1	
2	Parallel reductions in flowering time from <i>de novo</i> mutations enable
3	evolutionary rescue in colonizing lineages
4	Fulgione and Neto et al.
5	
6	

7 Supplementary Method 1. Sample collection

8 We collected plants over a series of field expeditions between 2012 and 2019 on Santo Antão 9 and Fogo, the two islands where *Arabidopsis thaliana* had been recorded in herbarium records 10 (personal communication, Wolfram Lobin). In addition, we explored possible locations in the 11 two other islands with the most similar landscape (Santiago and São Nicolau) but found no 12 evidence of A. thaliana there, consistent with a lack of herbarium records. In total, we present 13 data for 335 accessions from the Cape Verde Islands (Supplementary Data 1), including 189 14 accessions from 26 stands across four regions in Santo Antão (Cova de Paúl, Lombo de Figueira, 15 Pico da Cruz and Espongeiro), and 146 accessions from 18 stands across three regions in Fogo 16 (Lava, Monte Velha and Inferno).

17

18 Supplementary Method 2. Climate data

19 We downloaded gridded data for climatic and bioclimatic variables at 30 second resolution (~ 20 1 km²) in GeoTiff file format for the temporal range of 1970-2000 from WorldClim version 2.1¹, 21 including monthly climate data for average temperature and precipitation (12 data layers, i.e. 1) 22 layer for each month) and 19 bioclimatic variables (1 data layer each) which are temperature and 23 rainfall derived datasets. In addition, we downloaded a raster file for Aridity Index (1 data layer) 24 from CGIAR Consortium for Spatial Information (CGIAR-CSI)², which is the ratio of precipitation to potential evapotranspiration, where higher values correspond to more humid 25 26 conditions and lower to more arid conditions. In addition, we estimated growing season length

using the monthly average temperature and precipitation data obtained from WorldClim. Months for which mean temperature $\ge 4^{\circ}$ C and mean precipitation $\ge 2^{*}$ mean temperature were summed to produce an estimate of the growing season length³ using 'Raster Calculator' of ArcGIS. We extracted values for sites where CVI, Moroccan and Eurasian samples had been collected in ArcGIS and compared distributions of climate variables across regions using Mann Whitney Wilcoxon (MWW) tests.

33

34 Supplementary Method 3. Sequencing

35 We sequenced 335 newly collected Cape Verde Islands accessions and Cvi-0 using Illumina 36 Hi-Seq and HiSeq3000 machines. We extracted genomic DNA using DNeasy Plant Mini kits 37 (Qiagen), fragmented using sonication (Covaris S2), and prepared libraries with Illumina TruSeq 38 DNA sample prep kits (Illumina), NEBNext Ultra II FS DNA Library Prep Kit (New England 39 Biolabs) and NEBNext Ultra II DNA Library Prep Kit (New England Biolabs). Libraries were 40 immobilized and processed onto a flow cell with cBot (Illumina) and subsequently sequenced 41 with 2x 100-150 bp paired end reads. We assessed DNA quality and quantity via capillary 42 electrophoresis (TapeStation, Agilent Technologies) and fluorometry (Qubit and Nanodrop, Thermo Fisher Scientific). Due to changes in product availability over time, sample preparation 43 44 differed slightly between subsets of the sequenced accessions. Sample IDs 12766 to 35519 were 45 prepared with Illumina TruSeq DNA sample prep kits (Illumina, San Diego, CA), samples in 46 projects 4073 and 3968 were prepared with NEBNext Ultra II FS DNA Library Prep Kits 47 (Illumina, New England Biolabs) including four cycles of PCR amplification, and samples from

48 projects 3619, 3541, 3536, and 2876 were prepared with NEBNext Ultra II DNA Library Prep
49 Kit (Illumina, New England Biolabs) with five cycles of PCR amplification.

50

51 Supplementary Method 4. SNP identification and genotyping

52 For Illumina sequence data mapping and genotype calling we used the A. thaliana TAIR10 53 reference genome and we called variants with three different pipelines. Two pipelines were 54 previously used to call genotypes in the 1135 Eurasian and 64 Moroccan sequences⁴. Here, we 55 used the same parameters, settings, and software versions in order to analyse the Cape Verdean 56 and worldwide sequences in a common framework (https://github.com/HancockLab/CVI). Specifically, in the SHORE pipeline⁵ we pre-processed the reference genome with the 57 58 subprogram 'preprocess' and parameters < -C --indexes BWA, SuffixArray > and with bwa v0.7.5a⁶, command 'index', parameters < -a bwtsw >. We trimmed adapters with adapterremoval 59 60 $v2.1.2^7$, parameters < --trimqualities >. We aligned reads to the reference genome with the bwa program 'aln', parameters < -n 0.1 > and 'sample', parameter < -a 500 >, and we 61 62 imported trimmed fastq files using the SHORE subprogram 'import' with parameters < --63 application genomic --importer Fastq --shore-filter --max-Ns 10% --lowcomplexity >. Finally, 64 we called variants with the empirical scoring matrix approach implemented in the SHORE 65 subprogram 'consensus' with parameters < -b 0.9 -g 4 -h 6 -i 0.5 -N >. We used this pipeline for 66 all analyses except MSMC, GWAS and BSA. The second, more conservative pipeline is 67 implemented as a custom program in java v.1.8, and it was used to reduce false positive variant 68 calls due to indels for the MSMC analysis, which is sensitive to linked errors⁴. This pipeline

69 excludes repetitive genomic regions, such as regions where the same base is repeated five or 70 more times, as well as the adjacent ten bases, it excludes the first and last positions of each read, 71 it removes bases with quality < 30 and coverage < 5x, and it eliminates positions with coverage 72 greater than twice the average coverage to remove potential duplications. For variant calling, the pipeline calls the reference allele with a calling ratio of 0.0 to 0.2, and a mismatch to the 73 74 reference with a calling ratio of 0.8 to 1.0. To avoid strand-specific errors, mismatches are called 75 only if they are supported by at least one read aligned on both the forward and reverse strand. To 76 call short indels, we used a modified version of the GATKv.4.1.3.0⁸ best practices workflow for 77 germline short variant discovery and genotyping. We included biallelic variants only and 78 converted heterozygous sites to missing data to mask possible false positives. Further, we 79 converted genomic regions with coverage higher than twice the genomic average to missing data, 80 to eliminate false variant calls due to duplications not represented in the reference genome. In the 81 three pipelines, file conversions between fastq, bam and sam formats relied on picard v. 2.21.1 and samtools v. 1.9⁹, vcf merging and subsetting relied on bcftools vl.9¹⁰ and vcftools v. 0.1.14¹¹. 82 83 Average coverage across samples was 19.4x (range from 9.3x to 51.7x; Supplementary Data 1) 84 after alignment to the reference.

85

For all downstream analyses, we retained variants with coverage greater than 3 and base
quality greater than 25 in the SHORE calls, and 2 and 30 in the GATK calls
(<u>https://github.com/HancockLab/CVI</u>). To call S-locus haplogroups we followed the procedure
used in ⁴. We added to the reference genome (TAIR10), which represents haplogroup A, the

sequences of S-locus haplogroups B and C (from Cvi-0¹² and Lz-0¹³, respectively). We called
variants against this modified reference and assigned to each CVI sample the S-locus haplogroup
that had the highest proportion of sites with non-zero coverage, after quality filtering.

93

94 Supplementary Method 5. Plant growth and phenotyping

95 In the flowering time experiment, we scored flowering time, bolting time, time to anthesis, number of days until the stem reached 3 cm, and the number of rosette leaves at bolting, as in 96 Salomé et al.¹⁴. We measured correlation between the four flowering traits scored in the 97 98 simulated CVI conditions experiment using the function cor() implemented in R 99 (https://github.com/HancockLab/CVI). These phenotypes were strongly correlated, with 100 Spearman's rho of at least 0.96 in Santo Antão and Morocco between flowering time and bolting 101 time. In Fogo, where flowering time is more difficult to score due to differences in petal 102 morphology, the correlation was somewhat lower (rho=0.83), likely due to increased error for the 103 flowering time trait here. Therefore, bolting time results were used as a proxy for flowering time 104 in downstream analyses.

105

106 Supplementary Method 6. Linkage disequilibrium

107 Linkage disequilibrium (LD) was assessed between pairs of SNPs with minor allele

108 frequency greater than 5% were calculated using the command < --ld-window 999 --ld-window-

109 kb 10 --ld-window-r2 0 --r2 --snps-only > in PLINK¹⁵. The numbers of sites on which LD was

110	estimated were 55, 645 for Santo Antão, 56, 173 for Fogo and 1, 435, 763 for Morocco.
111	Calculations were made between pairs of SNPs up to a distance of 10 kb. LD decay analyses
112	were conducted by division of marker pairs within the 10-kb region into bins of 1 kb and r^2
113	values within each bin were averaged. To visualize the result, the r ² values were sorted and
114	plotted against the physical distance, using loess smoothing.

115

Supplementary Method 7. Demographic reconstruction 116

Using MSMC-CCR¹⁶, we inferred split times by computing the mean across combinations of 117 118 sets of samples and the confidence interval of the mean (± 1.96 *standard error of the mean). For 119 the split between Santo Antão and Fogo, we used a total of 63 combinations of eight accessions 120 from each island. For the split to Morocco, we used 357 combinations (separately for the High 121 Atlas, South and North Middle Atlas Moroccan populations). For splits within Santo Antão, we 122 used 12 combinations to examine pairwise splits between the Figueira, Cova, Espongeiro, and Pico da Cruz populations. As suggested in ¹⁶, we inferred split times when CCR reached 0.5, 123 124 with an uncertainty interval between $0.25 \le CCR \le 0.75$.

125

For the inference of split parameters in dadi v.2. 1.0^{17} , we used joint site frequency spectra 126 127 (JSFS) based on intergenic SNPs, which are less likely to evolve under strong selection than 128 coding regions. We estimated parameters between the two Cape Verde islands and between CVI 129 and Morocco using four demographic models: 1) a simple two-population split model with no

migration and constant population size (N_e) ; 2) the same model with a bottleneck at the split; 3) a split with exponential changes in N_e after the split and no migration; and 4) an isolation with migration model (IM): a split with exponential changes in N_e and asymmetric migration.

133

134 For each demographic model and population pair, we replicated the analysis 1000 times with 135 a maximum of 50 iterations. In each replicate run we used starting values for all parameters drawn randomly from predefined ranges. The parameter boundaries were $(10^{-3} \times N_{ref}, 20^{\circ} N_{ref})$ for 136 effective population sizes (Ne), (0; 20/Nref) for migration rate, and (0; 10*Nref) for the split time, 137 138 where N_{ref} is the size of the ancestral population. Among the 1000 runs per model, we selected 139 the parameter combination that resulted in the highest likelihood. We identified the model with 140 the best support using the Akaike information criterion (AIC), and for each resulting best model, 141 we calculated confidence intervals for parameters using 100 000 bootstrapped data sets and the 142 Godambe Information Matrix implemented in dadi. For the best-supported models, the 5% runs 143 with highest likelihood all converged to the same parameter set.

144

We inferred colonization time by obtaining an upper bound based on the minimum
coalescence time between CVI and Morocco, and a lower bound based on the maximum
coalescence time within the CVI clade. First, we ran coalescent simulations of the CVIMoroccan split in msprime v.0.4.0¹⁸ with split times drawn from a uniform distribution of times
between 5-50 kya. To account for the confounding effect of purifying selection, which reduces
the rate at which new mutations are introduced in the genome, we scaled mutation rate across

151 simulated genomic windows as $\mu_{scaled} = \theta_{local}/4 N_e$, where θ_{local} was estimated as θ_{π} in the 152 Moroccan population within each window and Ne was fixed to the genome-wide average $(N_e = \theta_{genome}/4*\mu)$ so that $\mu_{scaled} = \mu^*(\theta_{local}/\theta_{genome})$. Then, we inferred coalescence times between 153 154 simulated and observed CVI and Moroccan genomes across genomic windows based on the 155 density of mutations. We obtained 95% confidence intervals based on the standard error (SE) 156 estimated by non-parametric bootstrap resampling of observed and simulated data. By fitting the 157 simulated cumulative proportion of genomic windows with different inferred ages to observed 158 data, we obtained a conservative estimate of the upper bound of colonization time. We inferred 159 coalescence times within Cape Verde, across genomic windows (0.1 Mbp, non-overlapping), based on the density of mutations and used the 95th percentile as a lower bound for colonization 160 161 time.

