Genome-wide scans of selection highlight the impact of biotic and abiotic constraints in natural populations of the model grass *Brachypodium distachyon*.

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22 **Summary**

23 Grasses are essential plants for ecosystem functioning. Quantifying the selective
 24 pressures that act on natural variation in grass species is therefore essential regarding
 25 biodiversity maintenance. In this study, we investigate the selection pressures that act
 26 on two distinct populations of the grass model *Brachypodium distachyon* without
 27 prior knowledge about the traits under selection. We took advantage of whole-
 28 genome sequencing data produced for 44 natural accessions of *B. distachyon* and used
 29 complementary genome-wide scans of selection (GWSS) methods to detect genomic
 30 regions under balancing and positive selection. We show that selection is shaping
 31 genetic diversity at multiple temporal and spatial scales in this species and affects
 32 different genomic regions across the two populations. Gene Ontology annotation of
 33 candidate genes reveals that pathogens may constitute important factors of positive
 34 and balancing selection in *Brachypodium distachyon*. We eventually cross-validated
 35 our results with QTL data available for leaf-rust resistance in this species and
 36 demonstrate that, when paired with classical trait mapping, GWSS can help
 37 pinpointing candidate genes for further molecular validation. Thanks to a near-base
 38 perfect reference genome and the large collection of freely available natural
 39 accessions collected across its natural range, *B. distachyon* appears as a prime system
 40 for studies in ecology, population genomics and evolutionary biology.

41

42

43 **Introduction**

44 Grasses cover more than 40% of the world land area (Gibson, 2009) and dominate a
 45 wide variety of ecosystems, from tropical to temperate regions (Clayton, 1981;
 46 Gibson, 2009). Grasses also play a key role in eco- and agrosystem functioning as
 47 they provide habitats for many animal species (Groves, 2000) and represent the main
 48 source of grain and forage (Stromberg 2011). Increasing crop production to meet the
 49 food and energy requirements of the world’s growing population is, however, putting
 50 great pressure on natural grasslands (Wallace, 1997; Helm *et al.*, 2009; Ceballos *et*
 51 *al.*, 2010). Faced with constant deterioration and fragmentation due to human
 52 activities (Kiviniemi, 2002), these ecosystems are highly endangered (Ceballos *et al.*,
 53 2010), but little is known about their evolutionary resilience. Assessing the genetic
 54 basis of adaptation and quantifying the selection pressures that act on natural variation
 55 in grass species is therefore crucial with respect to biodiversity maintenance and food
 56 security.

57 To date, reciprocal transplant experiments have been extensively used to test for
 58 adaptive differentiation across populations (for review see Savolainen *et al.*, 2013).
 59 Based on a “home vs. foreign” effect on fitness, reciprocal transplants are indeed
 60 powerful to unravel overall genotype by environment (GxE) interactions and
 61 demonstrated the prevalence of local adaptation in grasses and plants in general (for
 62 review see Bischoff *et al.*, 2006; Wadgymar *et al.*, 2017). However, reciprocal
 63 transplant experiments use information such as survival, vegetative growth or seed
 64 production to measure the effect of the habitat on fitness (Bischoff *et al.* 2006). Hence
 65 they provide little insight into the functional and genetic bases of adaptation, unless
 66 combined with trait mapping such as quantitative trait locus (QTL) analyses and
 67 genome-wide association studies (GWAS) (Latta 2009). QTL analyses and GWAS,
 68 on the other hand, are largely constrained by the effort and time required for high-
 69 resolution mapping. In grasses, while these trait-by-trait approaches have been
 70 valuable to decipher the genetic architecture of important characters with regard to
 71 crop genetic improvement (Huang *et al.* 2002; Barbieri *et al.* 2012; Morris *et al.* 2013;
 72 Slavov *et al.* 2014), they remain of limited value to grasp the overall selective forces
 73 that act on natural populations.

74 An efficient alternative to provide insights about evolutionary forces in natural
 75 populations consists in identifying genes under selection at a whole genome scale,

76 then describing their function and the type of selection acting on them (Mitchell-Olds
77 et al. 2007). For instance, new mutations that are beneficial in some populations can
78 be positively selected and are more likely to quickly increase in frequency. Such so-
79 called selective sweeps tend to reduce genetic diversity, increase differentiation
80 among populations, and lead to extended haplotypes in the vicinity of the locus under
81 selection due to genetic hitchhiking (Nielsen 2005; Hermisson 2009). Various
82 genome-wide selection scans (GWSS) methods have been developed to detect such
83 footprints of positive selection (Tang et al. 2007; Gautier et al. 2012; Stamatakis et al.
84 2013; Messer 2015) and GWSS are now emerging as complementary approaches to
85 classical trait mapping.

86 A first class of GWSS is based on cross-population comparison. These methods scan
87 the genome by comparing the lengths of haplotypes across populations and are
88 powerful to identify hard selective sweeps (Tang *et al.*, 2007), i.e. regions in the
89 genome where one haplotype has undergone recent and near-complete fixation in one
90 of the studied populations in response to selection (Figure 1A). Yet, if the
91 advantageous mutation has been present in the population for long enough and
92 recombined before selection took place, then several different haplotypes may
93 increase in frequency (Figure 1B). Such events (“soft sweeps”), as well as partial hard
94 sweeps, are less efficiently detected by GWSS based on cross-population comparison
95 (Schlump et al. 2016) since they do not erase polymorphism to the same extent as
96 completed hard sweeps. Soft-sweeps can be yet detected by a second class of GWSS
97 which are also based on extended haplotypes but are applied at the within population
98 level. These scans, by measuring mean pairwise differences in haplotype length along
99 the genome in a given population, can indeed identify loci under selection
100 independently of the number of haplotypes (Figure 1B) or of the completeness of the
101 sweep (Schlump et al. 2016). Therefore, within-population GWSS can detect not only
102 soft-sweeps, but also mutations that are undergoing selection and did not reach
103 complete fixation within a population.

104 Scans for positive selection are nonetheless not exempt of shortcomings and, as any
105 computational approach, can lead to the detection of false positives. First, these tests
106 often rely on the assumption that regions above a given threshold for the test statistics
107 should be enriched in selected genes. However, defining which threshold should limit
108 the amount of false positives is not straightforward (Pavlidis et al. 2012). In addition,

demographic events such as bottleneck may also lead to the fixation of long haplotypes and mimics signal of selection (Schrider & Kern, 2016). This is especially true when the bottleneck is recent and that recombination did not have enough time to break haplotype blocks. While some GWSS are now able to incorporate bottlenecks or expansions in their models (Schrider & Kern, 2016), disentangling the effects of selection from demography remains notoriously challenging as it implies to precisely model population size evolution over time. Comparing and validating cross- and within-population GWSS with approaches that take into account the demographic history of each population may therefore constitute the best way to identify genes under different regimes of positive selection with high confidence.

Furthermore, while local adaptation is commonly associated to positive selection on new advantageous polymorphisms, recent studies have demonstrated that balancing selection is also playing an important role in this evolutionary process (Mitchell-Olds et al. 2007; Rasmussen et al. 2014; Wu et al. 2017). The term balancing selection is an “umbrella” concept (Fijarczyk & Babik, 2015) which describes the maintenance of genetic diversity over longer periods of time through adaptation to spatial heterogeneity, heterozygote advantage and negative frequency-dependent selection (Mitchell-Olds et al. 2007; Rasmussen et al. 2014). This process is more difficult to detect than positive selection (Fijarczyk & Babik, 2015) since older alleles had more time to recombine and may lead to narrow signatures around selected sites (Teixeira et al. 2014). As a consequence, the effect of balancing selection is still largely overlooked in genome scans, which remain strongly biased towards the detection of recent positive selection (Hassl & Payseur, 2016). It is now possible, however, to model the coalescent process along chromosomes and to identify genomic regions with unusually long times to the most recent common ancestor (TMRCA, Figure 1C), a signature which can arise through balancing selection (Rasmussen et al. 2014).

In this study, we capitalize on the near base-perfect quality of the reference genome of the Mediterranean grass *Brachypodium distachyon* (<https://phytozome.jgi.doe.gov>) to investigate how both positive and balancing selection are shaping diversity in this species. In the last decade, *B. distachyon* has been developed as a powerful model for research on temperate grass species as it is closely related to major crop cereals and to some of the grasses used for biofuel production (The International Brachypodium Consortium 2010). Entirely sequenced, its small diploid genome (272Mb) is fully

142 assembled into five chromosomes and has been exhaustively annotated (The
143 International Brachypodium Consortium 2010). In addition, *B. distachyon* is broadly
144 distributed around the Mediterranean rim (Dell'Acqua *et al.*, 2014; Gordon *et al.*,
145 2014; Tyler *et al.*, 2016), providing access to natural populations from contrasting
146 habitats for which a large collection has been collected. It constitutes therefore a
147 unique and prime system to investigate the genetic basis of local adaptation in natural
148 grass populations, opening the way to further fundamental and applied research.

