulating winter (SDV), most genotypes flowered within a narrow time
307 period, with only BIS2 displaying significantly later flowering (Figure 4c). This experiment
308 demonstrated that there is considerable variation in how the 12 Cologne genotypes regulate
309 flowering time. The magnitude of these differences depended on photoperiod and vernalization.

310 The genotypes also differed in how they regulated germination (Table S6). After three months of
311 seed storage at room temperature, germination assays revealed that the genotypes differed in
312 primary and secondary dormancy. When seeds were untreated, their germination rates indicated
313 levels of primary dormancy (Baskin & Baskin, 2004; Finkelstein et al., 2008). Untreated seeds of BER,
314 BIS2, MIL1, and MIL2 plants had high germination rates, whereas seeds of plants from the other
315 genotypes had a less than 50% germination rate (χ^2, df=11, N=48, $p<2.2e^{-16}$; Figure 5a). Incubating
316 non-dormant seeds at very low (-21°C) or high (35 °C) temperatures induced variable levels of
317 secondary dormancy in Cologne genotypes, as indicated by a significant interaction between
318 genotypes and germination conditions (likelihood ratio test, χ^2= 83.2, df=24, $p=1.81e^{-8}$, Figure 5c and
319 supplemental Figure S6).

320

321 **Outdoor common garden experiments confirm differences in germination and flowering regulation**
322 **under field conditions**

323 The 12 genotypes were further assayed in an outdoor common garden located in Cologne, and the
324 genetic variation underlying their differences in survival and fertility was quantified. Here again, we
325 included the widely used genotype Col-0 in the experiment as a reference (Table S7a-b). Since
326 phenological variation is known to depend on the timing of germination, seeds were sown directly
327 into pots at a field site in late August, mid-September, early November (6 weeks, 9 weeks, and 3
328 months after harvest), and the end of February. As pots were not watered artificially, germination
329 and further development depended on natural rainfall and temperature. Germination rates among
330 genotypes strongly differed in the August and September sowing cohorts. In the August cohort, 43
331 days after seeds were sown, only Col-0 plants had germinated to 100%; BER and MIL1 showed 43 and
332 28 % germination, respectively, 10 % or fewer of the seeds of the other genotypes had germinated
333 (differences between the genotypes: $F_{12,195}$=16.97, p<2e-16, Figure 5c). In the September cohort, 15
334 days after seeds were sown, most genotypes had germinated at 80% or more, and only HLU1, GHU,

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335 and SGY had germinated at less than 60% (after 22 d: $F_{12,195}$=15.84, p<2e-16; Figure 5d). Eventually,
336 most seed germinated, but the most delayed was the August cohort (supplemental figure 7a-d).
337 Later germination in this cohort can be explained by heat-induced secondary dormancy. In contrast,
338 differences in germination for the November and February cohorts were minor (supplemental figure
339 7e,f).

340 More than 95 % of the germinated seedlings survived until seed set. Most flowered in spring with the
341 exception of the Col-0 and BER plants from the August cohort, which germinated almost immediately
342 and flowered already in late autumn/winter. Differences in flowering time, which were observed for
343 each of the four cohorts ($F_{12,195}$>13.33, p<2 e^{-16}), revealed a significant interaction between genotype
344 and sowing date ($F_{34,755}$= 20.89, p<2e^{-16}, Table 2, figure 4d, supplemental figure 8). Flowering times
345 observed for the 12 genotypes were generally not correlated across the various common garden
346 cohorts grown outdoor, with the exception of the February and September cohorts (supplemental
347 Figure 9). Fertility among the genotypes, determined by the number of fruits per pot, differed
348 between the genotypes for the August, September, and November cohorts ($F_{12,195}$=3.072, p=0.00054;
349 $F_{12,194}$=7.721, p=1.15e^{-12}; and $F_{12,195}$=4.702, p=1.01e^{-06}). Again, a significant interaction between
350 genotype and seed-sowing date was observed ($F_{24,584}$=3.534, p= 4.75e^{-08}). For the August cohort, only
351 two genotypes (RKO and SGY) were less fertile than most of the genotypes (Table 2, supplemental
352 Figure 10). Fitness differences for the plants grown from seeds sown in February, all of which
353 germinated within a week in early March and flowered 23-39 days later, were not significant. With
354 52% of the plants dying after an unusually warm dry spell in May, the average fitness of the spring
355 cohort was low .

356

357 **Table 2.** Flowering dates and fertility of Cologne lines and Col-0 grown in common garden
358 experiments (averages and standard deviation). Seeds were sown on dry soil on August 23,
359 September 20, November 8, or February 6, and grown without artificial watering. Letters indicate
360 significant differences at a level p<0,05.

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Genotype	flowering date (day of year)				fruits/pot			fruits/plant
	August	September	November	February	August	September	November	February
BER	59.3 (13.0)[b]	79.1 (2.3)[ab]	101.0 (0.4)[a]	126.1 (2.3)[abc]	176.9 (109.2)[abc]	255.5 (59.8)[d]	72.2 (11.2)[b]	27.5 (45.9)[a]
BIS1	79.6 (4.2)[a]	69.1 (3.3)[def]	92.7 (1.7)[ef]	126.1 (1.1)[abc]	209.4 (80.7)[abc]	301.3 (68.6)[bcd]	75.8 (9.6)[b]	42.8 (34.1)[a]
BIS2	83.5 (3.6)[a]	81.7 (2.0)[a]	95.1 (1.5)[cd]	127.2 (1.8)[a]	201.1 (97.0)[abc]	276.1 (103.8)[bcd]	93.4 (23.6)[ab]	30.0 (35.2)[a]
GHU	82.4 (17.5)[a]	81.1 (3.5)[a]	102.6 (0.8)[a]	124.1 (1.4)[bcd]	153.7 (98.2)[bc]	249.7 (94.5)[d]	77.8 (13.4)[b]	12.9 (23.9)[a]
HLU1	76.8 (6.1)[a]	70.6 (5.6)[cde]	91.1 (3.0)[f]	121.6 (3.1)[de]	183.1 (85.5)[abc]	253.6 (83.7)[d]	77.3 (26.1)[b]	33.8 (24.8)[a]
HLU2	76.6 (8.9)[a]	70.5 (3.9)[cde]	92.0 (2.6)[f]	125.8 (1.8)[abc]	161.7 (94.83[bc]	226.5 (49.7)[d]	75.9 (18.8)[b]	24.0 (33.9)[a]
KAD1	80.4 (6.5)[a]	64.4 (5.2)[f]	94.6 (0.8)[de]	118.6 (1.4)[f]	193.9 (95.7)[abc]	371.9 (114.2)[abc]	89.5 (21.5)[ab]	10.3 (18.8)[a]
KAD2	76.3 (5.1)[a]	66.3 (4.0)[ef]	94.8 (1.0)[cde]	123.4 (3.7)[cd]	295.1 (138.1)[a]	429.8 (118.8)[a]	108.4 (14.5)[a]	14.1 (22.1)[a]
MIL1	75.6 (8.7)[a]	72.9 (3.3)[c]	94.7 (1.0)[cde]	124.3 (1.0)[bc]	211.4 (128.3)[abc]	264.0 (69.9)[cd]	80.9 (11.1)[b]	27.8 (35.4)[a]
MIL2	81.9 (5.0)[a]	80.2 (3.6)[a]	97.0 (2.9)[bc]	126.9 (2.5)[ab]	244.0 (90.8)[abc]	261.8 (102.0)[d]	86.6 (11.2)[ab]	27.9 (38.0)[a]
RKO	73.3(10.4)[a]	51.8 (5.5)[g]	100.6 (1.9)[a]	119.3 (2.0)[ef]	140.5 (99.1)[c]	373.8 (60.1)[ab]	80.6 (25.4)[b]	39.4 (37.2)[a]
SGY	80.8 (9.8)[a]	74.9 (4.0)[bc]	94.6 (1.9)[de]	124.3 (2.3)[bc]	142.9 (92.6)[c]	220.9 (108.3)[d]	75.4 (25.8)[b]	22.8 (31.6)[a]
Col-0	54.6 (12.3)[b]	74.9 (3.9)[bc]	97.8 (2.2)[b]	125.0 (2.9)[abc]	273.5 (149.1)[ab]	261.7 (81.0)[d]	71.9 (12.3)[b]	38.8 (19.6)[a]