162

163 We constructed a time-calibrated chloroplast phylogeny to examine divergence in the 164 chloroplast genome and to compare these to patterns at nuclear loci. First, we aligned the 165 chloroplast sequences with outgroups from other Arabidopsis species, Capsella grandiflora, *Capsella bursa-pastoris* and *Camelina sativa*¹⁹, excluding the Inverted Repeat region. All indels 166 167 were removed. Identical sequences were excluded from the alignment and a maximum likelihood (ML) phylogenetic tree was reconstructed with RAxML v.8.1.16 20 using the GTR+F+I model of 168 169 rate heterogeneity and setting the clade of *Capsella* and *Camelina* as outgroup. Rapid 170 bootstrapping followed by a thorough ML search was applied with 1000 bootstrap replicates. 171

172 Divergence time was estimated using BEAST v.1.8.3²¹. Three secondary calibration points were included from the literature²²: the root height (split between genus *Arabidopsis* and the 173 174 *Capsella/Camelina* clade) was set to 8.1627 million years (my), the split between *Capsella* and 175 Camelina was set to 7.3572 my, and the crown age of genus Arabidopsis was set to 5.9685 my; a standard deviation of 1.0 was used for all three calibration points. The GTR+ Γ +I model of rate 176 177 heterogeneity with 4 Gamma categories was used as substitution model with an uncorrelated 178 relaxed lognormal clock²³ and tree prior Speciation: Birth-Death Process²⁴. Two independent MCMC runs with chain length $1x10^9$ were combined in LogCombiner v.1.8.3²¹, discarding the 179 180 first 10% of each run as burn-in, and the median heights from the remaining 18002 trees were 181 annotated onto the maximum clade credibility tree in TreeAnnotator v.1.8.3²¹.

182

183 Supplementary Method 8. Additional inference of demography within CVI

We used forward, individual-based simulations in SLiM $v.3.3.2^{25}$ to model the demographic 184 185 history within the archipelago, including the colonization events and consequent bottlenecks. 186 Under this model, the initial propagule founded a population on one island in the archipelago. 187 The population grew following an exponential function that varies across simulations (growth 188 rate varies with final size between 100 and 2500 individuals) until the time of the split between 189 islands (4.0 kya), when the second island was colonized from the first. Both populations grew 190 exponentially until they reached final N_e (10K, inferred from θ_{π}). On the second island, as inferred with dadi¹⁷, we simulated a 1000 year-long bottleneck with 400 individuals. 191

193 In order to determine which island was colonized first, we fit simulations to the observed 194 data using the difference in the proportion of variants that are fixed in one island and segregating 195 in the other (proportion of variants segregating in Santo Antão and fixed in Fogo minus the 196 proportion of variants segregating in Fogo and fixed in Santo Antão) as a summary statistic. The 197 value of this statistic is positive if Santo Antão was colonized first and negative if Fogo was 198 colonized first. In addition, we used three-way (Morocco, Santo Antão, Fogo) JSFS modelling in 199 dadi to compare the relative fit of Santo Antão-first and Fogo-first models and estimated the length of the Fogo bottleneck period¹⁷. For each demographic model we replicated the analysis 200 201 100 times with a maximum of 50 iterations. In each replicated run, we used starting values for all 202 parameters drawn randomly from predefined ranges. The parameter boundaries were $(10^{-3} * N_{ref})$ 20*N_{ref}) for effective population sizes (N_e), and (0; 10*N_{ref}) for split times, where N_{ref} is the size 203 204 of the ancestral population. We identified the model with the best support using the Akaike 205 information criterion (AIC).

206

207 Supplementary Method 9. Niche modelling

In the first step of niche modelling in MaxEnt²⁶, we produced a predictive model using collection locations in Morocco and the bioclimatic variables described above and listed in Supplementary Data 2. We considered supplementing the collection locations with information from herbarium collection records from GBIF (https://www.gbif.org), but only 'fuzzy matches'

212 existed in the data base and GPS coordinates were thus unreliable. We conducted climatic niche 213 modelling in Morocco using occurrence data based on²⁷. Data for the Moroccan region was 214 extracted using the 'extract by mask' function in ArcToolbox. To avoid overfitting, climatic 215 variables were pruned so that no two variables were correlated with Pearson correlation 216 coefficient > 0.75. The model we present uses a set of variables chosen based on ecological and 217 biological relevance, but the predicted suitability of CVI habitat for Moroccan accessions did not 218 change across these different variable selection regimes. We ran a Maxent under the standard 219 default parameters with jackknife resampling to estimate the importance of each variable on the 220 model. Model fit was inferred based on the area under the curve (AUC) for the model output and 221 a cross-validation approach in which the data were split into equal sized subsets. Then, we 222 projected this model onto the CVI landscape to predict the suitable range of Moroccan samples 223 in CVI. We further identified the regions within CVI that were most similar to the Moroccan A. 224 thaliana habitat. Since the approach we used for pruning variables is somewhat subjective and 225 the CVI suitability result was extreme, we also tried other approaches for pruning correlated 226 variables but found no change in suitability in CVI across variable selection regimes including 227 random selection of variables with Pearson's r < 0.75 and a variance inflation factor approach. 228 This robustness is likely due to the fact that nearly all climate variable values for CVI lie outside 229 those found in the Moroccan presence data.

230

231 Supplementary Method 10. Testing for evidence of adaptive evolution

To compute the d_{sel}/d_{neu} ratio, we used custom scripts written in java v.1.8 (<u>https://github.com/HancockLab/CVI</u>) and defined it as the rate ratio of 0-fold non-synonymous to 4-fold synonymous substitutions, scaled by the number of sites at risk for each category as in the following equation.

$$\frac{dsel}{dneu} = \frac{\frac{number of 0 - fold substitutions}{number of sites at risk for 0 - fold substitutions}}{\frac{number of sites at risk for 0 - fold substitutions}{number of 4 - fold substitutions}}$$
(1)

236

237 For this, we first constructed an artificial variant call format (VCF) file with all possible 238 variants at all sites in the genome and annotated them with $SnpEff v. 3.0.7^{28}$. Then we used a 239 custom script for the calculation of JSFS (https://github.com/HancockLab/CVI) to compute the 240 number of zero- and four-fold degenerate substitutions, as proxies for selected and neutral sites, 241 respectively. Note that this is a simplified approach to estimating dN/dS, which excludes 2- and 242 3-fold degenerate sites. We used this approach because estimating the expected changes at these 243 classes of sites is problematic due to asymmetries in substitution rates. We scaled these to the 244 number of sites in the genome at risk for each substitution type and deducted the positions with 245 more than 5% missing data. For continental clades, the spectra were polarized to A. lvrata 246 samples. Due to the long divergence time and genomic rearrangements between species, the 247 alignment of A. lyrata to A. thaliana reduced the number of bases for the analyses to 70676280. 248 For the CVI populations, we defined substitutions as variants derived in comparison to Morocco, 249 fixed in one island and absent from the other. To estimate uncertainty, we bootstrapped frequency spectra 500 times in polyDfe v.2.0²⁹ and calculated empirical *p*-values based on the 250 251 bootstrapped data. The large variance in the bootstrapped data stems from the low number of

total variants fixed in the two island populations. If the number of four-fold substitutions was
zero (in real or bootstrapped data), we conservatively added one to avoid dividing by zero. For
the Moroccan clade, we used *Arabidopsis lyrata* samples as an outgroup. We used the spectra at
zero- and four-fold degenerate sites to infer the distribution of fitness effects (DFE) and the
proportion of adaptive substitutions (alpha) with polyDfe v.2.0²⁹ using default parameters <-m C
o bfgs>. We ran the analysis independently for the two CVI islands (11 samples in Fogo and 13
in Santo Antão), and the four Moroccan clusters.

259

Supplementary Method 11. Evidence for ongoing multi-variate adaptation in Santo Antão

Since its collection 37 years ago³⁰, a single plant from CVI (Cvi-0) was studied extensively. 262 263 Many mapping studies have used recombinant inbred line (RIL) populations (Cvi-0 x Ler-0 and 264 Cvi-0 x Col-0)^{31,32}, and near inbred introgression lines (NILs) of Cvi-0 into the Ler-0 genome³³. 265 The island of origin of Cvi-0 was previously unknown, but we found it clusters tightly with the 266 Espongeiro population in Santo Antão, indicating that it was collected in this region 267 (Supplementary Fig. 1). We conducted a literature review of studies that used the Cvi-0 x Ler-0 268 RILs. We identified 47 QTL-mapping studies (Fig. 6a) that mapped 129 traits that we grouped 269 into 23 major trait-categories. These studies localized 717 QTL intervals. Based on these studies and follow-up fine mapping, we compiled a set of 135 candidate genes^{4914,31-62,62-102}. In eleven 270 271 cases, the actual mutation responsible for an effect on phenotype was found and validated (by

272 complementation tests, transgenics, sequence analyses). These variants include two large 273 deletions, three small indels (frameshifts) and six SNPs (non-synonymous amino acid changes 274 and truncating variants). To genotype large deletions in the natural population, we computed 275 average coverage across 100 bp windows overlapping the deletions and flanking regions. The 276 phenotypes affected by the functional variants range from flowering time and light signalling (FRI K232X⁸⁶, CRY2 V367M⁸⁴, GI L718F^{48,88}), circadian clock regulation (ZTL P35T⁸⁸), 277 278 stomatal aperture (MPK12 G53 R^{92}), freezing tolerance (*CBF2* promoter deletion¹⁰³), pathogen resistance (cPGK2 S78G¹⁰⁴ and *RPM1* whole gene deletion⁷⁸), chloroplast morphology (FtsZ2-2 279 G441fs⁸⁷), fructose signalling (ANAC089 S224fs⁵⁹), and innate immunity (FLS2 N452fs⁸¹). In 280 281 one case, a functional variant responsible for copper detoxification was identified (HMA5 282 N923T⁵⁶), but it likely arose in Cvi-0 in the laboratory, since it is completely absent from the 283 sampled natural population.

284

To assess the effects of the seven functional variants segregating in Santo Antão on fitness, we used forward-backward stepwise regression (i.e., sequential replacement) approach in a linear model framework using the R package caret v.6.0-86¹⁰⁵. For the forward case, we started with a model with no predictors (only an intercept), iteratively added functional variant predictors, and stopped when the improvement was no longer statistically significant based on the change in root mean squared error (RMSE). For the backward case, modelling started with the full model (intercept plus all functional variants), iteratively removed the predictors that contributed the

least, and stopped when all predictors were statistically significant. Significance of models wasassessed based on the root mean squared error (RMSE), by bootstrap resampling (1000 times).

294

295 To test whether the explanatory power of the seven functional variants was higher than 296 randomly selected genomic variants, we resampled 2000 sets of seven randomly chosen variants from an LD-pruned genome (PLINK¹⁵ command: <--indep-pairwise 50 10 0.1>) and conducted 297 298 stepwise regression on each of these sets, exactly as we had done on the seven functional variants. We calculated the model R^2 to produce a null distribution and obtained an empirical p-299 value by comparing the observed R² value to this using the formula: $(1 + sum(s \ge s_0)) / (N + 1)$, 300 where s is the R^2 value per draw, s₀ the observed R^2 value, and N the number of draws 301 302 (https://github.com/HancockLab).

303

304 Supplementary Method 12. Trait mapping

To assess flowering time segregation in Cape Verde, we generated three inter-island F2 populations (S5-10 x F13-8 (n=488), S15-3 x F3-2 (n=636), and Cvi-0 x F9-2 (n=598)). These were grown in Bronson climatic growth chambers, with settings to match CVI conditions: 20°C during the day and 14°C at night, with a 12h photoperiod and 70% humidity. We scored bolting and flowering time in all F2 individuals and 12 replicates per parental line, except for Cvi-0 and F9-2, for which only four replicates were grown.

312	To determine whether there was transgressive segregation in each of these populations
313	against their corresponding parental lines, we used the DunnettTest() function implemented in
314	the R package <i>DescTools</i> v.0.99.37 ¹⁰⁶ . We used Fisher's method ¹⁰⁷ to calculate a combined p -
315	value across the set of crosses, using the function <i>fisher.method()</i> implemented in the R package
316	metaseqR v.1.26.0 ¹⁰⁸ (https://github.com/HancockLab/CVI).

318 Bulked segregant analysis was done in an inter-island F2 population (S5-10 x F13-8, n=488), 319 in which the ancestral allele FRI K232 was fixed), grown under simulated CVI conditions. 320 Because early flowering segregated at approximately a 1:3 ratio (indicating a single recessive 321 locus), we sampled leaf tissue from the 25% early tail of the F2 (n=108). We extracted DNA 322 using a DNeasy Plant Mini kit (Qiagen), assessed DNA quality and quantity with Qubit and 323 Nanodrop (Thermo Fisher Scientific), prepared a single library using NEBNext Ultra II FS DNA 324 Library Prep Kit (New England Biolabs) and sequenced it to 50x coverage using the Illumina 325 HiSeq3000 platform. We called variants against the TAIR10 reference assembly using a GATK 326 pipeline⁸ (https://github.com/HancockLab/CVI), retaining only biallelic variants. We identified 327 window(s) where the median allele frequency was greater than 95% and annotated variants 328 within candidate region(s) using SnpEff v. 3.0^{28} .