149 We took advantage of whole-genome sequencing data produced for 44 *B. distachyon*
150 natural accessions originating mainly from Spain and Turkey (Gordon *et al.*, 2017).
151 Those accessions are genetically distinct, occur in the eastern and western outer range
152 of the species distribution and were selected to maximize genetic differentiation and
153 identify regional selective pressures that may have been important during
154 recolonization following the last glacial maximum (Stritt *et al.* 2018). Making use of
155 the 6 million SNPs we identified in these genomes, we combined a cross-population
156 (Rsb; Tang *et al.*, 2007) and a within-population scan (H-scan; Schlamp *et al.*, 2016)
157 to an integrated demography approach (diploS/HIC; Kern & Schrider, 2018) to detect
158 high-confidence genomic regions under different regimes of positive selection. We
159 also tested for regions under balancing selection with the program ARGweaver
160 (Rasmussen *et al.* 2014) and asked: i) At what time and geographical scale is selection
161 acting in *B. distachyon* populations? ii) What are the selective constraints that shape
162 diversity and adaptation in these populations? iii) Can we recover candidates for local
163 adaptation identified from QTL analyses performed in controlled conditions?

164

165 **Results**

166 *Population structure and genetic diversity*

167 In this study, we used whole-genome sequencing data (paired-end; Illumina
168 technology) with a 86-fold median coverage of 44 *B. distachyon* accessions
169 originating from Turkey, Iraq, Spain and France (Figure 2A, Table S1, Gordon *et al.*,
170 2017). After filtering, we identified 6,204,029 SNPs. An Admixture analysis, where
171 K=2 was identified as the best model (Figure S1), highlighted two distinct genetic
172 clusters, an eastern and a western one, with extremely little admixture between the
173 two (Figure 2B). For the rest of the study, and even though these accessions belong to

174 two genetic groups rather than *stricto sensu* populations, accessions from Turkey and
175 Iraq will be referred to as the eastern population while accessions from Spain and
176 France will be referred to as the western population.

177 The western population showed a lower level of nucleotide diversity (median π value
178 in east = 0.0021, median π value in west = 0.00186; Wilcoxon test; P-value < 2.2e-16,
179 Figure 2C) than the eastern one. Excluding the reference accession Bd21, which has
180 been artificially inbred before sequencing, the level of heterozygosity in these
181 accessions ranges from 4 to 17.4% (Table S1) but median values (7.8 and 6.3 in the
182 eastern and western population respectively) did not statistically differ between the
183 two populations (Wilcoxon test; P-value = 0.31). Eventually, linkage disequilibrium
184 (LD) decays faster in the western population than in the eastern one (Figure 2 D).

185

186 *Functional clustering of the genome of B. distachyon and subsequent filtering*

187 To assess whether the genome of *B. distachyon* harbors functional clusters of genes,
188 we first performed a GO annotation for the 32,712 genes annotated in the reference
189 genome. We then controlled for potential gene clustering by following the procedure
190 described in (Al-Shahrour *et al.*, 2010). Briefly, we split the genome into overlapping
191 windows of 50 consecutive genes and performed enrichment analyses on each
192 window. We identified 272 windows significantly enriched for at least one biological
193 process (Table S2). Several windows were enriched for processes that may be
194 associated to adaptation to local environmental conditions such response to stress and
195 defense response (Table S2).

196 A classical approach applied to analyze the results of GWSS consists in selecting
197 genomic regions containing top 1% outliers for signals of selection and then assessing
198 whether some biological functions or processes are significantly over-represented in
199 these regions through a Gene Ontology (GO) annotation. We anticipated that the non-
200 random organization of *B. distachyon* genome we observed could lead to an over-
201 representation of some biological pathways or functions in small genomic regions and
202 to an artificial enrichment for some GO terms in GWSS 1% outlier regions (Pavlidis
203 *et al.* 2012). This prompted us to narrow down the top 1% outlier regions by keeping
204 only the genes located at and in the immediate vicinity (-10% of the peak value) of
205 each of the peaks of selection for the cross- (Rsb test) and within-(H-scan) population
206 scans of selection presented in the following section. In contrast, the outputs of the

207 coalescence and demography approaches, which are both window-based (see
208 methods), were not filtered.

209

210 *Detection of hard selective sweeps through cross-population contrasts*

211 When new mutations providing a selective advantage rise in frequency through
212 positive selection, neutral mutations that are physically close tend to remain strongly
213 linked to them. Recent positive selection should therefore lead to a signature of long
214 haplotypes near selected mutations. We used the Rsb test (see methods) to contrast
215 the two populations (Tang et al. 2007) and to detect regions where one haplotype
216 (hard sweep, Figure 1A) reached near or complete fixation a recent past in one of the
217 two populations. By doing so, we identified 312 regions harboring 824 genes and 319
218 regions harboring 1212 genes potentially under selection in the eastern and western
219 populations respectively.

220

221 *Detection of soft or ongoing selective sweeps through a within-population scan*

222 The Rsb test is less efficient to detect soft-sweeps, i.e. regions where more than one
223 haplotype increased in frequency (Figure 1B), or to identify ongoing selective sweep
224 where not all individuals within the population display haplotype extension in the
225 region under selection. As an alternative, we used the program H-scan (see methods)
226 to detect loci associated to soft selective sweeps or undergoing positive selection
227 within each population. We identified 142 regions harboring 487 genes and 79
228 regions harboring 463 genes in the eastern and western population respectively. As
229 expected given the specificity of each test, only 49 and 58 genes were common to the
230 H-scan and Rsb approaches in the eastern and the western populations respectively.

231

232 *Integration of demography and detection of high-confidence gene sets under positive* 233 *selection*

234 As presented in the introduction, bottlenecks can mimic signals of selection as they
235 also lead to the fixation of long haplotypes. To alleviate this issue, we applied
236 diploS/HIC, a recently developed machine-learning approach to detect selective
237 sweeps and classify them as hard or soft (Schridder and Kern 2016; Kern and Schridder
238 2018) while integrating the demographic history of the eastern and western population

239 previously inferred by (Stritt et al. 2018). One of the major advantages of diploS/HIC
240 is that the algorithm is robust to demographic model misspecification (Schrider and
241 Kern 2018). By providing a confusion matrix, it also estimates false positive and false
242 negative rates and allows a better assessment of the confidence levels expected under
243 different scenarios of selection.

244 We first trained the program to identify regions under positive selection. To do so,
245 genomic windows were simulated under neutrality, with a hard sweep or with a soft
246 sweep (see methods). The algorithm was able to recover from the simulations 71%
247 and 91.5% of hard sweeps in western and eastern populations respectively (Figure S2
248 for an example of confusion matrix). For soft sweeps, these proportions were of 71%
249 and 83.7%. The proportion of neutral simulations being classified as hard sweeps was
250 low, accounting for 1.25% and 0.04% of the simulations in western and eastern
251 populations. These proportions were higher for soft sweeps, particularly in the
252 western population (11.28%) but still remained low in the eastern population (2.6%).
253 These results suggest a good sensitivity and specificity of diploS/HIC to detect
254 regions that are under selection, especially in the eastern population.

255 We then ran diploS/HIC on our actual dataset. Windows classified as selective sweeps
256 by diploS/HIC on this dataset recovered 34% (164 genes) and 38% (312 genes) of the
257 genes detected by the H-scan and Rsb tests in the eastern population, as well as 55%
258 (258 genes) and 53% (644 genes) of the genes detected by the H-scan and Rsb tests in
259 the western population. DiploS/HIC recovered more genes detected by the Rsb and H-
260 scan tests than expected by chance (Fisher test, all P-values < 1.8e-12). These four
261 new genes sets (intersects between diploS/HIC-Rsb East, diploS/HIC-Rsb West,
262 diploS/HIC-Hscan East, diploS/HIC-Hscan West) are considered as high-confidence
263 genes under positive selection (Table S3 for a list). Unless mentioned otherwise, those
264 sets will be referred to as genes under positive selection in the discussion.

