

Genomic basis and evolutionary potential for extreme drought adaptation in *Arabidopsis thaliana*

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One-sentence summary: “Future genetic changes in *A. thaliana* populations can be forecast by combining climate change models with genomic predictions based on experimental phenotypic data.”

Short title: “Genetic adaptation to extreme drought in *A. thaliana*”

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21 **Because earth is currently experiencing unprecedented climate change, it is important to predict**
22 **how species will respond to it. However, geographically-explicit predictive studies frequently**
23 **ignore that species are comprised of genetically diverse individuals that can vary in their degree**
24 **of adaptation to extreme local environments; properties that will determine the species' ability**
25 **to withstand climate change. Because an increase in extreme drought events is expected to**
26 **challenge plant communities with global warming, we carried out a greenhouse experiment to**
27 **investigate which genetic variants predict surviving an extreme drought event and how those**
28 **variants are distributed across Eurasian *Arabidopsis thaliana* individuals. Genetic variants**
29 **conferring higher drought survival showed signatures of polygenic adaptation, and were more**
30 **frequently found in Mediterranean and Scandinavian regions. Using geoenvironmental models,**
31 **we predicted that Central European populations might lag behind in adaptation by the end of**
32 **the 21st century. Further analyses showed that a population decline could nevertheless be**
33 **compensated by natural selection acting efficiently over standing variation or by migration of**
34 **adapted individuals from populations at the margins of the species' distribution. These findings**
35 **highlight the importance of within-species genetic heterogeneity in facilitating an evolutionary**
36 **response to a changing climate.**

37 Ongoing climate change has already shifted latitudinal and altitudinal distributions of many plant
38 species (1). Future changes in distributions by local extinctions and migrations are most commonly
39 inferred from niche models that are based on current climate across species ranges (2, 3). Such
40 approaches, however, ignore that an adaptive response can occur also *in situ* if there is sufficient
41 variation in genes responsible for local adaptation (4–6). The plant *Arabidopsis thaliana* is found
42 under a wide range of contrasting climates, making it distinctively suited to study evolutionary
43 adaptation to a changing climate (7–9). For the next 50 to 100 years, it is predicted that extreme
44 drought events, potentially one of the strongest climate change-related selective pressures (10),
45 will become pervasive across the Eurasian range of *A. thaliana* (2, 11). An attractive hypothesis is
46 that populations from the southern edge of the species' range (12) provide a reservoir of genetic
47 variants that can make individuals resistant to future, more extreme, climate conditions (12, 13). To
48 investigate the potential of *A. thaliana* to adapt to extreme drought events, we first linked genetic
49 variation to survival under an experimental extreme drought treatment (14–16). By combining
50 genome-wide association (GWA) techniques that capture signals of local and/or polygenic
51 adaptation (17, 18) with environmental niche models (8, 19), we then predict genetic changes of

52 populations under future climate change scenarios.

53 We began by exposing a high-quality subset of 211 geo-referenced natural inbred *A.*
54 *thaliana* accessions (18) to an experimental extreme drought event during the vegetative phase,
55 which killed the plants before they could reproduce (Table S1). After two weeks of normal growth,
56 plants were challenged by a terminal severe drought for over six weeks and imaged every 2-4
57 days (Fig. 1A) (see Supplementary Online Materials [SOM]). A polynomial linear mixed model was
58 fit to the time-series data to quantify the rate of leaf decay (Fig. 1B-D, Video S1). The genotype
59 deviations from the mean quadratic-term in the model provided the best estimate of this
60 survivorship trait in late stages (Fig. S3, see details in SOM), ranging from -5 to $+5 \times 10^{-4}$ green
61 pixels/day². The most sensitive plants survived only about 32 days, while the most resilient plants
62 survived about 15 days longer.

63 The amount of water available during the drought experiment translates to only about
64 30-40 mm of monthly rainfall, and as expected, accessions with higher survival come from
65 regions with low precipitation during the warmest season (correlation with climate variable bio18
66 [www.worldclim.org, ref. (20)]: Pearson correlation, $r=-0.19$, $p=0.005$), and specifically with low
67 precipitation during May and June ($r \leq -0.19$, $p \leq 0.005$) (see Fig. 2A) (21). To further exploit current
68 climatic data, we used 19 bioclimatic variables and random forest models (22) for environmental
69 niche modeling (ENM) to predict the geographic distribution of the drought-survival index across
70 Europe (Fig. 1C). Surprisingly, we found that individuals with higher drought survival were not only
71 from the Mediterranean, but also from the opposite end of the species' range in Sweden (Fig. 1C,
72 ENM cross-validation accuracy=89%, Table S10) (21). In contrast to the warm-dry Mediterranean
73 climate, Scandinavian dry periods occur on average at freezing temperatures (Fig. S12).
74 Consequently, precipitation might occur as snow, which is not accessible for plants and produces a
75 physiological drought response (23).

76 We then studied whether the different populations of *A. thaliana* are locally adapted (5) to
77 low precipitation regimes via an increased drought-survival. Using an extended panel of 762 *A.*
78 *thaliana* accessions (Table S1) we carried out genetic clustering (24) and studied population
79 trajectories (25) (Fig. 2). This corroborated the existence of a so-called 'relict' group (12) and ten
80 other derived groups of relict (e.g. Spanish groups) or other (e.g. Central Europe) origin; likely of the
81 result of complex migration and admixture processes (26). A generalized linear model indicated
82 that genetic group membership explains a significant amount of drought-survival variance (GLM:
83 $R^2=12.8\%$; $p=4 \times 10^{-5}$), with the North (N) Swedish and Northeastern (NE) Spanish groups each
84 having on average higher survival than the other groups (t-test $p \leq 0.01$). A population graph

84 estimated by Treemix (27) suggested a gene flow edge between the Mediterranean and
85 Scandinavian drought-resistant genetic groups, potentially indicative of historical sharing of
86 drought survival alleles (Fig. 2D). Finally, running an ENM of the genetic group membership with
87 climatic variables from the origin of plants confirmed that the most important predictive variable
88 is precipitation during the warmest quarter (bio18), followed by mean temperature of the driest
89 quarter (bio9), and minimum temperature of the coldest month (bio6) (ENM accuracy > 95%. Fig.
90 S8B and Table S10). As our results indicate that the deepest genetic structure parallels the local
91 precipitation regimes and the ability of populations to survive drought, we expect that areas with
92 the strongest decline in rainfall will see the most turnover in genetic diversity (see Fig.12 Fig. S8)
93 (11).

94 Because the potential of populations to adapt to drought will depend on the genetic
95 architecture of the selected trait, we identified drought-associated loci with EMMAX (28), a
96 genome-wide association (GWA) method. Although genotype-associated variance (28) h^2 was
97 50%, no individual SNP was significantly associated with drought survival (minimum $p \sim 10^{-7}$, after
98 FDR or Bonferroni corrections $p > 0.05$) (Fig. S5, Table S3). Significant associations in multiple
99 phenotypes have been detected in similarly powered *A. thaliana* experiments (29). While multiple
100 testing adjustment can over-correct p-values and obscure true associations, the absence of
101 significant associations may also be due to (i) polygenic trait architecture, with many small-effect
102 loci (30), and/or (ii) confounding by strong population structure, consistent with the association of
103 drought survival with genetic group membership.

104 To test for polygenic adaptation, we repeated the GWA analyses with a model that
105 specifically handles both oligo- and polygenic architectures, BSLMM (31). This model estimates,
106 among other parameters, the probability that each SNP comes from a group of major-effect loci.
107 Around half of the top non-significant EMMAX SNPs were found to have over 99% probability of
108 belonging to such a major-effect group (Fisher's exact test of overlap, $p=3 \times 10^{-7}$; see SOM). We
109 further tested the polygenic hypothesis using the population genetic approach of Berg & Coop
110 (32). The test is based on the principle that if populations diverge in drought-survival due to many
111 loci, there should be an orchestrated shift in their allele frequency. After testing some 60 groups
112 of EMMAX SNP hits of variable size and at different ranks, we detected the most significant signal
113 of polygenic adaptation with the group that included the 151 top SNPs (Table S9). The signal was
114 lost for ranks below the top 300-400 EMMAX SNPs (Table S9). We then compared summary
115 statistics of the top 151 SNPs with background SNPs matched in frequency to avoid GWA
116 discovery biases. The top 151 SNPs showed high F_{ST} values, consistent with allele frequency

117 differentiation between populations (Fig. S5). Tajima's D values were positive (U Mann-Whitney
118 p-value < 0.05), indicating intermediate allele frequencies at the GWA loci (Fig. S5), which could
119 be a result of selection favoring alternative alleles in different ecological niches of the species (33).
120 The top SNPs did not show any evidence for precipitous reductions of haplotypic diversity, as
121 would be expected for hard selective sweeps (34) (Fig. S5). Together these patterns fit the
122 expectations of local adaptation from a polygenic trait controlled by some hundreds loci (35) –
123 theoretically expected to enable a fast response to a new environmental shift (36)

124 During local adaptation, the relevant loci diverge due to natural selection across
125 populations, which generates a statistical correlation with population groups (37). In this situation,
126 the default correction of population structure applied in GWA might obscure some of the true
127 associations. While F_{ST} scans can be useful to identify overly divergent loci across populations,
128 elevated genome-wide F_{ST} due to strong population structure can difficult outlier detection (37),
129 as it is in our case (Fig. S4). In order to recover relevant variants that are deeply divergent across
130 populations, we can study the ancestry of each SNP. Using ChomoPainter (38), which relies on
131 linkage disequilibrium information, we segment each genome in question into its different
132 population ancestries (here 11 groups). The first outcome of this analysis was that individuals from
133 NW and NE Spain and, to lesser extent, the Southern Mediterranean (Fig. 2A), have inherited
134 many DNA segments from relict individuals (Fig. S7). Then, in a generalized linear model
135 framework, we test whether the ancestries of individuals at a SNP coincide with the observed
136 phenotypic differences in drought-survival. Performing this “ancestry” genome-wide association
137 (aGWA) and using a permutation correction of p-values (see SOM), we detected 8 distinct peaks
138 ($p < 0.001$, Fig. 3A) including over 1,000 significant SNPs (70 SNPs after linkage disequilibrium
139 pruning) (Table S4). The most prominent peak was located on chromosome 5 and explained over
140 20% of the variance in drought survival (Table S4). There was no overlap in top SNPs between
141 GWA and aGWA because they search for different association signals. Our aGWA resembles other
142 admixture mapping techniques (39), and might be most useful for associations in scenarios of
143 adaptive introgression and local adaptation. To understand the origin of aGWA-identified SNPs,
144 we constructed trees for all concatenated aGWA SNPs and for genome-wide background SNPs.
145 Although the individuals from both the warm (Iberia and relicts) and cold (Scandinavia) edges of
146 the species distribution are far apart in genome-wide SNPs, they are closely related in
147 drought-associated SNPs (Fig. 3B). Overall, this is consistent with a common Mediterranean origin
148 of drought-adaptive genetic variants of both Northern and Southern individuals (Fig. 2D, Fig. 3B),
149 and highlights the relevance of populations at the latitudinal extremes of the species range as a

150 possible genetic reservoir for future climate change adaptation (12).

151 Depending on the nature of the stress, different mechanisms for drought adaptation can
152 be most advantageous (23, 40, 41). Annual plants, including *A. thaliana*, typically adapt to water
153 stress deficit by accelerating the transition from germination to flowering (escape strategy) (14–16,
154 41) instead of increasing water use efficiency (avoidance strategy). Previous drought experiments
155 with *A. thaliana* showed variation in both strategies but concluded it predominantly utilizes the
156 drought escape strategy. Our extreme drought experiment focused in characterising the
157 avoidance strategy by means of the drought-survival index, which was linearly associated to
158 precipitation regimes (Fig. S11, Table S6). This trait was not correlated with flowering time of the
159 accessions in unstressed conditions (Pearson correlation, $r=0.07$, $p=0.12$). However, we found a
160 positive correlation between drought-survival and flowering time GWA summary statistics of the
161 top 151 SNPs (Pearson correlation, $r=0.51$, $p=1 \times 10^{-11}$, see SOM) – suggesting a weak genetic
162 trade-off (16). Interestingly, we did not find any associated between GWA or aGWA top SNPs and
163 known flowering time QTLs (14–16), but rather a weak enrichment with membrane transporters
164 (see SOM). Adjustment of osmotic balance through cell membrane transport is a drought
165 avoidance mechanism (42) that might also confer cross-tolerance to other abiotic stresses (43),
166 therefore it might be of relevance for Scandinavian *A. thaliana* accessions or other populations in
167 the niche extremes (Fig. S12) (21).

168 Increased survival to extreme abiotic stresses should confer an evolutionary advantage
169 given the predicted increase in drought frequency and intensity both around the Mediterranean
170 and in Europe, which will constitute a critical hazard for many plants, including *A. thaliana* (2, 11).
171 Environmental niche models (ENM), which have been developed to relate species distributions to
172 climate variables, can be used to predict future changes to species' ranges (2, 3). Ignoring
173 adaptation from standing variation (44–46), however, could lead to overestimates of extinction
174 rates (47–49). By fitting ENM of current climate with SNP data (19), using a similar rationale as in
175 Hancock and colleagues' "climate GWA" (7), we can predict the most likely genetic makeup under
176 current and future climate conditions. Using such an approach, we trained ENMs with 762
177 accessions and produced maps of the present distributions of the 151 GWA and 70 aGWA
178 drought-associated SNPs (all ENM 5CV accuracy >92%; Table S3–4, Fig. S13–16). Concatenating
179 the 221 maps, we inferred the most likely individual genotype at each location. At present,
180 individuals from both Northern and Southern edges of the distribution are predicted to harbor
181 more drought-survival alleles than those located in between (Fig. 3C, Fig. S15–16, with the
182 quadratic term in a regression of allele count on latitude being positive at $p=10^{-3}$), corroborating

183 our previous observations. Then, using the trained ENM, we forecast the distribution of the 221
184 drought-survival alleles in 2070 (rpc 8.5, IPCC, www.ipcc.ch, ref. (20)). While it was expected that
185 populations in the Mediterranean Basin would need to become more drought resistant (11), we
186 predicted a more robust increase in the total number of drought-survival alleles for Central Europe
187 (Fig. 3, Fig. S14-15). This is because rainfall in Central Europe will likely become more similar to that
188 in the Mediterranean by 2070 (2, 11) (Fig. S12).

189 Because some of the drought-survival alleles are currently not yet present in Central
190 Europe, we speculated that gene migration might be necessary to facilitate adaptation to future
191 conditions (50). An underlying assumption of the ENM is that allele presence only depends on
192 environmental variables, but this assumption, “universal migration”, may not be realistic for future
193 predictions if present distributions are geographically narrow. We therefore included two
194 geographic boundary conditions in the ENM to generate two models that were either more or less
195 “migration-limited” (see SOM). After fitting all possible models and predicting allele distributions
196 with future climate, we calculated the difference of predicted presence per map grid cell between
197 the naïve, free migration ENM and the two geographically constrained ones (Fig. 3D-E). If an allele
198 has currently a narrow distribution or is specific to a certain genetic background, its future
199 presence in an area might not be predicted by the constrained models, even though the climate
200 variables coincide with the SNP’s environmental range. Such a scenario seems to apply to Central
201 Europe, as the deficit in drought-survival alleles predicted by the free over the constrained models
202 was 8-30% (18-66 out of 221) (Fig. 3E; with the quadratic term in a regression of the allele count
203 difference on latitude being negative at $p < 10^{-10}$). Central European populations may therefore be
204 under threat of lagging adaptation by the end of the 21st century.

205 In the end, for a population to persist, not only the number of drought-survival alleles has
206 to increase, but it has to do so in actual individuals (51). The chance of this occurring will depend
207 on local allele frequencies and the natural selection favouring the drought-survival alleles.
208 Therefore, we studied current allele frequencies at three representative locations with the highest
209 sampling density in our dataset (40 samples within a 50 kilometer area): Madrid (Spain), Tübingen
210 (Germany) and Malmö (Sweden), which are at the southern edge, center and northern edge of the
211 range, respectively. Based on ENM predictions, we calculated allele frequencies from present to
212 2070. Frequencies are predicted to increase significantly only in the Tübingen population
213 (Student’s t test, $p < 10^{-16}$, Table S11), but not in Madrid and Malmö, indicating that these two
214 populations might be already adapted to the future local climate. Because the Tübingen
215 population already has most drought-associated alleles (53% of 70 aGWA SNPs and 90% of 151

216 GWA SNPs), increasing the number of total favorable alleles in individual genotypes should be
217 feasible, especially since there are single genotypes that have 63% (aGWA) and 90% (GWA) of
218 those alleles already present (see SOM). Starting 50-generations simulations at the present
219 Tübingen frequency of independent drought-survival alleles and assuming a range of selection
220 coefficients, we estimated that a 1-3% of fitness advantage on average would be necessary to
221 increase frequencies to match those of the adapted Madrid and Malmö populations (Fig. S17, see
222 SOM). Such selection could take place efficiently in large populations like the ones of a
223 highly-reproductive weed (51, 52).

224 Leveraging the model organism *A. thaliana*, we have begun to address key questions to
225 understand the burning issue of climate change effects on biodiversity. We provide evidence for
226 the possibility of adaptive genetic variation to extreme drought events. Harnessing the power of
227 methods that allow polygenic genetic architecture and testing evolutionary hypotheses of natural
228 selection, we detected that relevant genetic variants had been under polygenic local adaptation
229 and were more abundant at the edges of the species range. Extreme adaptation at range edges
230 might indeed be critical for a species' persistence under climate change. Although many aspects of
231 future adaptation are not considered here, namely non-drought related or seasonal climate
232 change (51), biotic interactions, phenotypic plasticity, or novel adaptive mutations (53), our
233 spatially explicit analyses emphasize the potential of adaptive evolution from standing variation
234 to ameliorate climate change's detrimental effects.

235 REFERENCES

- 236 1. C. Parmesan, G. Yohe, A globally coherent fingerprint of climate change impacts across
237 natural systems. *Nature*. **421**, 37–42 (2003).
- 238 2. W. Thuiller, S. Lavorel, M. B. Araújo, M. T. Sykes, I. C. Prentice, Climate change threats to plant
239 diversity in Europe. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 8245–8250 (2005).
- 240 3. T. Jezkova, J. J. Wiens, Rates of change in climatic niches in plant and animal populations are
241 much slower than projected climate change. *Proc. R. Soc. B.* **283**, 20162104 (2016).
- 242 4. R. D. H. Barrett, D. Schluter, Adaptation from standing genetic variation. *Trends Ecol. Evol.* **23**,
243 38–44 (2008).
- 244 5. G. Turesson, The Plant Species in Relation to Habitat and Climate. *Hereditas.* **6**, 147–236
245 (1925).
- 246 6. J. Hereford, A quantitative survey of local adaptation and fitness trade-offs. *Am. Nat.* **173**,
247 579–588 (2009).
- 248 7. A. M. Hancock *et al.*, Adaptation to climate across the *Arabidopsis thaliana* genome. *Science*.