329

330 Supplementary Method 13. FLC RNA quantification

331	We measured FLC expression in a representative set of eight Cape Verdean, and six
332	Moroccan accessions. We also measured FLC expression in the Col-0 reference strain, as well as
333	a modified Col-0 with a functional FRI introgressed (Col-0 FRI-Sf2, shown as Col-0 FRI+FLC+),
334	since FRI affects FLC mRNA levels ^{109,110} , and Col-0 FRI-Sf2 with an FLC knock-out (Col-0
335	<i>FRI</i> -Sf2 <i>flc-3</i> , shown as Col-0 <i>FRI</i> ⁺ <i>FLC</i> ⁻) ¹¹¹ . We grew three replicates of each genotype under
336	CVI simulated conditions (12h light, 20°C day, 14°C night). We collected and immediately froze
337	2-3 rosette leaves each from 2-week-old plants and ground them with the TissueLyser II
338	(Qiagen). We extracted RNA with TRIzol (Invitrogen) and treated $2\mu g$ with the DNA-free DNA
339	Removal Kit (Invitrogen) for 1h at 37°C. We generated cDNA using the Superscript II reverse
340	transcriptase (Invitrogen) together with oligo(dT) primer for 2h at 42°C. We assessed mRNA
341	levels by qRT-PCR on a LightCycler 480 instrument (Roche) with the EvaGreen dye (Biotium)
342	using the $2^{-\Delta\Delta Ct}$ method (Applied Biosystems) and <i>PP2A</i> (AT1G13320) as a reference gene.
343	Primers used in this experiment are listed in Supplementary Table 9. Differences in FLC
344	expression between genotypes were tested with the Kruskal-Wallis method implemented in the R
345	package agricolae v.1.3-2 ¹¹² (<u>https://github.com/HancockLab/CVI</u>).

347 Supplementary Method 14. FLC complementation test

348 We performed genetic complementation tests for *FLC* by crossing four individuals from

349 Fogo (each with the FLC 3X allele) to Col-0 *FRI*-Sf2 plants with and without a functional *FLC*

- allele (Col-0 *FRI*-Sf2, referred to as Col-0 *FRI*⁺*FLC*⁺, and Col-0 *FRI*-Sf2 *flc-3*¹¹¹, referred to as
- 351 Col-0 FRI⁺FLC⁻, respectively). We also crossed the mutants (Col-0 background) to obtain a

heterozygous F1 at *FLC*. We grew four replicates of each parent and F1 per cross and scored
bolting and flowering time in 12h standard greenhouse conditions.

354

355 Supplementary Method 15. Historical reconstruction of evolution of FRI and

356 FLC loci and fit to models of adaptation

We used RELATE v1.1.4¹¹³ to infer the genealogical trees for the derived alleles FRI 232X 357 (Chr4:269719) and FLC 3X (Chr5:3179333). We used bcftools v1.9¹¹⁴ to filter the VCF file for 358 359 quality, removed non-biallelic SNPs, retained segregating sites, and filtered out missing data 360 with the following execution

bcftools view -m2 -M2 -v snps -min-ac=1 -i 'MIN(FMT/DP)>3 361 & MIN(FMT/GQ)>25 & F MISSING=0'>. For FLC 3X, because the derived allele is fixed in 362 Fogo, we included S1-1 from Lombo de Figueira, Santo Antão, as the outgroup. Within 363 RELATE, we used the command RelateFileFormats (using --mode ConvertFromVcf) to convert 364 the VCF file into haplotype and sample files. To infer the genome-wide genealogies, we first ran 365 the command Relate (using -mode All) per chromosome and defined parameters of the 366 recombination map (--map), mutation rate (--m), and the coalescence file (--coal) for N_e over time. The estimated mutation rate $(7x10^{-9})$ for A. thaliana¹¹⁵ was corrected for the percentage of 367 missing data for each region and -m set to 2.512x10⁻⁹ for FRI 232X and 2.1x10⁻⁹ for FLC 3X. 368 We used a published recombination map¹¹⁶ corrected for the estimated outcrossing rate of 5% 369 370 estimated in natural populations¹¹⁷ by dividing the genetic distances by 20. We then used the 371 output to estimate coalescence rates using the script EstimatePopulationSize.sh across all 5

372 chromosomes with generation time set to one year, running the algorithm for 10 iterations. To

373 obtain 95% confidence intervals for RELATE-inferred coalescence rates, we used genome-wide

374 genealogies and coalescence rates as inputs into the COLATE package¹¹⁸

375 (<u>https://github.com/leospeidel/Colate</u>), which uses a block bootstrap (100x) over genomic regions.

376

To infer the local genealogies for FRI 232X and FLC 3X, we ran RELATE and used the genome-wide coalescence rates (--coal) inferred previously. To produce genealogical trees for FRI 232X and FLC 3X variants with confidence intervals for the estimated ages based on 200 samples from the MCMC (derived using SampleBranchLengths.sh --format a, and using default settings), we used the script TreeViewSample.sh, with 10*N steps (N is the number of haplotypes) and 1000 burn-in iterations.

383

We used CLUES¹¹⁹ to infer the frequency trajectory and selection coefficient for the derived 384 385 FRI 232X (Chr4:269719) and FLC 3X (Chr5: 3179333) alleles. CLUES uses importance 386 sampling over trees generated in RELATE to produce a posterior distribution of trees from 387 which a frequency trajectory can be inferred. From the output from RELATE, we used the 388 command <./SampleBranchLengths.sh --format b> to obtain 200 samples from the MCMC. For 389 FRI 232X, we integrated a pseudo-ancestor individual (from our inferred CVI ancestral states) as 390 an outgroup for the Santo Antão population. Then, we inferred genome-wide and local 391 genealogies and conducted importance sampling in RELATE after adjusting mutation rate (-m) to 3.24×10^{-10} and 4.193×10^{-10} based on the proportion of missing data removed for the *FRI* and 392

393	FLC regions, respectively. We obtained estimates of the posterior distributions of allele
394	frequencies over time using a recessive model (dom 0): <inference.pypopfreq 0.7513<="" td=""></inference.pypopfreq>
395	tCutoff 5000coal relate.popsize.coalsMax 1df 100dom 0 > for FRI 232X, and
396	<inference.pypopfreq 0.99tcutoff="" 0<="" 100dom="" 1df="" 7000coal="" relate.popsize.coalsmax="" td=""></inference.pypopfreq>
397	> for FLC 3X (<u>https://github.com/HancockLab/CVI</u>). As in other analyses, we assumed one
398	generation per year. We inferred selection coefficients jointly across two-time bins (epochs) for
399	FRI 232X (0-2 and 2-4 kya) and three-time bins for FLC 3X (0-2, 2-4 and 4-6 kya) between the
400	present day and the time in the past when the variants arose.

We calculated the fit to strong selection weak mutation (SSWM) and weak selection strong
mutation (WSSM) models of evolution^{120–122} using an estimate of the genome-wide mutational
target size based on molecular studies^{109,123–126} and inferences from our population genetic
analyses. The logic and details can be found in the Supplementary Notes.

406

We conducted forward simulations in SLiM²⁵ under a Wright-Fisher model based on parameter estimates from the Fogo population to examine the probabilities of fixation of an adaptive variant (i.e., one that abolishes the vernalization requirement for flowering) taking into account the stochastic effects of drift. The (constant) population size was set to N=48 based on the estimate from RELATE and the selection coefficient was set to *s* = 0.09273 based on the estimate from CLUES under a model where the reconstructed N_e was used in RELATE/CLUES. The final number of generations depended on when the variant fixed with a maximum of 6000

414 generations. We simulated two genomic elements: one of size 1.5 Mbp where neutral mutations 415 could arise and another of 1 bp where the selected variant could arise. We used three different 416 plausible estimates for the degree of selfing (90%, 95% and 99%) based on estimates from 417 natural population¹¹⁷ and conducted 200 simulations for each case. From these, we calculated the 418 proportion of runs where populations adapted, the proportions of potentially adaptive variants 419 that are lost or fixed in all runs, and the times to fixation or loss.

420

421 Supplementary Note 1. Population history reconstruction

We used Chromopainter¹²⁷ to identify the closest ancestor to CVI across the genome by 422 423 matching haplotypes to sequenced African and European individuals^{4,128}. We found that the 424 Moroccan High Atlas population was the closest relative for the majority of the genome (approx. 425 61%), followed by the North Middle Atlas population (approx. 7%) and then other Moroccan 426 and European populations (Supplementary Fig. 4). Some of the variance visible in 427 Supplementary Fig. 4 matching across populations may be due to the lack of a strong (close) 428 match, so that multiple nearly equivalent distant matches can be often found. We next examined 429 two specific large-scale loci (the chloroplast and the S-locus), where interpretation of ancestral 430 sharing may be simpler due to the very low probability of recombination at these loci. At the 431 chloroplast, we found that Cvi-0 clusters most closely with individuals from the South Middle 432 Atlas population (Supplementary Fig. 4-5).

433

434	The S-locus is a well-characterized region responsible for self-incompatibility in A. thaliana.
435	In the species, three deeply diverged haplogroups segregate (A, B, C) as well as an ancient
436	recombinant (A/C) haplotype ^{$129-131$} . Due to the deep divergence between the major haplogroups,
437	recombination between these is suppressed and thus exceedingly rare in this region ¹³² so that
438	matching to major haplogroups is clear. We classified S-locus haplogroups in all CVI samples
439	following ⁴ and found that they all carried haplogroup B (Supplementary Fig. 4). In the
440	continental sample, this haplogroup is present only in three samples and only in the northernmost
441	Moroccan population in the Rif Mountains ^{4,133} .
442	
443	Taken together, these patterns show that while Morocco is the continental population
444	genetically closest to CVI, there is no single Moroccan sample or population that is consistently
445	closest to CVI across the genome. Instead, our findings suggest that CVI was colonized by a
446	'ghost' population that is not represented well by any modern-day sampled population.
447	
448	Previously, based on the timing of coalescence events, we inferred that Moroccan
449	populations expanded and contracted over time ⁴ . This could have led to loss and/or re-sorting of
450	lineages among populations and could help to explain why we were unable to identify a single
451	best representative of the colonizing population ⁴ .
452	

453 Next, we narrowed down the CVI colonization time by obtaining an upper bound based on
454 the minimum coalescence time between CVI and Morocco and a lower bound based on the
455 maximum coalescence time within the CVI clade.

456

457 To estimate the split time between Moroccan and CVI populations, and therefore the upper 458 bound of colonization time, we calculated the relative ratio of between-group coalescence events 459 to within-group coalescences (i.e., the cross-coalescence rate (CCR) statistic) implemented in MSMC^{16,134}. Given that we are unable to identify the closest Moroccan population, we expected 460 461 this analysis to overestimate the divergence time from the actual continental ancestor. The CVI 462 population exhibited initial divergence from all present-day Moroccan populations at 463 approximately 40-60 kya (Supplementary Fig. 7), which we interpret to represent the split time 464 between the present-day Moroccan population and the 'ghost' ancestor of the CVI populations.

465

466 CCR between CVI and High Atlas shows a somewhat different pattern compared to other 467 Moroccan populations. The trajectory of the statistic does not monotonically decay as would be 468 expected under a simple split model but rather inflects and reaches a local maximum between 10 469 and 20 kya (Supplementary Fig. 7). As a result, the 0.25 - 0.75 CCR quantiles for the CVI-High 470 Atlas split are consistent with a wide range of split times from 60 kya until as recently as 10 kya. 471 This inflection could potentially be explained by a complex relationship between the ancestors of 472 the Moroccan and CVI lineages, i.e., secondary contact between the ancestors of the 'ghost' and

the High Atlas populations. This resulted in the presence of some haplotypes across the HighAtlas genomes that represent the population that originally colonized CVI.

475

476 Although we were unable to identify a close outgroup population to CVI, we can use 477 information about the coalescences within CVI to obtain a lower bound on the colonization time. 478 We examined historical coalescence events in Santo Antão, Fogo and between the two islands 479 based on haplotype coalescences. Coalescence rates spike around 10 kya (Supplementary Fig. 7) 480 and decline sharply in Santo Antão starting at approximately 7 kya, when we infer population 481 structure begins to develop within this island (Supplementary Fig. 8d). In agreement with this 482 time estimate, and based on the density of mutations, 95% of genomic windows between islands 483 (0.1 Mb, non-overlapping) coalesced by 7.1 kya (Supplementary Fig. 8b). Then, at 484 approximately 4-5 kya, there is a strong signature of reduced coalescence between islands, 485 consistent with a split at this time (Supplementary Fig. 8d). 486 487 The long gap between the coalescence of lineages within CVI (5-7 kya) and the coalescence 488 times between present-day Moroccan and CVI populations (40-60 kya) is consistent with a 489 model in which a now extinct or unsampled 'ghost' population was the actual founding 490 population of CVI. Based on our analyses, we hypothesize that this population split from the 491 present-day Moroccan population approximately 35-50 kya (Supplementary Fig. 7) and that 492 island colonization likely occurred approximately 7 kya, when population structure becomes 493 apparent in CVI (Supplementary Fig. 7).

495	To further explore the colonization dynamics and to assess evidence for colonization by a
496	'ghost' population in different time frames, we compared the distribution of ages of genomic
497	windows (haplotypes) to those from simulations of a 'ghost'-CVI split at different time points (5,
498	10, 20, 30, 40 and 50 kya). These simulations were conducted under the assumption that variable
499	mutation rate and purifying selection reduce diversity by the same extent as divergence (the same
500	rationale as in the HKA test) and allow for testing for secondary contact between the ancestors of
501	the Moroccan and 'ghost' populations. In that way, we were able to capture the genomic
502	variance in the combined $N_e\mu$ parameter by scaling the simulations to diversity in Morocco
503	across genomic regions. This approach was based on the logic that the inflection in the MSMC-
504	CCR plot (Supplementary Fig. 7) could be due to secondary contact between the 'ghost'
505	population and the Moroccan population (i.e., gene flow back into the Moroccan population from
506	the 'ghost'). In this case, we could use the distribution of window ages (inferred based on the
507	density of SNP variants) to estimate the timing of the secondary contact event. This timing
508	inference would better represent the split between CVI and the 'ghost', although it is still likely
509	to be an overestimate. We found that the cumulative tail of recent coalescence times in observed
510	data fits best with a 10 ky old split and upper bound of colonization time (Supplementary Fig. 7).