265 The high-confidence gene set diploS/HIC-Rsb of the eastern population displayed a
266 significant enrichment for genes involved in stress response and defense response
267 (Table 1). Among other GO term enrichments, the eastern high-confidence gene set
268 diploS/HIC-Hscan was significantly enriched for gene involved in response to
269 cadmium ion (see Table 1 for more significant GO terms). As we observed that some
270 of the well-known types of plant resistance genes (i.e. R-genes harboring NBS-LRR
271 domains) were not associated with the process of defense response in the GO

analysis, we specifically counted how many genes containing NBS-LRR domains were present in the four high-confidence gene sets and compared those to the number of genes with NBS-LRR domains annotated in the whole genome. Both high-confidence gene sets (diploS/HIC-Hscan and diploS/HIC-Rsb) of the eastern population displayed a significant enrichment for R-genes (P-value = 1.7×10^{-6} and 1.3×10^{-5} respectively). This was not the case for the high-confidence gene sets of the western population (both P-value > 0.6).

Genomic regions affected by positive selection

The regions common to diploS/HIC-Hscan contained slightly more genes in the western than in the eastern population (Wilcoxon test, P-value=0.04; Figure 3A), while there was no significant difference between the sizes of the diploS/HIC-Rsb regions in the eastern and western populations (Wilcoxon test, P-value=0.07; Figure 3A).

We also tested whether distinct loci/genomic regions were affected by recent positive selection in the two populations using linear models. While we found a significant association between the density of genes under positive selection (S/HIC-Rsb and S/HIC-Hscan outliers combined) in 100 kb windows in the eastern and western populations on all chromosomes, all R^2 were inferior to 1.307×10^{-6} . This result indicates that positive selection mostly affects distinct loci across the two populations (as depicted on Figure 3B). Interestingly, we observed that some genomic regions harbor higher density of genes under selection than the rest of the genome. Thus, genes under positive selection appear to be non-randomly distributed along chromosomes in the two populations (Figure 3B).

Genes with extremely long coalescence time

Balancing selection can be detected with coalescence approaches, as ancient alleles are associated with older coalescence times (Charlesworth 2006). We used the software ARGweaver to detect footprints of balancing selection, a method that has robustly identified signatures of balancing selection in recently expanded human populations (Rasmussen et al. 2014). Briefly, ARGweaver models the coalescent process along chromosomes and across non-recombining haplotype blocks to infer

304 their evolutionary history (see methods). It allows recovering several statistics such as
305 times to the most recent common ancestor (TMRCA, Figure 1C), which should be
306 increased near ancient alleles such as those under ancient balancing selection. By
307 doing so, we identified 72 regions harboring 115 genes under what will be referred to
308 as long-term balancing selection in the following (Table S3 for a list). This gene set
309 harbors a significant enrichment for genes involved in phosphorylation (Table 1).

310

311 *Identification of candidate genes in known QTL regions*

312 Combining association mapping and analyses of selection constitutes a powerful
313 approach to identify candidate genes and to address their selective regime. We
314 identified many candidate regions harboring resistance genes. As a proof of concept,
315 we therefore aimed at assessing whether regions identified as resistance loci against
316 known pathogens were also highlighted in our selection scans. In *B. distachyon*, the
317 genetic basis of resistance to the rust fungus *Puccinia brachypodii* has been
318 deciphered through a QTL mapping (Barbieri et al. 2012) which showed that leaf-rust
319 resistance is controlled by three main QTL located on chromosome 2 (from
320 nucleotide 37,949,269 to 40,903,216), 3 (from nucleotide 13,943,000 to 14,512,222)
321 and 4 (from nucleotide 9,649,152 to 10,679,750). For the rest of the study, these
322 regions will be referred to as QTL_{rust-2}, QTL_{rust-3} and QTL_{rust-4}.

323 We screened these three regions for evidence of selection and found signals of
324 positive selection in QTL_{rust-3} and QTL_{rust-4}. In QTL_{rust-3}, we found that one gene
325 (Bradi3g16331: 14,502,928-14,503,290) belonged to the eastern high-confidence
326 gene set diploS/HIC-Rsb (Figure 4A, left panel). This gene is located 60 kb upstream
327 the QTL peak identified in this region (Barbieri et al. 2012) and codes for an
328 unknown protein. It is flanking a serine/threonine phosphatase (Bradi3g16320:
329 14,486,831- 14,488,838; Figure 4A, left panel) which was also identified as being
330 under selection in the eastern population with the Rsb test. The latter gene was
331 however not recovered by diploS/HIC but classified as a locus being linked to a
332 selected site (see methods). This 16kb region spanned by Bradi3g16331 and
333 Bradi3g16320 may therefore be considered as a candidate for rust resistance.

334 The two adjacent Rsb signals in QTL_{rust-4} reach their highest point into two R-genes
335 (Bradi4g10153: 9,807,879-9,812,927; and Bradi4g10171: 9,828,236-9,835,003;
336 Figure 4A, right panel). These two genes are located in the near vicinity of the QTL

337 peak (estimated to be located at 9,806,000 kb) and both belong to the eastern Rsb-
338 diploS/HIC high-confidence gene set (Figure 4A, right panel). Bradi4g10153 and
339 Bradi4g10171 constitute therefore further prime candidates for rust resistance.
340 Congruent with the signal detected by the Rsb test, we observed extended haplotypes
341 in the eastern population in both QTL_{rust-3} and QTL_{rust-4} (Figure 4B). The large
342 majority of the eastern accessions display the extended haplotype in these regions,
343 indicating a nearly completed selective sweep, especially in QTL_{rust-4} (Figure 4B).

344 QTL_{rust-4} shows a striking enrichment for genes involved in defense response (P-value
345 = 8E-09) and in immune signaling processes such as phosphorylation (P-value =
346 5.6E-06). Among the 113 genes covered by QTL rust-4, 40 correspond either to a
347 gene with a NBS-LRR, a receptor-like protein kinase (RLK) or a F-box domains,
348 three types of genes that can confer resistance in plants. The presence of such a large
349 gene clusters, as found in other regions of the genome (Table S2), further
350 demonstrates the importance of narrowing down candidate regions under selection to
351 top outlier genes for unbiased GO annotation.

352

353 Discussion

354 Assessing the time and spatial scales at which selection acts is a key to understand
355 how genetic diversity is maintained or lost through adaptation (Stinchcombe and
356 Hoekstra 2008; Fuller et al. 2015). In plants, and especially in grasses, this question
357 has been largely restricted to crops, which biases our understanding of evolutionary
358 processes that have shaped genomes in their natural ancestors and extant relatives. In
359 this study, we investigated the selective forces influencing adaptation in two
360 populations consisting of 44 natural accessions of the wild Mediterranean grass
361 *B. distachyon*. We found that ancient balancing and recent positive selection left
362 distinct signatures on specific gene categories, and that positive selection affects
363 distinct loci across the two populations. Importantly, our results support a role for
364 pathogens in driving population differentiation and confirm that GWSS constitute
365 effective approaches to pinpoint candidate genes as a complement to classical trait
366 mapping.

367 Time- and space-varying selection is shaping diversity in *B. distachyon*

368 The ending of the last glaciation period 10,000 years ago led to drastic and recent
369 changes of plant communities in Eurasia (Svenning et al. 2008; Binney et al. 2017).

370 At that time, climate warmed and species expanded over Europe (Hewitt 1999).
 371 Pollen-based studies show that vegetation expansion was fast, reaching up to 2km per
 372 year for some species (Hewitt 1999). To our knowledge, no fossil pollen records are
 373 available for *B. distachyon*, which prevents reconstructing the geographical
 374 distribution of this species before and during the last ice age. Yet a previous study
 375 showed that the two populations analyzed here experienced population size reduction
 376 during the last glaciation followed by a rapid expansion within the last 10,000 years
 377 (Stritt *et al.*, 2018). Even though unraveling the history of populations in southern
 378 peninsulas is more complex than in northern regions (Hewitt 2000; Feliner 2011),
 379 these results are congruent with the recent global postglacial recolonization of Europe
 380 by plants (Hewitt 1999; Svenning *et al.* 2008; Binney *et al.* 2017) and imply that
 381 *B. distachyon* populations had to adapt to newly colonized habitats in the recent past.

382 Balancing selection may have maintained ancestral polymorphisms over long periods
 383 of time in natural populations of *B. distachyon*. As a support of balancing selection,
 384 we identified an old set of shared polymorphisms with a coalescence approach.
 385 *B. distachyon* populations encounter heterogeneous habitats (Lopez-Alvarez *et al.*,
 386 2015) which could have provided selective pressure strong enough to maintain
 387 polymorphisms over long periods of time within each population. Whether balancing
 388 selection results from spatial heterogeneity or from negative frequency-dependent
 389 selection remains yet to be investigated. Our results nevertheless suggest that
 390 adaptation to climate after recolonization does not necessarily involve *de novo*
 391 mutations, even after bottlenecks.