361

362

363 In summary, plants from the 12 Cologne genotypes displayed remarkable genetic differences in their
364 ability to control germination and flowering in common garden experiments. Flowering times
365 measured in controlled growth chamber and in the common garden were significantly correlated,
366 but correlation depended on the various experimental indoor conditions and/or the seed dispersal
367 times characterizing the different common garden cohorts (supplemental Figure 9).

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

370 The phenotypes of urban *A. thaliana* individuals in situ correlates with the ecological characteristics
371 of the sites in which they are monitored. We further tested whether the distribution of genetic
372 differences revealed in common gardens and under lab conditions is independent of the ecological
373 characteristics at the sites of origin. We found that genetic variation in the rate of germination under
374 late summer conditions in the August cohort or after cold treatment correlates positively with
375 variation in the severity of disturbance (r=0.763, p=0.0039 and r=0.816, p=0.0012, respectively).
376 Furthermore, in the absence of vernalization, genetic variation in the regulation of flowering
377 correlates negatively with variation in disturbance severity and temperatures (e.g., indicator value T
378 (flowering date in LD or SD): r=-0.814 and -0.806; p=0.0013 and 0.0016; Figure 6, supplemental
379 Figure 11). Genetic variation in phenology is thus not randomly distributed across habitats. Its
380 distribution matches the disturbance gradient along which the *A. thaliana* plant populations
381 monitored in this study are distributed.

382 Both genetic variation for the regulation of flowering time in LD conditions and phenotypic variation
383 for flowering time *in situ* were correlated with disturbance frequency, but the slope of the
384 relationship changed signed ($F_{1,20}$=14.6984, p=0.001, Figure 6, supplemental Figure 12). We conclude

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385 that genetic variants accelerating flowering in the absence of vernalization are found in the most
386 disturbed sites, where plants are observed to flower at a later date.

387

388 **Discussion**

389 Urban habitats form a mosaic of diverse environments that pose serious challenges to plant growth.
390 By combining plant community and trait-based ecological measurements with analysis of genetic
391 diversity at the genomic and phenological levels, we provide compelling evidence that the genetic
392 variation in *A. thaliana* is not randomly distributed across environmentally diverse urban habitats. *A.*
393 *thaliana* populations sampled here confirm previous reports about urban populations (Bomblies et
394 al., 2010): Each habitat patch is colonized by one, sometimes two, clonal lineages. An analysis of
395 genomic variation showed that these lineages belong to the broadly distributed gene pool of
396 northwestern European *A. thaliana* genotypes, supporting the notion that urban city patches were
397 established from a pool of regionally diverse migrants. Furthermore, our study shows that the urban
398 habitats hosting spontaneously occurring *A. thaliana* stands are heterogeneous. The advantage of
399 ecological characteristics based on community indicators, which leverage the particularly extensive
400 information available for the German flora, is that they provide robust measures of environmental
401 variation (Diekmann, 2003; Fanelli et al., 2006). Here, these characteristics reveal that *A. thaliana*
402 habitats form a continuum of varying levels of humidity and nutrients, as well as varying frequencies
403 of disturbance. Environmental variation is thought to help shape the diversity of plant communities
404 by filtering out ill-suited species (Guo et al., 2018; Kraft et al., 2015; Laliberté et al., 2014; Yang et al.,
405 2022). The ecological differences we quantified were clearly associated with the phenotypic
406 differences displayed by plants *in situ,* as plants growing under better edaphic conditions (more
407 nutrients, more water) were larger, and plants growing in disturbed habitats bolted later and bore
408 more fruits. However, plant plasticity in response to environmental variation, as well as to
409 environmental filtering, may explain the patterns of covariation we found (Aguilar-Trigueros et al.,
410 2017; Cadotte & Tucker, 2017; Thakur & Wright, 2017).

411 Focusing on the 12 clonal lineages identified by genomic analysis, we thus explored whether genetic
412 variation underpins the phenological variation observed *in situ*. By comparing the lineages forming
413 each population patch under common growth conditions, we found strong genetic differences in
414 how they regulate seed germination and flowering. In total, we relied on at least seven indoor and
415 outdoor common garden conditions to show that the 12 clonal lineages varied in their requirements
416 for vernalization and their ability to flower during shortening daylength, as well as in the ability of
417 their seeds to delay germination after exposure to cold and heat. We further showed that differences

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418 observed in controlled growth chambers also manifested in the more realistic conditions of an
419 outdoor common garden (Wilczek et al., 2009).

420 Importantly, we observed that the genetic differences between clonal lineages were not randomly
421 distributed across the contrasting environmental conditions that characterize the eight sites studied
422 here. Considering that the assessment of variation *in situ* and at the genetic level constrained us to
423 the study of only 8 populations, our findings suggest that urban environmental heterogeneity has a
424 strong filtering power on the genetic basis of intraspecific trait variation. By contrasting variation in
425 plant traits expressed *in situ* with *ex situ* observations made in common garden settings, we showed
426 that genetic variation in phenology tends to buffer the effect that environmental heterogeneity has
427 on trait variation. Indeed, we found that genotypes that flowered early under LD or SD conditions (*ex*
428 *situ*) were found in sites that were more disturbed, but not in the less disturbed one where plants
429 flowered early *in situ* (Figure 6). Indeed, the timing of flowering in LD conditions and *in situ* showed
430 opposite patterns of covariation with disturbance frequency (Figure 6). In other words, genotypes
431 growing in habitats with particularly harsh environmental conditions (characterized by resource
432 stress, frequent disturbance, and a delay in development) tend to have traits genetically tailored to a
433 life cycle that is shorter in the absence of vernalization. We hypothesize that this particularly ruderal
434 strategy is promoted in habitats where the onset of the life cycle is delayed by human-driven
435 disturbances, such as mowing and weeding, or where unfavorable abiotic conditions slow plant
436 growth. Here we reveal a pattern of counter-gradient adaptation, according to which genetic
437 variation for flowering in LD helps decrease the extent of phenological variation that would
438 otherwise typify the heterogeneous environments hosting *A. thaliana* in the city (Conover & Schultz,
439 1995).