511

512 The site frequency spectrum provides complementary (largely independent) information 513 from signatures of haplotype coalescences. We ran dadi to infer the split time between Morocco 514 and the ancestor of the CVI population. We used five demographic models including 1) a simple

515 split model, 2) a model that included an exponential population size change in CVI after the split. 516 3) an isolation-with-migration model, 4) a model that included a bottleneck in CVI after the split, 517 followed by an instantaneous size change, and 5) a model that included bottlenecks in both the 518 CVI and Moroccan populations after the split and a subsequent size change in CVI. Details of the model parameters are shown in Supplementary Fig. 7. The best performing model (based on 519 520 AIC) is the two-bottleneck model. This model includes a bottleneck in CVI after the split, 521 followed by an instantaneous population increase (Supplementary Fig. 7). Similar to the 522 haplotype coalescence analysis, the parameter estimates under this JSFS-based model capture the 523 signal of an early split between the ancestor of the CVI colonist ('ghost' population) and the 524 ancestor of the current Moroccan population, placing this split time at 49.7 kya, with a 43.5 kya 525 bottleneck. This scenario fits well with the inferences from haplotype coalescence times 526 (Supplementary Fig. 7) where the long-term effective population size of the 'ghost' population 527 was small in the interval between the split of the parental populations and expansion within CVI. 528 In the JSFS-based model, the time when the CVI population is inferred to increase in size (6 kya) 529 is consistent with an expansion beginning sometime after the colonization of the islands 530 (Supplementary Fig. 7d-e). Neither the haplotype coalescence approach nor the JSFS approach 531 between Morocco and CVI can reveal specific information about the propagule size to CVI due 532 to confounding with the 'ghost' population. However, the very low number of shared variants 533 between present-day Moroccan populations and CVI (0.1%) suggests that the colonizing 534 population was depleted of variation and that current trait variation in the islands occurred via 535 new variants.

537 Next, we wanted to determine the order of island colonization. Isolation by distance leads to 538 a reduction in variation with distance from the starting population and to a pattern in which a 539 proportion of derived variants that segregate in one (parent) population will be fixed in the child population^{135,136}. Therefore, we focused on the subset of variants that are segregating in one 540 541 island and fixed derived in the other to infer colonization order. First, we examined our power 542 for this approach using simulations. Here, we found that as long as the time between the 543 colonization of the first and second island, or the size of the island colonized first is large enough 544 (larger than approximately 500 individuals at the split), the island colonized first will have a 545 lower proportion of fixed mutations that segregate in the other island, independent of the number 546 of colonizers. In the observed data, the Fogo population has a higher proportion of fixed 547 mutations that segregate in Santo Antão compared to the converse (a positive statistic in 548 Supplementary Fig. 8c), supporting initial colonization of Santo Antão, followed by Fogo (from 549 Santo Antão). When we fit the statistic estimated from data to forward simulations, we inferred 550 that population size in Santo Antão grew quite slowly from colonization until the split from 551 Fogo, with a final size of only about 1000 plants when Fogo was colonized.

552

553 Further, we used dadi¹⁷ to fit three-population models to the observed join site frequency 554 spectra. The models were simple 3-populations splits with constant population sizes, but they 555 differed in which island was colonized first. In the first model, Santo Antão was colonized from 556 Morocco, and later Fogo was colonized from Santo Antão. In the second model, Fogo was

colonized from Morocco, and later Santo Antão was colonized from Fogo. The model that best
fit observed data (lowest AIC, 612 vs 628; highest likelihood, -301.3 vs -309.1) was the one in
which Santo Antão was colonized first from Morocco, and Fogo was colonized later from Santo
Antão.

561

562 Next we inferred the split time between islands and other aspects of historical population 563 dynamics using the JSFS (dadi¹⁷). We estimated parameters under a range of models (simple 564 split, exponential, isolation with migration, bottleneck) and compared model AICs to identify the 565 best fitting model of the historical dynamics of the Fogo population. We found that the best 566 model was one in which the island colonization event was accompanied by a bottleneck lasting 567 930 years. The population size (N_e) during the bottleneck period was approximately 400 568 individuals (Supplementary Fig. 8a, Supplementary Table 3). We did not attempt to estimate the 569 number of founders separately from the bottleneck size because diffusion models generally do 570 not perform well, such as dadi, generally does not perform well to infer the N_e before a 571 bottleneck¹⁷.

572

573 The estimated split time is in rough agreement with a simpler estimate based on the 574 distribution of pairwise differences across windows within and between islands (Supplementary 575 Fig. 8b). We calculated the mean pairwise differences among CVI individuals across 100 kb 576 windows of the genome and found that coalescence time between islands was centred around 4.5 577 kya (mean: 4.6 kya median: 4.5 kya) and that 95% of the windows coalesced by 7.1 kya. The

complete distribution of pairwise differences is shown in Supplementary Fig. 8b. Variation
within Santo Antão was centred around 3.0 kya (mean: 3.0 kya; median: 3.0 kya) with 95% of
windows coalescing by 4.8 kya and in Fogo around 2.4 kya (mean: 2.5; median: 2.4) with 95%
of windows coalescing by 3.9 kya.

582

The bottleneck duration (T_s) agrees with a simple calculation based on the proportion of fixed variants in Fogo. The Fogo population carries 135 fixed derived mutations out of a total of 23 Mbp of sequence. Using $T_s =$ number of fixed mutations/(μ *L), we can estimate about 840 generations, in which Fogo remained a single connected population (about 840 years) after colonization before structure built up preventing mutations from fixing in the island. The same calculation based on intergenic sites only, results in a very similar bottleneck duration (36 fixed derived intergenic mutations out of 4.8 Mbp: about 1070 years).

590

The complete lack of population structure for approximately 870-1070 years for the Fogo population is striking. Seed-dispersed plant populations tend to be highly structured¹³⁷ including *A. thaliana* populations^{138–141}. However, we find no evidence of structure accumulating in the long post-colonization bottleneck period in Fogo. This implies that the nascent population was poorly adapted to its new environment and therefore severely limited in size during this waiting period until one or more necessary mutations arose that increased fitness and allowed the population to expand.

599 Secondary migration events after initial colonization are unlikely. Allowing for migration 600 (gene flow) between the Moroccan and 'ghost' populations after the split led to a poor model fit 601 (based on AIC; Supplementary Fig. 7), implying a lack of migration after the split. This is not 602 surprising given that multiple independent migration events into CVI would likely lead to much 603 higher genetic variation than we observe in the archipelago. The average pairwise differences 604 between two Moroccan individuals is 82.4-fold higher relative to the average pairwise differences within CVI (θ_{π} (Morocco) = 5.38x10⁻³; θ_{π} (CVI) = 6.53x10⁻⁵). Even within a single 605 606 Moroccan region, the average pairwise differences is 54.2-fold higher than in CVI (on average θ_{π} (Moroccan regions) = 3.54×10^{-3} ; Supplementary Table 1). Further, we observed extremely low 607 608 sharing of variation between CVI and Morocco (0.1%). Based on this, it is difficult to imagine a 609 scenario in which multiple independent dispersal events could have contributed to the present 610 CVI populations.

611

Similarly, given the almost complete lack of shared variants genome-wide between Santo Antão and Fogo (0.6%), it is highly unlikely there was any subsequent migration after the initial colonization of Fogo. In further support of this assertion, a single chloroplast haplotype is fixed in CVI, with only 18 variants segregating there. Similar to clustering from genomic variation, a chloroplast network clearly separates the two islands (Supplementary Fig. 6). Consistent with the lack of secondary migration between islands, demographic inference with dadi finds the best support for a model with no migration (Supplementary Fig. 8c-d). Further, given the inferred
small initial population size in Fogo, a secondary migration event from Santo Antão would likely
result in much higher shared variation between islands than observed.

621

622 Supplementary Note 2. Population history within islands

623 Trajectories of coalescence rates within Santo Antão and Fogo (MSMC) as well as matching 624 to patterns of polymorphism in simulations (Supplementary Fig. 8d) imply that Santo Antão was 625 colonized first and Fogo second from Santo Antão. Trajectories of coalescence rates over time 626 for individual Santo Antão populations and inferred split times with MSMC-CCR show that the 627 Cova de Paúl population best represents the early colonists (Supplementary Fig. 8d). Based on 628 CCR analysis, we estimated that Cova de Paúl split from Lombo de Figueira at approximately 7 629 kya. At this time, the coalescence (MSMC) trajectory for Santo Antão enters a period of intense 630 reduction (Supplementary Fig. 7b), which likely corresponds to the formation of population 631 structure as *Arabidopsis* expanded its range. The most recent population splits are between 632 Espongeiro and Pico da Cruz. In Fogo, the more arid island, we found evidence for a bottleneck 633 that lasted approximately 930 years after colonization (Supplementary Fig. 8a, Supplementary 634 Table 3). Once the population did begin to expand in Fogo, it dispersed to three regions (Monte 635 Velha, Inferno and Lava) and structure developed among these.

637 Supplementary Note 3. Niche modelling to predict suitability of Cape Verde 638 habitat based on Moroccan distribution

639 The variables in the final Maxent model were the length of the growing season,

640 isothermality, maximum temperature of the warmest month, minimum temperature of the coldest 641 month, temperature annual range, mean temperature of the wettest quarter and precipitation 642 seasonality. When we projected the Moroccan niche model onto the Cape Verde archipelago, we 643 found that there was no habitat predicted to be suitable for colonization from Morocco. As is 644 generally the case in niche modelling, correlations between environmental variables across the 645 range result in alternative possible models. The model we present uses a set of variables chosen 646 based on ecological and biological relevance. However, predicted suitability of CVI habitat for 647 Moroccan accessions did not change across different variable selection regimes: none predicted 648 suitable habitat for Moroccan A, thaliana establishment in CVI. This is likely due to the fact that 649 the climate variable values for CVI lie outside or nearly outside the distribution of most 650 Moroccan climatic variables. As a result, the predicted (dis-)similarity may be more informative, 651 which shows that much of Santo Antão and a band around the highest altitude Bordeira region of 652 Fogo has the highest predicted similarity. These most similar regions encompass locations where 653 we found populations of Arabidopsis in CVI.

654

655 Supplementary Note 4. Evidence of positive selection in CVI

656 We computed the relative rate of fixation of 0-fold non-synonymous to 4-fold synonymous 657 variants (d_{sel}/d_{neu}) for the Moroccan and CVI lineages. In the absence of selection $d_{sel}/d_{neu}=1$, as 658 non-synonymous and synonymous mutation have the same probability to arise and fix. Purifying 659 selection reduces the probability of fixation of non-synonymous mutations resulting in $0 \le 1$ $d_{sel}/d_{neu} < 1$. A relaxation of purifying selection will move the d_{sel}/d_{neu} ratio from $0 \le d_{sel}/d_{neu} < 1$ 660 661 towards neutrality, $d_{sel}/d_{neu}=1$. Positive selection is in principle the only force that can result in 662 higher substitution rates of functional compared to neutral mutations, resulting in $d_{sel}/d_{neu} > 1$. 663 However, multiple forces are likely to be acting at any one time across loci in the genome, so 664 that the observed d_{sel}/d_{neu} is expected to represent a composite of the neutral evolution, purifying 665 selection and adaptive evolution. Therefore, $d_{sel}/d_{neu} > 1$ implies that there are many 666 advantageous mutations fixed on the branch leading to the clade.

667

668 Consistent with the large-scale effect of purifying selection in continental A. thaliana, the 669 dsel/dneu ratio in Morocco, polarized to A. lyrata, is 0.18 (Fig. 5a). For Santo Antão and Fogo, we 670 analysed the long branch of divergence from the continents, on which mutations are found fixed 671 derived in CVI as a whole, as well as the two short branches separating the two islands, where 672 mutations are fixed derived in one or the other island. The long branch of divergence mainly 673 represents the continental history in the 'ghost' population but it is also confounded with the 674 early history in CVI after colonization. In this case, we obtained a d_{sel}/d_{neu} ratio of 0.276, slightly 675 higher than the Moroccan population. Then we examined the short branches separating the two

676 islands, which correspond to the past 4-10 ky of evolution in isolation within Santo Antão and Fogo. In this case the genome-wide d_{sel}/d_{neu} ratios (Santo Antão: $d_{sel}/d_{neu}=2.2$, Fogo: 677 678 $d_{sel}/d_{neu}=1.7$) are much higher than for the Moroccan population and also higher than unity, 679 consistent with strong positive selection acting across the genomes of the island populations. Due 680 to the very shallow history within each island, few mutations had the time to arise and fix in each 681 functional category; as a consequence, confidence intervals around these estimates are 682 necessarily high, but nonetheless consistent with exceptionally high d_{sel}/d_{neu} values in CVI. To further investigate these patterns, we used the software polyDfe²⁹ to estimate the statistic alpha, 683 684 the proportion of non-synonymous substitutions driven by positive selection or linked to 685 positively selected alleles. Consistent with the high d_{sel}/d_{neu} ratio, we estimate that in Santo 686 Antão 70.7% and in Fogo 62.8% of non-synonymous substitutions were driven to fixation by 687 positive selection or by linkage to positively selected alleles.