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- 249 **334**, 83–86 (2011).
- 250 8. A. Fournier-Level *et al.*, A map of local adaptation in *Arabidopsis thaliana*. *Science*. **334**,
251 86–89 (2011).
- 252 9. J. R. Lasky *et al.*, Characterizing genomic variation of *Arabidopsis thaliana*: the roles of
253 geography and climate. *Mol. Ecol.* **21**, 5512–5529 (2012).
- 254 10. A. M. Siepielski *et al.*, Precipitation drives global variation in natural selection. *Science*. **355**,
255 959–962 (2017).
- 256 11. A. Dai, Increasing drought under global warming in observations and models. *Nat. Clim.*
257 *Chang.* **3**, 52–58 (2012).
- 258 12. A. Hampe, R. J. Petit, Conserving biodiversity under climate change: the rear edge matters.
259 *Ecol. Lett.* **8**, 461–467 (2005).
- 260 13. J. A. Lee-Yaw *et al.*, A synthesis of transplant experiments and ecological niche models
261 suggests that range limits are often niche limits. *Ecol. Lett.* (2016), doi:10.1111/ele.12604.
- 262 14. J. A. Bac-Molenaar, C. Granier, J. J. B. Keurentjes, D. Vreugdenhil, Genome-wide association
263 mapping of time-dependent growth responses to moderate drought stress in *Arabidopsis*.
264 *Plant Cell Environ.* **39**, 88–102 (2016).
- 265 15. M. El-Soda, W. Kruijjer, M. Malosetti, M. Koornneef, M. G. M. Aarts, Quantitative trait loci and
266 candidate genes underlying genotype by environment interaction in the response of
267 *Arabidopsis thaliana* to drought. *Plant Cell Environ.* **38**, 585–599 (2015).
- 268 16. A. M. Kenney, J. K. McKay, J. H. Richards, T. E. Juenger, Direct and indirect selection on
269 flowering time, water-use efficiency (WUE, $\delta^{13}C$), and WUE plasticity to drought in
270 *Arabidopsis thaliana*. *Ecol. Evol.* **4**, 4505–4521 (2014).
- 271 17. B. Brachi *et al.*, Investigation of the geographical scale of adaptive phenological variation and
272 its underlying genetics in *Arabidopsis thaliana*. *Mol. Ecol.* **22**, 4222–4240 (2013).
- 273 18. 1001 Genomes Consortium, 1,135 Genomes Reveal the Global Pattern of Polymorphism in
274 *Arabidopsis thaliana*. *Cell*. **166**, 481–491 (2016).
- 275 19. J. A. Banta *et al.*, Climate envelope modelling reveals intraspecific relationships among
276 flowering phenology, niche breadth and potential range size in *Arabidopsis thaliana*. *Ecol. Lett.*
277 **15**, 769–777 (2012).
- 278 20. R. J. Hijmans, S. E. Cameron, J. L. Parra, P. G. Jones, A. Jarvis, Very high resolution interpolated
279 climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978 (2005).
- 280 21. J. P. Mojica *et al.*, Genetics of water use physiology in locally adapted *Arabidopsis thaliana*.
281 *Plant Sci.* **251**, 12–22 (2016).
- 282 22. L. Breiman, Random Forests. *Mach. Learn.* **45**, 5–32 (2001).
- 283 23. J. Ingram, D. Bartels, The Molecular Basis of Dehydration Tolerance in Plants. *Annu. Rev. Plant*
284 *Physiol. Plant Mol. Biol.* **47**, 377–403 (1996).
- 285 24. D. H. Alexander, J. Novembre, K. Lange, Fast model-based estimation of ancestry in unrelated

- 286 individuals. *Genome Res.* **19**, 1655–1664 (2009).
- 287 25. S. Schiffels, R. Durbin, Inferring human population size and separation history from multiple
288 genome sequences. *Nat. Genet.* **46**, 919–925 (2014).
- 289 26. C.-R. Lee *et al.*, On the post-glacial spread of human commensal *Arabidopsis thaliana*. *Nat.*
290 *Commun.* **8**, 14458 (2017).
- 291 27. J. K. Pickrell, J. K. Pritchard, Inference of population splits and mixtures from genome-wide
292 allele frequency data. *PLoS Genet.* **8**, e1002967 (2012).
- 293 28. H. M. Kang *et al.*, Variance component model to account for sample structure in
294 genome-wide association studies. *Nat. Genet.* **42**, 348–354 (2010).
- 295 29. S. Atwell *et al.*, Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana*
296 inbred lines. *Nature.* **465**, 627–631 (2010).
- 297 30. G. Gibson, Rare and common variants: twenty arguments. *Nat. Rev. Genet.* **13**, 135–145 (2011).
- 298 31. X. Zhou, P. Carbonetto, M. Stephens, Polygenic modeling with bayesian sparse linear mixed
299 models. *PLoS Genet.* **9**, e1003264 (2013).
- 300 32. J. J. Berg, G. Coop, A Population Genetic Signal of Polygenic Adaptation. *PLoS Genet.* **10**,
301 e1004412–e1004412 (2014).
- 302 33. P. W. Hedrick, Genetic Polymorphism in Heterogeneous Environments: The Age of Genomics.
303 *Annu. Rev. Ecol. Evol. Syst.* **37**, 67–93 (2006).
- 304 34. P. Pavlidis, D. Živkovic, A. Stamatakis, N. Alachiotis, SweeD: likelihood-based detection of
305 selective sweeps in thousands of genomes. *Mol. Biol. Evol.* **30**, 2224–2234 (2013).
- 306 35. J. K. Pritchard, J. K. Pickrell, G. Coop, The genetics of human adaptation: hard sweeps, soft
307 sweeps, and polygenic adaptation. *Curr. Biol.* **20**, R208–15 (2010).
- 308 36. K. Jain, W. Stephan, Rapid adaptation of a polygenic trait after a sudden environmental shift.
309 *arXiv [q-bio.PE]* (2016), (available at <http://arxiv.org/abs/1610.05478>).
- 310 37. E. B. Josephs, J. R. Stinchcombe, S. I. Wright, What can genome-wide association studies tell
311 us about the evolutionary forces maintaining genetic variation for quantitative traits? *New*
312 *Phytol.* (2017), doi:10.1111/nph.14410.
- 313 38. D. J. Lawson, G. Hellenthal, S. Myers, D. Falush, Inference of population structure using dense
314 haplotype data. *PLoS Genet.* **8**, e1002453 (2012).
- 315 39. D. Shrinier, A. Adeyemo, E. Ramos, G. Chen, C. N. Rotimi, Mapping of disease-associated
316 variants in admixed populations. *Genome Biol.* **12**, 223 (2011).
- 317 40. F. Tardieu, Any trait or trait-related allele can confer drought tolerance: just design the right
318 drought scenario. *J. Exp. Bot.* **63**, 25–31 (2012).
- 319 41. F. Roux, P. Touzet, J. Cuguen, V. Le Corre, How to be early flowering: an evolutionary
320 perspective. *Trends Plant Sci.* **11**, 375–381 (2006).
- 321 42. K. M. Jarzyniak, M. Jasiński, Membrane transporters and drought resistance - a complex issue.

- 322 *Front. Plant Sci.* **5**, 687 (2014).
- 323 43. W. R. Swindell, The association among gene expression responses to nine abiotic stress
324 treatments in *Arabidopsis thaliana*. *Genetics*. **174**, 1811–1824 (2006).
- 325 44. S. U. Pauls, C. Nowak, M. Bálint, M. Pfenninger, The impact of global climate change on
326 genetic diversity within populations and species. *Mol. Ecol.* **22**, 925–946 (2013).
- 327 45. J. L. Brown *et al.*, Predicting the genetic consequences of future climate change: The power of
328 coupling spatial demography, the coalescent, and historical landscape changes. *Am. J. Bot.*
329 **103**, 153–163 (2016).
- 330 46. M. C. Fitzpatrick, S. R. Keller, Ecological genomics meets community-level modelling of
331 biodiversity: mapping the genomic landscape of current and future environmental
332 adaptation. *Ecol. Lett.* **18**, 1–16 (2015).
- 333 47. R. A. Catullo, S. Ferrier, A. A. Hoffmann, Extending spatial modelling of climate change
334 responses beyond the realized niche: estimating, and accommodating, physiological limits
335 and adaptive evolution. *Glob. Ecol. Biogeogr.* **24**, 1192–1202 (2015).
- 336 48. C. Moritz, R. Agudo, The future of species under climate change: resilience or decline? *Science*.
337 **341**, 504–508 (2013).
- 338 49. A. A. Hoffmann, C. M. Sgrò, Climate change and evolutionary adaptation. *Nature*. **470**,
339 479–485 (2011).
- 340 50. S. N. Aitken, M. C. Whitlock, Assisted Gene Flow to Facilitate Local Adaptation to Climate
341 Change. *Annu. Rev. Ecol. Evol. Syst.* **44**, 367–388 (2013).
- 342 51. A. Fournier-Level *et al.*, Predicting the evolutionary dynamics of seasonal adaptation to novel
343 climates in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* **113**, E2812–21 (2016).
- 344 52. F. Roux, S. Giancola, S. Durand, X. Reboud, Building of an experimental cline with *Arabidopsis*
345 *thaliana* to estimate herbicide fitness cost. *Genetics*. **173**, 1023–1031 (2006).
- 346 53. M. Exposito-Alonso *et al.*, The rate and effect of de novo mutations in a colonising lineage of
347 *Arabidopsis thaliana*. *bioRxiv* (2016), p. 050203.

348

349 SUPPLEMENTAL MATERIALS

350 Methods and any associated references are available in the online version of the paper. [[link](#)]

351 Supplementary text

352 Figs. S1 to S17

353 Video S1

354 Tables S1 to S1

355 References (* to *)

356 **URLs** Code for image analysis pipeline available at
357 <http://github.com/MoisesExpositoAlonso/hippo>. Code for ancestryGWA available at
358 <http://github.com/MoisesExpositoAlonso/aGWA>. Bioclimatic data used in the paper accessible
359 through an R package stored in <http://github.com/MoisesExpositoAlonso/rbioclim>. Phenotypic
360 datasets available at dryad [[link here](#)]. Genomes are available at
361 <http://1001genomes.org/data/GMI-MPI/releases/v3.1/>.

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368 **Author contribution** MEA conceived and designed the project. GW and FV helped and advised on
369 image phenotyping and FV provided additional phenotypes. MEA and WD performed
370 chromosome painter analyses. MEA performed the drought experiment, processed the image
371 data, and designed and carried out the statistical analyses. DW and HAB advised and oversaw the
372 project. MEA wrote the first draft and together with HAB and DW wrote the final manuscript with
373 input from all authors.

374

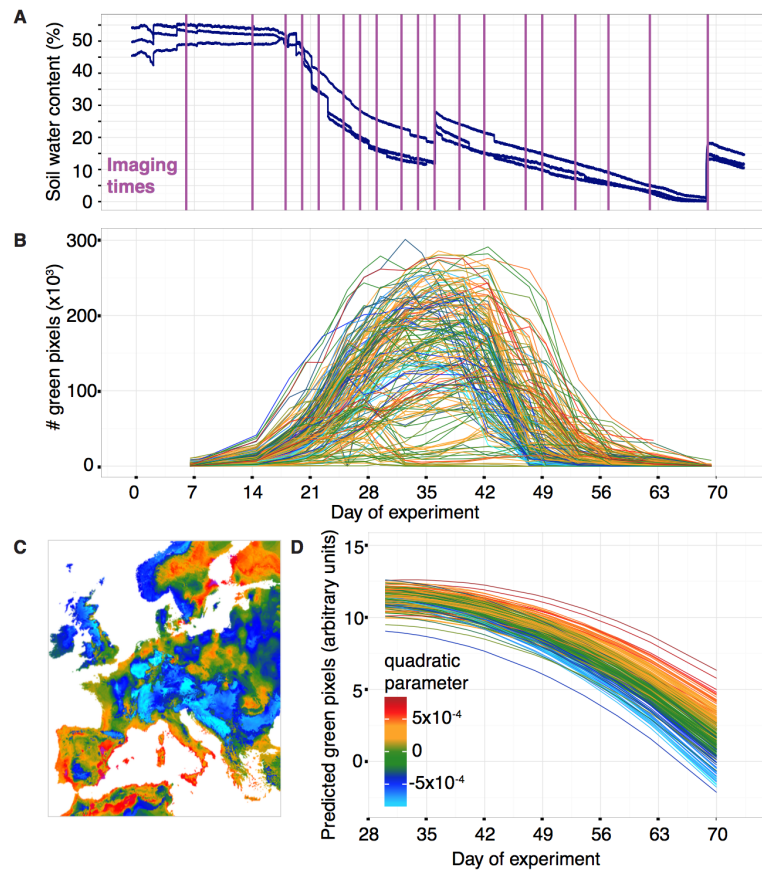
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376

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FIGURE LEGENDS

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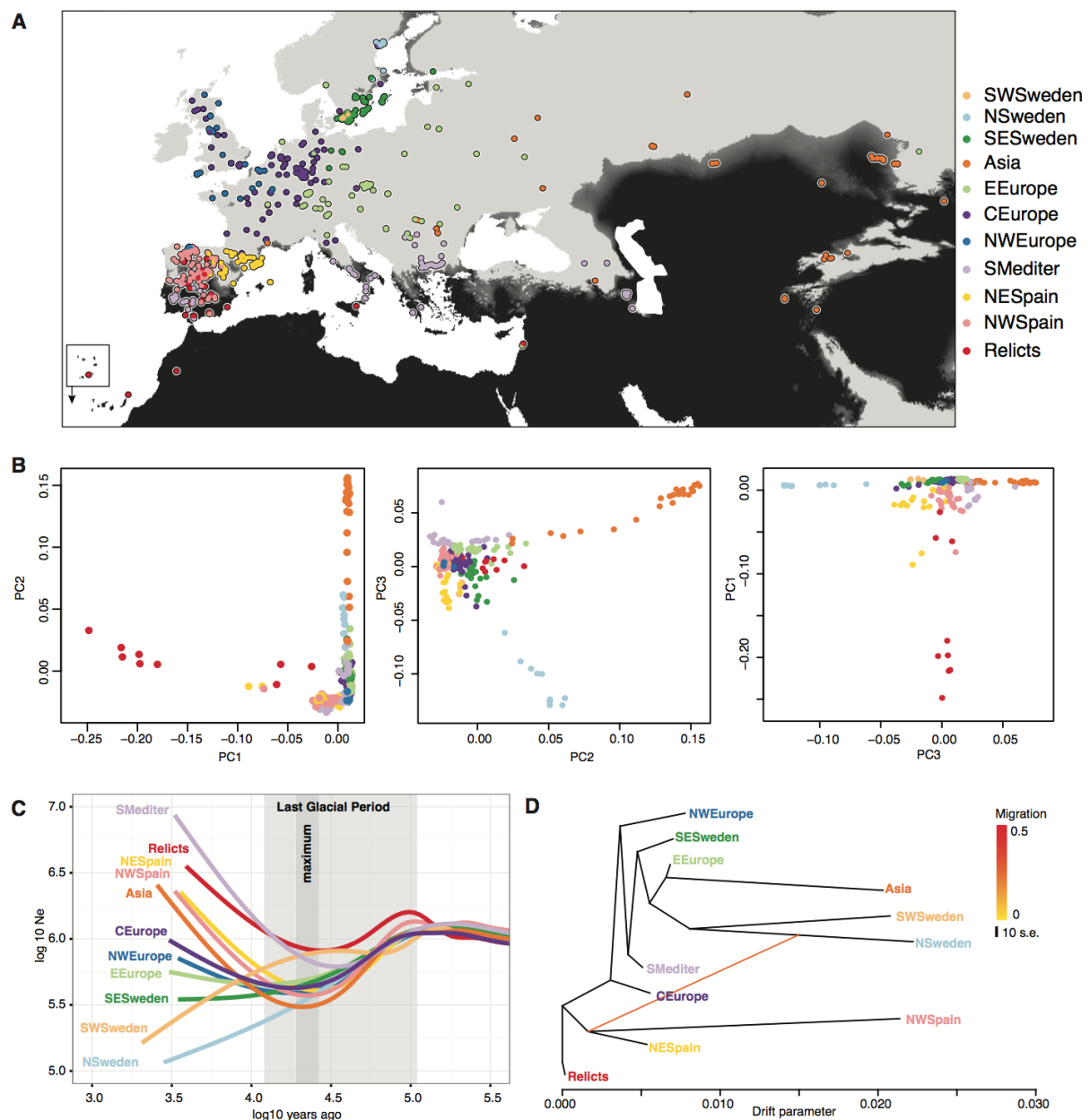
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380 (A) Soil water content from three sensors placed in three experimental trays regularly distributed
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385 with genotypes as random factors, from the average maximum day of green pixels until the end of
386 the experiment. Each line corresponds to one genotype.

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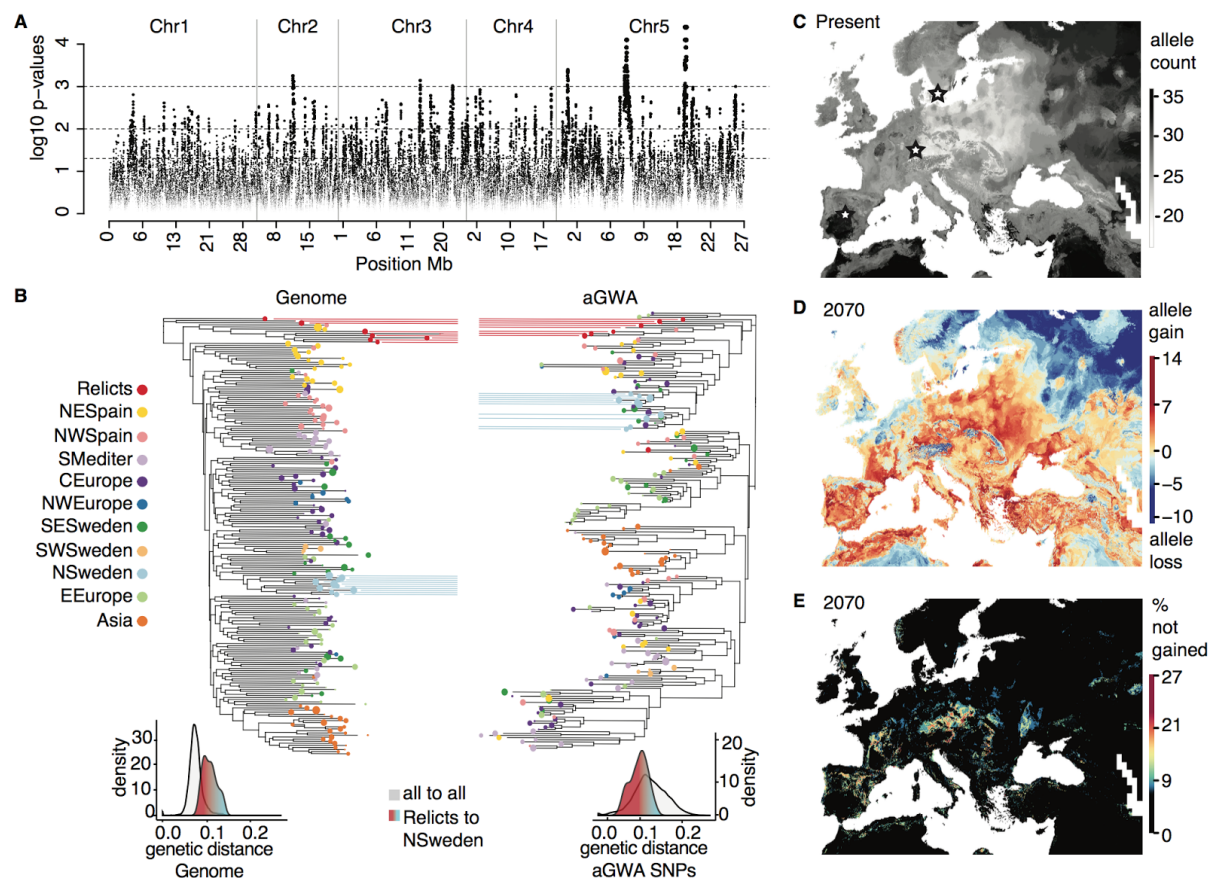
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Supplementary Information for:

Genomic basis and evolutionary potential for extreme drought adaptation in *Arabidopsis thaliana*

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105 SUPPLEMENTARY TEXT

106 **1. Experimental design and biological material**

107 **1.1 Choice of accessions from the 1001 Genomes resource**

108 The 1001 Genomes project has released resequencing data for 1,135 natural inbred lines, also
109 called accessions (<http://1001genomes.org>). We applied several filters to select the most
110 informative, least biased accessions for our experiment. (i) The first filter removed 176 accessions
111 with low quality genome information, < 10X genome coverage and < 90% congruence of SNPs
112 called from Max Planck Institute and Gregor Mendel Institute pipelines (1). (ii) The second filter
113 removed 244 nearly-identical accessions, many from N. America. For this, we calculated pairwise
114 genome-wide identity-by-state differences using PLINK v1.9 (2). When pairs differed in less than <
115 0.01 changes per polymorphic site, we randomly removed one member of the pair. The overlap
116 between (i) and (ii) was 762 accessions ([Fig. S1](#), [S2](#), Table S1). For geographic analyses in the native
117 range (e.g. environmental niche models), we used the 729 accessions that were within 50°W to
118 100°E longitude, i.e. Eurasia (see [section 4.2.1](#)). For the terminal drought experiment, we used 211
119 of these 729 accessions. The seeds were progeny of the 1001 Genomes collection seed stocks
120 obtained from the ABRC Stock Center (CS78942).

121 **1.2 Greenhouse terminal drought experiment**

122 The 211 accessions included both vernalization-requiring, slow-flowering and
123 vernalization-independent, fast-flowering ones. Because the phenotype of interest in our study
124 was the variance in survival during vegetative stages under terminal drought conditions, we
125 applied a terminal severe drought without prior vernalization. Because of the difficulties
126 associated with disentangling drought-induced mortality and reproduction-associated
127 senescence at the end of the plant life cycle, our study focused on drought stress during the
128 vegetative stage. (Note that onset of flowering, or flowering time, was not a confounding factor.
129 See [section 2.2](#)).

130 Seeds were aliquoted in Eppendorf tubes, suspended in 1% agar solution, stratified in a
131 4°C dark room for 5 days to promote germination, and then pipetted into pots filled with sieved
132 soil (CL-P, Einheitserde Werkverband e.V., Deutschland). When multiple seeds germinated per
133 pot, all but one were removed at random. We sowed 8 replicates per genotype in 49 trays of 8x5
134 cells (5.5 x 5.5 x 10 cm) using a randomized incomplete block design. We excluded corner cells,

135 where edge effects are strongest.

136 During the first two weeks after sowing (defined as day 0), trays were watered close to soil
137 saturation once every 3 days, with temperature maxima from 20 to 25°C under 16 hours natural
138 and supplemental light. After this period, seedlings were challenged with a terminal drought, with
139 “recovery waterings” after 3 and 6 weeks, in order to increase the variance in survival. The overall
140 watering during the drought period (4 l in each tray of 40 x 60 cm), corresponded to
141 approximately 33 mm of rainfall ($4,000 + 4,000 \text{ cm}^3 \text{ water} / 2400 \text{ cm}^2 \text{ surface} = 3.3 \text{ cm}$). We
142 monitored water content using moisture sensors (Parrot SA, Paris, France) (see water content
143 graph in Fig. 1A). We monitored rosette green area by imaging at 20 time points (Fig. 1A) using a
144 customized system (see below).