440 Quantifying variation in *in situ* and *ex situ* phenology variation highlights both the ecological role of
441 genetic variation for the regulation of flowering time under long day conditions and the importance
442 of interdisciplinary approaches in ecology, quantitative genetics, and genomics. For example,
443 although genomic sequencing sometimes helps verify the occurrence of local adaptation, such an
444 approach would not benefit this study (Hancock et al., 2011; Savolainen et al., 2013). In contrast to
445 phenological variation, genome-wide nucleotide diversity is randomly distributed, and clonal
446 propagation precludes the application of genome-wide association studies (Hancock et al., 2011;
447 Herden et al., 2019). Similarly, common garden experiments can distinguish plasticity from genetic
448 variation (Kawecki & Ebert, 2004), but the genetic diversity that can be quantified *ex situ* cannot
449 directly predict the functional role of either factor in plants grown under natural conditions. It is
450 ultimately the relationship between environmental variables and *in situ* trait variation that clarifies
451 the functional role of genetic variation in the regulation of phenology. Individuals with a genetic
452 make-up that delays flowering time under long-day conditions did not grow in habitats where

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453 environmental conditions happened to delay flowering. Such delay may be due to a delay in the
454 timing of germination in these populations, but it could as well result from slower plant development
455 in the most disturbed locations.

456 Environmental filters imposed by habitat heterogenetiy in cities have been shown to diversify plant
457 communities (Aronson et al., 2016). Indeed, cities can preserve species diversity, depending, for
458 example, on the size and connectivity of habitats within cities (Beninde et al., 2015; Hahs et al.,
459 2009). To the best of our knowledge, this study is the first to show that environmental filtering also
460 has the potential to maintain functionally diverse and locally adapted genetic lineages. Such lineage
461 form *bona fide* ecotypes, a term often used in the literature but seldom scrutinized. The dynamics of
462 adaptation in the populations of this study are possible only because diverse phenological variants
463 are present in the regional pool of migrants. It is tempting to speculate that this process may
464 prefigure how plant populations will adapt in the anthropocene.

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468 **Authors' Contributions**

469 AL, JM, GS, AS conceived the ideas and designed the methodology; JM, GS and AL wrote the
470 manuscript; AF, GS, HD, GC collected and/or analyzed the data; all authors contributed to the draft
471 and gave final approval for publication. Sequencing data was deposited as submission numbers
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474

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698 **Figure Legends**

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

707 **Fig. 2:** Phenological stages and plant functional traits of the eight urban *A. thaliana* populations
708 measured in-situ, with a) differences in bolting ($F_{7,149}$=17.81, p<2e^{-16}); b) differences in maximum
709 rosette diameter ($F_{7,163}$=10.92, p=2.85e^{-11}). Letters indicate significant differences at p<0.05. c)
710 correlations of populations' average phenological stages and functional traits with abiotic habitat
711 characteristics given by the accompanying plant community (L -light, T -temperature, K -
712 continentality, F -humidity, R -pH, N –nitrogen content) and disturbance regimes (disturbance
713 frequency DF, severity DS, herb-layer structure DV).

714 **Fig. 3:** a) Analysis of genetic distance between progeny of 85 individual plants from 8 sampling sites
715 in Cologne determined by RAD-sequencing. Individual plants are colour coded according to their
716 origin. b) Principal component analysis of genetic differences between Cologne plants from the 8
717 sampling sites (blue), plants from additional sites of the sampling area (red) and selected lines of the
718 Arabidopsis 1001 genome set (grey). Origin of 1001 genome lines is indicated for countries
719 represented by higher number of accessions (AUT-Austria, CZE-Czech Republic, ESP-Spain, FRA-
720 France, GER-Germany, ITA-Italy, NED-The Netherlands, SUI-Switzerland, SWE-Sweden, UK-United
721 Kingdom). c) Magnification of the yellow marked part of the plot b).

722 **Fig. 4:** Flowering time (days after sowing) of plants grown in controlled conditions: long days (16 h
723 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
724 followed by vernalisation (8 h light/4 °C; 16 h dark/4 °C) and shift to long days at day 64 (c). Flowering
725 time (days of following year) of plants sown on dry soil on September 20th and grown without
726 artificial watering in the botanical garden (d). Letters indicate significant differences at a level p<0,05.

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

735 **Fig. 6:** Opposite variation of phenology in urban A. thaliana genotypes, as observed within urban
736 habitat patches in the City of Cologne (in-situ; blue), and in a common-garden experiment in the
737 same city (ex-situ; red). Populations are sorted by the disturbance frequency in urban habitat
738 patches (average DF values). The timing of flowering (bolting) in-situ increases with disturbance
739 frequency, whereas the opposite trend is observable for the timing of flowering in indoor common
740 garden conditions (red).

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742

743 # Journal of Ecology Supporting Information

744 **Environmental filtering allows local adaptation of life-history traits of *Arabidopsis thaliana* urban**
745 **populations**

746 **Authors:** Gregor Schmitz, Anke S. K. Frank, Hannes Dittberner, Andrea Schrader, Karl-Heinz Linne von
747 Berg, George Coupland, Juliette de Meaux, Anja Linstädter

748 **Supplementary information:**

749 **Bioinformatics pipeline and identification of A. thaliana population structure in the city of Cologne**

750 Genomic DNA sequence was characterized by RAD-Seq as described by Dittberner at al. (2019) using
751 10 pools with 20 plants each. The mapping of sequenced reads and the determination of population
752 structure is described in the following.

753 Reads were trimmed with TRIMMOMATIC and low quality reads were sorted out using FastQC. Reads
754 were subsequently mapped to the *A. thaliana* reference genome TAIR10 (Berardini *et al.* 2015).
755 Minimum coverage for SNP calling was 15. Forty-five individuals with missing data in more than 70%
756 of loci were removed from the main dataset (see below). Loci with more than 20% missing data were
757 discarded. The RAD genotype dataset was merged with the 1001 genomes SNP dataset (Alonso-
758 Blanco *et al.* 2016) using *bcftools(Li 2011)* and filtered to include only loci genotyped in the RADseq
759 dataset. Genetic distances between pairs of individuals were calculated using a custom python script.
760 A heatmap of genetic distances was created using the *heatmap3* package (Zhao et al., 2015). Genetic
761 distance (Cavalli-Sforza & Edwards 1967) among populations was calculated using the *hierfstat*
762 package (Goudet & Jombart 2015). For each environmental factor, pairwise Euclidean distance
763 among populations was calculated. Correlation between environmental and genetic distance as well
764 as between geographic and genetic distance was tested using a Mantel test with 999 permutations.