392 On the other hand, we also found evidence for positive selection acting on younger
 393 polymorphisms. Our analysis also revealed that positive selection is targeting
 394 different loci in the two populations and that these loci appear to be non-randomly
 395 distributed along chromosomes. As recombination rate is relatively high in
 396 *B. distachyon* (Huo *et al.* 2011) and since we observed a rather moderate linkage
 397 disequilibrium in the two populations, we do not believe that this pattern is due to
 398 extended linkage disequilibrium and the subsequent process of background selection
 399 along large genomic regions (Cutter and Payseur 2013; Slotte 2014). Rather, the
 400 peaks of selection we identified were narrow and allowed to pinpoint genes,
 401 indicating that while *B. distachyon* is predominantly inbreeding, outcrossing events
 402 must occur every now and then to limit extended linkage disequilibrium. This is also

403 congruent with the slightly higher level of heterozygosity we observed here in
 404 *B. distachyon* compared to other selfing plants such as *A. thaliana* (Platt et al. 2010).
 405 Interestingly, the regions displaying signs of soft-sweep or ongoing selection tended
 406 to be larger in the western population than in the one of the eastern cluster despite
 407 slower LD decay in this latter group. This also indicates that selection may have acted
 408 more recently in the western population, possibly due to the modern and rapid
 409 expansion of these lineages in the Spanish peninsula (Stritt *et al.* 2018).

410 *B. distachyon* occurs exclusively around the Mediterranean rim which constitute a
 411 mosaic of landscapes. Our results reveal that this variation in habitats lead to natural
 412 selection which affected different genes and genomic regions across populations. As
 413 we obtained enrichments for different GO terms in the eastern and western
 414 populations, we also imply that contrasting abiotic and biotic factors are shaping
 415 natural population diversity in different regions of the genome of *B. distachyon*
 416 through positive selection. Even though we identified more genes under positive than
 417 under balancing selection, it would be daring to conclude that the former selection
 418 regime, less challenging to detect (Delph & Kelly, 2014), is a predominant process
 419 shaping diversity in this species. Rather, we believe that we provide here genomic
 420 evidence that balancing selection also leads to the adaptation of *B. distachyon*
 421 populations and that selection acted at different temporal scale.

422

423 *Pathogens as a potential driving force of population evolution*

424 Host-pathogen interactions lead to a strong coevolutionary dynamics and are
 425 considered a major factor shaping diversity (Fumagalli et al. 2011; Karasov et al.
 426 2014; Krattinger and Keller 2016a). Two main types of interaction have been
 427 proposed. Under an arms race model, repeated innovation from both sides results in
 428 repeated fixation of advantageous alleles (Brown and Tellier 2011). This interaction
 429 can therefore lead to positive selection that can be detected by tests focusing on
 430 extended haplotypes. The other type of interaction is often referred to as Red Queen
 431 dynamics or trench warfare, where alleles involved in the interaction are recycled
 432 through negative-frequency dependence and can therefore subsist for long periods of
 433 time in populations through balancing selection (Brown and Tellier 2011).

434 The plant immune system machinery is complex and is composed of two tiers of
 435 extracellular and intracellular receptors (Krattinger and Keller 2016a; Krattinger and
 436 Keller 2016b) that efficiently detect the presence of pathogens (for review Greeff *et*
 437 *al.*, 2012; Couto & Zipfel, 2016; Eckardt, 2017). In this study, we found a significant
 438 enrichment of signals of selection at genes involved in this first level of defense in the
 439 eastern population. More specifically, we found many of well-characterized R-genes,
 440 i.e. genes with NBS-LRR domains and involved in disease resistance (Mchale *et al.*,
 441 2006; Jacob *et al.*, 2013; Liu *et al.*, 2014; Couto & Zipfel, 2016; Eckardt, 2017;
 442 Ooijen *et al.*, 2017), to be under ongoing or completed positive selection in the
 443 eastern population. On the other hand, we also found additional R-genes and genes
 444 involved in phosphorylation, a process especially important for immune signaling
 445 response in plants (for review Park *et al.*, 2012), to be under balancing selection. Our
 446 results are thus consistent with the two classical models of host-pathogens
 447 coevolution (Mondragón-palomino *et al.* 2002; Mchale *et al.* 2006; Gos *et al.* 2012;
 448 Mace *et al.* 2014; Zhong *et al.* 2015; Wu *et al.* 2017). Overall, and as shown in other
 449 organisms (Karasov *et al.* 2014; Fumagalli *et al.*, 2011; Krattinger & Keller, 2016a,b;
 450 Bourgeois *et al.*, 2017), our genome-wide approach suggests that pathogens may
 451 constitute an important driving force of population and genome evolution in
 452 *B. distachyon*.

453 The significant enrichments for R-genes and for genes associated to the process of
 454 defense response in the gene sets under positive selection were only observed in the
 455 eastern population. As we incorporated the demographic history to our analyses, we
 456 conclude that the bottlenecks experienced by both populations did not extensively
 457 bias our results and did not interfere with our conclusions on the importance of host-
 458 pathogen interaction in the eastern population. The geographical origin of the
 459 populations could, however, play a role in this observation. The Middle East is indeed
 460 the center of origin of many grasses, including *B. distachyon*, and of their associated
 461 pathogens (Wyand & Brown, 2003; Stukenbrock *et al.*, 2005; Opanowicz *et al.*, 2008;
 462 Hovmøller *et al.*, 2011). Many studies found that both resistance genes in plants and
 463 effector genes in pathogens can be organized in clusters evolving through an arm race
 464 and resulting in a gene birth-and-death process (Michelmore and Meyers 1998; Dong
 465 *et al.* 2015; Singh *et al.* 2015). Because centers of origin are usually associated to
 466 higher diversity, it is therefore possible that a higher level of pathogen diversity drove

467 selection at a larger number of resistance genes in the eastern population.

468

469 *GWSS as complementary approach to QTL and GWAS*

470 Disentangling the mechanisms that promote or prevent adaptation requires more
471 integrated studies, using both experimentations in controlled conditions and methods
472 to characterize genetic diversity in natural populations (Feder and Mitchell-olds 2003;
473 Stinchcombe and Hoekstra 2008; Flood and Hancock 2017). Several studies used
474 GWSS to validate genes functionally characterized or previously identified through
475 GWAS and to propose stronger hypotheses on the mode of selection operating on
476 traits relevant for adaptation (Roulin *et al.* 2016; Tang *et al.*, 2007; Fumagalli *et al.*,
477 2011; Bourgeois *et al.* 2017). Following this idea, we inspected three QTL regions
478 responsible for the resistance of *B. distachyon* to the leaf-rust fungus *Puccinia*
479 *brachypodii*, a natural pathogen of *B. distachyon* expected to exert selection on
480 natural accessions.

481 For two of these QTL regions on chromosomes 3 and 4, we found signs of positive
482 selection and reduced haplotype diversity in the eastern cluster in the vicinity of the
483 QTL peaks, but no sign of balancing selection. These results strongly indicate that
484 positive selection is shaping rust resistance in *B. distachyon* natural populations, as
485 suggested in other species (Dodds & Thrall, 2009; Chavan *et al.*, 2015). On
486 chromosome 3, our analysis identified a 16kb region spanned by an unknown protein
487 and a serine/threonine phosphatase, a class of genes known for their role in defense
488 response and stress signaling (País *et al.* 2009; Durian *et al.* 2016), to be putatively
489 involved in resistance to rust. The region identified on chromosome 4 is more
490 complex and consists of a cluster of resistance and stress signaling genes.
491 Nevertheless, and while other genes display evidence of positive selection, two R-
492 genes that belong to the eastern high-confidence gene set co-localize with the peak of
493 the QTL. Such genes have been shown to confer resistance to rust in other species
494 (Bettgenhaeuser *et al.* 2014) and constitute further prime candidates for functional
495 characterization.

496 *B. distachyon* is closely related to major crop cereals as well as to grass species used
497 for biofuel production. Translating research from *B. distachyon* to plants of
498 agronomical and economical interest will require a deeper understanding of the
499 genetic architecture of traits involved in the response to environmental stresses. The

500 molecular basis of tolerance to various abiotic stresses such as drought, salt and cold
501 has been investigated in this species (Luo et al. 2011; Manzaneda 2013; Carmo and
502 Charron 2014; Gordon et al. 2014; DesMarais and Juenger 2015; Sun et al. 2015; Mur
503 and Bosch 2016). Here, we also highlighted cadmium pollution as a potential factor of
504 positive selection in the eastern population. As pollution with heavy metals including
505 cadmium has been reported in Turkey in regions where accessions were collected for
506 this study (Bakirdere and Yaman 2008; Mor and Ceylan 2017), our results suggest
507 that *B. distachyon* could be used to investigate the tolerance to this stress. As genetic
508 transformation is highly efficient in this species relative to other grasses, we anticipate
509 that combining classical trait mapping analyses with GWSS will assist allele mining
510 for additional eco-responsive traits.