145 **1.3 Experiment under optimal growing conditions**

146 In a first experiment, we grew the same 211 genotypes under optimal watering and nutrient
147 conditions and monitored vegetative growth by image analysis (Vasseur et al. submitted [*link to*
148 *preprint or in press article will be added here*]) (see Table S5 for a description of the 24 traits
149 extracted from the images). This set of traits was used to investigate whether variation of
150 drought-survival index was correlated with growth under optimal conditions.

151 **2. Drought phenotyping**

152 **2.1 Image analysis pipeline**

153 Images were taken using a Panasonic DMC-TZ61 digital camera and a customized closed black box
154 at a distance of 40 cm from the tray. This produced very consistent images in terms of
155 illumination (only from in-camera flash) and focal distance to the plants.

156 We extracted leaf area per plant over time using the imaging module Open Computer
157 Vision in Python (3) ([Video S1](#)), with these steps: (i) 5 pixel mean denoise of the whole-tray image.
158 (ii) Fixed Hue Saturation Value (HSV) segmentation of “green” values. The threshold values were
159 determined heuristically by comparing the HSV ranges of leaves at five timepoints (i.e. early and
160 mature). (iii) Cropping of each pot to extract individual plant images. (iv) Counting of green pixels
161 (for more specific details and code visit <http://github.com/MoisesExpositoAlonso/hippo>). Pots with
162 green pixels but without plants were excluded after careful visual inspection of all images.

163 **2.2. Drought index**

164 After determining the peak of green area for the majority of pots, we modeled the daily

165 number of green pixels per pot. Several different models, including up to third order polynomial
166 models, several error correction factors, either raw or genotype averages, were tested. All models
167 were ranked based on parameter convergence in an MCMC walk and AIC values. The final chosen
168 model was a generalized linear mixed model with Poisson link of the form:

$$169 \quad y = i + s t + q t^2 + \epsilon_{gi} + \epsilon_{gs} + \epsilon_{gq} + \epsilon_{tray} + \epsilon_{pos} + \epsilon$$

170 where green area, y , was the response variable, and an intercept (i), slope (s), and quadratic
171 coefficients (q) with time (t) were fitted as fixed effects. Genotypes were treated as random
172 factors, that is allowed to deviate from the main trends, following a normal distribution (0 ,
173 σ_g). Tray block and position within the tray grid were fitted as random factors following also a
174 normal distribution. To estimate these parameters, we performed 10,000 iterations in a Monte
175 Carlo Markov Chain (MCMC) and 1,000 burn-in using the glmmMCMC R package (4).

176 The variance from all genotype-dependent components relative to the total phenotypic
177 variance was ~10%. This might be an underestimate since certain variance is due to inaccurate
178 phenotyping: either optical and illumination distortion, or overlap of neighboring plants. Genotype
179 values of the three parameters of interest (intercept, slope, and quadratic coefficient) were used
180 for Genome Wide Association (GWA) and downstream analyses. Additive genetic variance was
181 estimated from linear mixed models using a kinship matrix (see GWA section (3.3)). The intercept,
182 slope and quadratic deviations had narrow-sense heritabilities (h^2 , or kinship-associated variance)
183 of 0%, 0%, and 50%; respectively. We chose the latter as the drought-survival index. This
184 parameter informed about survival during the late stage of the experiment, as can be observed
185 from a high correlation between the drought-survival index and the the raw green pixels in the
186 final monitored days (Fig. S3).

187 Because the drought-survival index could depend on the developmental stage of the
188 plants when the drought treatment started, we computed the pixel decay polynomial model with
189 and without a covariate of flowering time under optimal conditions (indicative of developmental
190 speed; see source of the phenotype in section 1.3 and phenotypic correlations in section 3.4.1).
191 The small effect of flowering time in the model (0.01% of the variance) confirmed that the
192 measurements were not biased, therefore we removed flowering time from the final model.

193 In order to provide an intuitive understanding of the drought survival index, we looked at
194 the relationship between the index and the last day on which a plant was clearly alive, defined as
195 the last day with at least 5,000 green pixels left. The relationship between the drought-survival
196 index and the last living day was highly significant ($p < 10^{-16}$). The most sensitive plants survived

197 for 32 days, and the most resistant were alive for 15 days longer.

198 **3. Quantitative genetics analyses**

199 **3.1 Population structure**

200 From the vcf file containing SNP calls from the 1001 Genomes project (
201 <http://1001genomes.org/data/GMI-MPI/releases/v3.1/>), we identified SNPs with a genotype calling
202 rate >95%, which resulted in ~4M SNPs in 762 accessions ([section 1.1](#)). We defined genetic clusters
203 with ADMIXTURE v1.2 (5) (Table S2). As a model-free alternative to ADMIXTURE, we used PCA
204 implemented in PLINK v1.9 (2). The first three axes explained 16.0%, 9.6% and 7.9% of the total
205 genetic variance. ADMIXTURE clustering and PCA were used to understand population structure
206 and to relate it to phenotype variables. We assessed population splits and migrations with a
207 population ancestral graph using TreeMix v. 1.12 (6), a tree based on genome-wide allele frequency
208 differences across populations. Additionally, we calculated a proxy of local genetic diversity (7) per
209 location using the number of polymorphic sites in the geographically closest genome.

210 **3.1.1 Association of genetic group membership with drought**

211 Using the ADMIXTURE membership probabilities of each genome, we carried out univariate linear
212 regressions with the drought survival phenotype. The groups that yielded positive relationships
213 were NE Spanish ($p < 0.05$), Mediterranean ($p = 0.06$), and the N. Swedish groups ($p < 0.001$). The
214 groups negatively associated were Central Europe ($p = 0.06$), Asia ($p < 0.001$), and E. Europe
215 ($p < 0.001$). This broadly coincided with the map of drought-survival prediction (Fig. 1D, [S11](#)). We
216 found that only PC3 was significantly associated with the drought survival phenotype (GLM
217 $R^2 = 0.076$; $p = 5.15 \times 10^{-5}$). The N. Swedish and NE Spanish groups showed particularly low values in
218 PC3 than the rest ([Fig. S8](#)).

219 **3.2 Coalescent rates over time**

220 Only the accessions with $\geq 90\%$ of membership probability in one of the genetic groups were
221 used. Using MSMC software (8) (<http://github.com/stschiff/msmc>), we performed within- and
222 cross-genetic group coalescent analyses by contrasting two pairs from different genetic groups. In
223 total 333 analyses were performed, with each genetic group being tested at least 3 times. The
224 results were summarized using a smoothed generalized additive model in R (Fig. 2C).

225 3.3 Genome wide associations (GWA)

226 3.3.1 Linear Mixed Models (LMMs) with EMMAX

227 We used 879,654 biallelic SNPs with a minimum allele frequency (MAF) of 5% for genome wide
228 association (GWA) using EMMAX (9). We carried out GWA for all climatic variables and 11
229 phenotypes (Table S5). The GWA is based on linear mixed models that test, one by one, each of
230 the SNPs, and correct the results by population structure using a random factor with a
231 variance/covariance kinship matrix built from genome-wide SNPs. This is an appropriate method
232 to correct for coancestry in *Arabidopsis thaliana* (10).

233 To rule out the possibility that drought survival measurements were dependent on the
234 developmental stage of the plant during the experiment, we carried out the GWA with and
235 without a covariate of flowering time that had been scored in controlled conditions (proxy of
236 developmental speed; [section 1.3](#)). The top SNP hits were the same with or without this covariate,
237 and we only show results without the covariate. To account for familywise error in GWA we used
238 Bonferroni correction (p value x number of SNPs) and the Benjamin-Hochberg false discovery
239 rate correction (11). The kinship-associated variance of drought-survival – an approximation of
240 narrow sense heritability, h^2 , was 49%. When we fit a kinship calculated from only the 151 top
241 polygenic GWA SNPs (see [section 3.5.2](#)), the estimate of h^2 was 52%. This is probably a better
242 estimate than that from the genome-wide-based kinship matrix, as the putatively causal SNPs are
243 better “tagged” in the 151 SNPs kinship matrix.

244 After calculating genome-wide F_{st} (12) and Tajima’s D (13) with PLINK v1.9 (2) and
245 likelihood of a selective sweep with SweeD (14), we investigated the enrichment of the top SNPs
246 in the upper tail of the distributions of those statistics ([Fig. S4](#)) (Table S3, rank columns).

247 3.3.2 Bayesian Sparse Linear Mixed Models (BSLMMs) with GEMMA

248 The Bayesian Sparse Linear Mixed model (BSLMM) implemented in GEMMA (15) accommodates
249 both poly- and oligogenic architectures in a GWA framework. It models two effect
250 hyperparameters, a basal effect, *alpha*, that captures the fact that many SNPs contribute to the
251 phenotype, and an extra effect, *beta*, that captures the stronger effect of only a subset of SNPs.
252 The parameter measuring the probability of having another extra effect, *gamma*, can be used to
253 prioritize SNPs (personal instructions from the author X. Zhou). Over 40% of the top 151 SNPs
254 from EMMAX were found to have over 99% percentile of the gamma inclusion probability in
255 GEMMA (Fisher’s exact test odds ratio = 17.21, $p=3 \times 10^{-7}$). The estimate of realized heritability with
256 BSLMM was 50%, which is in agreement with the EMMAX analyses. The 95% highest posterior

257 density (95%HPD) from 1,000 MCMC steps ranged from 25-85%.

258 **3.4 Multivariate analyses of phenotypes and GWA summary statistics**

259 For a description and sources of all variables used, see Table S5.

260

261 3.4.1 All pairwise correlations

262 We computed all-against-all Pearson product-moment correlation coefficients among accession
263 line means (n= 211 accessions) of phenotypic and climate variables (Table S5, S6). To study genetic
264 correlations, we performed the same analyses with SNP effect sizes (n= 151 drought-associated
265 SNPs) estimated from multiple GWA (Table S7).

266 The phenotype correlations (Table S6) showed that the drought-survival index was
267 negatively correlated with reproductive allocation and number of seeds ($r < -0.16$, $p < 0.02$),
268 suggesting a fitness trade-off between stressful and optimal growth environments.
269 Drought-survival was not correlated with flowering time ($r=0.07$, $p=0.12$) nor plant size (rosette
270 area and dry mass, $r < 0.12$, $p > 0.07$).

271 Drought-survival SNP effects negatively correlated with the SNP effect sizes of most
272 precipitation variables separately ($r < -0.4$; $p < 10^{-8}$, Table S7), indicating that alleles that increased
273 drought survival were found in more arid geographic regions, i.e. regions with high temperatures
274 and lower precipitation at different times of the year. Drought-survival SNP effects were also
275 positively correlated with SNP effects of rosette area, dry mass, and flowering time (Table S7).
276 These analyses have two-fold interests: (1) GWA-estimated effect have been corrected by
277 population structure, thus correlations should not reflect phenotypic differences caused by drift of
278 populations. (2) SNPs can have pleiotropic effects and this can limit adaptation due to genetic
279 constraints (see [section 4.2.4.3](#)) (16).

280 3.4.2 Canonical Correlation Analysis (CCA)

281 We further utilized Canonical Correlation Analysis (CCA) to decompose environment-phenotype
282 associations of SNP effects. This was done for all genome-wide SNPs (n=800,000) and for the
283 151 drought-associated SNPs (Table S8).

284 CCA of genome-wide SNPs revealed the first canonical correlation axis (CC1) to be driven
285 by lower flowering time (T_repro, loading=-0.77), lower rosette dry mass (loading=-0.76) and
286 higher annual temperature (bio1, loading=0.5). CC2 indicates that lower plant photosystem stress
287 (FvFm, loading=0.60) is related to higher mean temperature of the wettest quarter and higher

288 precipitation seasonality (bio8, bio15, loadings>0.25). CC3 shows that lower drought survival
289 (loading=-0.58) effects are related to higher precipitation in the driest (bio17, loading=0.44) and
290 warmest quarters (bio18, loading=0.35).

291 CCA of 151 top GWA SNPs yielded a first canonical correlation coefficient of 0.99, with a
292 phenotype canonical variate driven by lower drought survival, higher rosette area and dry mass
293 (loadings >0.75), and a climatic canonical variate dominated by higher precipitation during the
294 wettest month (bio13) and wettest quarter of year (bio16) (loadings >0.75).

295 **3.5 Polygenic adaptation signal**

296 **3.5.1 Classic Q_{st} - F_{st} comparison**

297 Q_{st}/F_{st} ratios have been proposed as an appropriate indicator of local adaptation in *A. thaliana* (17)
298 and related species (18). Genome-wide F_{st} across the eleven population was computed from 211
299 genomes using vcfTools (v0.1.12b) (19). We estimated the mean and confidence intervals based on
300 the standard error of the mean, obtaining a mean $F_{st} = 0.042$ (95% cumulative distribution =
301 0.360). We calculated Q_{st} for the drought-survival index as the between-genetic group variance
302 divided by the total variance. We used the MCMCglmm function in R with a 10,000 steps chain,
303 1,000 steps burn-in, and fitting the genetic group as random effect. This resulted in a $Q_{st} = 0.143$
304 (90%HPD = 0.052 - 0.338). When the variance across populations was done using the NE
305 Spanish and the NSweden (population groups hypothetically under local adaptation) against the
306 rest, we obtained $Q_{st} = 0.377$ (0.047 - 0.987). We thus concluded that a significant $Q_{st} > F_{st}$
307 signal is only observable at the individual level when the hypothetical populations that underwent
308 local adaptation were oriented in the calculation of the variance.

309 **3.5.2 Berg & Coop methodology**

310 We tested for a polygenic adaptation signature following Berg & Coop (20), an extension of the
311 Q_{st}/F_{st} ratio test based on SNP frequency per population and effect sizes as estimated from a
312 GWA analysis. We used different groups and numbers of ranked SNPs after pruning linked SNPs
313 ($r^2 > 0.6$), to learn about the robustness of this test and the apparent number of SNPs that
314 contribute to the signal (Table S9). Since this test does not use direct phenotypes but calculates
315 the average phenotype per population based on allele frequencies of GWA SNPs, we could
316 perform the test with 762 high quality accessions. Since results did not vary between 762 and 211
317 sample analyses, we only report the analyses with the 762 genomes (Table S9).

318 **3.6 ChromoPainter and ancestry GWA**

319 We ran ChromoPainter version 2.0.7 (available at <http://paintmychromosomes.com>) (21) on the
320 762 genomes dataset, after imputing missing genotypes with Beagle version 3.3.2 (22) using
321 default parameters.

322 ChromoPainter analyses require a “training” run to estimate several hyperparameters. We
323 ran 10 expectation maximization iterations on chromosome 2 (the smallest chromosome). We
324 informed ChromoPainter with a published recombination map of *Arabidopsis thaliana* (23) that we
325 reshaped to our SNP dataset. We used the command:

```
326 ChromoPainterv2 -i 10 -in -iM -j -g haplotypefile -r recombinationfile -a 0 0 -t labelfile
```

327 We used the output hyperparameters to run ChromoPainter on all chromosomes in an
328 unsupervised all-to-all genomes mode, with the command:

```
329 ChromoPainterv2 -n 4.737068 -M 0.000421 -j -g haplotypefile -r recombinationfile -a 0 0 -t  
330 labelfile
```

331 **3.6.1 Global proportion of ancestral chromosome segments**

332 To study the ancestry relationships of each of the genetic groups, we counted the number
333 of chromosomal segments (termed “chunks” in the original ChromoPainter paper (21)) that each
334 genome “received” from all other genomes. The segment varied in size depending on local
335 recombination rates and between genomes, but *a posteriori* analyses indicated that the median
336 size was in the order of magnitude of kilo and megabases. To make the counts more
337 informative, we show boxplots per ADMIXTURE group rather than counts per individual (Fig. S7
338 A-K). This showed, for example, that NW Spain, NE Spain and S. Mediterranean (the latter to a
339 lower degree), were “painted” mostly by relict DNA segments. Next, we tried to infer how well the
340 drought survival of an individual correlated with the number of segments inherited from a certain
341 ancestry. This indicated that only N. Sweden and relicts passed DNA segments that were
342 correlated with the drought-survival index of the receiving individual (Fig. S7L). The Pearson
343 correlation coefficient was calculated excluding the individuals from the same admixture group as
344 the predictor.

345 **3.6.2 aGWA for admixture mapping**

346 If populations are locally adapted, F_{st} outlier scans can be used to identify genetic variants under
347 divergent selection (24, 25). However, when populations get isolated and diverge genetically, as it

348 is the case in *Arabidopsis thaliana*, F_{st} values are shifted to high values across the entire genome
349 even when subsequent admixture happen, making the identification of outliers difficult (Fig. S4)
350 (24). Thus, we must rely on linkage disequilibrium and identity by descent to find DNA segments
351 characteristic of the different populations. If subsequent but incomplete admixture occurred
352 between the locally adapted populations, it is expected that the individuals that retained the DNA
353 segments responsible for local adaptation, would show the largest phenotypic differences. This is
354 the principle of admixture mapping (26).

355 With the above rationale, we developed an admixture mapping technique (26)
356 repurposing the output of ChromoPainter. The “painted” genome matrix produced by
357 ChromoPainter has 762 states (one per individual in the analysis) and we repainted it into a
358 genome matrix of 11 states (the genetic groups from ADMIXTURE analysis, which are
359 geographically and environmentally separated). We then computed a regression of the
360 drought-survival phenotype on the population group specific to a SNP as:

$$361 \quad y_i = \mu + Ab + \epsilon$$

362 Where y is a vector of $i=1...211$ individual's phenotypes, μ is the mean phenotype, B is the 211
363 x 11 design sparse matrix of the ancestry states, b is a 1 x 11 vector of effects of each ancestry has in
364 the mean phenotype, and ϵ is the uncorrelated random residuals assumed to be normal. This
365 model was repeated for each SNP in our dataset (~2 million imputed and ‘painted’ SNPs, see
366 [section 3.6](#)). We report R square of and p-value of each SNP model (Table S4). Since we already
367 knew that the phenotype is associated with the membership assigned per individual, we expected
368 that the membership of any random SNP would be on average also associated, because of linkage
369 resulting from common ancestry. Therefore, we implemented an empirical p-value distribution
370 correction to only detect those SNPs whose ancestry explained an even larger proportion of
371 variance than the whole-genome ancestry. The permutation was done within each individual
372 genome shuffling the SNP states at a distance of 1,000 to 10,000 SNP positions — defined from
373 analysing the typical size of “homogeneously painted DNA segments” (code are available at
374 <http://github.com/MoisesExpositoAlonso/aGWA>). We permuted the dataset 1,000 times and
375 repeated this “aGWA” analysis to build p-value distributions. Since the nature of the associations is
376 very different from that of a standard GWA analysis, we did not expect and did not find any
377 overlap of top aGWA SNPs with the top SNPs from regular GWA. The closest was a regular GWA
378 SNP that was 8 kb away from an aGWA SNP. The closest gene to both encodes a defensin-like
379 protein; a family of proteins with broad anti-fungal and anti-bacterial activity (27).

380 Our approach is conceptually related to admixture mapping in humans, which has focused

381 on local enrichment of Neandertal- and Denisovan-like variants, and which has led to the
382 identification of in TLR immunity genes (28) as adaptive. It has also helped to increase the power
383 for detection of background-dependent disease risk in humans with mixed ancestries, e.g.
384 African-American individuals (29), or other more complex mixtures (30). Such approaches
385 constitute a powerful tool for understanding the genetic basis of local adaptation when complex
386 demographic scenarios of admixture exist.

387 3.6.2.1 Phylogeny of aGWA SNPs

388 To learn about the distribution and shared ancestries of the drought-related alleles, we computed
389 a neighbour joining phylogeny of all concatenated SNP hits from aGWA ($p < 0.001$) and compared
390 it with a genome background phylogeny of 1,000 randomly chosen SNPs (Fig 3B). This revealed
391 that the N. Swedish groups and Mediterranean relicts were across the genome more distant from
392 each other than the average between-group distance (Student's t test, $p < 2 \times 10^{-16}$) (Fig. 3B), much
393 closer than the average when considering only the aGWA SNPs (Student's t test, $p < 2 \times 10^{-16}$). The
394 same analyses showed also higher affinity of N. Swedish and NE Spanish populations (Student's t
395 test, $p < 10^{-10}$).

396 3.7 Test for annotation enrichment

397 Using the TAIR10 gene annotation of *Arabidopsis thaliana* (available at
398 arabidopsis.org/portals/genAnnotation/functional_annotation/), we tested whether there was a
399 specific annotation class enriched in our GWA and aGWA hits. The most suggestive genes
400 overlapping with the 151 top GWA hits were the nitrate transporter gene *NRT1.8*, which among
401 other functions mediates cadmium tolerance and is related to ABA transport (31–33), the
402 *CATION/CARNITINE TRANSPORTER 4 (OCT4)*, which mediates homeostasis of metabolites and
403 promotes lateral root formation (34), and the sugar transporter gene *SWEET8*, which is
404 upregulated during salt stress (35). On the other hand, the strongest peak fell inside the *CATION*
405 *EXCHANGER 9*, a gene important for homeostasis of K^+ , Na^+ and Mn^{++} that confers salt tolerance
406 when introduced into yeast (36). An empirical distribution test based on random draws of genes
407 showed, however, only marginal enrichment. The 30 genes defined by the 151 top GWA SNPs
408 were weakly enriched for cell membrane transport (6/30; $p = 0.01$), and the 23 genes defined by
409 the 70 top aGWA SNPs were only very marginally enriched for membrane transport (7/23;
410 $p = 0.06$). Testing for overrepresentation with PANTHER (www.pantherdb.org) and including genes
411 adjacent to the GWA and aGWA SNPs revealed weak enrichment of aGWA genes for ferredoxin
412 metabolic process ($p = 0.03$) and vesicle-mediated transport ($p = 0.05$), and of GWA genes for
413 growth-related functions ($p = 0.0007$) and metabolite biosynthetic processes ($p = 0.0002$). It is

414 difficult to know what to conclude from this, but the most noteworthy finding is probably that
415 there was no link to flowering time, in contrast to previous QTL and GWA studies of *A. thaliana*
416 response to drought (37–39).