765 To relate Cologne genotypes to the larger collection of accessions of the 1001-genome project, we
766 included accessions from Germany and from countries close to Germany in western, northern or
767 southern direction (France, Spain, Switzerland, Netherlands, United Kingdom and Italy) with at most
768 40 accessions per country. Accessions from countries with more than 40 accessions in the dataset
769 were randomly subsampled and relict accessions (Alonso-Blanco *et al.* 2016) were removed. Cologne
770 genotypes from both sample sets ('populations from the eight habitat patches' and from 'scattered
771 sites') were subsampled to remove genetically identical individuals, resulting in a set of 51
772 genotypes. In order to visualize similarity between plant genomes, a principal component analysis
773 was conducted using the *vcfR* package (Knaus & Grünwald 2017) and the *adegenet* package
774 (Kollmann & Fischer 2003) with missing variants scaled to the mean.

775 To assign genotypes to the 45 individuals with low coverage, we called SNPs again allowing for
776 minimum coverage of 2. We removed all SNPs with more than 50% missing data. We used the
777 software *SNPmatch* (Pisupati *et al.* 2017) to create a database of the high quality SNP dataset and
778 find the closest match to the database for each of the 45 low-coverage individuals. If the 3 best
779 matches were the same genotype and identity was over 97%, we assigned that genotype to the
780 individual.

781 **References for supplementary methods**

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804 **SUPPORTING TABLE LEGENDS**

805 **Table S1:** GPS coordinates of the sites monitored in the study

806 **Table S2:** Plant community present in the sites of the study and associated Ellenberg and disturbance
807 indicator values (EIV and DIV, respectively).

808
809 **Table S3:** Summary of RAD-sequencing data. First generation progeny of plants described below in
810 Table S4 were used for DNA isolation. Links to sequencing data are given for plants with high
811 sequencing coverage. Assignements of individual plants to genotypes is described in material and
812 methods.

813 **Table S4:** Phenology data of individual plants from the 8 monitored sites of Table S1. Each plant has a
814 unique identifier containing the name of the sub-site of sampling.

815 **Table S5:** Flowering time data for individual plants grown in controlled conditions (indoor common
816 garden). Data show the days when the first flower has open petals and is calculated from the day of
817 sowing. NA- plants were lost during the experiment. Plants that did not flower within 105 days were
818 assigned “>105”.

819 **Table S6:** Germination in controlled conditions (indoor common garden). Data indicate the fraction
820 of germinated seeds after 10 d incubation at 20°C. Pre-treatments are described in material and
821 methods.

822 **Table S7:** a- Summary of common garden experiments for plants sown in August, September and
823 November and b- in February (see methods). Germination was recorded at different time points after
824 sowing. For the February cohort, density was reduced to 1 plant per pot, for the other cohorts all
825 plants were left to grow. For these experiments, flowering time is the average of flowering time of
826 surviving plants.

827

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830 **SUPPORTING FIGURE LEGENDS**

831 **Figure S1:** Characteristics of eight urban *Arabidopsis thaliana* populations and their habitat
832 conditions in the city of Cologne, with (a) differences between habitat patches based on plant
833 species' Ellenberg indicator for humidity (F), (b) light intensity (L) index , and (c) Herb layer structure-
834 based disturbance index. Letters indicate significant differences at $p<0.05$.

835 **Figure S2:** Significant correlations between Ellenberg Indicator Values (EIVs) and Disturbance
836 Indicator Values (DIVs) for the eight *A. thaliana* habitat patches in the City of Cologne. EIVS are light
837 regime (L), temperature (T), continentality of climate (K), soil moisture (F), soil reaction (R), and
838 nutrient availability (N); DIVs are Disturbance frequency index (DF), Disturbance strength index (DS),
839 and Herb layer structure-based disturbance index (DV)

840 **Figure S3:** Development of phenological stages over time observed at the eight Cologne populations
841 shown as percentage of plants reaching the stage of bolting, onset of flowering and onset of fruiting
842 .

843 **Figure S4:** Correlation of average phenological stages and growth phenotypes between urban
844 populations (only significant correlations are shown).

845 **Figure S5:** Pairwise genetic distance of genotypes sampled in the eight urban habitat patches (BER,
846 BIS, GHU, HLU, KAD, MIL, SGY, RKO) and from additional scattered sites of the study area.

847 Genetic distance between genotypes, as measured by the average number of pairwise nucleotide
848 differences p, ranged from 0.0017 to 0.0034 with a mean of 0.003. Genetic similarity allowed
849 clustering genotypes into three groups: Group 1 comprised RKO and BER, group 2 KAS-1, KAS-2, and
850 BIS-2, group 3 consisted of HLU-1, HLU-2, and SGY. Populations displayed no signal of isolation by
851 geographic distance (Mantel test $p>0.05$) or isolation by environmental distance (Mantel test $p>0.05$
852 for all environmental factors; see below). In the additional populations, 44 individual genotypes were
853 found among the 65 sequenced, and no genotype was found at more than one site. Five accessions
854 (RUS16, RUS17, ROD8, RUS23, RUW1) deviated from the rest and co-localized with Swedish
855 accessions. Interestingly, we also found four individuals originating from a single site (ROD-1, ROD-2,
856 ROD-3, ROD-4), which were virtually identical (1 SNP) to the accession TueWa1-2 from Tübingen
857 (Germany), which was described in the Arabidopsis 1001 Genomes Project. This lineage might result
858 from long-range migration.

859 **Figure S6:** Germination frequency of seeds from the 12 different Cologne genotypes and Col-0
860 controls. Seeds were germinated on filter paper at 20 °C in petri dishes following stratification for 7d
861 at 4 °C and demonstrating that seeds are viable (a) or an incubation at -21 °C in the dark (b). (4
862 replicates per genotype).

863 **Figure S7:** Germination frequency of seeds from the 12 different Cologne genotypes and Col-0
864 controls germinated in common garden conditions. Seeds were sown on dry soil on August 23rd (a,
865 b), September 20th (c, d), November 8th (e), or February 6th (f). Germination was scored on October
866 5th (a, c), October 31st (b, d) for the August and September sowing. Note that at the second time
867 point, more than 50% of seeds germinated, but GHU, SGY, KAS1, and RKO still had approximately
868 20% lower germination rates compared to the other genotypes. Germination was scored on
869 December 13th (e), and April 5th (f) for the November and February cohorts, respectively.
870 Differences between a) and c) can be explained by high temperature induced secondary dormancy.