511

512 *Conclusion*

513 Our results revealed widespread signatures of natural selection at genes involved in
514 adaptation in *B. distachyon* and provide the community with a list of candidate genes
515 displaying strong signs of selection in natural populations. We also found that
516 pathogens may constitute an important driving force of genetic diversity and
517 evolution in this system. While we limited our analysis to classical point mutations,
518 recent studies showed that copy number variants (CNVs) and transposable element
519 polymorphisms are abundant across *B. distachyon* populations (Gordon *et al.*, 2017;
520 Stritt *et al.*, 2018). Hence, the important genomic resources currently developed in
521 this species open new avenues of research to further investigate the role of structural
522 variation in natural population evolution and adaptation. To date, *B. distachyon*
523 remains a classical model for research on grass genomics with a strong orientation
524 towards applied research. Thanks to the high quality of its reference genome and the
525 existence of large collections of freely available natural accessions collected from the
526 species native range, it also constitutes a prime system for studies in ecology,
527 population genomics and evolutionary biology.

528

529

530

531

532 **Experimental procedures**

533 *SNP calling, population structure and genetic diversity*

534 We used paired-end Illumina sequencing data generated for 44 accessions of
535 *B. distachyon* (Gordon *et al.*, 2017; Table S1 for information about the origin of the
536 accessions and sequencing effort) originating from Spain (N=16), France (N=1),
537 Turkey (N=23) and Iraq (N=4). Reads were aligned to the reference genome v2.0
538 with BWA-MEM (standard settings; Li, 2013). After removing duplicates with
539 Sambamba (Tarasov *et al.* 2017), single nucleotide polymorphisms (SNPs) were
540 called with Freebayes (Garrison and Marth 2016). The output was then filtered by
541 removing SNPs with more than 10 missing genotypes or more than 2 alleles, a quality
542 lower than 20 and a mean depth lower than 20 or higher than 200. Data were phased
543 using the software BEAGLE V4 (Browning and Browning 2007) using default
544 settings.

545 We then used the program Admixture (Alexander and Novembre 2009) to identify the
546 genetic structure of the two populations. The analysis was run for K values from 1 to
547 5, and the best model was determined as the model with the lowest cross-validation
548 error. Summary statistics such as within-population nucleotide diversity (π) were
549 computed with the R package PopGenome (Pfeifer *et al.* 2014). Levels of
550 heterozygosity were calculated with VCFtools (Danecek *et al.* 2011).

551

552 *Detecting positive selection associated to nearly completed sweeps*

553 We used the Rsb test (Tang *et al.* 2007) to detect signatures of recent or almost
554 completed hard sweeps. This test detects haplotypes that are positively selected in one
555 population by estimating the length of haplotypes around each allele at a core SNP
556 and then comparing these lengths between populations. While the output of the test
557 provides P-value of significance, it also indicates in which population a given allele is
558 under selection. Rsb statistics were computed for each SNP with the R package
559 rehh2.0 (Gautier *et al.* 2012) with default settings.

560

561 *Detecting ongoing positive selection and soft-sweeps within populations*

562 We used the software H-scan (Schlamp *et al.* 2016) to detect soft-sweep or loci under
563 incomplete ongoing positive selection. To do so, we calculated average pairwise
564 haplotypes lengths using the number of segregating sites spanned by each tract within

each population. The H-scan statistics is expected to be larger as the number of extended haplotypes increases in a population. We ran the method on eastern and western accessions independently to detect soft- or incomplete selective sweeps within each geographical group.

Testing for functional clustering and subsequent filtering of GWWS results

We first performed a GO annotation for the 32,712 genes annotated in the reference genome (version 2.1, <https://phytozome.jgi.doe.gov>) with Blast2GO (Conesa et al. 2005). We then controlled for potential gene clustering by following the procedure described in (Al-Shahrour *et al.*, 2010). The entire gene set of the reference genome was split into windows of 50 consecutive genes. Windows were moved along chromosomes in steps of 25 genes to allow for half-window overlaps. Enrichment analyses of biological processes were then performed for all the generated windows with the R package GOstats (Falcon and Gentleman 2017) using Fisher's exact test. P-values were subsequently adjusted for multiple testing with a Benjamin-Hochberg correction. Regions were considered significantly enriched for a biological process when they displayed a corrected P-value ≤ 0.01 and also harbored at least five genes associated to the given process.

Both the H-scan and the Rsb tests compute statistics at each SNP. To limit false positives, we first selected 10 kb windows displaying at least four significant SNPs within the top 1% outliers. Overlapping significant windows were merged. To limit the impact of gene clustering on the GO analysis, we then narrowed down the selected windows by keeping only the genes located at and around (-10% of the peak value) each of the top 1% peaks of selection. These filtering criteria, however, were not applied to the outputs of ARGweaver and diploS/HIC, which are window-based approaches.

Demographic history and filtering false positives

We used the recently developed machine-learning algorithm diploS/HIC (Kern & Schrider, 2018) to further control for the potentially confounding effects of demography on our outlier approach. To do so, genomic windows were simulated under neutrality, with a hard sweep or with a soft sweep. We trained the algorithm using a set of 3,000 coalescent simulations obtained with discoal (Kern and Schrider

2016). We simulated 330kb windows each divided into 22 subwindows. Hard and soft sweep examples consisted in windows with a sweep occurring in the two central 30kb subwindows. The adjacent subwindows are assigned as linked to selection.

We performed simulations for each of the two populations using demographic trajectories inferred in a previous study (Stritt et al. 2018). Note that we rescaled these estimates with a mutation rate of 1.4×10^{-9} /bp/generation instead of the previously used 7×10^{-9} /bp/generation. We estimated this new mutation rate by aligning the orthologs of 100 genes and using rice as an out-group (divergence estimated at 40My; The International Brachypodium Consortium, 2010). We sampled selection coefficients from an uniform prior $2N_s \sim (250, 25000)$ for the eastern population and $2N_s \sim (2000, 200000)$ for the western population, to take into account the largest estimate for present effective population size (N) in the western cluster ($N \approx 4,000,000$) compared to the eastern one ($N \approx 500,000$). We used a truncated exponential prior for recombination rates encompassing the range 5.9×10^{-9} - 1.18×10^{-7} , values that are between ten times lower and two times higher the rate of 5.9×10^{-8} estimated from controlled crosses (Huo et al. 2011).

To take into account the likely heterogeneity in effective population sizes and mutation rates along the genome, we used priors for present effective population sizes between twice lower and five times higher than the values estimated from the SMC++ analysis for present times (Stritt et al. 2018). We conditioned on sweep completion occurring between present time and shortly before populations expansion, around 10,000 generations ago. For soft sweeps, we used uniform priors on the initial frequency of the adaptive variant of (0,0.2). We took into account inbreeding by assuming each individual contained two copies of the same haplotypes.

This set of simulated datasets was then used to train a supervised machine-learning algorithm to differentiate between each category. Predictions on the actual genomic dataset were then performed over subwindows. Since the statistics computed by diploS/HIC are normalized across subwindows, they can remain flat if selection is strong and erases diversity over the whole region to be classified. We therefore classified genomic subwindows as selected, linked or neutral using two sizes (15 and 50kb), in order to detect genomic regions displaying the strongest signals of selection. The algorithm as well as a detailed tutorial are available at <https://github.com/kern-lab/diploSHIC>.

We finally used bedtools intersect (Quinlan and Hall 2010) to identify high-confidence regions recovered by both the Rsb test and diploS/HIC or by both the H-scan test and diploS/HIC. Genes present in such high-confidence regions were retrieved with bedtools intersect using the annotation of the genome of *B. distachyon* v2.1 (<https://phytozome.jgi.doe.gov>).

Distribution of genes under positive selection along chromosomes

To compare the distribution along chromosomes of candidate genes for recent positive selection (DiploS/HIC-Hscan and DiploS/HIC Rsb candidate genes combined) in each population, we used linear models where the density of selected genes along each chromosome identified in the eastern and western populations (100,000 bins per chromosome) were entered as variables. We eventually used circos (Krzywinski et al. 2009) to visualize the density of genes under positive selection along each chromosome.