417 **4. Environmental and forecasting analyses**

418 **4.1 Environmental data**

419 The environmental data comprised the Last Glacial Period (LPG, ~22,000 years ago), recent
420 averages from 1960-1990, and two 2070 climate projections of contrasting socio-economic
421 scenarios, the 2.6 and 8.5 CO₂ representative concentration pathways (rcp) (40, 41). The data was
422 retrieved from www.worldclim.com v.1.4 (ref. (42)). It consists of 19 bioclimatic variables at 2.5
423 minutes geographic resolution (code to retrieve and process data available at
424 <http://github.com/MoisesExpositoAlonso/rbioclim>).

425 **4.2 Environmental Niche Models (ENM)**

426 We carried out ENM with a number of response variables (for summary statistics see Table S10),
427 namely the drought-survival phenotype, flowering time, the genomic principal component axes,
428 the discrete population groups, the local genetic diversity, and the SNPs identified in GWA and
429 aGWA analyses .

430 4.2.1 Geographic areas used and niche limits

431 To train ENM, we removed accessions from Japan and from N. America, as they are recent
432 introductions (43) and might not reflect long-term climate adaptation. In addition, the sampled
433 locations used to trained the models were within 15 to 63° N and 23°W to 88°E longitude, but we
434 only predicted in a reduced area, from 34 to 63° N and -10.5 to 35°E, to avoid extrapolation of
435 data. Predictions for the last glacial maxima were masked in those areas that were likely tundra or
436 ice sheet at the time (<5°C and <0°C annual temperature, respectively), as they are presumably
437 artifacts.

438 Because the sampling in the 1001 Genomes project was not even across the species
439 range, predictions for underrepresented regions such as N. Africa, the Middle East, or Russia, must
440 be taken with caution. In order to be explicit about for which areas we could make the most robust
441 predictions, we show the sampling density per 1° x 1° latitude x longitude grid, which varies from
442 around 1 to 60 individuals (Fig. S8D), and plot trends of predicted values against other variables,
443 such as latitude or climate variables (e.g. Fig. S9-S11), only at those locations where there is at least

444 one sample.

445 Finally, it is worth noting that even for the most pessimistic climate change scenario (rcp
446 8.5), the values of annual precipitation (bio1) and the precipitation during the warmest season
447 (bio18) were always above the present minimum precipitation values where *A. thaliana* is currently
448 found (see [Fig. S12](#)). Therefore, we expect that transgressive phenotypes are not required to
449 survive future climates.

450 4.2.2 Random Forest models

451 After trying different methodologies, including generalized linear models, MaxEnt, and
452 linear discriminant models, we opted for random forest models because they are nonparametric,
453 nonlinear, allow both continuous and discrete response variables, and are computationally
454 efficient (44). Additionally, the implementation of an “importance” parameter of each predictor
455 variable available in the *randomForest* R package makes ranking of variables straightforward. To
456 mitigate the overfitting problem typical of machine learning methods, a 5-fold cross validation
457 procedure was used. We randomly divided the dataset in five parts, used four parts as training
458 dataset and one part as testing dataset, and repeated this five times. Reported accuracy from
459 cross validation was the R^2 of a linear model between observed and predicted values for
460 continuous variables, and the rate of successful assignment of categories relative to the total
461 number of observations for discrete variables. To build the final forest, a total of 50 classification
462 or regression trees per cross-validation set were used, and six variables were tested for each
463 classification split.

464 4.2.3 ENM of genetic groups

465 We modeled the presence of population structure as a discrete response variable in ENM;
466 either using eleven genetic groups as states, or the two relict and non-relict states.

467 In order to formally quantify the relevance of genetic group membership, we calculated
468 the percentage of map grid cells that each genetic group occupies. For this we only considered
469 areas where at least one genome per 1° x 1° latitude x longitude was observed ([Fig. S8A](#)) and where
470 tundra or ice sheet are not expected (important for LGM comparisons).

471 When we used the present-data trained relict/non-relict ENM with past climate data from
472 the last glacial maxima, we found that relicts likely occupied almost a quarter of the non-glaciated
473 areas, compared to less than 2% today ([Fig. S8D](#)), in agreement with genomic inferences of
474 higher effective population size in the past ([Fig. 2C](#)). The reason that the relicts' environmental
475 niche is predicted with 100% accuracy under 5-fold cross-validation (5CV) is likely that the local

476 number of relicts individuals is low, 26 accessions out of 762, and because their niche is very
477 restricted.

478 Under a future high CO₂ increase socio-economic scenario, the ENM with 11 genetic
479 groups predicts that the S. Mediterranean group will expand most dramatically into
480 Central-European areas, replacing groups currently occupying these areas (Fig. S8 C, F). Although
481 these models are not mechanistic, they illustrate that genetic groups from the Mediterranean and
482 from temperate areas have contrasting environmental niches and thus might replace each other
483 under future climate warming.

484 4.2.4 Genome Environment Models (GEMs)

485 All 151 GWA and 70 aGWA SNPs were modeled as a bivariate discrete variable (drought-sensitive
486 and drought-survival allele) in a random forest. The prediction accuracy and the most important
487 predictor for each model below is shown per SNP in Table S3 and S4.

488 After modeling presence/absence of each drought-survival SNP, we projected the present
489 inferred allele distributions in a map and then summarized all maps by intersecting them. In this
490 way, we generated a continuous map surface of the total number of drought-resistant alleles in a
491 given location. Ancestral GWA and GWA models showed overall similar patterns (Fig. S13-14), but
492 the latter were more biased to Western areas. This is likely due to GWA SNPs suffering from
493 high-frequency bias, making them more likely to be present in geographic areas with more
494 samples (Fig. S8). After we had trained the models with present data, we used them to predict
495 allele distributions in 2070 under low and high CO₂ increase scenarios. While patterns were
496 similar in both scenarios, for further analyses we used the most “pessimistic” high CO₂ increase
497 scenario to be able to show main trends more clearly.

498 4.2.4.1 Migration assumptions

499 For each SNP we trained three models in order to overcome the “universal (or free) migration”
500 assumption, implicit when using a current climate-trained ENMs with future climate data (e.g.
501 (45)). Although typically the free-migration model may not be entirely appropriate for predictions,
502 it might be more realistic for cosmopolitan species with continental-scale migrations in the recent
503 past, as is the case of *A. thaliana* (43). Nevertheless, we designed two additional models to
504 account for limited migration. The free model includes only the 19 bioclimatic variables as
505 predictors of the drought-survival alleles. The first geographically-controlled model includes in
506 addition the first three PC genomic axes as predictors (Fig. S8G-I), in an attempt to limit
507 prediction allele presence to geographic areas where the genomic background that they reside on

508 is present today. The second geographically-controlled model, which is even more restrictive,
509 includes latitude and longitude together with the 19 bioclimatic variables. For all models we not
510 only show the predicted maps (Fig. S13-14) but also provide residuals of predicted vs observed
511 (empirical) number of alleles in the locations where we have a sample. We also show their
512 relationship with latitude (Fig. S15-16).

513 4.2.4.2 Allele frequency change predicted by GEMs

514 We took 40 individuals approximately within 50 km of each other at three locations with the
515 highest density of samples in our dataset: Madrid (Spain), Tübingen (Germany) and Malmö
516 (Sweden) (Fig. 3C, Fig. S8). We tested overall future allele frequency changes of all SNPs per
517 population as well as SNP-specific allele frequency changes.

518 First, we calculated the mean allele frequency differences between future (rcp 8.5, 2070)
519 and present predictions. This proved to be significant in most locations and models (Table S11),
520 although the direction of change was different between the two edge populations, Madrid and
521 Malmö, and the Tübingen population from the center of the range. The former showed a decrease
522 or a steady state in allele frequency, and the latter showed a highly significant increase in all
523 models and SNP subsets (Table S11).

524 Secondly, we calculated the differences in frequency (F) between present (*pres.*) and
525 future (2070) populations per SNP and tested the difference using a Student's t test and a pooled
526 standard error (se) of the frequency measurements:

$$527 \quad t = \frac{F_{SNP\ 2070} - F_{SNP\ pres}}{\sqrt{se_{SNP\ 2070}^2 + se_{SNP\ pres}^2}}$$

528 This not only revealed the main frequency change trend, but also the distribution of
529 differences in alleles (see histograms in Fig. S13-14). It corroborated the general trend observed for
530 all SNPs (Table S11) and in addition showed that the global distribution of allele frequency changes
531 in Tübingen is skewed to the right in some SNPs (increase of drought allele frequency).

532 4.2.4.3 Possible genetic trade offs of drought survival and flowering time

533 Contrary to our expectations, there were areas in the Mediterranean that were predicted to lose
534 drought-survival alleles under climate change (Fig. S9-10). These are areas that already suffer
535 today from low precipitation (reached the zero in summer, Fig. S12) and will probably not become
536 much drier in summer. On the other hand, temperatures will keep increasing, which likely will

537 demand an acceleration of flowering time (in trade-off with drought avoidance). Predictions at the
538 phenotypic level (Fig. S9-10) showed this trend: drought-survival will increase only in the
539 transition areas from Mediterranean and temperate regions (Fig. S9) and might decrease in areas
540 that are already dry (Fig. S11). On the other hand, flowering time was predicted to decrease in the
541 Mediterranean (Fig. S10-11). We note that the SNP effects on drought survival and flowering time
542 were positively correlated, as disclosed by Canonical Correlation Analyses (section 3.4).

543 4.2.4.4 Population genetics simulations

544 The predicted an allele in 2070 does not directly inform about the actual possibility of adaptation.
545 This depends on (i) the frequency of the alleles and haplotypes in the population, (ii) the
546 recombination rate, and (iii) the strength and efficiency of selection. Indeed, geographic
547 predictions of alleles are probably bad predictors of allele frequency because random forest
548 models tend to predict either one allele or another in a certain region. That is why we do not
549 compare empirical present allele frequencies with frequency calculated from future predicted
550 presence of alleles in different locations of Tübingen, Madrid and Malmö.

551 To obtain further insights into population dynamics required for adaptation, we simulated
552 allele frequency changes in a Wright-Fisher population under a mutation-selection balance with
553 inbreeding, as *Arabidopsis thaliana* is a selfer (code available at
554 <http://github.com/MoisesExpositoAlonso/popgensim>). We started the simulations with the
555 present frequencies of drought-related alleles of the 221 aGWA/GWA SNPs, with (codominant)
556 selection coefficients (s) ranging from 0.01 to 20% fitness advantage. We considered SNPs as
557 independent, that is, we did not include linkage disequilibrium information nor a recombination
558 rate (see next section).

559 We carried out forward-in-time simulations for 50 generations, the approximate number
560 of generations that natural populations of *A. thaliana* from today until 2070 at an average
561 generation time of around 1.5 years (46). We assumed a mutation rate (μ) calculated from
562 laboratory mutation accumulation lines (43, 47) and a selfing coefficient (ψ) of 99%, a
563 conservative lower bound estimate from natural populations' heterozygosity (48). The population
564 size (N) was estimated from the genomic diversity in our dataset: The 40 genomes within
565 Tübingen area had a genome-wide nucleotide diversity of 0.004 (section 3.1). With the equation:

$$566 \quad 4N_e \times \mu = \pi$$

567 ,we solved for effective population size (N_e) and transformed it into population size

568 following the the relationship (49):

$$569 \quad N_e = \frac{1}{1 + F} \times N = \frac{2 - \psi}{\psi} \times N$$

570 This yielded a $N = 300,000$ plants, which might be reasonable given that we consider an
571 area of 50 km around Tübingen.

572 After running the simulations, we asked what selection coefficient would be needed to
573 reach quasi-fixation frequencies of each allele (>0.9 frequency) or to match the drought-allele
574 frequencies in Madrid or Malmö (assuming that those populations are adapted in comparison with
575 the Tübingen one). When a specific allele frequency was higher in the Tübingen population than in
576 Madrid or Malmö, we assumed selection would not be necessary and the coefficient was assumed
577 zero. The results indicated that selection coefficients should be strong (but see (50)) for alleles to
578 become fixed (Fig. S17). However, the distribution of selection coefficients were centered around
579 1-3% fitness advantage for Tübingen allele frequencies to match Malmö or Madrid (Fig. S17) (but
580 see next section 4.2.4.5). We did not simulate different degrees of drift in our analyses as when
581 the inequality: $N_e s > 1$, holds, the weight of drift relative to selection is thought to be
582 imperceptible (7).

583 4.2.4.5 Considerations regarding recombination

584 As stated above, assessing whether a population can adapt depends on the frequency of
585 drought-resistant individuals in the population, the rate of recombination to shuffle advantageous
586 alleles, and the strength of selection. In our simulations above we did not work with haplotypes of
587 SNPs in linkage disequilibrium as they are found in individuals, but considered SNPs to be
588 independent. This can be seen *a priori* as a strong assumption. Simulations including whole
589 haplotypes could inform about processes such as Hill-Robertson effect, hitchhiking, or
590 background selection, which could be achieved with more complex approaches in the future (51).

591 Of relevance is that even in Tübingen there are already some individuals that already have
592 many of the 151 GWA drought-resistant alleles, with one exceptional individual having 123/151
593 drought-survival alleles. The three next best individuals have 107, 105, and 99 alleles. Fifteen of
594 the 28 drought-resistance alleles are not present in the Tübingen population and will have to be
595 imported by migration. Therefore, to produce a hypothetical “fully adapted haplotype” with 136
596 alleles from the current standing variation (123 alleles are 90% of all 136 present alleles), only 13
597 drought-resistant alleles would have to be recombined and introgressed in the already
598 advantageous haplotype. Such introgression events might not be limited by low frequencies of

599 the advantageous alleles, as some were found at intermediate or as high as 90-95% frequency.
600 Furthermore, in a scenario with a haplotype in the population with 123 alleles already present,
601 simple individual-based simulations show that already with selection coefficients on the order of
602 0.5% advantage per allele, the 123 alleles haplotype will become completely fixed in the
603 population within 50 generations (simulations not shown). Results of aGWA indicate similar
604 patterns (24 alleles are 63% of all 32 present alleles) but more alleles are missing in the Tübingen
605 population, as their frequency is lower and geographic distributions are narrower than GWA
606 alleles.

607 We also used a series of approximate calculations to ascertain how many recombination
608 events are required to generate a hypothetical “fully adapted haplotype”. In *A. thaliana*, there are
609 on average 1.4 meiotic crossovers for each of the five chromosomes (52). Together with
610 independent segregation of the five chromosomes, the parental haplotypes are rearranged at
611 around 12 positions. A population of ~300,000 individuals (N) with a lower bound outcrossing
612 rate of 1% (=1-F) over 50 generations (g), could thus undergo around ~2 million recombination
613 events (= $N * (1-F) * r * g$), or about 1 event per 50 bp. Note that this could be a conservative
614 estimate as rates of outcrossing in geographically close plants can be above 10% (53). This result
615 suggests that recombination might be less limiting than expected *a priori*.

616 4.2.4.6 GEM limitations

617 There is a long list of factors that we did not take into account and that will influence future plant
618 response to climate change. We briefly enumerate them here:

- 619 A. We only focus on adaptation to drought, but other environmental stresses could have
620 similar detrimental effects such as extremely high temperatures or ecosystem destruction.
621 In addition, fluctuation in selection gradients and seasonal environmental variation are
622 other possible consequences of climate change (54, 55).
- 623 B. We only can explain ca. 50% of the drought survival variance with 221 SNPs.
- 624 C. We evaluated drought survival in a controlled greenhouse experiment, but the
625 extrapolation to natural conditions may be difficult. This would require field experiments
626 assessing fitness *in situ* to confirm that the identified SNPs actually report a fitness
627 advantage (56).
- 628 D. Because the high narrow sense heritability suggests a mostly-additive genetic architecture,
629 we carry out predictions with allele counts. However, we acknowledge that there is
630 variation of the magnitude of the SNP effects (Table S3-4), and epistatic effects might
631 exist.

- 632 E. All of our predictions are based on existing diversity, but de novo mutations are likely to
633 make a contribution as well, especially in species with high reproduction rate, short
634 generation time, and large population sizes (43, 57, 58).
- 635 F. Biotic interactions can also play a relevant role of population dynamics, which we ignored
636 (59–61).
- 637 G. In addition, although long-term evolution should be driven by genetic adaptation, it is
638 expected that phenotypic plasticity will partially buffer the detrimental consequences of
639 environmental change (62).
- 640 H. The existence of a seed bank in *A. thaliana* (46, 63) would cause longer generation times
641 and overlapping generations, and both alter the speed and dynamics of allele frequencies
642 (64).
- 643 I. Although our rough calculations suggest that recombination would not be a limitation for
644 future adaptation in *A. thaliana* populations, we have not incorporated such processes in
645 our modeling, as it is not a trivial matter (51, 65). This ignores phenomena such as
646 background selection or hitchhiking effects that could arise from phenotypic trade-offs
647 and the currently realized composition of haplotypes in the population.

648 REFERENCES

- 649
- 650
- 651 1. 1001 Genomes Consortium, 1,135 Genomes Reveal the Global Pattern of Polymorphism in
Arabidopsis thaliana. *Cell*. **0** (2016), doi:10.1016/j.cell.2016.05.063.
- 652 2. S. Purcell *et al.*, PLINK: a tool set for whole-genome association and population-based linkage
653 analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
- 654 3. G. Bradski, The opencv library. *Doctor Dobbs Journal*. **25**, 120–126 (2000).
- 655 4. J. D. Hadfield, MCMC methods for multi-response generalized linear mixed models: the
656 MCMCglmm R package. *J. Stat. Softw.* **33**, 1–22 (2010).
- 657 5. D. H. Alexander, J. Novembre, K. Lange, Fast model-based estimation of ancestry in unrelated
658 individuals. *Genome Res.* **19**, 1655–1664 (2009).
- 659 6. J. K. Pickrell, J. K. Pritchard, Inference of population splits and mixtures from genome-wide
660 allele frequency data. *PLoS Genet.* **8**, e1002967 (2012).
- 661 7. B. Charlesworth, D. Charlesworth, *Elements of Evolutionary Genetics* (Roberts and Company
662 Publishers, 2010).
- 663 8. S. Schiffels, R. Durbin, Inferring human population size and separation history from multiple
664 genome sequences. *Nat. Genet.* **46**, 919–925 (2014).
- 665 9. H. M. Kang *et al.*, Variance component model to account for sample structure in
666 genome-wide association studies. *Nat. Genet.* **42**, 348–354 (2010).

- 667 10. S. Atwell *et al.*, Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana*
668 inbred lines. *Nature*. **465**, 627–631 (2010).
- 669 11. Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful
670 approach to multiple testing. *J. R. Stat. Soc. Series B Stat. Methodol.*, 289–300 (1995).
- 671 12. B. S. Weir, C. C. Cockerham, Estimating F-Statistics for the Analysis of Population Structure.
672 *Evolution*. **38**, 1358–1370 (1984).
- 673 13. F. Tajima, Statistical method for testing the neutral mutation hypothesis by DNA
674 polymorphism. *Genetics*. **123**, 585–595 (1989).
- 675 14. P. Pavlidis, D. Živkovic, A. Stamatakis, N. Alachiotis, SweeD: likelihood-based detection of
676 selective sweeps in thousands of genomes. *Mol. Biol. Evol.* **30**, 2224–2234 (2013).
- 677 15. X. Zhou, P. Carbonetto, M. Stephens, Polygenic modeling with bayesian sparse linear mixed
678 models. *PLoS Genet.* **9**, e1003264 (2013).
- 679 16. T. Mitchell-Olds, Pleiotropy Causes Long-Term Genetic Constraints on Life-History Evolution
680 in *Brassica rapa*. *Evolution*. **50**, 1849–1858 (1996).
- 681 17. E. Porcher, T. Giraud, I. Goldringer, C. Lavigne, Experimental demonstration of a causal
682 relationship between heterogeneity of selection and genetic differentiation in quantitative
683 traits. *Evolution*. **58**, 1434–1445 (2004).
- 684 18. C.-R. Lee, T. Mitchell-Olds, Complex trait divergence contributes to environmental niche
685 differentiation in ecological speciation of *Boechera stricta*. *Mol. Ecol.* **22**, 2204–2217 (2013).
- 686 19. P. Danecek *et al.*, The variant call format and VCFtools. *Bioinformatics*. **27**, 2156–2158 (2011).
- 687 20. J. J. Berg, G. Coop, A Population Genetic Signal of Polygenic Adaptation. *PLoS Genet.* **10**,
688 e1004412–e1004412 (2014).
- 689 21. D. J. Lawson, G. Hellenthal, S. Myers, D. Falush, Inference of population structure using dense
690 haplotype data. *PLoS Genet.* **8**, e1002453 (2012).
- 691 22. B. L. Browning, S. R. Browning, Genotype Imputation with Millions of Reference Samples. *Am.*
692 *J. Hum. Genet.* **98**, 116–126 (2016).
- 693 23. K. Choi *et al.*, *Arabidopsis* meiotic crossover hot spots overlap with H2A.Z nucleosomes at
694 gene promoters. *Nat. Genet.* **45**, 1327–1336 (2013).
- 695 24. E. B. Josephs, J. R. Stinchcombe, S. I. Wright, What can genome-wide association studies tell
696 us about the evolutionary forces maintaining genetic variation for quantitative traits? *New*
697 *Phytol.* (2017), doi:10.1111/nph.14410.
- 698 25. F. C. Jones *et al.*, The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*.
699 **484**, 55–61 (2012).
- 700 26. D. Shriener, A. Adeyemo, E. Ramos, G. Chen, C. N. Rotimi, Mapping of disease-associated
701 variants in admixed populations. *Genome Biol.* **12**, 223 (2011).
- 702 27. M. Tesfaye *et al.*, Spatio-Temporal Expression Patterns of *Arabidopsis thaliana* and *Medicago*
703 *truncatula* Defensin-Like Genes. *PLoS One.* **8**, e58992 (2013).