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871 **Figure S8:** Flowering time (days of year) of plants from the 12 different Cologne genotypes and Col-0
872 controls grown in common garden conditions. Seeds were sown on dry soil on February 6th (a),
873 August 23rd (b), September 20th (c), or November 8th (d). Letters indicate significant differences at a
874 level $p<0,05$.

875 **Figure S9:** Correlation among averages for flowering time phenotypes measured in controlled
876 laboratory conditions and in common garden experiments. Flowering times between the 4 planting
877 cohorts show little correlation

878 **Figure S10:** Common garden experiments - number of siliques of single plants sown in February (a),
879 or number of siliques per pot with 8 seeds sown in August (b), September (c), or November (d).
880 Letters indicate significant differences at a level $p<0,05$.Scales differ for the plots.

881 **Figure S11:** Correlation of averages of phenotypes of plants in controlled lab conditions, outdoor
882 common garden experiments (cohorts) and averages of Ellenberg and disturbance indicator values
883 of the sites of their origin (L- Light, T- Temperature, K- Continentality, R- pH, N- nutrients, DF-
884 disturbance frequency, DS- disturbance severity, DV- structure-based disturbance index).
885 Germination rates were monitored at several time points after sowing. Flowering time was scored in
886 controlled growth chamber under long days, short days, and in a sequence combining short days,
887 vernalization and then long days (vernalization conditions). Germination was scored in controlled
888 conditions on untreated 2-month-old seeds and after stratification. Secondary dormancy was
889 determined by scoring germination in stratified seeds exposed to either freezing conditions or heat
890 (see methods for details). Fertility was measured in all four outdoor common garden cohorts, but not
891 in growth chambers.

892 **Figure S12:** Correlation of averages of phenotypes of plants in their original sites, in controlled lab
893 conditions and in common garden experiments. Germination rates were monitored at several time
894 points after sowing. Flowering time was scored in controlled growth chamber under long days, short
895 days, and in a sequence combining short days, vernalization and then long days (vernalization
896 conditions). Germination was scored in controlled conditions on untreated 2-month-old seeds and
897 after stratification. Secondary dormancy was determined by scoring germination in stratified seeds
898 exposed to either freezing conditions or heat (see methods for details). Fertility was measured in all
899 four outdoor common garden cohorts, but not in growth chambers.

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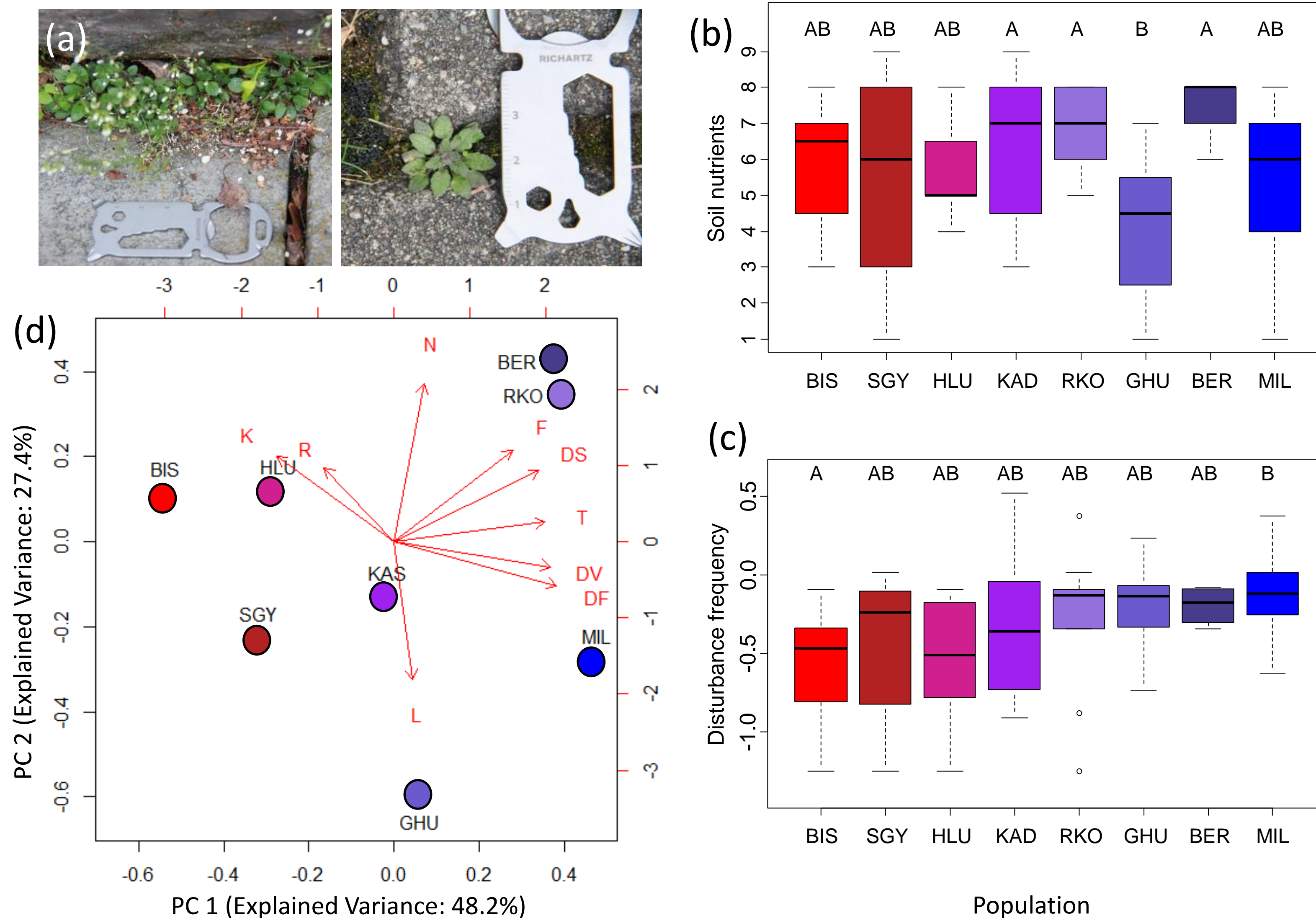


Fig. 1: Characteristics of eight urban *Arabidopsis thaliana* populations and their habitat conditions in the city of Cologne, with (a) differences in size and phenological stage between *A. thaliana* from two adjacent habitats (both photographs from 2017-04-03); (b) differences between habitat patches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