Detecting ancient balancing selection with ancestral recombination graphs (ARG)

We used the software ARGWeaver to detect additional candidate regions for balancing selection with a coalescence approach (Rasmussen et al. 2014). We included in the analysis a subset of 12 accessions with high sequencing depth and covering the largest geographical range (6 accessions from each population) to limit computation time. We used a mutation rate of 1.4×10^{-9} /bp/generation and a recombination rate of 5.9×10^{-8} /bp/generation. Note that we subsequently used an outlier approach to identify the oldest polymorphisms present in the two populations (top 1% outliers). Therefore, potential biases inherent to the use of a molecular clock do not affect our analysis. ARGWeaver is also flexible with regard to recombination rate as it reconstructs ancestral recombination graphs and accommodates variable recombination rates, effective population sizes and genealogies along the genome. The algorithm was run for 1000 iterations, using 20 discretized time steps, a maximum coalescence time of 3 million generations and a prior effective population size of 100,000 individuals. This size was of the same order of magnitude as the harmonic mean of effective population sizes over the last 500,000 generations (spanning the recent population declines and expansions), as estimated in a previous study (Stritt et al. 2018) using the algorithm SMC++ (Terhorst et al. 2017).

665 *GO annotation of genes under selection*

666 For each high-confidence gene set and the gene set under balancing selection, we
667 extracted the genes located in the filtered regions with bedtools (Quinlan and Hall
668 2010). We then examined potential enrichment for biological processes for each of
669 the selected gene sets with the R package GOstats (Falcon and Gentleman 2017)
670 using the Fisher's exact test. Gene sets were considered significantly enriched for a
671 biological process when they displayed a P-value ≤ 0.01 and harbored at least five
672 genes associated to the given process. The ancestor and child terms of each significant
673 process were determined using QuickGO (<http://www.ebi.ac.uk/QuickGO>) and used
674 to simplify Fisher test outputs and keep non-redundant terms.

675 We also retrieved from each gene set under selection the number of genes containing
676 a NBS-LRR domain, and annotated as R-genes in version 2.1 of the genome. We then
677 estimated whether these gene sets were enriched for R-genes compared to the rest of
678 the genome with Fisher's exact test in R (R development core team, 2017).

679

680 *QTL for leaf-rust resistance validation*

681 A QTL analysis performed in *B. distachyon* revealed three genomic regions involved
682 in the resistance to *P. brachypodii* (Barbieri et al. 2012). The coordinates of these
683 three QTL were extracted from (Barbieri et al. 2012) from v.2.1 of the *B. distachyon*
684 reference genome. We then assessed whether those three regions were identified as
685 outliers in at least one of the tests of selection or in the high-confidence gene set. We
686 further visualized the extension of haplotypes at candidate regions using the
687 `bifurcation.diagram()` function of the `rehh2.0` R package.

688

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698 **Availability of data and materials**

699 The raw vcf file and the raw outputs of each GWWS will be archive upon acceptance
700 of the manuscript. All whole-genome sequences data are available at the NCBI
701 Sequence Read archive (SRA available in Gordon *et al.*, 2017).

702

703 **Conflict of interest**

704 There is no conflict of interest issue related to this work.

705

706 **Supporting Information**

707 **Figure S1: Admixture analysis**

708 Cross-validation plot.

709 **Figure S2: Confusion matrices for diploS/HIC.**

710 The trained algorithm was applied on datasets simulated under a coalescent
711 framework, and the proportion of simulations correctly assigned to their category was
712 estimated. The location of the classified subwindows relative to the sweep is shown
713 vertically and the predicted class for each subwindow is shown horizontally. Each
714 row corresponds to 3,000 simulations.

715 **Table S1:** Geographical coordinates of the 44 accessions and sequencing effort.

716 **Table S2:** Significant GO terms in each 50 gene-window of the reference genome.

717 **Table S3:** List of genes under selection.

718

719 References

- 720 **Al-Shahrour, Minguez P, Marques-Bonet T, Gazave E, Navarro A, Dopazo J.**
721 **2010.** Selection upon Genome Architecture: Conservation of Functional
722 Neighborhoods with Changing Genes. *PLoS computational biology* **6**: e1000953.
- 723 **Alexander DH, Novembre J. 2009.** Fast Model-Based Estimation of Ancestry in
724 Unrelated Individuals. *Genome research* **19**: 1655–1664.
- 725 **Bakirdere S, Yaman M. 2008.** Determination of lead , cadmium and copper in
726 roadside soil and plants in Elazig , Turkey. *environmental monitoring Assessment*
727 **136**: 401–410.
- 728 **Barbieri M, Marcel TC, Niks RE, Francia E, Pasquariello M, Mazzamurro V,**
729 **Garvin DF. 2012.** QTLs for resistance to the false brome rust Puccinia
730 brachypodii in the model grass. *Genome* **163**: 152–163.
- 731 **Bettgenhaeuser J, Gilbert B, Ayliffe M, Moscou MJ. 2014.** Nonhost resistance to
732 rust pathogens – a continuation of continua. *frontiers in plant science* **5**: 1–15.
- 733 **Binney H, Edwards M, Macias-fauria M, Lozhkin A, Anderson P, Kaplan JO,**
734 **Andreev A, Bezrukova E, Blyakharchuk T, Jankovska V, et al. 2017.**
735 Vegetation of Eurasia from the last glacial maximum to present: Key
736 biogeographic patterns. *Quaternary Science Reviews* **157**: 80–97.
- 737 **Bischoff A, Crémieux L, Smilauerova M, Lawson CS, Mortimer SR, Dolezal J,**
738 **Edwards AR, Brook AJ, Macel M, Leps JAN, et al. 2006.** Detecting local
739 adaptation in widespread grassland species – the importance of scale and local
740 plant community. *journal of ecology* **94**: 1130–1142.
- 741 **Bourgeois Y, Roulin AC, Müller K, Ebert D. 2017.** Parasitism drives host genome
742 evolution: Insights from the *Pasteuria ramosa* - *Daphnia magna* system. *Evolution*
743 **71**:1106–13.
- 744 **Brown JKM, Tellier A. 2011.** Plant-parasite coevolution: bridging the gap between
745 genetics and ecology. *Annual Review of Phytopathology* **49**: 345–67.
- 746 **Browning SR, Browning BL. 2007.** Rapid and Accurate Haplotype Phasing and
747 Missing-Data Inference for Whole-Genome Association Studies By Use of
748 Localized Haplotype Clustering. *the american journal of human genetics* **81**:
749 1084–1097.
- 750 **Carmo S Do, Charron J. 2014.** Comparative analysis of the cold acclimation and
751 freezing tolerance capacities of seven diploid Brachypodium distachyon
752 accessions. *Annals of botany* **113**: 681–693.
- 753 **Ceballos, G., Davidson, A., List, R., Pacheco, J., Manzano-Fisher, P., Santos-**
754 **Barrera, G., Cruzado J. 2010.** Rapid Decline of a Grassland System and Its
755 Ecological and Conservation Implications. *PloS one* **5**: e8562.
- 756 **Charlesworth D. 2006.** Balancing selection and its effects on sequences in nearby
757 genome regions. *PLoS Genetics* **2**: e64.
- 758 **Chavan S, Gray J, Smith SM. 2015.** Diversity and evolution of Rp1 rust resistance
759 genes in four maize lines. *Theoretical and Applied Genetics* **128**: 985–998.
- 760 **Clayton WD. 1981.** Evolution and Distribution of Grasses. *Annals of the Missouri*
761 *Botanical Garden* **68**: 5–14.