- 704 28. M. Dannemann, A. M. Andrés, J. Kelso, Introgression of Neandertal- and Denisovan-like
705 Haplotypes Contributes to Adaptive Variation in Human Toll-like Receptors. *Am. J. Hum. Genet.*
706 **98**, 22–33 (2016).
- 707 29. D. C. Rife, Populations of hybrid origin as source material for the detection of linkage. *Am. J.*
708 *Hum. Genet.* **6**, 26–33 (1954).
- 709 30. H. Qin *et al.*, Interrogating local population structure for fine mapping in genome-wide
710 association studies. *Bioinformatics.* **26**, 2961–2968 (2010).
- 711 31. J.-Y. Li *et al.*, The Arabidopsis nitrate transporter NRT1.8 functions in nitrate removal from the
712 xylem sap and mediates cadmium tolerance. *Plant Cell.* **22**, 1633–1646 (2010).
- 713 32. A. Gojon, F. Gaymard, Keeping Nitrate in the Roots: An Unexpected Requirement for
714 Cadmium Tolerance in Plants. *J. Mol. Cell Biol.* **2**, 299–301 (2010).
- 715 33. K. M. Jarzyniak, M. Jasiński, Membrane transporters and drought resistance - a complex issue.
716 *Front. Plant Sci.* **5**, 687 (2014).
- 717 34. C. Lelandais-Brière *et al.*, Disruption of AtOCT1, an organic cation transporter gene, affects
718 root development and carnitine-related responses in Arabidopsis. *Plant J.* **51**, 154–164 (2007).
- 719 35. S. Ma, Q. Gong, H. J. Bohnert, Dissecting salt stress pathways. *J. Exp. Bot.* **57**, 1097–1107
720 (2006).
- 721 36. M. P. Apse, J. B. Sottosanto, E. Blumwald, Vacuolar cation/H⁺ exchange, ion homeostasis, and
722 leaf development are altered in a T-DNA insertional mutant of AtNHX1, the Arabidopsis
723 vacuolar Na⁺/H⁺ antiporter. *Plant J.* **36**, 229–239 (2003).
- 724 37. J. T. Lovell *et al.*, Pleiotropy of FRIGIDA enhances the potential for multivariate adaptation.
725 *Proceedings of the Royal Society of London B: Biological Sciences.* **280**, 20131043 (2013).
- 726 38. J. A. Bac-Molenaar, C. Granier, J. J. B. Keurentjes, D. Vreugdenhil, Genome-wide association
727 mapping of time-dependent growth responses to moderate drought stress in Arabidopsis.
728 *Plant Cell Environ.* **39**, 88–102 (2016).
- 729 39. F. Vasseur, T. Bontpart, M. Dauzat, C. Granier, D. Vile, Multivariate genetic analysis of plant
730 responses to water deficit and high temperature revealed contrasting adaptive strategies. *J.*
731 *Exp. Bot.* **65**, 6457–6469 (2014).
- 732 40. R. H. Moss *et al.*, The next generation of scenarios for climate change research and
733 assessment. *Nature.* **463**, 747–756 (2010).
- 734 41. J. Guiot, W. Cramer, Climate change: The 2015 Paris Agreement thresholds and
735 Mediterranean basin ecosystems. *Science.* **354**, 465–468 (2016).
- 736 42. R. J. Hijmans, S. E. Cameron, J. L. Parra, P. G. Jones, A. Jarvis, Very high resolution interpolated
737 climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978 (2005).
- 738 43. M. Exposito-Alonso *et al.*, The rate and effect of de novo mutations in a colonising lineage of
739 Arabidopsis thaliana. *bioRxiv* (2016), p. 050203.
- 740 44. L. Breiman, Random Forests. *Mach. Learn.* **45**, 5–32 (2001).
- 741 45. W. Thuiller, S. Lavorel, M. B. Araújo, M. T. Sykes, I. C. Prentice, Climate change threats to plant

- 742 diversity in Europe. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 8245–8250 (2005).
- 743 46. M. Falahati-Anbaran, S. Lundemo, H. K. Stenøien, Seed dispersal in time can counteract the
744 effect of gene flow between natural populations of *Arabidopsis thaliana*. *New Phytol.* **202**,
745 1043–1054 (2014).
- 746 47. S. Ossowski *et al.*, The rate and molecular spectrum of spontaneous mutations in *Arabidopsis*
747 *thaliana*. *Science*. **327**, 92–94 (2010).
- 748 48. A. Platt *et al.*, The scale of population structure in *Arabidopsis thaliana*. *PLoS Genet.* **6**,
749 e1000843 (2010).
- 750 49. M. Nordborg, P. Donnelly, The Coalescent Process with selfing. *Genetics*. **146**, 1185–1195 (1997).
- 751 50. F. Roux, S. Giancola, S. Durand, X. Reboud, Building of an experimental cline with *Arabidopsis*
752 *thaliana* to estimate herbicide fitness cost. *Genetics*. **173**, 1023–1031 (2006).
- 753 51. B. C. Haller, P. W. Messer, SLiM 2: Flexible, Interactive Forward Genetic Simulations. *Mol. Biol.*
754 *Evol.* **34**, 230–240 (2017).
- 755 52. P. A. Salomé *et al.*, The recombination landscape in *Arabidopsis thaliana* F2 populations.
756 *Heredity*. **108**, 447–455 (2012).
- 757 53. K. Bomblies *et al.*, Local-scale patterns of genetic variability, outcrossing, and spatial structure
758 in natural stands of *Arabidopsis thaliana*. *PLoS Genet.* **6**, e1000890–e1000890 (2010).
- 759 54. A. Fournier-Level *et al.*, Predicting the evolutionary dynamics of seasonal adaptation to novel
760 climates in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* **113**, E2812–21 (2016).
- 761 55. L.-M. Chevin, M. E. Visser, J. Tufto, Estimating the variation, autocorrelation, and
762 environmental sensitivity of phenotypic selection. *Evolution*. **69**, 2319–2332 (2015).
- 763 56. P. de Villemereuil, O. E. Gaggiotti, M. Mouterde, I. Till-Bottraud, Common garden experiments
764 in the genomic era: new perspectives and opportunities. *Heredity*. **116**, 249–254 (2016).
- 765 57. R. I. Colautti, J. A. Lau, Contemporary evolution during invasion: evidence for differentiation,
766 natural selection, and local adaptation. *Mol. Ecol.* **24**, 1999–2017 (2015).
- 767 58. K. M. Dlugosch, S. R. Anderson, J. Braasch, F. A. Cang, H. D. Gillette, The devil is in the details:
768 genetic variation in introduced populations and its contributions to invasion. *Mol. Ecol.* **24**,
769 2095–2111 (2015).
- 770 59. T. L. Karasov *et al.*, The long-term maintenance of a resistance polymorphism through diffuse
771 interactions. *Nature*. **512**, 436–440 (2014).
- 772 60. K. Jakob *et al.*, *Pseudomonas viridiflava* and *P. syringae*—Natural Pathogens of *Arabidopsis*
773 *thaliana*. *Mol. Plant. Microbe. Interact.* **15**, 1195–1203 (2002).
- 774 61. M. B. Araújo, M. Luoto, The importance of biotic interactions for modelling species
775 distributions under climate change. *Glob. Ecol. Biogeogr.* **16**, 743–753 (2007).
- 776 62. L. M. Chevin, R. Lande, When do adaptive plasticity and genetic evolution prevent extinction
777 of a density-regulated population? *Evolution*. **64**, 1143–1150 (2010).
- 778 63. S. Lundemo, M. Falahati-Anbaran, H. K. Stenøien, Seed banks cause elevated generation

- 779 times and effective population sizes of *Arabidopsis thaliana* in northern Europe. *Mol. Ecol.* **18**,
780 2798–2811 (2009).
- 781 64. B. Charlesworth, Selection in populations with overlapping generations. VI. Rates of change
782 of gene frequency and population growth rate. *Theor. Popul. Biol.* **6**, 108–133 (1974).
- 783 65. N. Li, M. Stephens, Modeling linkage disequilibrium and identifying recombination hotspots
784 using single-nucleotide polymorphism data. *Genetics.* **165**, 2213–2233 (2003).
- 785 66. J. J. Berg, G. Coop, A Coalescent Model for a Sweep of a Unique Standing Variant. *Genetics.*
786 **201**, 707–725 (2015).
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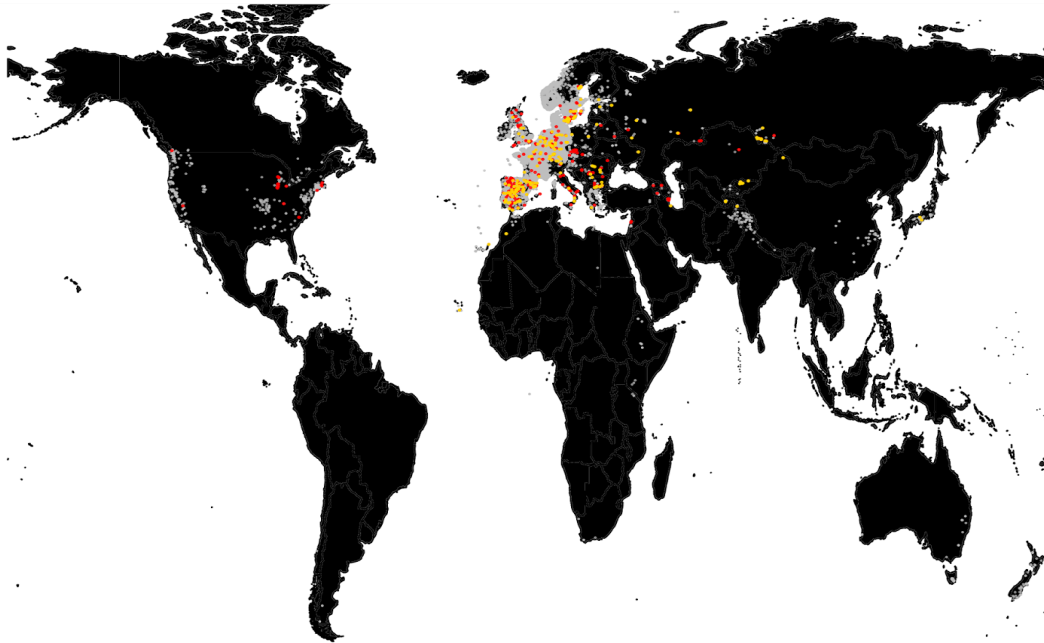
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SUPPLEMENTARY FIGURES & MEDIA

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Figure S1. Extent of *Arabidopsis thaliana* distribution

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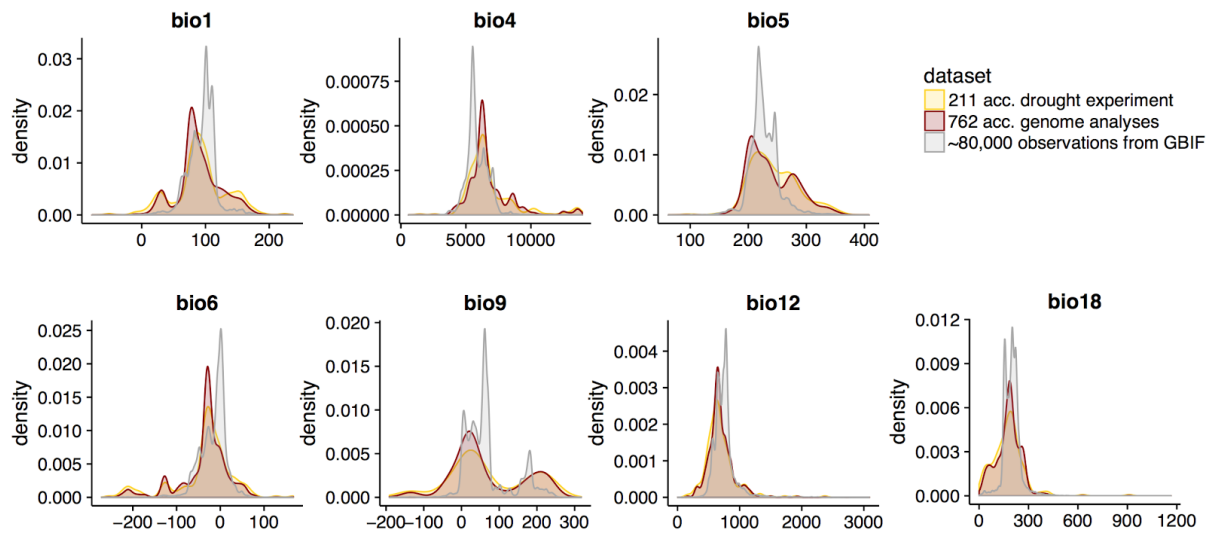
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The global map shows ca. 80,000 records from the Global Biodiversity Information Facility (GBIF, www.gbif.org) (grey), the 762 global accessions used for genetic analyses (red), and 210 accessions used for phenotyping experiments (yellow).

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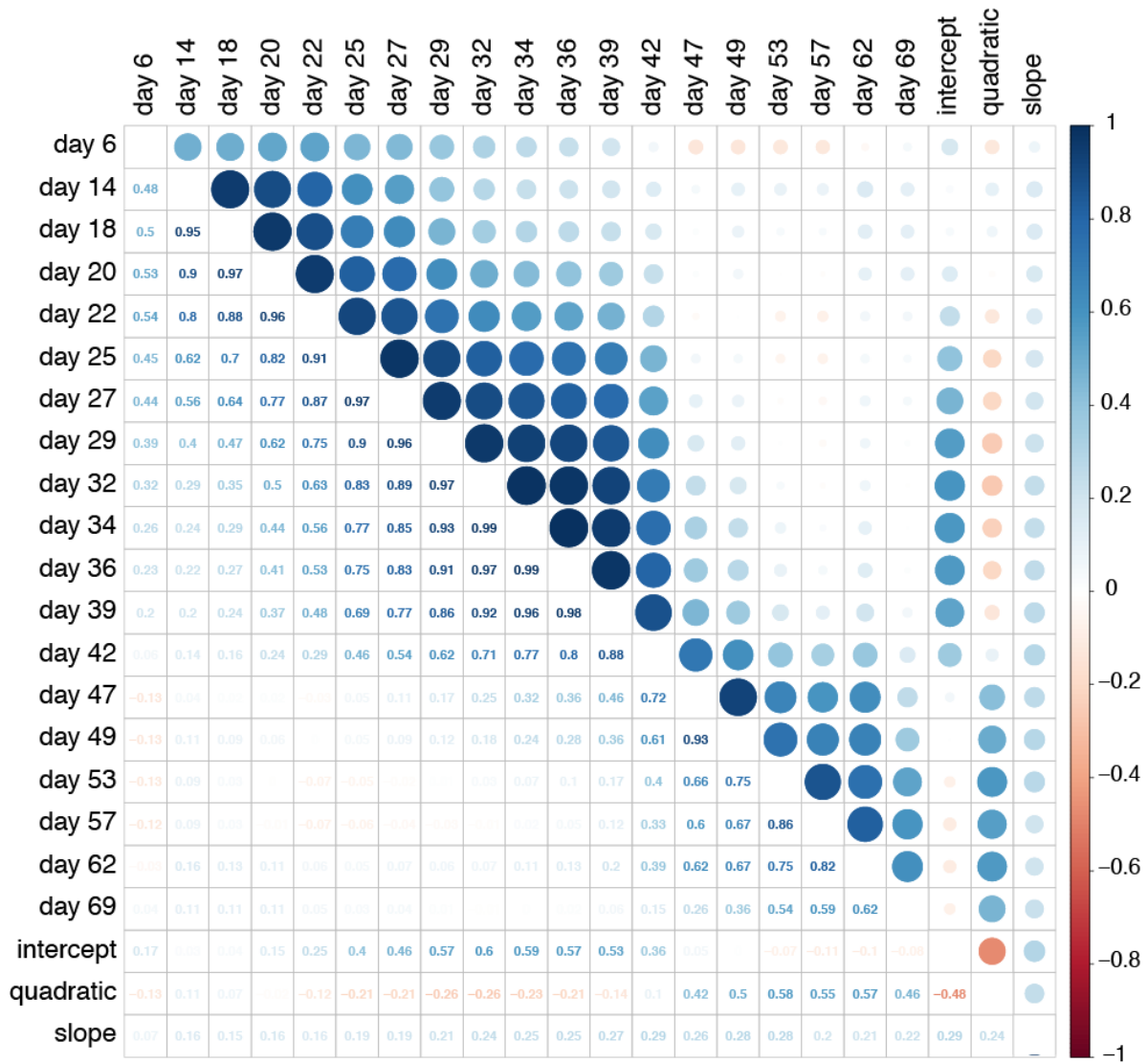


806 **Figure S2. Environmental ranges of *Arabidopsis thaliana***

807 We show the range in key environmental variables for the three datasets in Fig. S1. The set of
808 accessions used in our analyses not only covered the range of the species as estimated from GBIF
809 data, but also showed that these accessions have a more even distribution throughout the
810 environmental ranges. The bioclimatic variables are: annual precipitation (bio12), precipitation of
811 the warmest quarter (bio18), annual mean temperature (bio1), temperature seasonality (bio4),
812 maximum temperature of the warmest month (bio5), minimum temperature of the coldest
813 month (bio6), and mean temperature of the driest quarter (bio9). See Table S5 for more
814 information.

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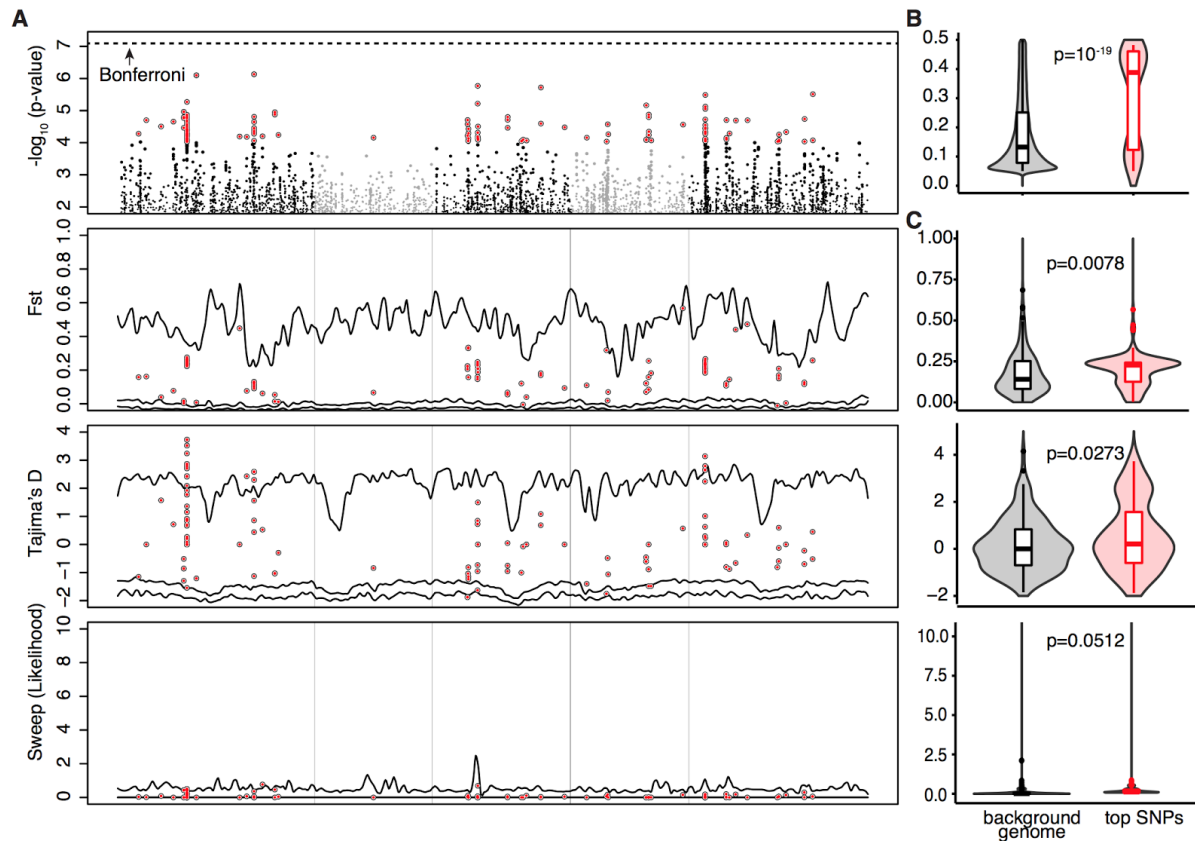
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817 **Figure S3. Correlation between raw green pixels in plant images and model parameters**
 818 Pearson product-moment correlation coefficients between the three drought-index parameters
 819 and the 'raw' number of green pixels per pot. Sizes of circles indicate strength and colors signs of
 820 association, shown as numbers in the lower triangle.