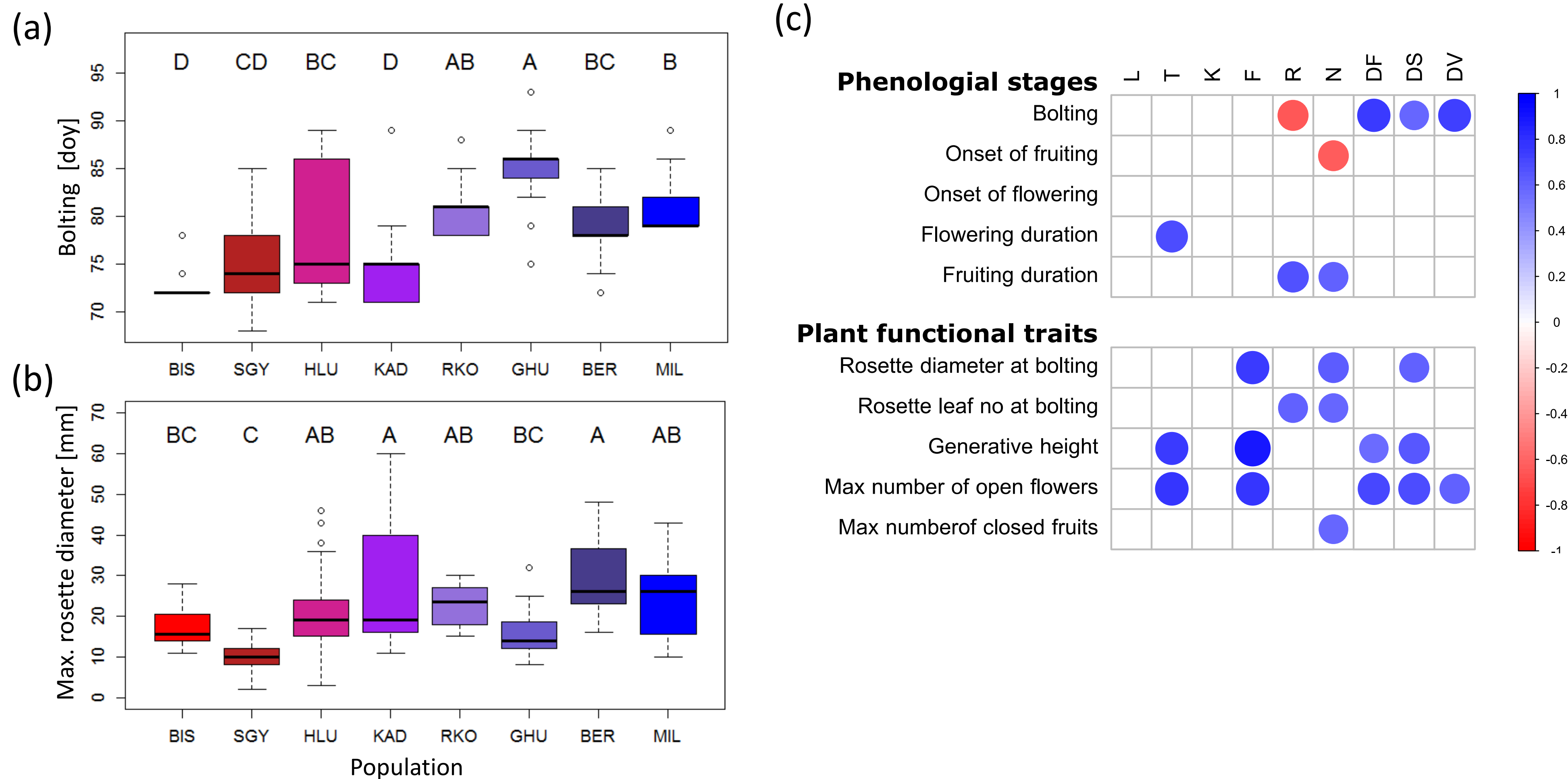


Fig. 2: Phenological stages and plant functional traits of the eight urban *A. thaliana* populations measured in-situ, with a) differences in bolting ($F_{7,149}$=17.81, p<$2e^{-16}$); b) differences in maximum rosette diameter ($F_{7,163}$=10.92, p=$2.85e^{-11}$). Letters indicate significant differences at p<0.05. c) correlations of populations' average phenological stages and functional traits with abiotic habitat conditions (L, T, K, F, R, N) and disturbance regimes (DF, DS, DV).

(a)

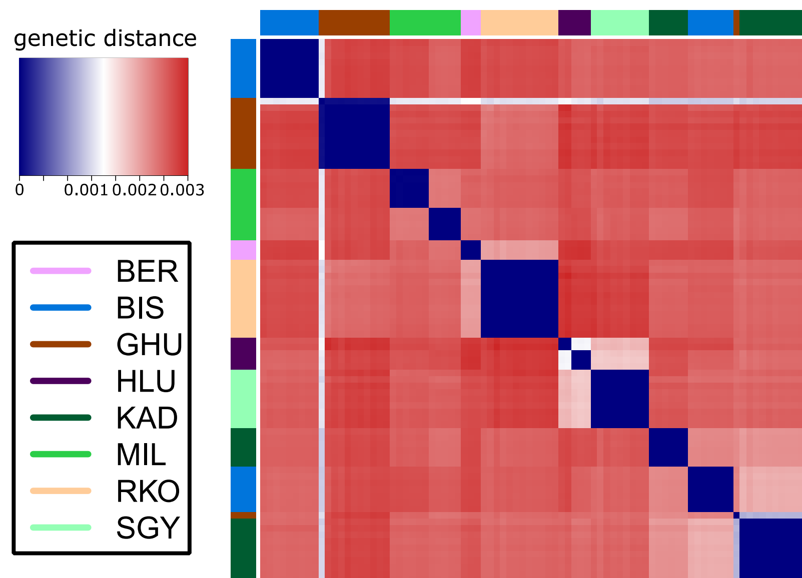
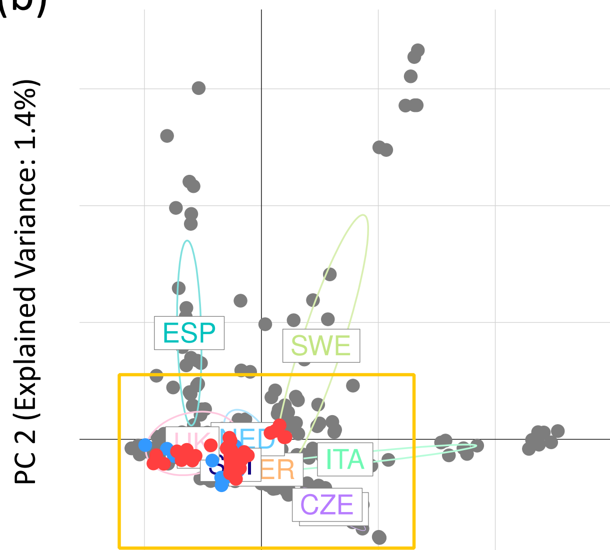
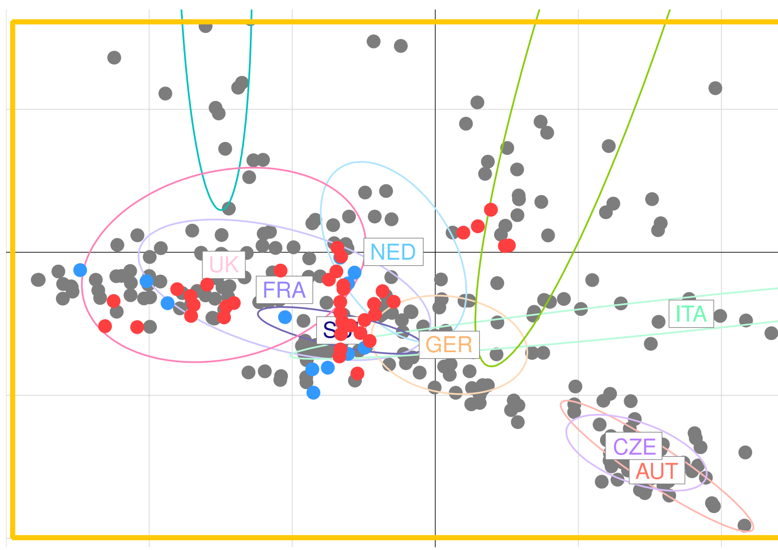