688

689 Additionally, we estimated the distribution of fitness effects (DFE) of variants segregating in the CVI and Moroccan populations with polyDfe²⁹. The DFE in the two Cape Verde islands are 690 691 enriched both in neutral and slightly deleterious variants (-1, 0 category), as well as in variants of 692 large positive effects on fitness (all positive categories) compared to Moroccan populations (Fig. 693 5b). This is consistent with a reduction in efficiency of purifying selection relative to the 694 continent combined with strong positive selection in response to the novel CVI environment. The 695 two islands differ somewhat in the inferred parameters of positive selection. In Santo Antão, the 696 percentage of variants estimated to have a positive effect on fitness (p_b) was 2.9%, with an

697 average effect of $S_b = 54.7$. In Fogo, the percentage of beneficial variants was greater, $p_b = 698$ 19.7%, but with a smaller average inferred effect on fitness, $S_b = 6.8$.

699

700 Supplementary Note 5. Parallel adaptation by reduced time to flowering

701 We mapped flowering time in Santo Antão using GWAS, with a linear mixed model (LMM) 702 that controls for population stratification¹⁴². We used the median per genotype across replicates 703 as phenotype, since no block effect was detected in the simulated CVI conditions experiment 704 (Supplementary Fig. 9). All typed variants in the genome explained 99.997% of the observed 705 phenotypic variance (PVE; also known as 'chip heritability' or 'SNP heritability') and we 706 identified one clear genome-wide significant peak on top of chromosome 4. This peak included FRIGIDA (*FRI*, AT4G00650; likelihood ratio test, *p*-value = 5.468×10^{-35}), a major flowering 707 708 time determining gene. The nonsense mutation FRI K232X in Cvi-0 truncates the protein and 709 was previously shown to strongly reduce flowering time⁸⁶. Adding FRI K232X to our model as a 710 covariate allowed us to quantify the association with the phenotype. We found that when FRI 711 K232X was added to the model, the percentage of phenotypic variance explained by all 712 remaining markers decreased to 53.59%, with an estimated effect size for the covariate of -35.27 713 \pm 1.50 days. This means that this single SNP is able to reduce flowering time in this population 714 in about 35 days and explain 46.41% of the phenotypic variance. Concordant with this estimate, 715 in the natural population, FRI 232X is associated with a decrease in flowering time of 34 days (MWW test, W = 7, *p*-value < 2.2x10⁻¹⁶), and a 140-fold increase in seed number (+387 seeds: 716
717 MWW test, W = 4541, *p*-value = 7.179x10⁻¹⁴; Fig. 6e). In the Col-0 background, under CVI

conditions, a non-functional *FRI* allele was responsible for a decrease in flowering time of 27

719 days (MWW test, W = 0, *p*-value = 0.00384) and an increase in fitness of 669 seeds (MWW test,

720 W = 37.5, *p*-value = 0.008856; Fig. 6e).

721

722 In Fogo, we did not find any statistically significant association in GWAS for flowering time 723 (Supplementary Fig. 11), suggesting that the genetic variant(s) underlying the uniformly reduced 724 flowering time were fixed or at high frequency (mean=28.72 days, SD=3.76). Taking this into 725 consideration, we scored flowering time in an inter-island F2 population in which the ancestral 726 allele FRI K232 was fixed. We observed early flowering individuals segregating at 727 approximately 1:3 ratio, indicating that a single recessive locus is causing the early flowering 728 phenotype. After bulking and sequencing the early tail of this distribution (n=108), we identified 729 a single region on chromosome 5 between 2 Mbp and 3.3 Mbp where the frequency of the Fogo 730 alleles was greater than 95% in the sequenced pool. Among the 33 variants in this region 731 (Supplementary Data 6), 14 are at a frequency greater than 90% in the natural population and 732 therefore they are stronger candidates to explain the uniform early flowering observed in Fogo. Of these 14, two are predicted by SnpEff²⁸ to have moderate impact, and one to have high 733 734 impact.

736	The two moderate impact variants affect AT5G09930, an ABC transporter protein, causing a
737	missense mutation (V193F), and AT5G07520, a glycine-rich protein expressed only in flowers
738	during a specific developmental stage (flower stage 12), with an 11 amino acid deletion
739	(A202_A213del). The high impact variant is predicted as causing a premature stop codon in
740	AT5G10140 (R3X). AT5G10140 is FLOWERING LOCUS C (FLC), a MADS-box protein
741	central to the flowering time pathway. FLC is regulated by FRI and vernalization, contributes to
742	temperature compensation of the circadian clock, and acts as a repressor of floral transition ^{143,144} .
743	Due to its central function in flowering time, FLC is the best candidate for the early flowering
744	observed in Fogo.

Although the flowering time gene *FLC* contains a premature truncation variant at the third amino acid fixed in Fogo, the gene could be functional, e.g., due to an alternative start codon or transcriptional read-through. To functionally characterize the nonsense mutation in *FLC* in Fogo, we quantified *FLC* mRNA levels in the natural population, and compared them to *FLC* mRNA levels in Santo Antão, Morocco, the Col-0 reference strain, and Col-0 *FRI*-Sf2 (functional *FRI* in Col-0 background) with and without a functional FLC¹¹¹ (noted as *FRI*+*FLC*+ and *FRI*+*FLC*, respectively; Supplementary Fig. 12a).

753

754 Compared to FRI^+FLC^+ , transcript levels of FLC were reduced in Fogo individuals 755 (Kruskal-Wallis, *p*-value < 1x10⁻⁴), as expected if FLC R3X results in non-functional FLC.

756	Similarly, <i>FLC</i> transcription was reduced in <i>FRI</i> ⁺ <i>FLC</i> ⁻ and Col-0 wild-type (non-functional FRI
757	and functional FLC) compared to FRI^+FLC^+ (Kruskal-Wallis, <i>p</i> -value < 1x10 ⁻⁴), which was
758	consistent with previous findings ¹⁰⁹ . In contrast, all accessions from Santo Antão showed high
759	levels of <i>FLC</i> expression (comparable to FRI^+FLC^+ ; Kruskal-Wallis, <i>p</i> -value > 0.6783). This
760	result is in agreement with previous work showing a higher baseline expression of FLC in Cvi-
761	$0^{86,109}$. Within Santo Antão, the single sample tested with a functional FRI (S5-10) had higher
762	FLC expression compared to the other samples from Santo Antão (Kruskal-Wallis, p-values:
763	Cvi-0=0.0006; S1-1=0.0039; S15-3=0.0008), consistent with an effect of <i>FRI</i> on <i>FLC</i> expression
764	even in the CVI genetic background ^{86,109} . In Moroccan accessions, we observed high levels of
765	FLC expression across populations, with much larger variation.

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766

767 The two loci, *FRI* and *FLC*, explain the differences in bolting time in the tested accessions 768 (Supplementary Figure 12b). In the Col-0 background, when both loci have functional alleles 769 (*FRI*⁺*FLC*⁺), the plants bolted later than when either of the loci had one non-functional allele 770 (non-functional FRI in Col-0 and non-functional FLC in FRI+FLC; Kruskal-Wallis, p-value = 771 0.0047 and *p*-value = 0.0002, respectively). In Santo Antão, all individuals bolted early, 772 comparable to Col-0 (Kruskal-Wallis, for S1-1, S15-3, Cvi-0: *p*-value = 1) and *FRI*⁺*FLC*⁻ 773 (Kruskal-Wallis, for S15-3, Cvi-0: *p*-value = 1, for S1-1: *p*-value = 0.2376), as all accessions 774 carry a non-functional FRI. The exception was S5-10, which bolted later (Kruskal-Wallis, p-775 values: Cvi-0=0.0005; S1-1=0.5811; S15-3=0.0026) due to a functional FRI allele. This 776 accession also showed higher levels of *FLC* expression, consistent with a role of regulation of

777	FLC mRNA levels by FRI ^{86,109} . In Fogo, the non-functional FLC present in all accessions is
778	consistent with the early bolting and the low levels of FLC mRNA, both comparable to the non-
779	functional FLC mutant in the Col-0 background (Kruskal-Wallis, for F10-1-3, F13-8, F9-2: p-
780	value = 1, for F3-2: <i>p</i> -value = 0.2014). In Morocco, the levels of <i>FLC</i> mRNA did not completely
781	explain the bolting time, suggesting that other loci may influence this trait in this population.
782	
783	To further test whether FLC is responsible for the early flowering phenotype in Fogo, we
784	conducted a genetic complementation test, by crossing 4 individuals from Fogo (all with a

potential non-functional FLC allele) to Col-0 FRI-Sf2 with and without a functional FLC (noted

as *FRI*⁺*FLC*⁺ and *FRI*⁺*FLC*^{- (111)}, respectively; Supplementary Fig. 12). 786

787

785

788	If the allele in Fogo was non-functional, when crossed to FRI+FLC, all F1 individuals would
789	flower as early as <i>FRI</i> ⁺ <i>FLC</i> ⁻ , as they would carry non-functional <i>FLC</i> alleles from both parents.
790	In this case, F1 individuals homozygous for the null-allele at FLC would also flower earlier than
791	heterozygous individuals (F1 from the cross between <i>FRI</i> ⁺ <i>FLC</i> ⁺ and <i>FRI</i> ⁺ <i>FLC</i> ⁻), given that the
792	functional FLC allele should be dominant ^{109,111} . On the other hand, if the FLC allele in Fogo was
793	functional, the F1 individuals from this cross would have one functional allele and flower later
794	than FRI+FLC, and similar to the heterozygous individuals (F1 from the cross between
795	FRI^+FLC^+ and FRI^+FLC^-). In the inverse cross – between Fogo and FRI^+FLC^+ – if the Fogo

allele is non-functional, we expect the F1 to be heterozygous and flower as late as FRI^+FLC^+ and similarly to the F1 resultant from the cross between FRI^+FLC^+ and FRI^+FLC^- .

798

799 For the crosses between Fogo individuals and FRI^+FLC^- , we found that all F1 individuals flowered as early as the parental line FRI^+FLC^- (MWW test, W = 42, *p*-value = 0.3611), much 800 801 earlier than the FRI^+FLC^+ (MWW test, W = 0, *p*-value = 0.00248) and the F1 heterozygous between FRI^+FLC^+ and FRI^+FLC^- (MWW test, W = 4, *p*-value = 0.0002342). On the other hand, 802 803 all the F1 individuals from the cross between Fogo and FRI^+FLC^+ flowered as late as the FLC 804 functional parental line FRI^+FLC^+ (MWW test, W = 34, *p*-value = 0.8814), much later than the Fogo parents (MWW test, W = 256, *p*-value = 1.301×10^{-6}) and also later than the F1 between 805 806 Fogo and FRI^+FLC^- (MWW test, W = 116.5, *p*-value = 0.001227). These results together suggest 807 that the FLC 3X allele found in the natural population in Fogo is indeed non-functional.

808

Next, we inferred the coalescent genealogies of FRI 232X and FLC 3X using RELATE¹¹³.
The coalescent tree for FRI 232X is bifurcated rather than hierarchical at the time when the
variant is estimated to have arisen, indicating that some branches were likely lost (extinct) or
unsampled in the modern populations. The time of the bifurcation is estimated at approximately
2.9 kya, with 95% CI estimated from 200 samples from the MCMC approximately 2.14 kya –
3.74 kya. The tMRCA, which represents the lower bound on the age, is 2.14 kya (95% CI: 1.622.72 kya). Based on the coalescent reconstruction for FLC 3X and 200 sampled trees, the allele

arose between the tMRCA at 3.3 kya (95% CI: 2.82-3.96 kya) and the split from the outgroup at
4.72 kya (95% CI: 3.56-6.66 kya).

818

819 We inferred the frequency trajectories for FRI 232X and FLC 3X using CLUES¹¹⁹, which 820 uses importance sampling over trees generated in RELATE to infer the frequency trajectories 821 and selection coefficients for the functional variants. For each variant, we defined time bins 822 (epochs) between the present and the emergence of the allele (based on the inferred age of the 823 allele in RELATE when approximately 97.5% of the coalescent trees for the region support the 824 existence of the allele). For FRI 232X, these epochs were 0-2 kya and 2-4 kya. For this variant, 825 we found that s was maximized in the epoch 2-4 kya years ago, with a selection coefficient of 826 4.56% (Supplementary Table 6). For FLC 3X, these epochs were 0-2 kya, 2-4 kya, and 4-6 kya, 827 with the inferred selection coefficient maximized 4-6 kya with s = 9.27% (Supplementary Table 828 7).