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Figure S4. GWA with drought survival and population genetic statistics

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(A) Manhattan plot of drought resistant GWA, F_{st} , Tajima's D, and selective sweeps. (B) Violin and box plots of allele frequency, and (C) F_{st} , Tajima's D, and selective sweeps of the top 150 SNPs (red) vs frequency-matched 150 SNPs from a random genome background (grey). GWA was calculated using EMMAX. F_{st} across populations (see Fig. 1) and Tajima's D were calculated using PLINK. Sweep likelihood was calculated using SWEED software. Median p-values from Wilcoxon tests with 100 bootstrap replicates are indicated.

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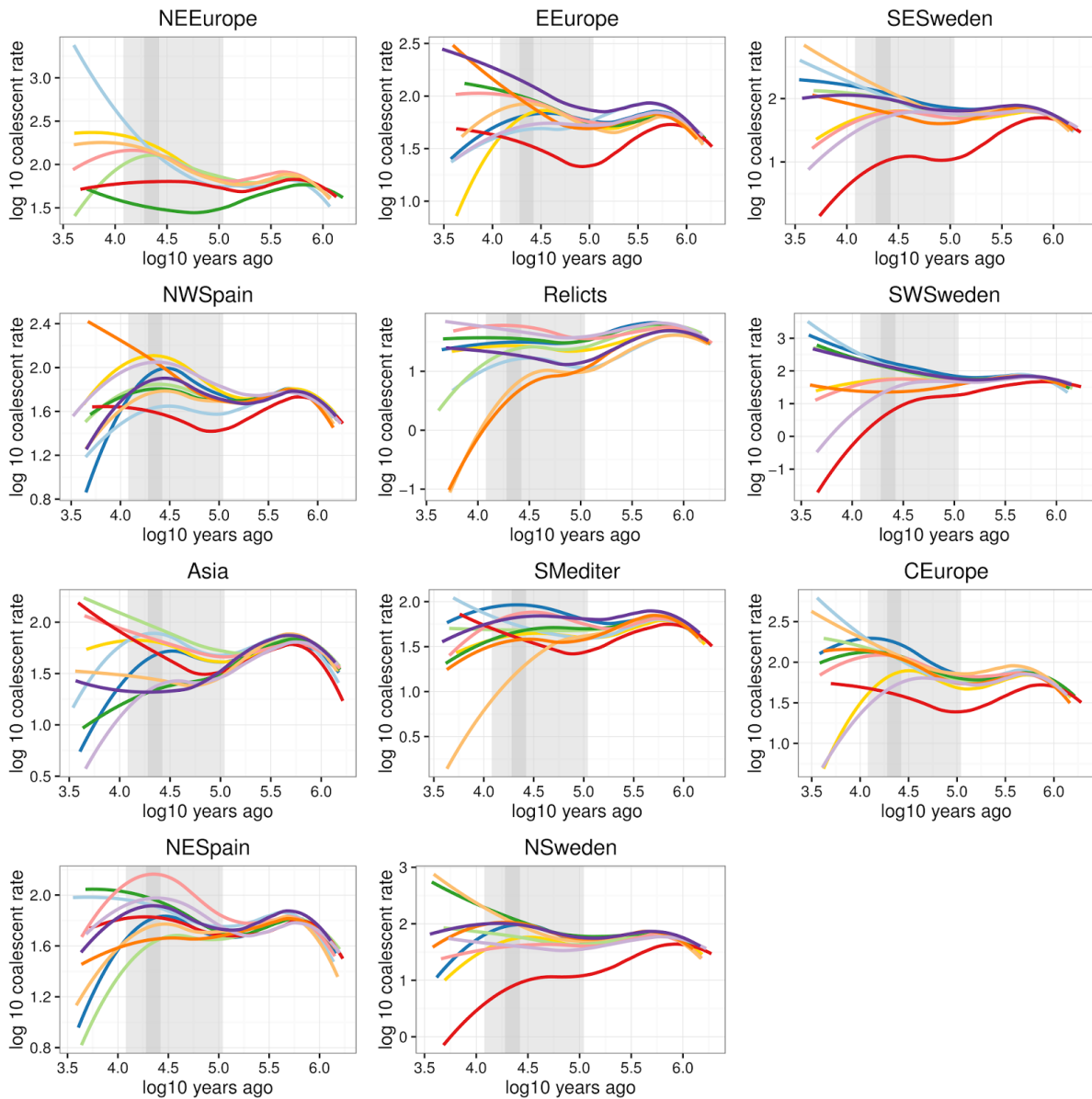
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Figure S5. Cross-coalescent rates between populations inferred by MSMC

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Joint coalescent rates of each of the 11 ADMIXTURE genetic groups are (see Fig. 1 and Fig. S12)

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compared to the other groups. Each line is a smoothed loess of 6 replicated runs. Light grey area

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indicates the extent of the last glacial maxima (100-10 kya) and dark grey area the peak of the last

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glaciation (22 kya). Analyses between certain groups failed (e.g. NE Europe with Asia), likely due to

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proximity between genomes. Note that the N. Swedish group is the first to separate from the

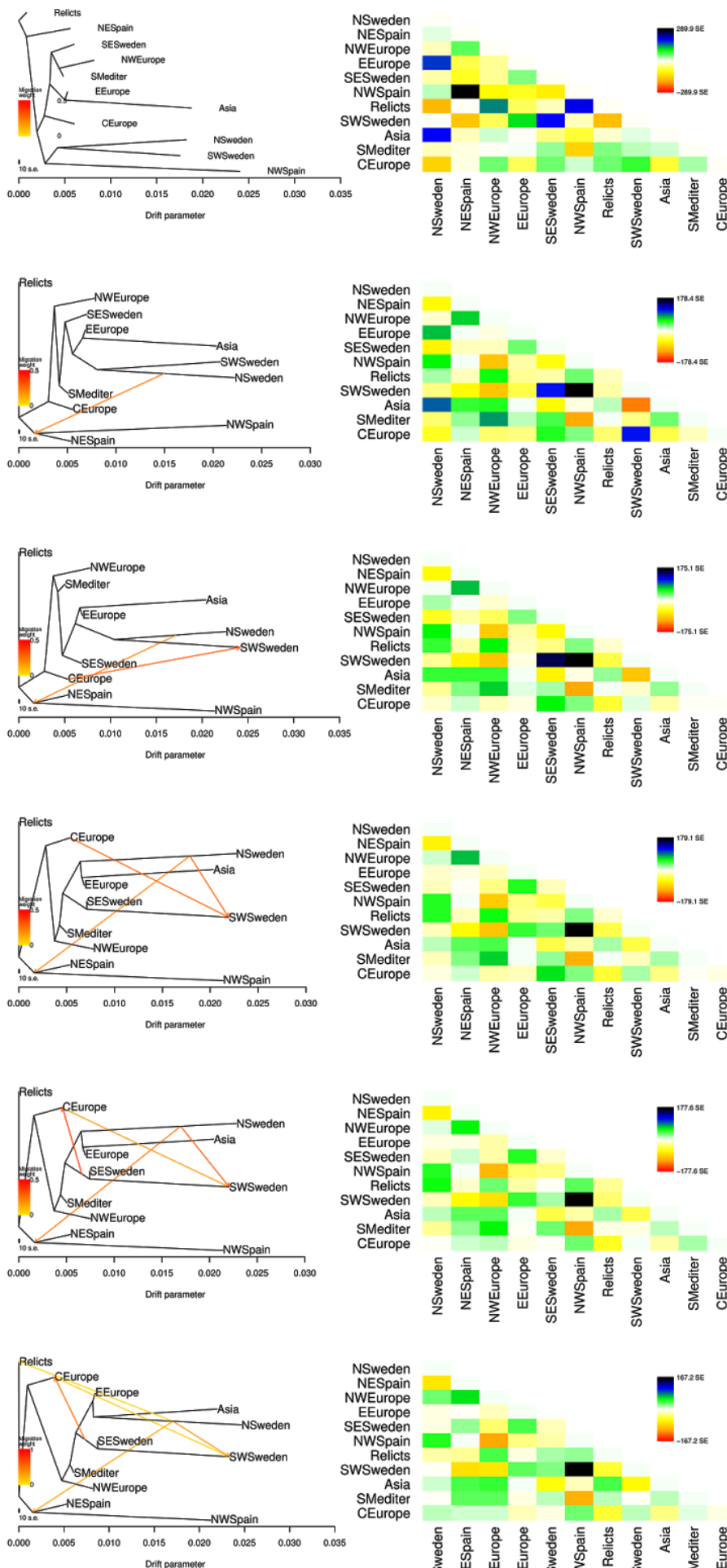
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relicts.

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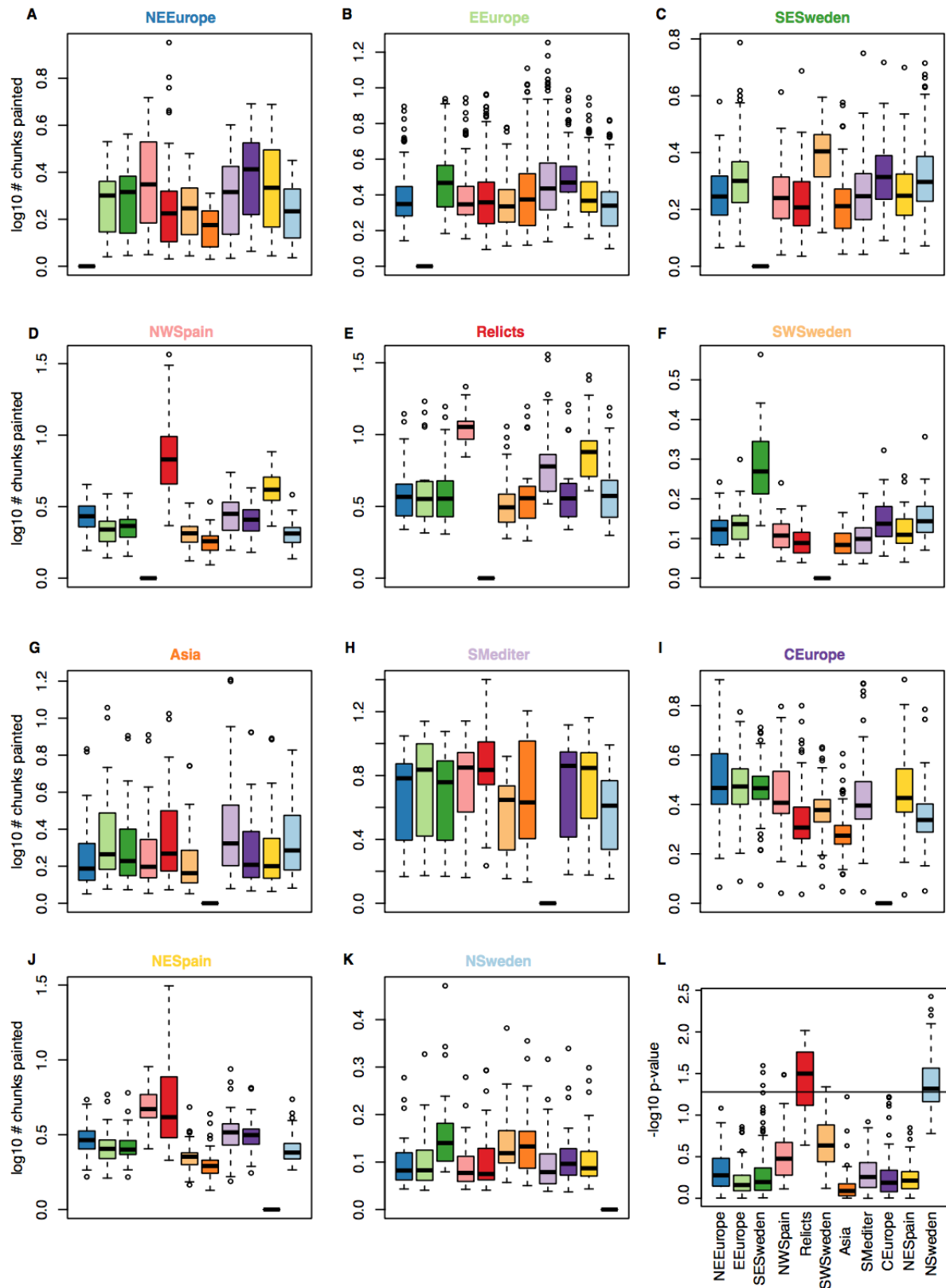
844 **Figure S6. Treemix with different migration rates**

845 Maximum likelihood (ML) population trees from Treemix (left). Analyses with zero to five
846 migration edges are presented. Heatmaps with the residual fit of the ML trees are shown on the
847 right. Note that the unexpected closeness of NW Spain and Sweden without migration is resolved
848 with one migration edge. With this, a more parsimonious tree that adheres to geographic locations
849 is uncovered.

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854 **Figure S7. Genomic ChromoPainter chunks per population**
855 (A-K) Summary of the number of ChromoPainter chunks inherited from other genomes that had

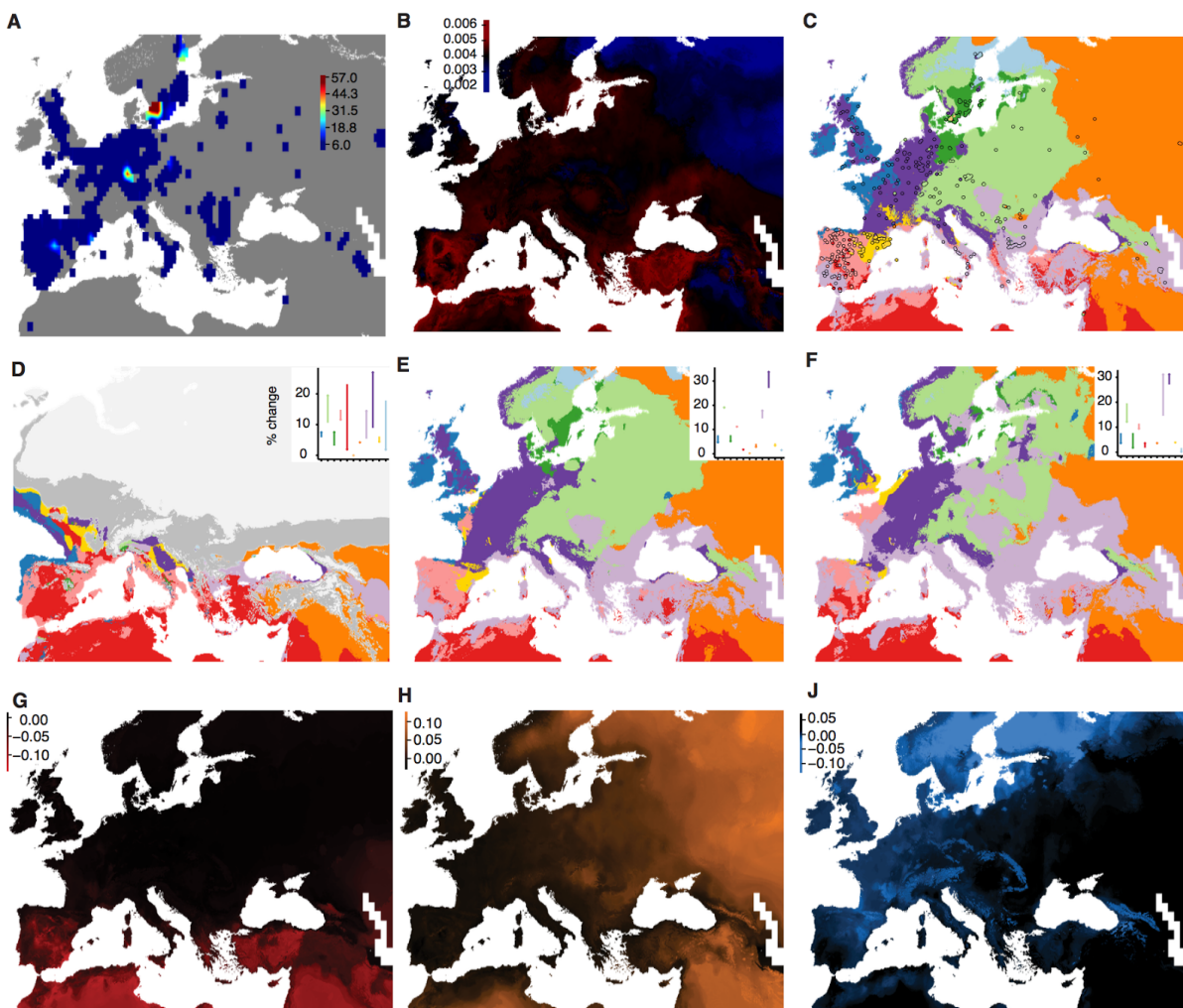
856 been assigned to ADMIXTURE groups. Each graph summarises the information of all the genomes
857 from an admixture group. (L) The p-value of the Pearson correlation test between an accession's
858 drought survival index and the number of chunks received from another genome. The p-value
859 distributions of genomes from the same ADMIXTURE group are grouped in a box plot. Intuitively
860 this can be interpreted as how well the number of chunks inherited from a specific donor predicts
861 the drought survival of the receiver. The black line indicates the 5% significance threshold, which is
862 passed by most relict and N. Sweden groups. Therefore, chunks that have N. Swedish and/or relict
863 ancestry explain the drought survival of other individuals well.

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868 **Figure S8. Environmental niche model of genetic diversity and population structure**

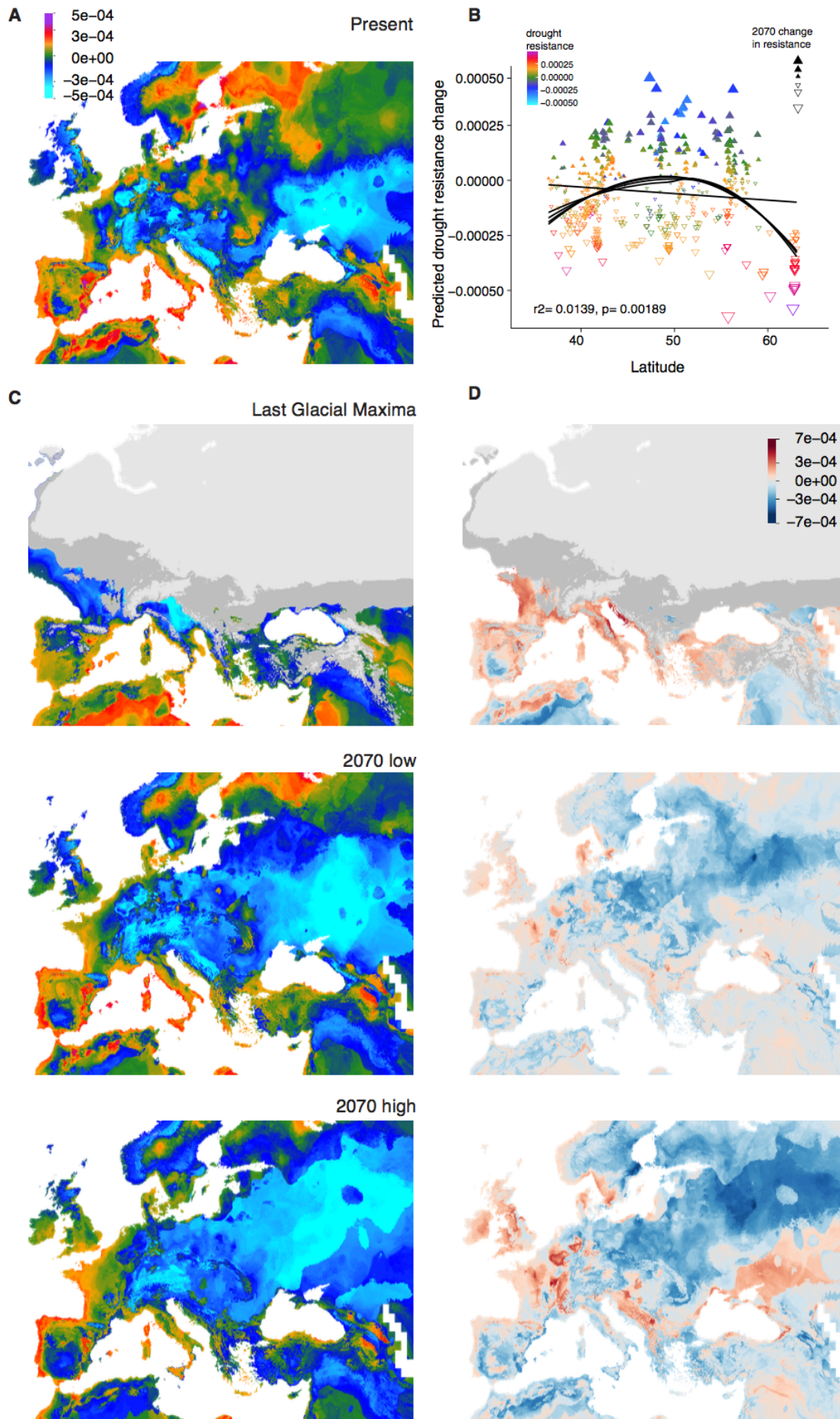
869 (A) Distribution of 762 accessions from the 1001 Genomes project used for environmental niche
870 modeling of genetic diversity and analysis of population structure. Colors indicate number of
871 accessions within a 1°x1° latitude x longitude grid. (B) Random forest environment niche models
872 using estimates of pairwise nucleotide diversity (π) diversity of each accession with its closest
873 10 geographic neighbours. The trained model was used to predict diversity based on
874 environmental data. (C) Random forest environment model of the 11 genetic groups (see Fig. 1).
875 Locations with accessions are shown as points filled with the actual genetic group assigned, and
876 are used for model training as in (B). The trained model was used to predict a raster of
877 environmental variables and is shown in the background. When the circle is filled with the same
878 color as the background, the model succeeds in the prediction. The trained model was also used
879 to predict presence of different genetic groups at the Last Glacial Maxima (D) and for 2070 under
880 low (E) and high (F) CO₂ concentration scenarios. (see Fig. 1 for color keys). (G-J) The first three
881 genome-wide principal components from Fig. 2 were modeled based on environmental variables.