- 762 **Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M. 2005.**
763 **Blast2GO: a universal tool for annotation, visualization and analysis in functional**
764 **genomics research. *Bioinformatics* 21: 3674–6.**
- 765 **Couto D, Zipfel C. 2016. Regulation of pattern recognition receptor signalling in**
766 **plants. *nature reviews immunology* 16: 537–552.**
- 767 **Cutter AD, Payseur BA. 2013. Genomic signatures of selection at linked sites□:**
768 **unifying the disparity among species. *Nature reviews Genetics* 14: 262–274.**
- 769 **Danecek P, Auton A, Abecasis G, Albers CA, Banks E, Depristo MA, Handsaker**
770 **RE, Lunter G, Marth GT, Sherry ST, *et al.* 2011. The variant call format and**
771 **VCFtools. *Bioinformatics* 27: 2156–2158.**
- 772 **Dell'Acqua MD, Zuccolo A, Tuna M, Gianfranceschi L, Pè ME. 2014. Targeting**
773 **environmental adaptation in the monocot model *Brachypodium distachyon*□: a**
774 **multi-faceted approach. *BMC Genomics* 15: 801.**
- 775 **Delph LF, Kelly JK. 2014. On the importance of balancing selection in plants Lynda.**
776 ***New phytologist* 201: 1–22.**
- 777 **Des Marais DL, Juenger TE. 2015. Brachypodium and the Abiotic Environment. In:**
778 **Vogel J, editor. *Genetics and Genomics of Brachypodium*, 291–311.**
- 779 **Dodds P, Thrall P. 2009. Recognition events and host–pathogen co-evolution in**
780 **gene-for- gene resistance to flax rust. *Functional plant biology* 36: 395–408.**
- 781 **Dong S, Raffaele S, Kamoun S. 2015. ScienceDirect The two-speed genomes of**
782 **filamentous pathogens□: waltz with plants. *Current Opinion in Genetics &***
783 ***Development* 35: 57–65.**
- 784 **Durian G, Rahikainen M, Alegre S, Brosché M. 2016. Protein Phosphatase 2A in**
785 **the Regulatory Network Underlying Biotic Stress Resistance in Plants. *frontiers in***
786 ***immunology* 7: 1–17.**
- 787 **Eckardt NA. 2017. The Plant Cell Reviews Plant Immunity□: Receptor-Like**
788 **Kinases, ROS-RLK Crosstalk, Quantitative Resistance, and the Growth / Defense**
789 **Trade-Off. *Plant cell* 29: 601–602.**
- 790 **Falcon SÃ, Gentleman R. 2017. Using GOstats to test gene lists for GO term**
791 **association. *Bioinformatics* 23: 257–258.**
- 792 **Feder ME, Mitchell-olds T. 2003. Evolutionary and ecological functional genomics.**
793 ***Nature reviews Genetics* 4: 649–655.**
- 794 **Feliner GN. 2011. Southern European glacial refugia□: A tale of tales. *taxon* 60:**
795 **365–372.**
- 796 **Fijarczyk A, Babik A. 2015. Detecting balancing selection in genomes□: limits and**
797 **prospects. *Molecular ecology* 24: 3529–3545.**
- 798 **Flood J, Hancock AM. 2017. ScienceDirect The genomic basis of adaptation in**
799 **plants. *Current opinion in plant biology* 36: 88–94.**
- 800 **Fuller ZL, Niño EL, Patch HM, Bedoya-Reina OC, Baumgarten T, Muli E,**
801 **Mumoki F, Ratan A, McGraw J, Frazier M, *et al.* 2015. Genome-wide analysis**
802 **of signatures of selection in populations of African honey bees (*Apis mellifera*)**
803 **using new web-based tools. *BMC genomics* 16: 518.**
- 804 **Fumagalli M, Sironi M, Pozzoli U, Ferrer-admettla A, Pattini L. 2011. Signatures**

- 805 of Environmental Genetic Adaptation Pinpoint Pathogens as the Main Selective
806 Pressure through Human Evolution. *PLoS genetics* **7**: e1002355.
- 807 **Garrison E, Marth G. 2016.** Haplotype-based variant detection from short-read
808 sequencing. Available at: <https://arxiv.org/abs/1207.3907>
- 809 **Gautier M, Vitalis R, Baillarguet D, Cedex F-M. 2012.** rehh: an R package to
810 detect footprints of selection in genome-wide SNP data from haplotype structure.
811 *Bioinformatics* **28**: 1176–1177.
- 812 **Gibson DJ. 2009.** *Grasses and grassland ecology* (Oxford university press, Ed.).
- 813 **Gordon SP, Contreras-Moreira B, Woods DP, Des Marais DL, Burgess D, Shu S,
814 Stritt C, Roulin AC, Schackwitz W, Tyler et al. 2018.** Extensive gene content
815 hetlvariation in the Brachypodium distachyon pan-genome correlates with
816 phenotypic variation. *Nature Communications* **8**: 2184
- 817 **Gordon SP, Priest H, Marais DL Des, Schackwitz W, Figueroa M, Martin J,
818 Jennifer N, Tyler L, Lee C, Bryant D, et al. 2014.** Genome diversity in
819 Brachypodium distachyon: deep sequencing of highly diverse inbred lines. *Plant*
820 *Journal* **79**: 361–374.
- 821 **Gos G, Slotte T, Wright SI. 2012.** Signatures of balancing selection are maintained
822 at disease resistance loci following mating system evolution and a population
823 bottleneck in the genus *Capsella*. *BMC Evolutionary Biology* **12**: 152.
- 824 **Greeff C, Roux M, Mundy J, Petersen M. 2012.** Receptor-like kinase complexes in
825 plant innate immunity. *frontiers in plant science* **3**: 1–7.
- 826 **Groves RH. 2000.** Temperate grasslands of the southern hemisphere. In: Jacobs
827 SWL, Everett J, editors. *Grasses Systematics and Evolution*, 3781–3791.
- 828 **Hassl RJ, Payseur BA. 2016.** Detecting selection in natural populations: Making
829 sense of genome scans and towards alternative solutions. *Molecular Ecology* **25**:
830 5–23.
- 831 **Helm A, Oja T, Saar L, Takkis K, Talve T, Pa M. 2009.** Human influence lowers
832 plant genetic diversity in communities with extinction debt. *journal of ecology* **97**:
833 1329–1336.
- 834 **Hermisson J. 2009.** Who believes in whole-genome scans for selection? *Heredity*
835 **103**: 283–284.
- 836 **Hewitt GM. 1999.** Post-glacial re-colonization of European biota. *Biological journal*
837 *of the Linnean society* **68**: 87–112.
- 838 **Hewitt G. 2000.** The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.
- 839 **Hovmøller MS, Sørensen CK, Walter S, Justesen AF. 2011.** Diversity of *Puccinia*
840 *striiformis* on Cereals and Grasses. *Annual Review of Phytopathology* **49**: 197–220.
- 841 **Huang J, Pray C, Rozelle S. 2002.** Enhancing the crops to feed the poor. *Nature*
842 **418**: 678–684.
- 843 **Huo N, Garvin DF, You FM, Luo SMM, Gu YQ, Lazo GR, Philip J. 2011.**
844 Comparison of a high-density genetic linkage map to genome features in the model
845 grass *Brachypodium distachyon*. *Theoretical and Applied Genetics* **123**: 455–464.
- 846 **Jacob F, Vernaldi S, Maekawa T. 2013.** Evolution and conservation of plant NLR
847 functions. *frontiers in immunology* **4**: 1–16.

- 848 **Karasov TL, Horton MW, Bergelson J.** ScienceDirect Genomic variability as a
849 driver of plant – pathogen coevolution? *Current Opinion in Plant Biology* **18**:
850 24–30.
- 851 **Kern AD, Schrider DR.** 2016. Discoal: flexible coalescent simulations with
852 selection. *Bioinformatics* **32**:3839–3841.
- 853 **Kern AD, Schrider DR.** 2018. diploS/HIC: An Updated Approach to Classifying
854 Selective Sweeps. *G3; Genes|Genomes|Genetics* **8**: 1959-1970.
- 855 **Kiviniemi K, Eriksson O.** 2002. Size-related deterioration of semi-natural grassland
856 fragments in Sweden. *diversity and distribution* **8**: 21–29.
- 857 **Krattinger SG, Keller B.** 2016a. Tansley review Molecular genetics and evolution
858 of disease resistance in cereals. *New phytologist* **212**: 320–332.
- 859 **Krattinger SG, Keller B.** 2016b. Trapping the intruder — immune receptor domain
860 fusions provide new molecular leads for improving disease resistance in plants.
861 *Genome Biology* **17**:23.
- 862 **Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ,
863 Marra M a.** 2009. Circos: an information aesthetic for comparative genomics.
864 *Genome research* **19**: 1639–45.
- 865 **Latta RG.** 2009. Testing for local adaptation in *Avena barbata*: a classic example of
866 ecotypic divergence. *Molecular Ecology* **18**: 3781–3791.
- 867 **Li H.** 2013. Aligning sequence reads, clone sequences and assembly contigs with
868 BWA-MEM. preprint arXiv:1303.3997.
- 869 **Liu W, Frick M, Huel R, Nykiforuk CL, Wang X, Gaudet DA, Eudes F, Conner
870 RL, Kuzyk A, Chen Q, et al.** 2014. The Stripe Rust Resistance Gene Yr10
871 Encodes an Evolutionary-Conserved and Unique CC – NBS – LRR Sequence in
872 Wheat. *Molecular Plant* **7**: 1740–1755.
- 873 **Lopez-Alvarez D, Manzaneda AJ, Rey P, Giraldo P, Benavente E,
874 Allainguillaume J et al.** 2015. Environmental niche variation and evolutionary
875 diversification of the Brachypodium distachyon grass complex species in their
876 native. *American journal of botany* **102**: 1073–1088.
- 877 **Luo N, Liu J, Yu X, Jiang Y.** 2011. Natural variation of drought response in
878 Brachypodium distachyon. *physiologia plantarum* **141**: 19–29.
- 879 **Mace E, Tai S, Innes D, Godwin I, Hu W, Campbell B, Gilding E, Cruickshank
880 A, Prentis P, Wang J, et al.** 2014. The plasticity of NBS resistance genes in
881 sorghum is driven by multiple evolutionary processes. *BMC plant biology* **14**: 253.
- 882 **Manzaneda AJ, Rey PJ, Bastida JM, Weiss-Lehman C, Raskin E, Mitchell-Olds
883 T.** 2013. Environmental aridity is associated with cytotype segregation and
884 polyploidy occurrence in Brachypodium distachyon (Poaceae) Antonio. *New
885 phytologist* **193**: 797–805.
- 886 **Mchale L, Tan X, Koehl P, Michelmore RW.** 2006. Plant NBS-LRR proteins:
887 adaptable guards. *Genome Biology* **7**: 212.
- 888 **Messer PW.** 2015. H-scan: Detecting hard and soft sweeps in population genomic
889 data. **3**: 1–4.
- 890 **Michelmore RW, Meyers BC.** 1998. Clusters of Resistance Genes in Plants Evolve