829

830 Supplementary Note 6. Assessing the fit of adaptation in CVI to models of 831 selection

Theory predicts that when mutational input is low and selection is strong (SSWM regime), the first steps of adaptation are likely to occur through large effect mutations, whereas when mutational input is high and selection is weak (WSSM regime), adaptation is likely to occur through more, smaller effect variants^{120–122,145–148}. We examine the CVI case in the context of SSWM versus WSSM regimes. First, we approximate the genome-wide mutation rate for the adaptive phenotype (very early flowering through loss of vernalization) and then we apply the inferences we made about population history and selection coefficients to examine the fit of adaptation in each of the two Cape Verde islands to these two models.

840

841 The SSWM model is expected to hold when the total number of new mutations that enter a 842 diploid population each generation is small such that $4NU_b \ll 1$ (where N is population size and 843 U_b is the genome-wide beneficial mutation rate for the focal trait) and when selection is strong 844 $4Ns \gg 1$. In this scenario, single new beneficial mutations arise and overcome genetic drift (s>> 845 1/4N) and subsequently rise in frequency without interference (by linkage or epistasis) from beneficial alleles at other loci^{120–122}. Note that small population size (as in a founder population) 846 847 plays a double role. On one hand, it enables SSWM type adaptation by restricting the mutation 848 input; on the other hand, it can inhibit adaptation altogether unless selection is strong. The 849 combination of small population size and strong selection is expected to result in a "selective-850 sweep type" architecture of adaptation¹⁴⁹ where one or few variants with large effects underlie 851 the adaptive phenotype. Alternatively, adaptation in the WSSM regime occurs through small 852 frequency shifts at a large number of alleles with small individual effect. Theory shows that 853 $4NU_b = 1$ is indeed the threshold that separates the sweep-like from highly polygenic 854 architectures^{149,150}.

856 Time to flowering has been studied extensively in A. thaliana and much is known about its 857 molecular basis. We used available molecular and functional analyses of major flowering time 858 loci to produce rough approximations of U_b . First, we reviewed the literature to identify the loci 859 and mutational effects that lead to effects on flowering time. Many genes can contribute to 860 variation in flowering time (at least 174 genes are thought to be involved in the flowering time pathway (https://www.mpipz.mpg.de/14637/Arabidopsis flowering genes¹⁵¹⁻¹⁵⁴). However, 861 862 very few of these cause large or even moderate changes in flowering time. A more relevant 863 (specific) phenotype in this case is the loss of the vernalization requirement (i.e., cold period 864 needed to induce flowering), which results in a large reduction in flowering time. The loci most 865 often implicated in this trait are FRI and FLC, both of which are essential for vernalization 866 response. Loss of function of either of these genes results in loss of the vernalization requirement 867 for flowering. When diverse rapid flowering accessions were examined, 85% turned out to have clear evidence of functional mutations in FRI, FLC or both^{109,152}. In the absence of a 868 869 vernalization treatment, complete loss of function of these genes results in a reduction of 870 flowering time of approximately 35 days relative to wild type (Fig. 7e). In nature, variation in 871 FRI occurs primarily by loss of function mutations in coding regions^{109,123}, while most putative functional variation in FLC is found in the first intron^{124,125}, which contains a well-characterized 872 regulatory element¹²⁶. 873

Based on this information about the genetic basis of large effect changes in flowering time (vernalization), we can roughly estimate U_b, the genome-wide per individual mutation rate of beneficial mutations that act through the focal phenotype (the number of nucleotides whose changes would cause a large shift in phenotype).

879

First, we focus on loss of function mutations due to SNPs in coding regions of the genes involved in complete loss of vernalization (*FRI* and *FLC*). For these, the total length of the two genes is 805 amino acids. We estimate that on average three out of 64 mutations could lead to a premature stop codon. This results in a mutational target size for loss of function through SNPs in coding regions of 805*3/64. We assume a mutation rate by SNP changes of $7.1x10^{-9}$ (¹¹⁵). The rate of introduction of loss of function mutations by premature stop codons per individual and per generation is therefore:

887
$$U_{b (coding_{SNPs})} = 805 \text{ x } 3/64 \text{ x } 7.1 \text{ x} 10^{-9} = 2.68 \text{ x } 10^{-7}$$

We can additionally account for mutation by indels, using the mutation rate estimated from mutation accumulation lines. We used the per base mutation rate estimated for 1-3 bp long deletions, 4.0x10^{-10 (115)}. The mutational target size is then 805 amino acids * 3 nucleotides/amino acid. The rate of introduction of indel mutations in the coding region is

therefore:

893
$$U_{b(coding_indels)} = 805 \text{ x } 3 \text{ x } 4x10^{-10} = 9.66 \text{ x } 10^{-7}$$

Combining the probability of mutation by SNPs or indels in coding regions gives:

895
$$U_{b(coding)} = 2.68 x 10^{-7} + 9.66 x 10^{-7} = 1.23 x 10^{-6}$$

896 Regulatory mutations in *FLC* and *FRI* also have the potential to impact flowering time. To 897 include the possibility of regulatory mutations in our estimate of U_b, we estimated the probability 898 that a mutation has a major regulatory effect based on the literature. Regulatory elements can 899 include promoter regions as well as conserved non-coding elements upstream, downstream or 900 within introns. For FRI and FLC these are well-studied. Core promoters are on average approximately 75 bp in A. thaliana and are tightly packed with regulatory elements¹²⁴. Given a 901 902 moderate level of nucleotide-level functional redundancy, we assumed that one in 10 possible 903 changes in the core promoter could have major functional effects. In addition, we assumed that 904 larger 5' regions with interspersed transcription factor binding sites would add 300 bp to the core¹⁵⁵. In these regions regulatory motifs are generally more dispersed and redundancy is likely 905 906 to be rather high at the nucleotide level. We assumed that on average the probability of a 907 mutation resulting in a strong functional effect in these regions would be one in 50. In FLC there 908 is evidence that motifs exist within a 'vernalization response element' in the first intron (289 bp) that are crucial to function¹²⁶. Here, we assumed that one in 20 mutations may result in large 909 910 changes in flowering time.

911

While we attempted to come to a meaningful approximation of the number of variants
(mutational target size) that might have *major* effects on regulatory function of *FLC* or *FRI*,

assumptions about this mutational target size are necessarily less certain. However, the final

915 estimate would not change much if other biologically informed estimates were used.

916
$$U_{b(regulatory \ FRI/FLC_SNPs)} = (150 \ x \ 0.1 + 600 \ x \ 0.02 + 289 \ x \ 0.05) \ x \ 7.1 x 10^{-9} = 2.94 x 10^{-7}$$

917 And including indels in regulatory regions gives us:

918
$$U_{b(regulatoryFRI/FLC_indels)} = (41.45) \times 4*10^{-10} = 1.66 \times 10^{-8}$$

919 So that the combined probability of a major effect mutation in regulatory regions by SNPs or 920 indels is:

921
$$U_{b(regulatory)} = 2.94 \times 10^{-7} + 1.66 \times 10^{-8} = 3.11 \times 10^{-7}$$

And the combined probability of any mutation with a major effect on the vernalizationrequirement is:

924
$$U_b = 1.23 \times 10^{-6} + 3.11 \times 10^{-7} = 1.54 \times 10^{-6}$$

Using the estimate for U_b and an assumption about past population size, we can infer the waiting time for a mutation in the natural population. Then, using our inference about selection coefficients from the allele frequency trajectory in each island, we can assess how each estimate fits with SSWM and WSSM models. We initially focus on Fogo because the population history there is simpler and resolved with higher certainty. Then we turn to Santo Antão, and make some approximations and an estimate for that population. For N, we used the estimate of N_e from RELATE/COLATE in the Fogo population (N_e =48 individuals at colonization). With that, the per generation population mutation rate for a strong effect functional variant in *FRI* or *FLC* would be

934
$$\Theta_{(FRI/FLC)} = 4N_eU_b = 4 \times 48 \times 1.54 \times 10^{-6} = 2.95 \times 10^{-4} << 1.0$$

935 There is of course uncertainty in this result from our rough estimate of both U_b and N_e . 936 However, as our derivation leaves considerable room for error of almost four orders of 937 magnitude, the conclusion that adaptation of our focal trait (loss of vernalization requirement) is 938 mutation limited ($\Theta_{(FRI/FLC)} \ll 1$) appears to be highly plausible. Our estimate corresponds to an 939 expected waiting time for the occurrence of any mutation that abolishes function of the genes of 940 $1/\Theta = 3775$ generations. If we break this down into coding and non-coding we find that the 941 estimated waiting time for a coding change specifically is $(1/4N_eU_{b(coding FLC/FRI)}) = 4727$ 942 generations and the waiting time for loss or major reduction of function through a regulatory 943 change specifically is estimated at $(1/4N_eU_{b(regulatory FLC/FRI)}) = 18,694$ generations. Based on this, 944 if such a variant was required to escape eventual extinction, extinction risk would be very high. 945 Further, given the much lower non-coding adaptive mutation rate, an adaptive mutation from 946 variation in the coding region would be much more likely in this case.

947

948 Under the SSWM model, strong selection is required in order to escape drift. Here, we apply 949 a selection coefficient based on the reconstructed frequency trajectory of FLC 3X in the time just 950 after it is estimated to have arisen (s = 0.0927). If FLC 3X resulted in a population size increase,

this estimate would be highly conservative because the population size change is used as the null model here. Recall that SSWM is expected to hold when $4NU_b \ll 1$ and $s \gg 1/4N$. Even with the conservative estimate of *s*, we find that this is well above $1/4N_e$: s = 0.0927 and $1/4N_e =$ 5.21×10^{-3} . This implies that a new mutation that obliterates the vernalization requirement in a Fogo-like environment would tend to escape drift. We conclude that the scenario is consistent with the SSWM regime, where adaptation relies on sweeps of large-effect alleles^{120,147,149,150}.

957

958 Of course, variance on the estimates of the time for a variant to arise and fix in the 959 population would be high due to the stochastic nature of mutation and the uncertainty of 960 establishment. We conducted simulations under a model designed to fit the Fogo population to 961 quantify the probability of fixation under a constant-size Wright-Fisher model with three 962 plausible estimates of the selfing coefficient (90%, 95% and 99%). The model ignores the 963 possibility of extinction, a risk that may be high under individual and/or temporal (e.g., due to 964 climate) variance in reproductive success. After 6000 generations, 13.5 - 24% % runs resulted in 965 fixation of the adaptive variant (a variant that eradicates the vernalization requirement) 966 (Supplementary Table 8). The time of the simulated trajectories was inverted to a backwards in 967 time model to follow the same structure as the inferred trajectory from CLUES. Supplementary 968 Figure 14 shows the trajectories of functional variants that arise under the three different selfing 969 coefficients along with the inferred trajectory.

970

971 Santo Antão fits somewhat less well with the idealized model due to its more complex 972 history. The colonization event in Santo Antão is confounded with the much earlier split from the 973 'ghost' population. Further, population structure appears to have developed in Santo Antão well 974 before FRI 232X appeared, consistent with a more permissive landscape where late-flowering 975 plants could survive to reproduce potentially with moderate to high success in some years. We 976 consider a range of possible Ne from 500 to 1000 based on the estimated Ne in Santo Antão at the 977 time when FRI 232X arose. We examine the scenario with s set to 0.046 based on the selection 978 coefficient inferred from the frequency trajectory of FRI 232X in Santo Antão during the peak of 979 selection. U_b is the same as in the Fogo case.

980

With these assumptions $4N_eU_b$ would range from 3.08×10^{-3} to 6.16×10^{-3} , which is again much less than 1.0, indicating limited mutational input for adaptation. And *s* of 0.046 is much greater than $1/4N_e$, which ranges from 5×10^{-4} to 2.5×10^{-4} , consistent with expectations for a SSWM model, albeit less extreme than the Fogo case.

985

Given that most observed large changes in flowering time in nature can be attributed to changes in *FRI* and *FLC* (85%)^{109,152,156–159}, it seems reasonable to focus on these genes in our analysis of mutational target size. But there are many genes across the genome that act in the flowering time pathway and through which variation could affect flowering time. If we broadened the phenotypic definition to include variants with less extreme effects on flowering

991 time, the architecture of the trait would be more polygenic and U_b would be larger. A few other 992 genes are known to have fairly large effects on flowering time (ranging from 6 to 10 days) and 993 act through vernalization (e.g., FLM, genes in the MAF2-5 gene cluster, and SVP). We could 994 attempt to calculate expected waiting times for any change in flowering time under progressively 995 more polygenic architectures. While we have no estimate of s to which we could compare in 996 these cases, we can assume that it would be smaller, and that drift would then play a much more 997 important role in the probability that mutations in these genes would be established in the 998 population. In a larger population, where new variants were less susceptible to loss by drift, the 999 first steps of adaptation may be more likely to include variants in genes with weaker effects.