882 Later, these were used as covariates of Genome-Environment Models (Fig. S13-14).

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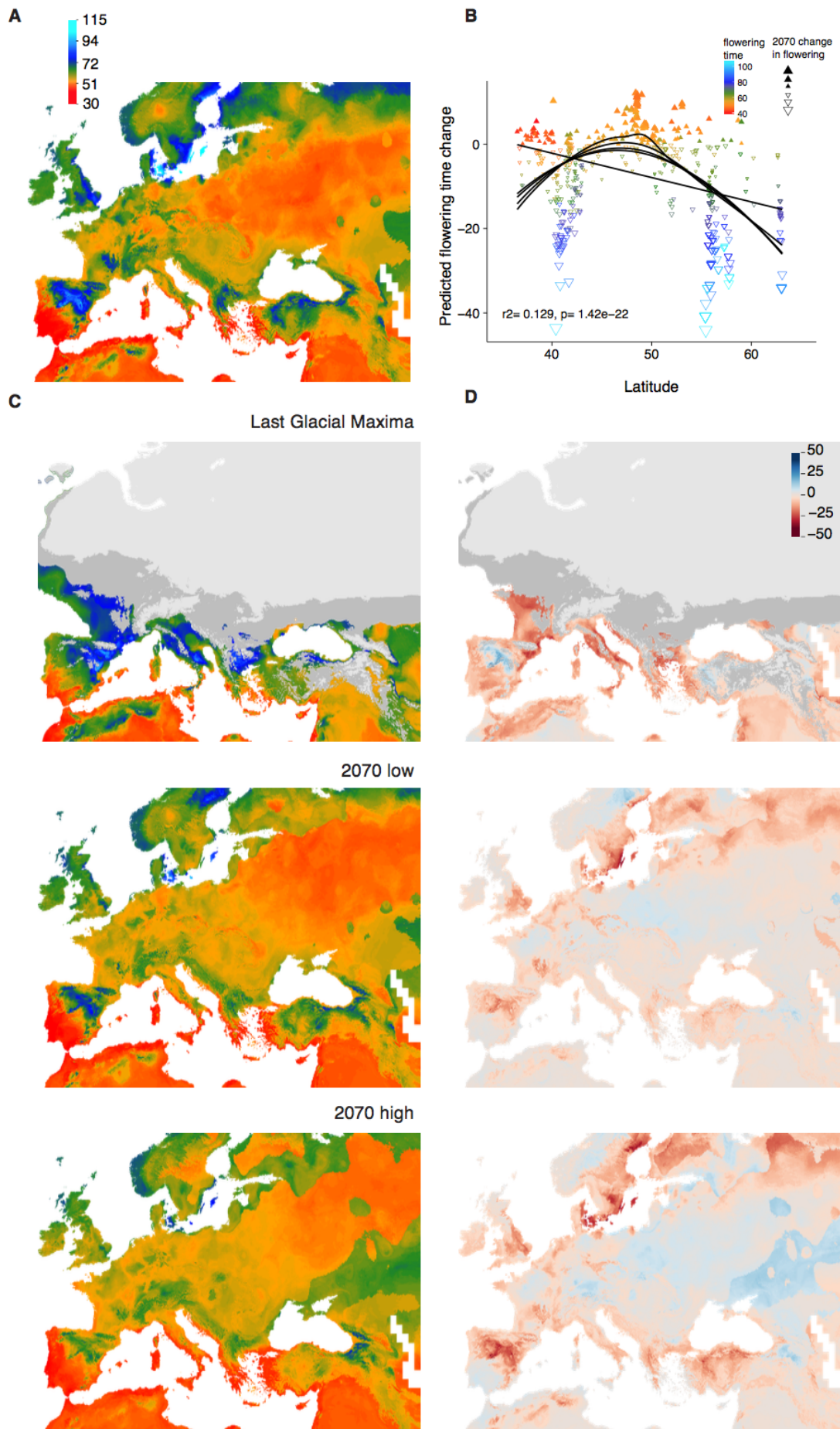
886 **Figure S9. Environmental niche model of drought index**

887 (A) Present geographic prediction of drought survival index from a random forest environment
888 niche model trained on experimentally determined phenotypes for 211 accessions. Note that the
889 highest drought survival values are inferred for the Mediterranean as well as N. Sweden. (B)
890 Correlation of phenotypic change in 2070 under a high CO₂ scenario with latitude; colors indicate
891 present drought survival values. (C) The trained model is also used to predict drought survival
892 index under the Last Glacial Maximum, and for two 2070 scenarios of low and high CO₂
893 concentrations. (D) For the three scenarios, the change is shown relative to the current date
894 prediction for easier comparison.

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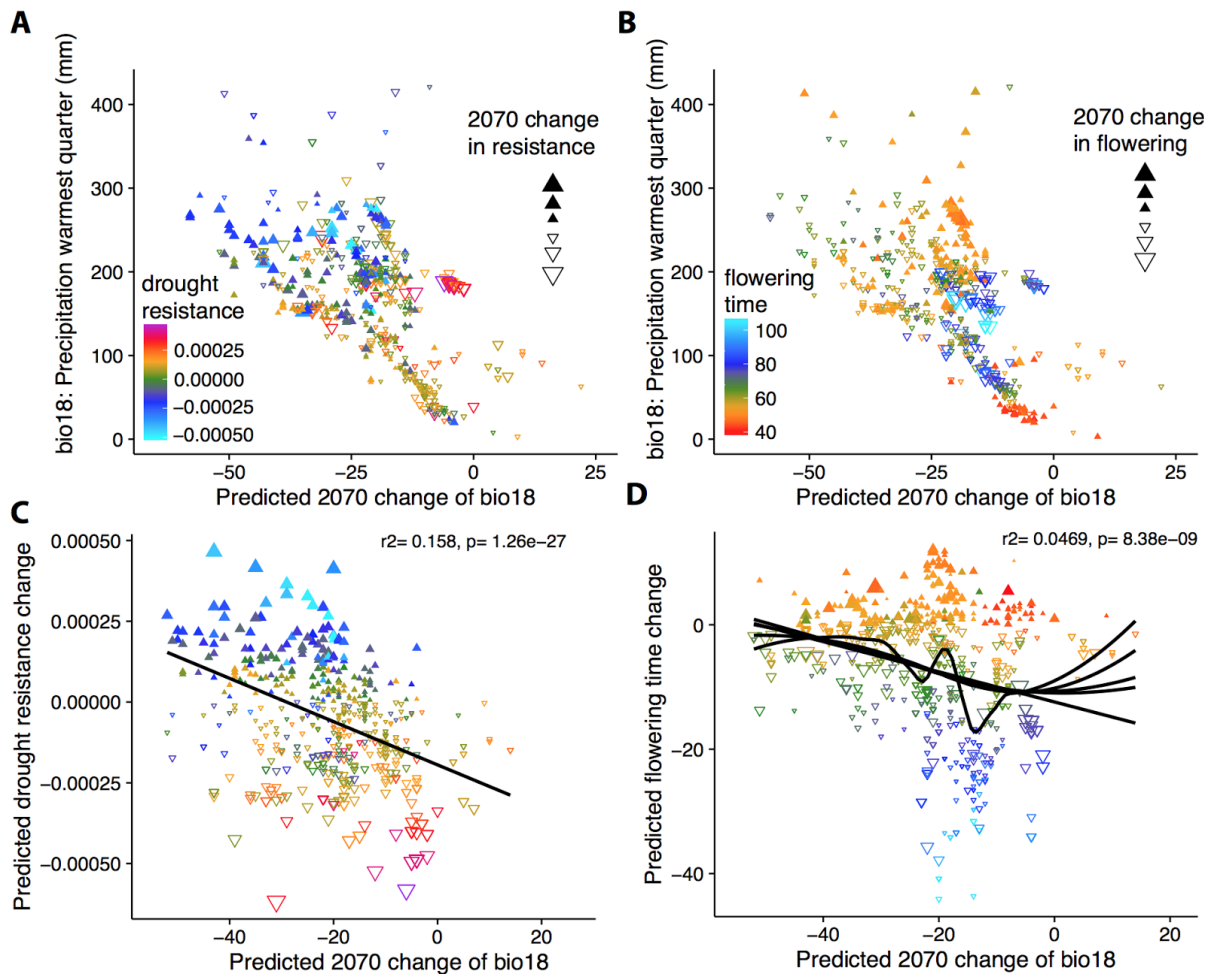
899 **Figure S10. Environmental niche model of flowering time**

900 Same models as in Fig. S9, but for flowering time.

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Figure S11. Profile of phenotypic change under climate change

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(A, B) Correlation of precipitation during the warmest quarter today and in 2070 under a high CO₂ scenario. Colors indicate current drought survival (A) or flowering time (B), and shapes indicate

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increase or decrease in trait values for 2070. (C, D) Regression of the predicted change in drought-survival (C) and flowering time (D) on the predicted change in precipitation in 2070.

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Note that areas with already low precipitation will not have large decreases in precipitation in 2070 (A-B). Note also the linear relationship between decreased precipitation in 2070 and

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predicted increase of drought-survival in (C). Flowering will be on average faster in 2070 (D), but the relationship between precipitation reduction and flowering time change is not linear, which

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suggests that areas with a moderate reduction in precipitation will have accelerated flowering (rather than increased drought survival).

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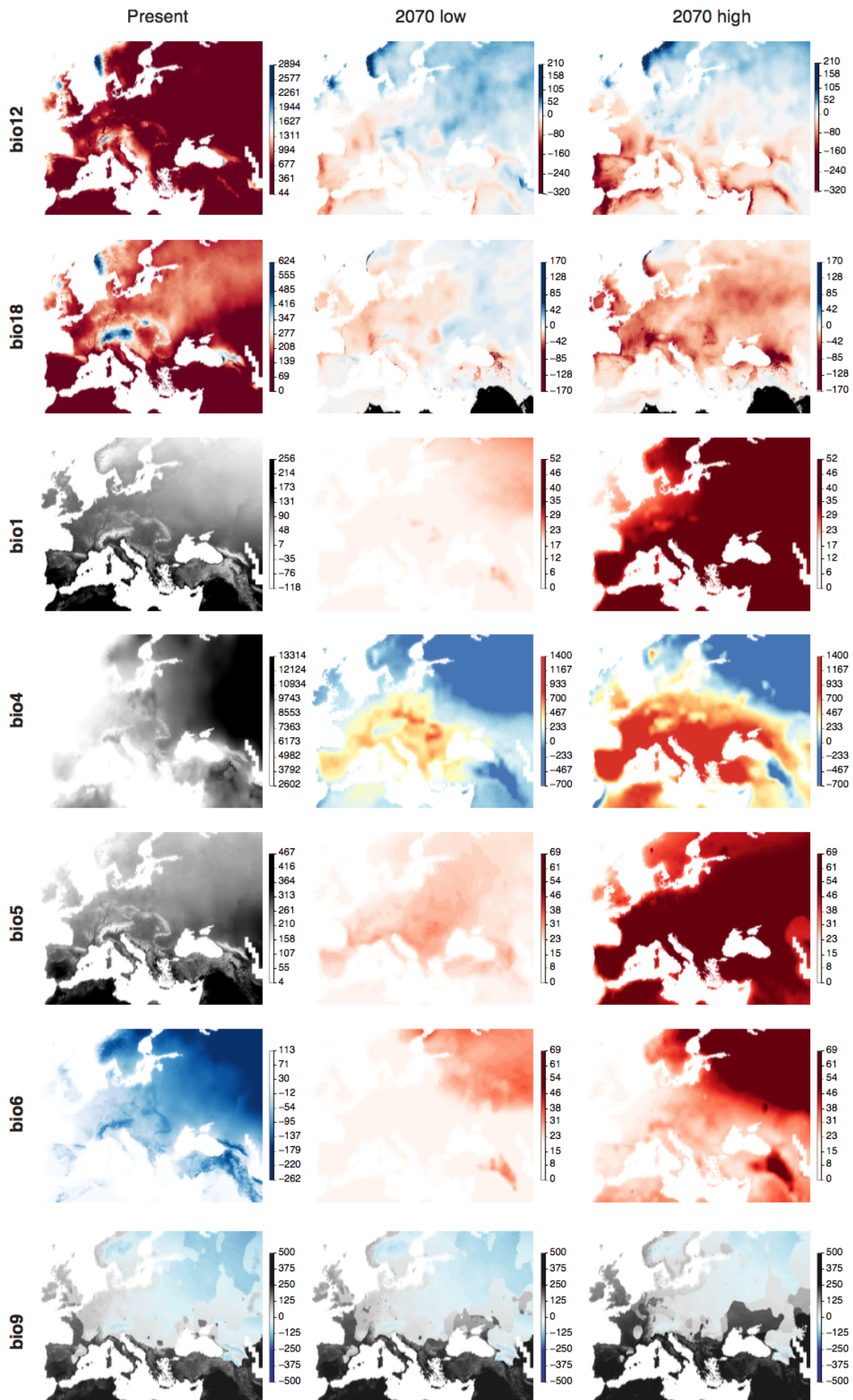
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917 **Figure S12. Maps of the most important climatic variables**

918 The bioclimatic variables (www.worldclim.org) that typically had more importance in phenotypic
919 and genome environmental models are shown as an aid for interpretation of the results from our
920 study. bioclim variable shown are annual precipitation (bio12), precipitation of the warmest quarter
921 (bio18), annual mean temperature (bio1), temperature seasonality (bio4), maximum temperature
922 of the warmest month (bio5), minimum temperature of the coldest month (bio6), and mean
923 temperature of the driest quarter (bio9). The columns show distributions at present, in 2070
924 under a scenario of low CO₂ concentration, and in 2070 under a scenario of high CO₂ scenario.
925 Except for bio9, the values for future scenarios were expressed as future-present difference to
926 highlight geographic areas that will change the most. Note the bimodality of bio9: areas in black
927 are summer drought (Mediterranean climate) areas, whereas blue areas are winter-drought. Also
928 note that bio18 is predicted to change mostly along the transition from the Mediterranean to
929 non-Mediterranean climate. In bio18, areas that will be under lower precipitation than any current
930 location of *A. thaliana* are shown in black, to highlight that most areas will remain within the range
931 of current precipitation across the species range.

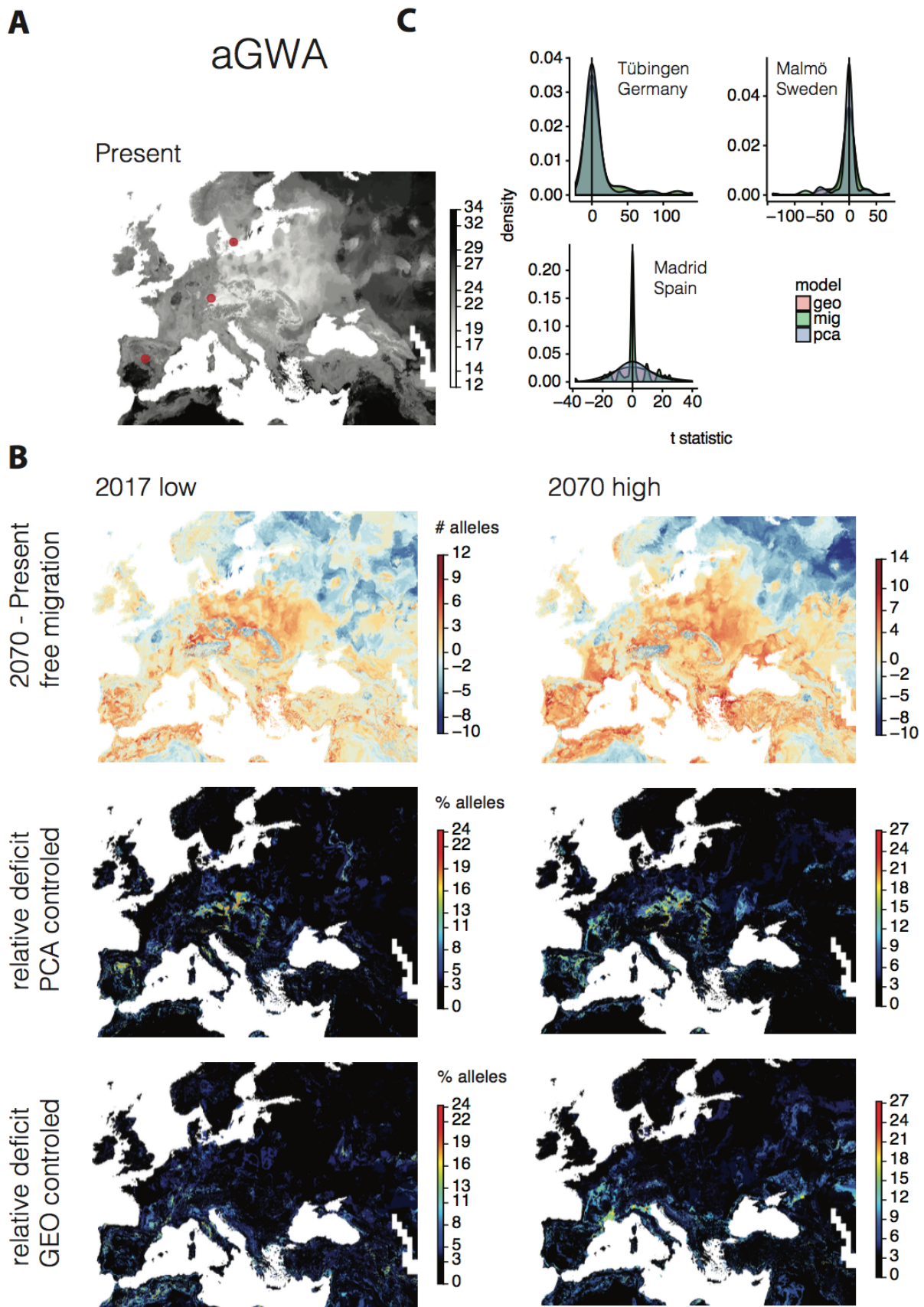
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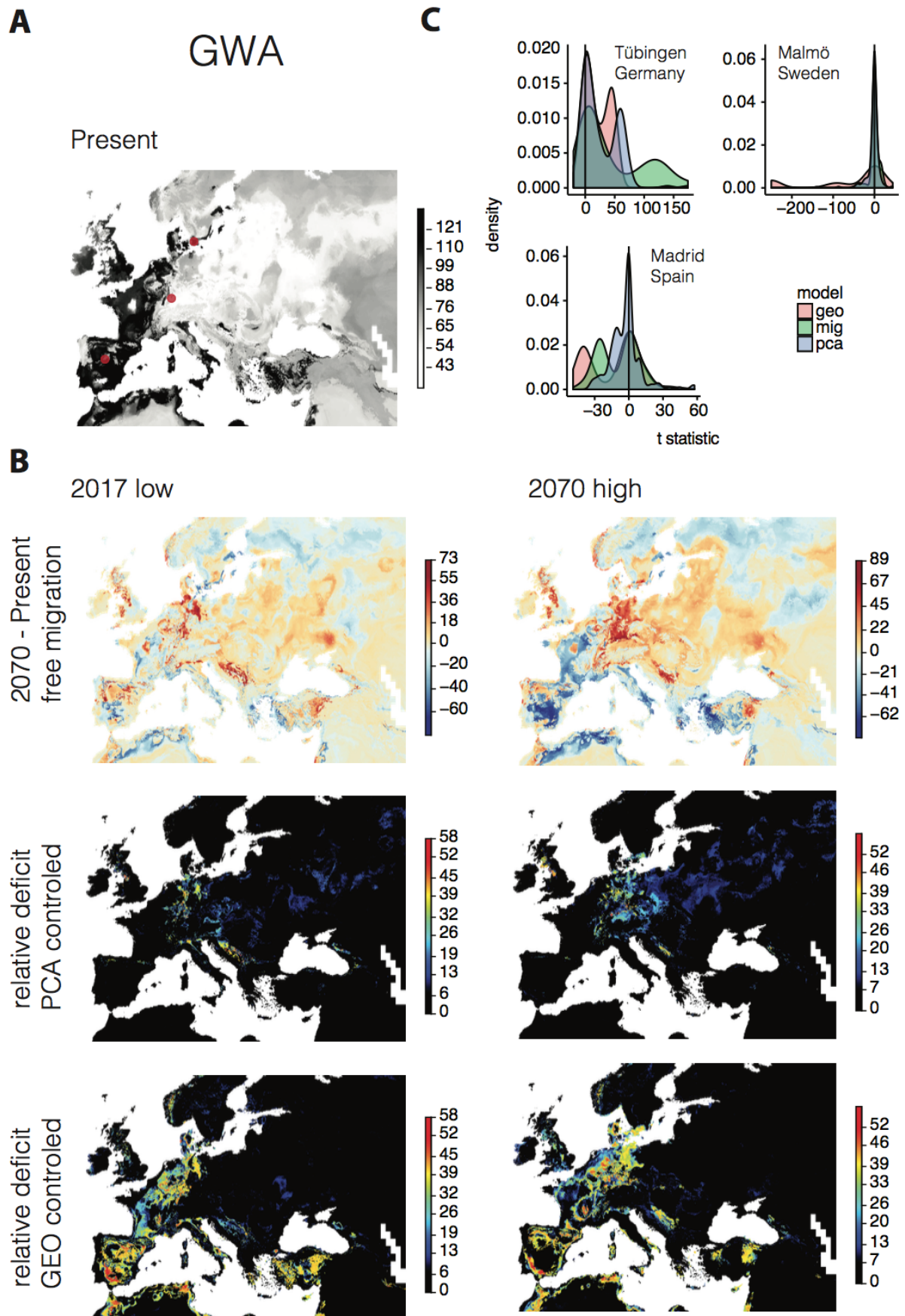
937 **Figure S13. aGWA Genome Environment Models (GEM)**