Fig. 3: a) Analysis of genetic distance between progeny of 85 individual plants from 8 sampling sites in Cologne determined by RAD-sequencing. Individual plants are colour coded according to their origin. b) Principal component analysis of genetic differences between Cologne plants from the 8 sampling sites (blue), plants from additional sites of the sampling area (red) and selected lines of the Arabidopsis 1001 genome set (grey). Origin of 1001 genome lines is indicated for countries represented by higher number of accessions (AUT-Austria, CZE-Czech Republic, ESP-Spain, FRA-France, GER-Germany, ITA-Italy, NED-The Netherlands, SUI-Switzerland, SWE-Sweden, UK-United Kingdom). c) Magnification of the yellow marked part of the plot b).

(b)



(c)



PC 1 (Explained Variance: 1.6%)

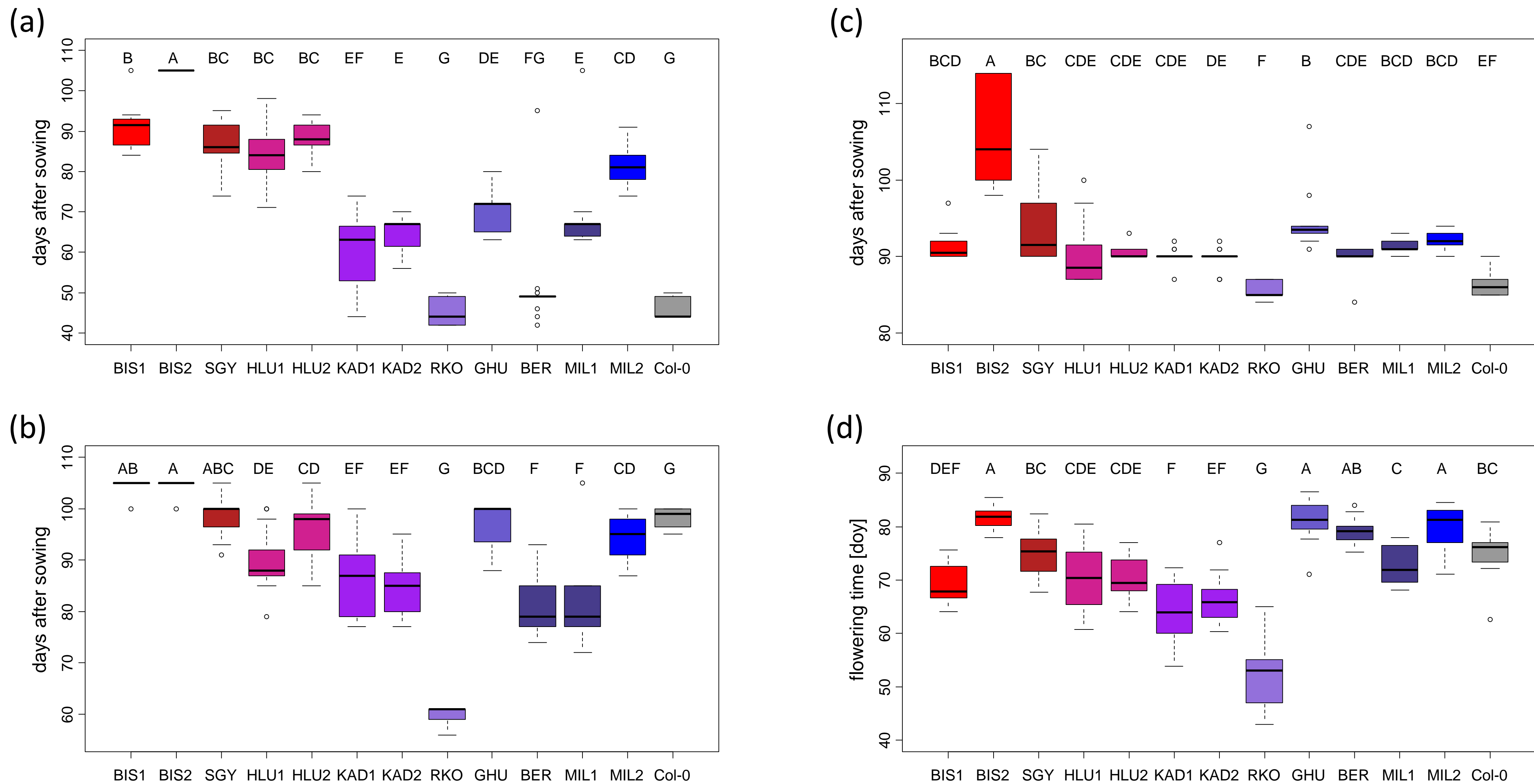


Fig. 4: Flowering time (days after sowing) of plants grown in controlled conditions: long days (16 h light/20 °C; 8 h dark/18°C) (a), short days (10 h light/18 °C; 14 h dark/14 °C) (b), or short days followed by vernalisation (8 h light/4 °C; 16 h dark/4 °C) and shift to long days at day 64 (c). Flowering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