1001 Supplementary Figure 1. Geographic locations and genetic clustering of CVI samples.

- 1002 Maps of sub-populations in **a**, Santo Antão and **b**, Fogo. Maps were created using Google Earth
- basemaps ^{160,161}. **c**, Neighbour-joining tree showing deep separation between islands and
- 1004 clustering of island sub-populations. Cvi-0 clusters with the Santo Antão population. Samples
- 1005 from Santo Antão are shown in blue, with sub-populations denoted as follows: Lombo de
- 1006 Figueira (triangles), Cova de Paúl (diamonds), Espongeiro (circles), and Pico da Cruz (squares).
- 1007 Samples from Fogo are shown in orange, with sub-populations denoted as Lava (diamonds),
- 1008 Ribeira Inferno (circles), and Monte Velha (triangles). Cvi-0 (1001) represents the Cvi-0
- 1009 sequenced in the 1001 Genomes Project for Arabidopsis thaliana, while Cvi-0 (new) represents
- 1010 the Cvi-0 re-sequenced in this study. Source data are provided as a Source Data file.





Eurasian sites. The three populations are shown on the x-axis, while the values for each climatic

1015 variable are shown on the y-axis. Each variable is presented with the respective unit. Boxplots

1016 show median, 1st and 3rd quartiles, and whiskers represent 95% CI. *P*-values for two-sided

- 1017 Wilcoxon tests are shown for Morocco (n=20) and Eurasia (n=1060) relative to CVI (n=56).
- 1018 Annual mean temperature: Eurasia: *p*-value $< 2x10^{-16}$, Morocco: *p*-value $= 4.6x10^{-8}$;
- 1019 Isothermality: Eurasia: p-value < $2x10^{-16}$, Morocco: p-value = $3.6x10^{-11}$; Temperature
- 1020 seasonality: Eurasia: p-value < $2x10^{-16}$, Morocco: p-value = $3.8x10^{-11}$; Mean temperature of the
- 1021 warmest quarter: Eurasia: p-value = 0.012, Morocco: p-value = 6.3×10^{-9} ; Annual precipitation:
- 1022 Eurasia: p-value < $2x10^{-16}$, Morocco: p-value = 0.082; Precipitation seasonality: Eurasia: p-value
- 1023 $< 2x10^{-16}$, Morocco: *p*-value = $3.8x10^{-11}$; Precipitation of the wettest quarter: Eurasia: *p*-value =
- 1024 2.9x10⁻¹⁴, Morocco: *p*-value = $5.7x10^{-5}$; Precipitation of driest quarter: Eurasia: *p*-value < $2x10^{-14}$
- 1025 ¹⁶, Morocco: *p*-value = 3.5×10^{-11} ; Aridity index: Eurasia: *p*-value < 2×10^{-16} , Morocco: *p*-value =
- 1026 $6x10^{-4}$; Growing season length: Eurasia: *p*-value < $2x10^{-16}$, Morocco: *p*-value = $4.1x10^{-12}$. Source
- 1027 data are provided as a Source Data file.



Supplementary Figure 3. Linkage disequilibrium (LD) decay in all three populations. Xaxis shows distance between SNPs in kbp, and the y-axis the corresponding r² value. Decay of
LD is shown for Santo Antao (blue), Fogo (orange) and Morocco (green). Lines were smoothed
with locally weighted scatterplot smoothing (LOESS) and the shaded grey area represents the
95% confidence interval.



1035

Supplementary Figure 4. Local ancestry of CVI genomes. a, The percentage of CVI genomes
for which the closest relative is found in each continental population, inferred with

- 1038 Chromopainter. Averages across runs are shown as coloured bars, 95% confidence interval (CI)
- 1039 as vertical black lines, one for each of 148 CVI genomes. Genome-wide, the Moroccan

1040 population in the High Atlas Mountain is the closest relative for 61% of CVI genomes. **b**, Region 1041 of the chloroplast phylogeny with Cvi-0 and worldwide accessions (Full phylogeny in 1042 Supplementary Fig. 5). Cvi-0 clusters with the Moroccan population from South Middle Atlas 1043 with an estimate of 150 ky divergence time from the closest Moroccan individual. Numbers at 1044 the nodes are divergence time in million years. Purple shades represent the 95% CI. c, Geographic distribution of S-locus haplogroups in Morocco and CVI. All individuals from the 1045 1046 islands carry haplogroup B, which is only found in the Rif population in Morocco. Different 1047 colours in the pie charts represent different haplogroups: A in blue, A/C in yellow, B in orange, 1048 and C in pink. Abbreviations: Mha, Moroccan High Atlas; Msma, Moroccan South Middle 1049 Atlas; Mnma, Moroccan North Middle Atlas; Mrif, Moroccan Rif; Ir, Iberian relicts; Weu, 1050 Western Europe; Inr, Iberian non-relicts; Ibc, Italy, Balkans and Caucasus; Ssw, South Sweden; 1051 Ger, Germany; Ceu, Central Europe; Cas, Central Asia; Nsw, North Sweden; SA, Santo Antão. Basemap was retrieved from the World Imagery map¹⁶² using leaflet¹⁶³. Source data are provided 1052 1053 as a Source Data file.



- 1055 Supplementary Figure 5. Time-calibrated chloroplast phylogeny showing the location of
- 1056 Cvi-0 relative to representatives of *A. thaliana*, and other *Arabidopsis* species, *Capsella*
- 1057 grandiflora, Capsella bursa-pastoris as well as Camelina sativa for calibration. Inset shows
- 1058 the location of Cvi-0.



1060 Supplementary Figure 6. Chloroplast network for all CVI accessions. The size of the nodes

1061 is proportional to the number of samples, with the corresponding cluster name. Blue represents

1062 haplotypes in Santo Antão, orange in Fogo.





1072 Cumulative proportion of genomic windows with different inferred ages based on the density of 1073 mutations. Simulations were run with a split at different times (5-50 kya) between a Morocco-1074 like and CVI-like population. Points represent observed data and averages across simulations. 1075 Whiskers represent 95% CI based on the SE estimated with ordinary non-parametric bootstrap 1076 for observed data and on the SE across simulations. Points were interpolated with cubic splines. 1077 Simulations with a split at 10 kya most closely match the observed data, supporting a 1078 colonization of CVI from a 'ghost' population more recent than 10 kya. d, Overview of models 1079 run in dadi (left) and best model (right). N_{ref}, size of the ancestral population; N_{1 bot}, N_{2 bot}, size 1080 of population one and two at the split, respectively; only in the bottleneck models, N_{1 bot} and 1081 N_{2 bot} remain constant for the duration of the bottleneck, T_{bot}; N_{1 end}, N_{2 end}, population sizes at 1082 present; T_{split}, split time; m₁₂, m₂₁, migration rates. Estimates of the split time with MSMC-CCR 1083 reveal a complex scenario, likely due to the absence of a direct outgroup to CVI in our sample. In 1084 these analyses, there is evidence of a split at 40-50 kya, followed by secondary contact or low-1085 level migration between 10 and 20 kya. This initial split and secondary contact likely happened 1086 on the continents between sampled Moroccan populations and an unsampled 'ghost' population 1087 (the true closest continental outgroup to CVI). Source data are provided as a Source Data file.



1089 Supplementary Figure 8. Modelling the dynamics within CVI. a, Overview of models run in 1090 dadi (left) and best model (right). Nref, size of the ancestral population; N1 start, N2 start, size of 1091 population one and two at the split, respectively; only in the bottleneck model, N₂ start remains 1092 constant for the duration of the bottleneck, Tbot; N1_end, N2_end, population sizes at present; Tsplit, split time; m₁₂, m₂₁, migration rates. **b**, Coalescence time within CVI across genomic windows 1093 1094 (size=0.1Mbp) provides a lower bound for colonization timing at approximately 7 kya (95th 1095 percentile of coalescence times between islands). Comparisons within island are in blue (Santo 1096 Antão) and orange (Fogo), and comparisons between islands in green. **c**, Forward simulations

1097 fitted to observed data support a scenario in which Santo Antão was colonized prior to Fogo. The 1098 percentage of fixed differences in Fogo compared to Santo Antão (SA) minus the percentage of 1099 fixed differences in Santo Antão compared to Fogo (y-axis) varies as a function of which island 1100 was colonized first (positive if Santo Antão, blue shade; negative if Fogo, orange shade) and of 1101 the population size at the split (x-axis). Circles represent the results from simulations (n=1516): 1102 blue if Santo Antão was colonized first, orange if Fogo was colonized first. Simulations in which 1103 Santo Antão was colonized first with a population size of approximately 1K at the time of the 1104 split, and in which Fogo was colonized later from Santo Antão, fit best the observed data 1105 (horizontal black line). d, Cross-coalescence rates (CCR) among populations in Santo Antão as 1106 inferred by MSMC-CCR. The estimated between-population split time corresponds to CCR=0.5 1107 (horizontal line). Dark red shows CCR between Santo Antão and Fogo for reference. Lines 1108 represent means across octets, shaded areas the 95% CI. Source data are provided as a Source 1109 Data file.

1110



1113 Supplementary Figure 9. Field climate data and simulated conditions. a, Field site 1114 measurements for precipitation (blue), humidity (green) and temperature (red) using loggers in 1115 the Espongeiro field site over two years (July 2016 to July 2018). Y-axis shows values in mm for 1116 precipitation, in percentage for humidity and in degrees Celsius for temperature. The dashed line 1117 highlights the period of time simulated in panel b. **b**, Chamber conditions during the experiment 1118 for humidity (green) and temperature (red). Watering is shown as blue vertical lines. Y-axis 1119 shows values in percentage for humidity and in degrees Celsius for temperature, and x-axis 1120 shows days after sowing (day 1: 1st Sept 2016). Source data are provided as a Source Data file.

1121



1124 Supplementary Figure 10. Evolutionary history of FRI K232X. Marginal genealogical tree 1125 estimated in RELATE for FRI (Chr4:269719). Individuals are shown across the x-axis with 1126 their ancestral and derived carriers coloured in mustard and grey, respectively. The estimated 1127 time to coalescence is shown on the y-axis. Error bars indicate the 0.025 and 0.975 quantiles of 1128 the posterior density of coalescence times and are shown by the vertical black lines on the tree. 1129 Red dots represent mapped SNPs on their corresponding branches. Abbreviations: SA, Santo 1130 Antão; Pi, Pico da Cruz; Fi, Lombo de Figueira; Es, Espongeiro; and Co, Cova de Paúl. Source 1131 data are provided as a Source Data file.



1133 Supplementary Figure 11. GWAS in Fogo. Manhattan plot showing results of GWAS for

1134 bolting time in the Fogo population (n=129). Dashed line represents the Bonferroni significance

1135 threshold. Source data are provided as a Source Data file.

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1139 Supplementary Figure 12. Functional characterization of *FLC* nonsense mutation. a, *FLC* 1140 effect on natural accessions from CVI and Morocco, and in mutant lines in the Col-0 1141 background. FRI K232X segregates among the Santo Antão individuals shown. Cvi-0, S1-1 and 1142 S15-3 carry the FRI 232X allele, while S5-10 carries the ancestral FRI K232 allele. All Fogo 1143 individuals carry the derived FLC 3X allele. Top: FLC mRNA expression levels, with y-axis 1144 showing the expression levels relative to functional FRI FLC (FRI^+FLC^+). Bottom: Days to 1145 bolting (shown on the y-axis). In both panels, dots represent replicates per genotype (n=3), and 1146 letters above boxplot represent statistical groups from Kruskal Wallis tests. The central line in 1147 the boxplots represents the median, box limits are first and third quartiles and whiskers the 95% 1148 CI. **b**, Bolting time differences in the complementation test. Each dot represents one replicate per 1149 genotype (n=4), and different symbols represent different accessions, with symbols matching 1150 between Fogo and F1 for the parental lines. The central line in the boxplots represents the 1151 median, box limits are first and third quartiles and whiskers the 95% CI. P-values are shown for 1152 two-sided Wilcoxon test. Throughout the figure, FRI+FLC+ represents the accession Col-0 FRI-1153 Sf2, with both functional FRI and FLC; FRI+FLC represents the accession Col-0 FRI-Sf2 flc-3, 1154 carrying a functional FRI and a non-functional FLC; F1 FRI+FLC+xFogo represents F1 1155 individuals from crosses between *FRI*⁺*FLC*⁺ and Fogo accessions; F1 *FRI*⁺*FLC*⁻xFogo 1156 represents F1 individuals from crosses between *FRI*⁺*FLC*⁻ and Fogo accessions; F1 Col-0 1157 represents F1 individuals from crosses between *FRI*⁺*FLC*⁺ and *FRI*⁺*FLC*⁻. Comparisons *p*-values: 1158 FRI^+FLC^+ vs. F1 FRI^+FLC^+ xFogo = 0.88, F1 FRI^+FLC^+ xFogo vs. Fogo = 1.3x10^{-6}, FRI^+FLC^+ 1159 vs. F1 FRI^+FLC -xFogo = 0.0025, F1 FRI^+FLC^+ xFogo vs. F1 Col-0 = 0.0012, Fogo vs.

- 1160 $F1_FRI^+FLC$ xFogo = 0.03, $F1_FRI^+FLC$ xFogo vs. FRI^+FLC = 0.36, $F1_FRI^+FLC$ xFogo vs.
- 1161 F1_Col-0 = 0.00023. For both panels, boxplots show median (centre), 1^{st} and 3^{rd} quartiles (lower
- and upper bound, respectively). Whiskers represent 95% CI. Source data are provided as a
- 1163 Source Data file.