938 (A) We ran GEMs to describe the geographic distribution of alleles at the 70 aGWA top loci.
939 Concatenating all maps, we produced a map of the count of all drought-survival alleles that a
940 genotype is expected to have in a given location today. (B) The trained model from (A) was used
941 to predict distribution of drought alleles in the future. The difference to numbers inferred for today
942 (A) corresponds to the alleles that will have been gained or lost in 2070 in a given location. Two
943 additional models were trained which included a genome background (PCA) correction and
944 latitudinal and longitudinal (GEO) correction of the allele distributions. The percentage of gained
945 alleles from the “free” model that were not present in the corrected models is shown as a deficit in
946 percentage. (C) For three highly sampled locations, Madrid (Spain), Tübingen (Germany) and
947 Malmö (Sweden), we calculated allele frequency differences between today and 2070 (under
948 high CO₂) and calculated a t-statistic to describe the effect size of the change. A skew towards the
949 right (increase) is observed for Tübingen only.

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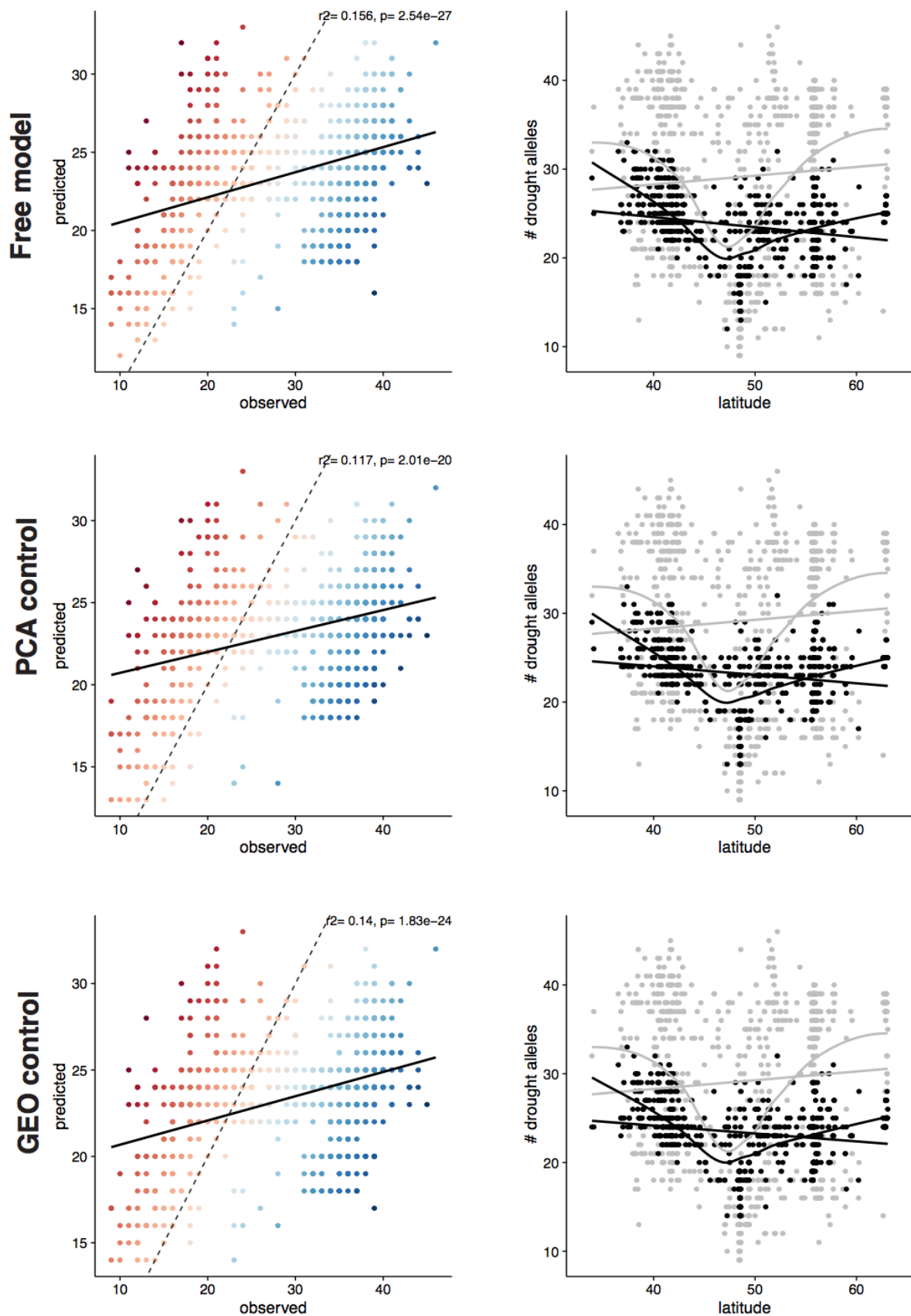
954 **Figure S14. GWA Genome Environment Models (GEM)**

955 See Fig. S13 for legend.

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aGWA



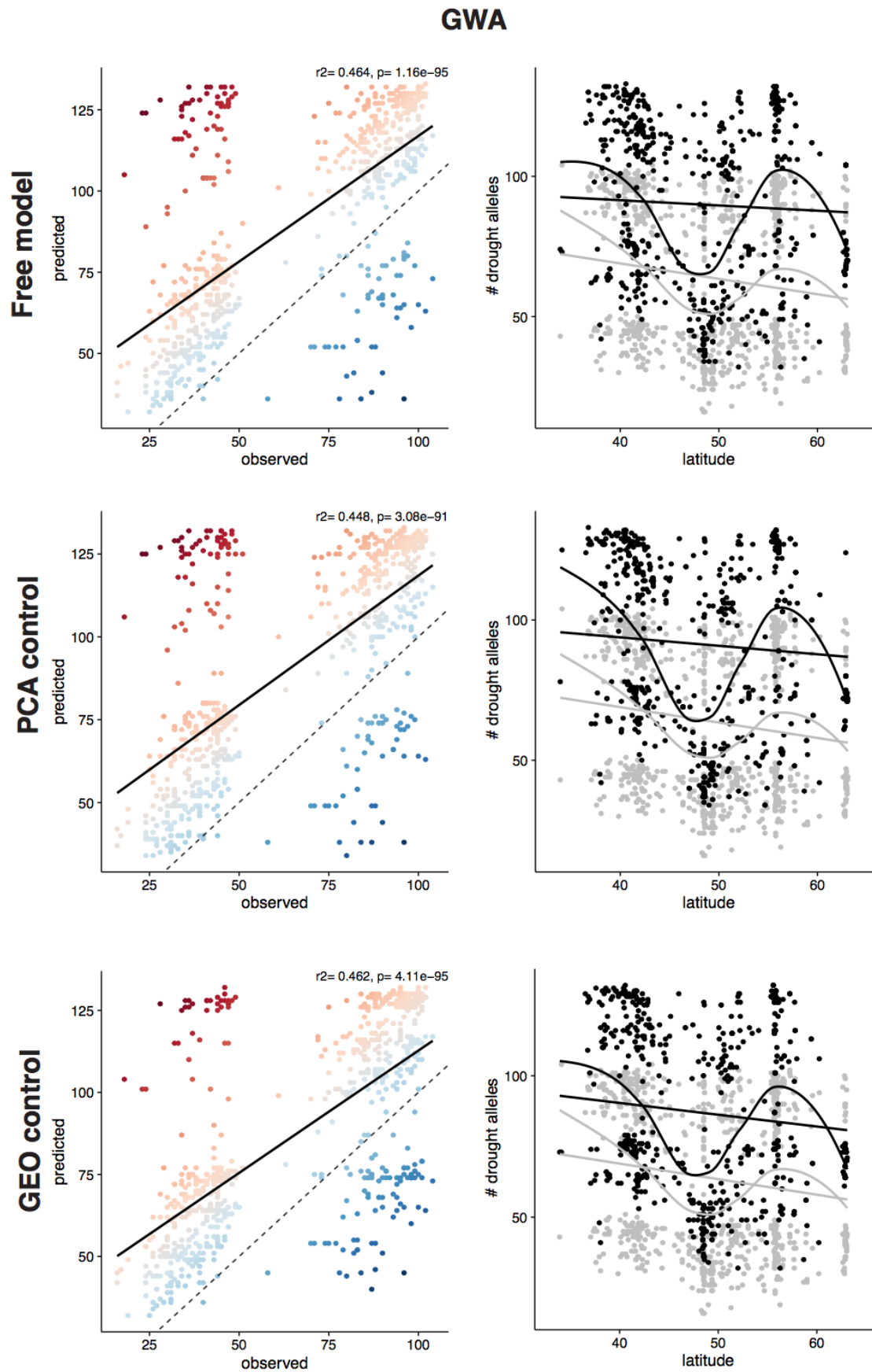
958 **Figure S15. aGWA GEM residuals**

959 For each GEM, we plot the predicted against the observed (empirical) number of
960 drought-associated alleles at each sampled locations. Red color indicates overestimation and blue
961 underestimation. Latitudinal trends of predicted (grey) and observed (black) are shown (right).
962 Note that the variance of predictions is larger than the empirical observations, probably due to the
963 discrete nature of random forests.

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967 **Figure S16. GWA GEM residuals**

968 See Fig. S15 for legend.

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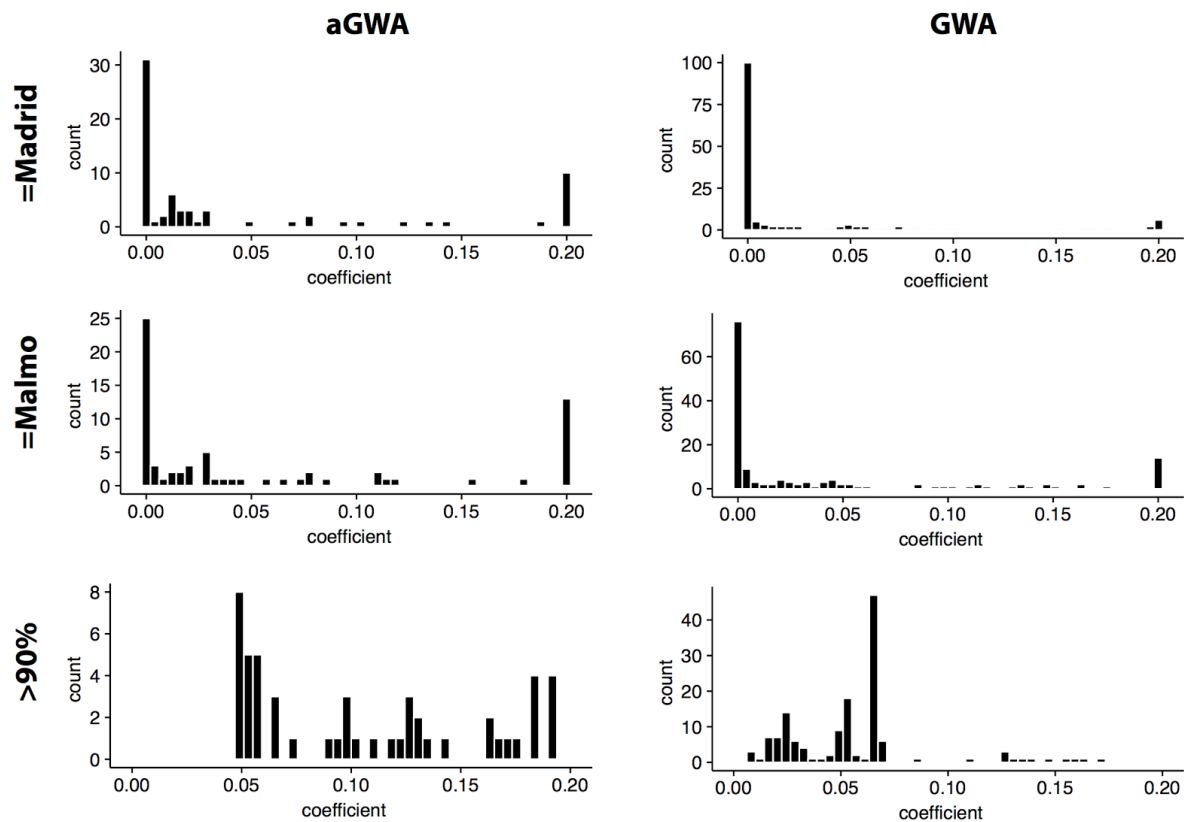
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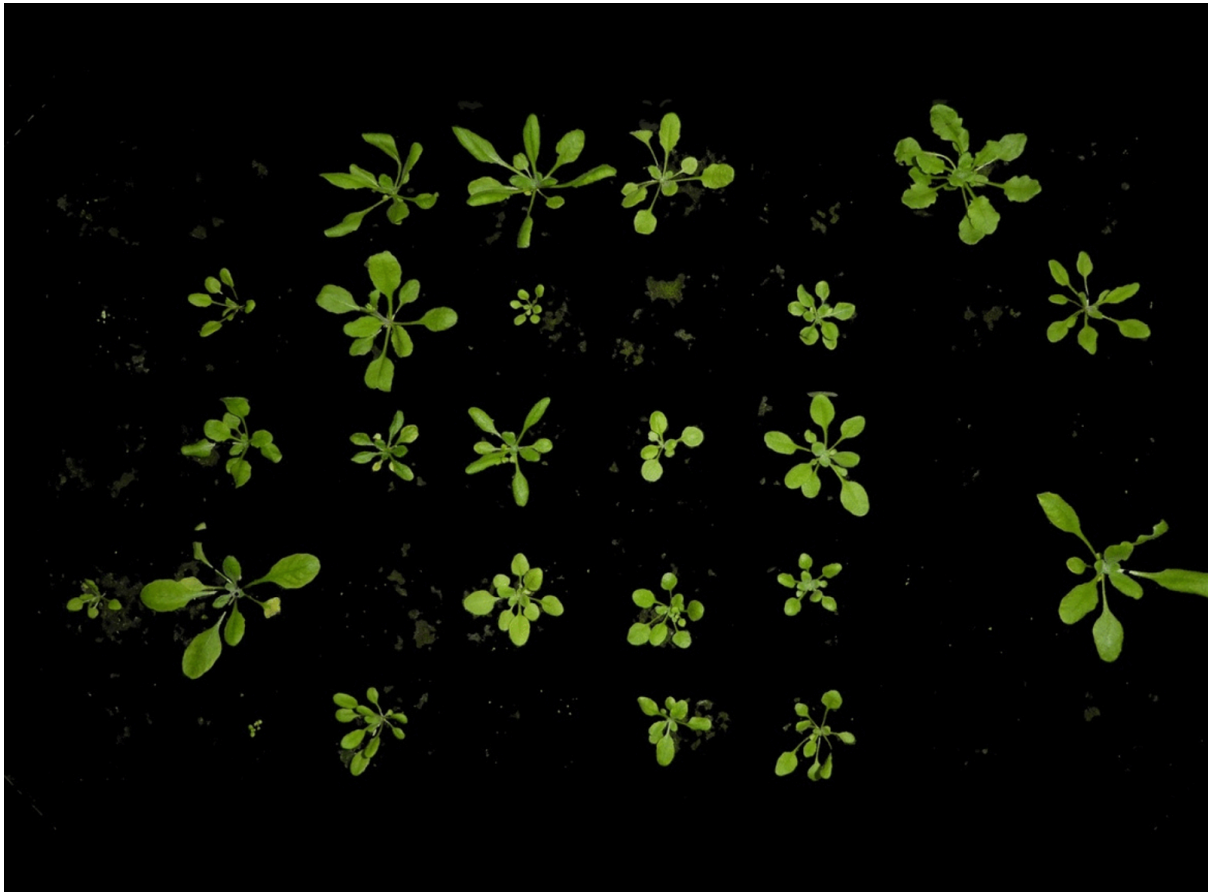
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975 **Figure S17. Population genetics simulations**

976 We ran Wright-Fisher population simulations of 70 (aGWA) or 151 (GWA) independent loci for 50
977 generations of evolution under mutation-selection balance, starting with the current allele
978 frequencies in the Tübingen population., and repeating each simulation with an array of selection
979 coefficients from 0.0001 to 0.2 (relative fitness advantage) for each locus. The distributions
980 shown correspond to the positive selection coefficients that are required for the drought -alleles
981 to rise to the frequency at which they are currently found in Malmö (top) or Madrid (center), or to
982 at least 90% (bottom), which is close to fixation.

983



984 **Video S1. Example of segmentation**

985 19-frames time series of green-segmented images for one exemplary tray. See the online file
986 Video_S1.gif (or [click here](#)).

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991 SUPPLEMENTARY TABLES & DATASETS

992 The combined tables can be downloaded as .xlsx file from:
993 [http://github.com/MoisesExpositoAlonso/Exposito-Alonso_2017_drought_Supplementary_Table](http://github.com/MoisesExpositoAlonso/Exposito-Alonso_2017_drought_Supplementary_Table_s)
994 [s](#) [here link to the journal's web upon publication]

995

996 **Table S1. Accession information.**

997 1001 Genomes IDs, common names, countries of origin, and geographical and environmental
998 information.

999

1000 **Table S2. ADMIXTURE cross-validation for all possible groups from 2 to 20.**

1001

1002 **Table S3. Selection signatures and annotation of top SNP hits from GWA.**

1003

1004 **Table S4. Annotation of top SNP hits from aGWA.**

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1006 **Table S5. Information on phenotypic and climate traits.**

1007

1008 **Table S6. Correlations between climate and phenotype variables per accession.**

1009 Pearson product-moment correlation coefficients between all phenotype and climate variables of
1010 Table S5. Lower triangle shows p-values, upper triangle correlation coefficients. The drought index
1011 parameter of choice (m1d_polqua) negatively correlates with the precipitation in the driest month
1012 and quarter, bio14 and bio18, respectively.

1013

1014 **Table S7. Correlations between different GWA effects of the 150 polygenic SNPs.**

1015 Pearson product-moment correlation coefficients between SNP effects estimated from GWA of a
1016 large subset of all phenotype and climate variables of Table S5.

1017

1018 **Table S8. Canonical Correlation Analysis.**

1019 CCA between GWA effects on different phenotypes and the SNP associations with climate
1020 variables.

1021

1022 **Table S9. Polygenic model at different top SNPs groups.**

1023 We applied the Berg & Coop model (66) of polygenic adaptation to different groups of top SNPs
1024 and report the value of Q_x statistics.

1025

1026 **Table S10. Importance of variables in Random Forest analyses.**

1027 For each random forest model, the importance of bioclimatic variables is reported. For
1028 classification random forest, importance is reported as the mean decreased accuracy (MDA) and
1029 for regression random forest, importance is reported as the mean square error (MSE). MDA is the
1030 number of misclassified observations when removing a variable and MSE is the increase of mean
1031 square error produced by removing a variable.

1032

1033 **Table S11. Allele frequency change**

1034 Student's t-test results of allele frequency changes in the locations of Madrid, Tübingen and
1035 Malmö under the three forecasting Genome Environment Models: free migration, principal
1036 components control, and geography control.

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