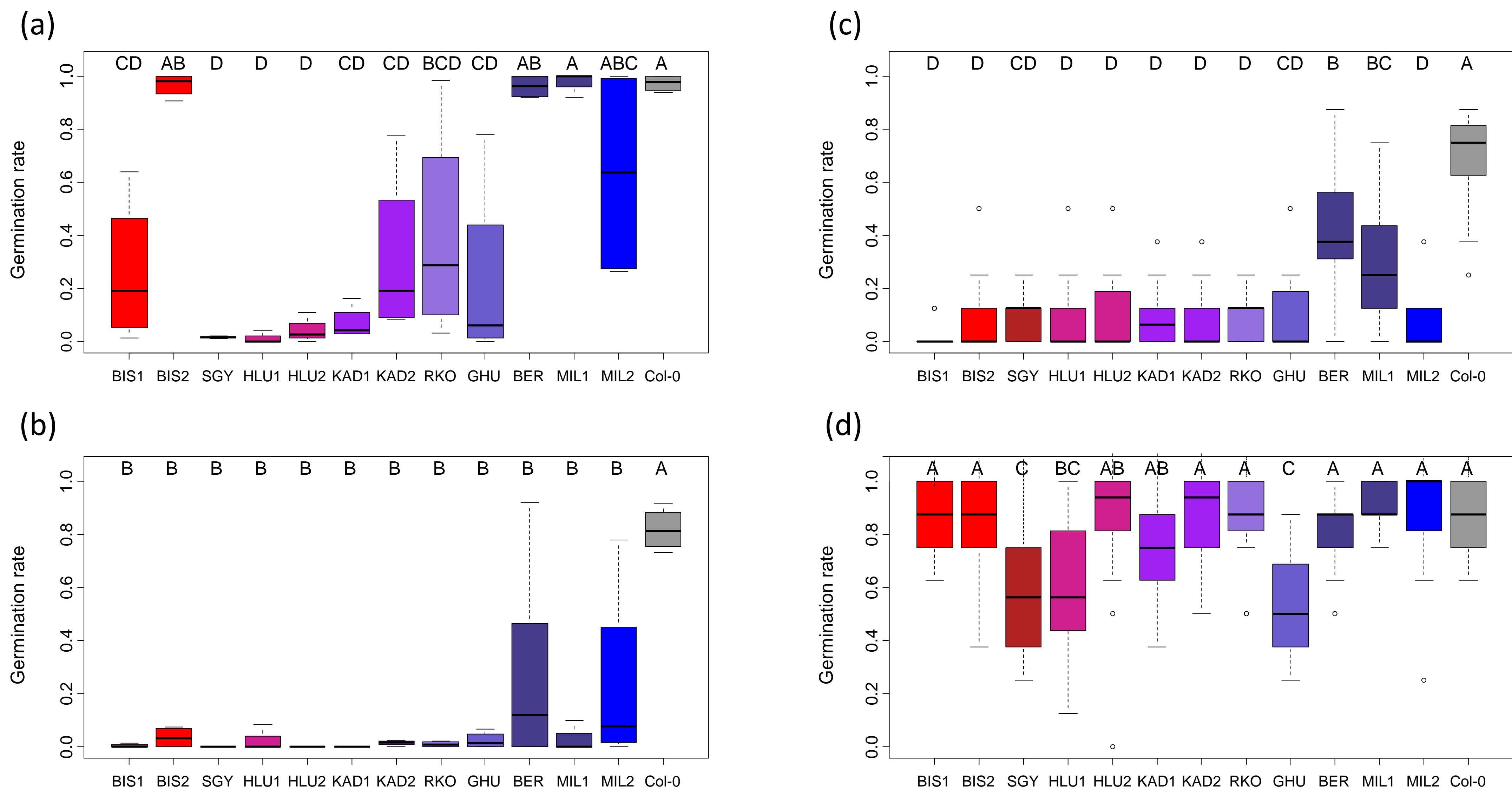


Fig. 5: Germination frequency of seeds from the 12 different Cologne genotypes and Col-0 controls. Seeds were germinated on filter paper at 20 °C in petri dishes after 3-4 moths of afterripening to test for primary dormancy (a) or incubated at 35 °C in the dark for 4 days before incubation at 20 °C to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

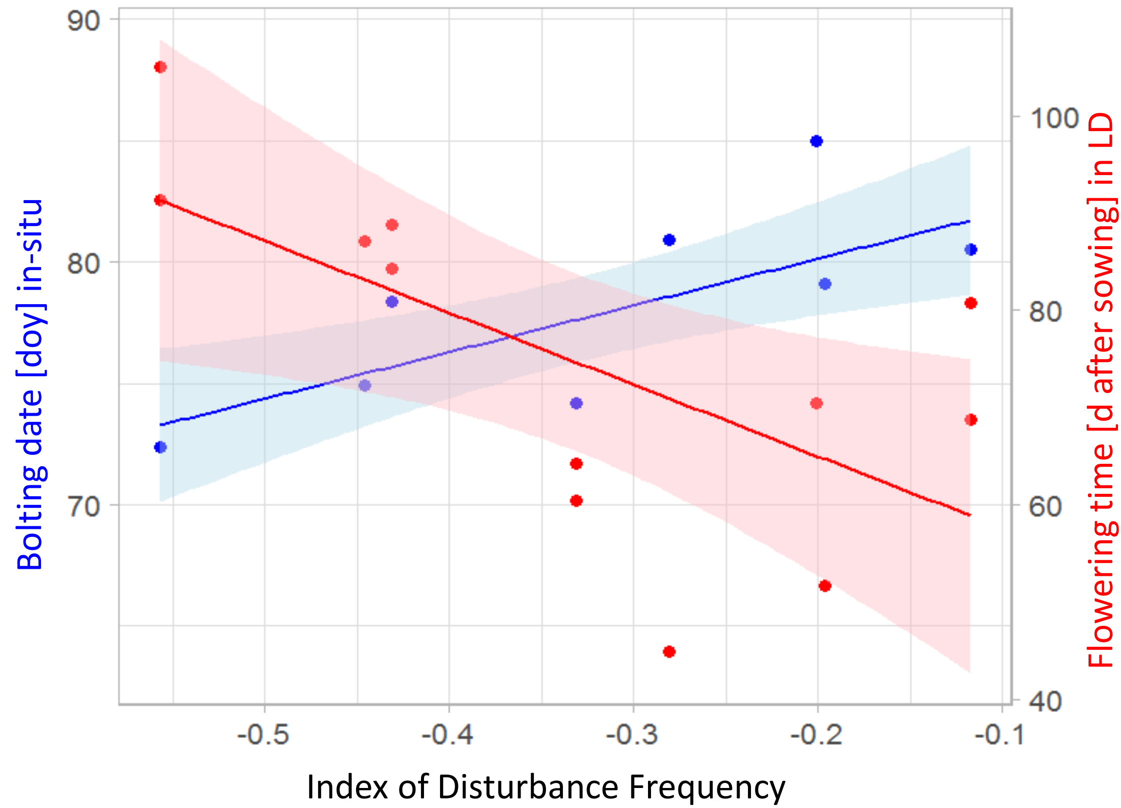


Fig. 6: Opposite variation of phenology in urban A. thaliana genotypes, as observed within urban habitat patches in the City of Cologne (in-situ; blue), and in a common-garden experiment in the same city (ex-situ; red). Populations are sorted by the disturbance frequency in urban habitat patches (average DF values). The timing of flowering (bolting) in-situ increases with disturbance frequency, whereas the opposite trend is observable for the timing of flowering in common garden conditions (red). Sowing in the latter was done at the time of natural germination.