1165 Supplementary Figure 13. Marginal genealogical tree estimated in RELATE for FLC 3X

1166 (Chr5: 3179333). Individuals are shown across the x-axis with the ancestral and derived carriers

1167 coloured in mustard and grey, respectively. The estimated coalescence times are shown on the y-

1168 axis. Error bars indicate the 0.025 and 0.975 quantiles of the posterior density of coalescence

- 1169 times and are shown by the vertical black lines on the tree. Red dots represent mapped SNPs on
- 1170 their corresponding branches. Abbreviations: FO, Fogo; MV, Monte Velha; La, Lava; In,
- 1171 Inferno; SA, Santo Antão; and S1-1 accession from Lombo de Figueira subpopulation in Santo
- 1172 Antão (used as the outgroup). Source data are provided as a Source Data file.



1175 Supplementary Figure 14. Allele frequency trajectories of variants arising in a simulated

Fogo colonizing population. Simulated allele frequency trajectories of variants arising in Fogo (starting population size=48) with selection coefficients of 9.2% (the estimate from CLUES with variable N_e). Each grey line represents a simulated trajectory, and the orange line represents the trajectory inferred from CLUES for FLC R3X, across selfing rates (left to right: 90%, 95%,

1180 99%).



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1182 Supplementary Figure 15. FLC 3X appeared in Fogo soon after colonization, and rose in

1183 frequency rapidly, consistent with evolutionary rescue. Top: reconstructed change in effective

1184 population size over time in Fogo. Bottom: inferred allele frequency trajectory of FLC 3X.

1185 Source data are provided as a Source Data file.

	θ_{π}^{1}	θ_{w}^{2}
CVI	6.53x10 ⁻⁵	1.48x10 ⁻⁴
Santo Antão	5.60x10 ⁻⁵	7.59x10 ⁻⁵
Fogo	4.51x10 ⁻⁵	8.93x10 ⁻⁵
Morocco	5.38x10 ⁻³	5.56x10 ⁻³
High Atlas	3.21x10 ⁻³	2.72x10 ⁻³
North Middle Atlas	2.74x10 ⁻³	2.27x10 ⁻³
South Middle Atlas	3.53x10 ⁻³	3.41x10 ⁻³
Rif	4.69x10 ⁻³	4.00x10 ⁻³
Eurasia	4.21x10 ⁻³	6.42x10 ⁻³
	60	

<u>Supplementary Table 1. Nucleotide diversity (θ) in Cape Verde, North Africa and Eurasia.</u>

1189 ¹ θπ: Tajima's estimator of θ ² θw: Watterson's estimator of θ.

1191 Supplementary Table 2. Modelling of the split between Morocco and CVI with dadi.

Model	MaxLik ¹	AIC ²	Theta ³	Nref ⁴	N1_start ⁵	N2_start ⁶	N1_end ⁷	N2_end ⁸	Tsplit ⁹	m12 ¹⁰	m21 ¹¹	Tbot ¹²
simpleSplit	-461	928	1878	239122	447059	2876	х	х	69802	х	х	х
Exp	-376	758	2039	259618	258831	787	55576	9 11967	55731	х	х	х
im	-1051	2112	1727	219908	4700	215208	44797	6 1468	1397384	7,96E-07	5,54E-06	х
bottleneck bottleneck-	-396	800	1849	235505	526073	253	52607	3 6652	50736	Х	Х	17041
2sided	-348	708	1943	247442	565501	1907	32469	7 12233	49668	х	х	43517

¹MaxLik: maximum log-likelihood obtained across dadi runs

¹193 ² AIC: Akaike Information Criterion

- ³ Theta: population mutation parameter inferred in dadi
- ⁴Nref: effective size of the ancestral population

⁵N1_start: effective size of population one at the split, respectively. In the bottleneck models, these sizes remain constant for the

1197 duration of the bottleneck

⁶N2_start: effective size of population two at the split, respectively. In the bottleneck models, these sizes remain constant for the

1199 duration of the bottleneck

1200 ⁷N1_end: effective population size of population 1 at present

1201 ⁸ N2_end: effective population size of population 2 at present

⁹ Tsplit: split time

- 1203 10 m12: migration rate from population 1 to 2
- 1204 ¹¹ m21: migration rate from population 2 to 1
- 1205 ¹² Tbot: duration of the bottleneck

AIC² Theta³ Nref⁴ N1 start⁵ N2 start⁶ N1 end⁷ N2 end⁸ Tsplit⁹ m12¹⁰ Model MaxLik¹ m21¹¹ Tbot¹² 784 5487 7750 x 4754 x simpleSplit -389 53 776 х х х Exp -313 635 142 2067 1579 488 9268 17166 3715 x х х 1841 9186 3995 4,28E-06 6,03E-07 x im -304 620 127 1427 413 17069 -282 574 99 1435 6434 397 bottleneck 6434 9511 3685 x Х 931

1206 Supplementary Table 3. Modelling of the split between Santo Antão and Fogo with dadi.

1207 ¹ MaxLik: maximum log-likelihood obtained across dadi runs

1208 ² AIC: Akaike Information Criterion

1209 ³ Theta: population mutation parameter inferred in dadi

1210 ⁴Nref: effective size of the ancestral population

⁵N1_start: effective size of population one at the split, respectively. In the bottleneck models, these sizes remain constant for the

1212 duration of the bottleneck

1213 ⁶N2_start: effective size of population two at the split, respectively. In the bottleneck models, these sizes remain constant for the

1214 duration of the bottleneck

1215 ⁷N1_end: effective population size of population 1 at present

1216 ⁸N2_end: effective population size of population 2 at present

⁹Tsplit: split time

1218 ¹⁰ m12: migration rate from population 1 to 2

- 1219 ¹¹ m21: migration rate from population 2 to 1
- 1220 ¹² Tbot: duration of the bottleneck

Variable	Variable Description	Percent contribution	Per imj	mutation portance
gs	Length of the growing season in months		38,7	28,5
bio3	Isothermality (BIO2/BIO7) (×100)		20,2	43
bio6	Min Temperature of Coldest Month		18,4	11,1
bio5	Max Temperature of Warmest Month		14,5	14,7
bio7	Temperature Annual Range (BIO5-BIO6) Precipitation Seasonality (Coefficient of		8	2,2
bio15	Variation)		0,1	0,5
bio8	Mean Temperature of Wettest Quarter		0,1	0

1222 Supplementary Table 4. Niche modeling with MaxEnt for the Moroccan region.

Supplementary Table 5. Model statistics for stepwise regression analysis. The best model
 (nvmax = 2) identifies FRI and GI as the main contributors to fitness.

nvmax ¹	RMSE ²	Rsquared ³	MAE^4	RMSE SD ⁵	Rsquared SD	MAE SD
1	486,6842	0,07352437	334,8117	62,62452	0,03778863	30,72803
2	446,8749	0,2203883	311,2951	61,00429	0,05345926	29,37821
3	450,3681	0,20845137	311,7726	60,74491	0,06327362	30,66107
4	452,386	0,20253587	309,7203	60,97669	0,05890219	32,23143
5	456,163	0,1890078	316,4986	61,53983	0,06234999	33,2347
6	458,9121	0,1796467	319,3417	61,4938	0,06423225	34,29386
7	447,3371	0,22692613	305,0492	58,34149	0,0512004	31,43282

1226 ¹ nvmax: number of variables in the model

² RMSE: root mean square error

¹²²⁸ ³Rsquared: squared value for R (correlation between the observed and predicted values)

1229 ⁴ MAE: mean absolute error

⁵ SD, standard deviation

epoch	S
0 - 2000	0,00112
2000 - 4000	0,04558

1232 Supplementary Table 6. Inferred selection coefficients over time for FRI 232X.

S ¹
-0,99679
-0,57297
0,09273

Supplementary Table 7. Inferred selection coefficients for FLC 3X.

- ¹ The timeframe of fixation corresponds with the epoch 4-6 kya. After fixation of the allele negative selection coefficients are generated.

selection coefficient (s)	Selfing rate	% fixed ¹	Mean gen. to fixation ²	% not fixed ³	Mean gen. to loss ⁴	% no mutations ⁵	Mean gen. to 1st segregating potentially adaptive variant ⁶	Mean gen. to adapt ⁷
0,0927	0,9	13,5	61,37	69,5	3,48	17	2061,63	2750,7
0,0927	0,95	16,00	64,87	67,5	3,02	16,5	1980,04	3210,21
0,0927	0,99	16,00	54,71	67,5	3,19	16,5	2002,59	3000,46
0,23	0,9	21,5	34,25	58,5	2,39	20	2096,52	3224,41
0,23	0,95	23,5	33,12	61,5	2,22	15	2201,36	2714,68
0,23	0,99	24,00	33,25	52,00	2,16	24	2203,79	2666,25

1238 Supplementary Table 8. Simulated trajectories. Each row corresponds to the summary of the simulations (n=200).

1239 ¹% fixed: the percentage of simulations where an adaptive variant arose and fixed in the population

²Mean gen. to fixation: the mean generations for the variant to fix after arising

1241 ³% not fixed: the percentage of simulations where a potentially adaptive variant arose but did not fix

⁴Mean gen. to loss: mean number of generations for a potentially adaptive variant to be lost, given that it is lost

⁵% no mutations: the percentage of simulations where no potentially adaptive mutation arose

⁶Mean gen. to 1st segregating potentially adaptive variant: mean number of generations for the first potentially adaptive variant to occur

⁷Mean gen to adapt: the mean number of generations for an adaptive variant to fix given that adaptation occurred, note that this differs

from the sum of columns H and D because in some cases potentially adaptive mutations are lost before an adaptive variant eventually

1248 fixes.

OligoName	OligoSequence
FLC_RqPCR1_F	CCGAACTCATGTTGAAGCTTGTTGAC
FLC_RqPCR1_R	CGGAGATTTGTCCAGCAGGTG
PP2A_qPCR2_F	AAATACGCCCAACGAACAAA
PP2A_qPCR2_R	CAGCAACGAATTGTGTTTGG
Oligo(dT)	TTTTTTTTTTTTTTTTTTT

1240 **D**_... 41.2 . . ~ . ~

Supplementary Table 10. Coalescence rate and effective population size (Ne) over time inferred in RELATE for FRI 232X in Santo Antão and with the CVI-Ancestor individual, used with the --coal argument in CLUES.

time	haploid.coalescence.rate	pop_size
0	1,61E-04	3113,65462
1000	2,48E-04	2019,43504
1389,5	6,70E-04	745,886807
1930,7	8,03E-04	622,579721
2682,7	6,21E-05	8048,17315
3727,59	1,16E-05	43164,8465
5179,48	0,00E+00	Inf
7196,86	0,00E+00	Inf
10000	2,60E-06	192187,206
13895	1,06E-05	46969,0849
19307	0,00E+00	Inf
26827	4,67E-04	1069,83681
37275,9	4,67E-04	1069,83681
51794,8	4,67E-04	1069,83681
71968,6	4,67E-04	1069,83681
1,00E+05	4,67E-04	1069,83681
138950	4,67E-04	1069,83681
193070	4,67E-04	1069,83681
268270	4,67E-04	1069,83681
372759	4,67E-04	1069,83681
517948	4,67E-04	1069,83681
719686	4,67E-04	1069,83681
1,00E+06	4,67E-04	1069,83681
1389500	4,67E-04	1069,83681
1930700	4,67E-04	1069,83681
2682700	4,67E-04	1069,83681
3727590	4,67E-04	1069,83681
5179480	4,67E-04	1069,83681
7196860	4,67E-04	1069,83681
1,00E+07	4,67E-04	1069,83681
1,00E+07	4,67E-04	1069,83681

Supplementary Table 11. Coalescence rate and effective population size (Ne) over time inferred from genome-wide data in RELATE and used with the --coal argument in CLUES.

time	haploid.coalescence.rate	pop_size
0	1,92E-04	2604,0446
1000	3,88E-05	12870,046
1389,5	1,07E-04	4662,74374
1930,7	2,30E-04	2177,89955
2682,7	1,17E-03	427,785525
3727,59	1,18E-03	424,358158
5179,48	1,36E-03	367,395825
7196,86	1,07E-01	4,68239326
10000	1,07E-01	4,68239326
13895	1,07E-01	4,68239326
19307	1,07E-01	4,68239326
26827	1,07E-01	4,68239326
37275,9	1,07E-01	4,68239326
51794,8	1,07E-01	4,68239326
71968,6	1,07E-01	4,68239326
1,00E+05	1,07E-01	4,68239326
138950	1,07E-01	4,68239326
193070	1,07E-01	4,68239326
268270	1,07E-01	4,68239326
372759	1,07E-01	4,68239326
517948	1,07E-01	4,68239326
719686	1,07E-01	4,68239326
1,00E+06	1,07E-01	4,68239326
1389500	1,07E-01	4,68239326
1930700	1,07E-01	4,68239326
2682700	1,07E-01	4,68239326
3727590	1,07E-01	4,68239326
5179480	1,07E-01	4,68239326
7196860	1,07E-01	4,68239326
1,00E+07	1,07E-01	4,68239326
1,00E+07	1,07E-01	4,68239326

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