- 891 by Divergent Selection and a Birth-and-Death Process. *Genome research* **530**:
892 1113–1130.
- 893 **Mitchell-Olds T, Willis JH, Goldstein DB. 2007.** Which evolutionary processes
894 influence natural genetic variation for phenotypic traits? *Nature reviews Genetics*
895 **8**: 845–56.
- 896 **Mondragón-palomino M, Meyers BC, Michelmore RW, Gaut BS. 2002.** Patterns
897 of Positive Selection in the Complete NBS-LRR Gene Family of *Arabidopsis*
898 *thaliana*. *Genome research* **12**: 1305–1315.
- 899 **Mor F, Ceylan S. 2017.** Cadmium and lead contamination in vegetables collected
900 from industrial , traffic and rural areas in Bursa Province, Turkey. *Food Additives*
901 *& Contaminants* **25**: 611–615.
- 902 **Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD. 2013.**
903 Population genomic and genome-wide association studies of agroclimatic traits in
904 sorghum. *Proceedings of the National Academy of Sciences* **110**: 453–458.
- 905 **Mur LAJ, Bosch M. 2016.** Linking Dynamic Phenotyping with Metabolite Analysis
906 to Study Natural Variation in Drought Responses of *Brachypodium distachyon*.
907 *frontiers in plant science* **7**: 1–15.
- 908 **Nielsen R. 2005.** Molecular Signatures of Natural Selection. Annual Review of
909 Genetics. **39**: 197–218.
- 910 **Ooijen G Van, Mayr G, Kasiem MMA, Albrecht M, Cornelissen BJC, Takken**
911 **FLW. 2017.** Structure – function analysis of the NB-ARC domain of plant disease
912 resistance proteins. *journal of experimental botany* **59**: 1383–1397.
- 913 **Opanowicz M, Vain P, Draper J, Parker D, Doonan JH. 2008.** *Brachypodium*
914 *distachyon*: making hay with a wild grass. *Trends in Plant Science* **13**: 172–177.
- 915 **País SM, Téllez-iñón MT, Capiati DA. 2009.** Serine / threonine protein
916 phosphatases type 2A and their roles in stress signaling. *Plant signaling and*
917 *behaviour* **4**: 1013–1015.
- 918 **Park C, Caddell DF, Ronald PC. 2012.** Protein phosphorylation in plant
919 immunity: insights into the regulation of pattern recognition receptor-mediated
920 signaling. *frontiers in plant science* **3**: 1–9.
- 921 **Pavlidis P, Jensen JD, Stephan W, Stamatakis A. 2012.** A Critical Assessment of
922 Storytelling: Gene Ontology Categories and the Importance of Validating
923 Genomic Scans Research article. *Molecular biology and evolution* **29**: 3237–3248.
- 924 **Pfeifer B, Wittelsbürger U, Ramos-Onsins SE, Lercher MJ. 2014.** PopGenome:
925 An efficient swiss army knife for population genomic analyses in R. *Molecular*
926 *Biology and Evolution* **31**: 1929–1936.
- 927 **Platt A, Horton M, Huang YS, Li Y, Anastasio AE, Mulyati NW, Bossdorf O,**
928 **Byers D, Donohue K, Dunning M, et al. 2010.** The Scale of Population Structure
929 in *Arabidopsis thaliana*. *plos genetics* **6**: e1000843.
- 930 **Quinlan AR, Hall IM. 2010.** BEDTools: a flexible suite of utilities for comparing
931 genomic features. *Bioinformatics* **26**: 841–842.
- 932 **R development core team. 2017.** R: A language and environment for Statistical
933 Computing.

- 934 **Rasmussen MD, Hubisz MJ, Gronau I, Siepel A. 2014.** Genome-Wide Inference of
935 Ancestral Recombination Graphs. *PLoS genetics* **10**: e1004342.
- 936 **Roulin A, Bourgeois Y, Stiefel U, Walser J-C, Ebert D.** A photoreceptor underlies
937 natural variation in *Daphnia magna* diapause induction. *Molecular Biology and*
938 *Evolution*. **12**:3194–3204.
- 939 **Savolainen O, Lascoux M, Merilä J. 2013.** Ecological genomics of local adaptation.
940 *Nature reviews Genetics* **14**: 807–820.
- 941 **Schlamp F, Van Der Made J, Stambler R. 2016.** Evaluating the performance of
942 selection scans to detect selective sweeps in domestic dogs. *Molecular Ecology* **25**:
943 342–356.
- 944 **Schrider DR, Kern AD. 2016.** S/HIC: Robust Identification of Soft and Hard
945 Sweeps Using Machine Learning. *PLoS genetics* **12**(3): 1–31.
- 946 **Singh S, Chand S, Singh NK, Sharma TR. 2015.** Genome-Wide Distribution ,
947 Organisation and Functional Characterization of Disease Resistance and Defence
948 Response Genes across Rice Species. *Plos one* **10**:e0125964
- 949 **Slavov GT, Nipper R, Robson P, Farrar K, Allison GG, Bosch M, Clifton-brown**
950 **JC, Donnison IS, Jensen E. 2014.** Genome-wide association studies and
951 prediction of 17 traits related to phenology, biomass and cell wall composition in
952 the energy grass *Miscanthus sinensis*. *New phytologist* **201**: 1227–1239.
- 953 **Slotte T. 2014.** The impact of linked selection on plant genomic variation. *briefings*
954 *in functional genomics* **13**: 268–275.
- 955 **Stamatakis A, Alachiotis N, Pavlidis P, Daniel Z. 2013.** SweeD: Likelihood-
956 Based Detection of Selective Sweeps in Thousands of Genomes. *Molecular*
957 *biology and evolution* **30**: 2224–2234.
- 958 **Stinchcombe JR, Hoekstra HE. 2008.** Combining population genomics and
959 quantitative genetics: finding the genes underlying ecologically important traits.
960 *Heredity* **100**: 158–170.
- 961 **Stritt C, Gordon SP, Wicker T, Vogel JP, Roulin AC. 2018.** Recent activity in
962 expanding populations and purifying selection have shaped transposable element
963 landscapes across natural accessions of the Mediterranean grass *Brachypodium*
964 *distachyon*. *Genome Biology and Evolution* **10**:304–318.
- 965 **Stromberg C. 2011.** Evolution of Grasses and Grassland Ecosystems. *Annual review*
966 *of earth and planetary science* **39**: 517–544.
- 967 **Stukenbrock EH, Banke S, Javan-nikkhah M, Mcdonald BA. 2005.** Origin and
968 Domestication of the Fungal Wheat Pathogen *Mycosphaerella graminicola* via
969 Sympatric Speciation. *Molecular biology and evolution* **24**: 398–411.
- 970 **Sun J, Hu W, Zhou R, Wang L. 2015.** The *Brachypodium distachyon* BdWRKY36
971 gene confers tolerance to drought stress in transgenic tobacco plants. *Plant cell*
972 *reports* **34**: 23–35.
- 973 **Svenning J, Normand S, Kageyama M. 2008.** Glacial refugia of temperate trees in
974 Europe: insights from species distribution modelling. *journal of ecology* **96**:
975 1117–1127.
- 976 **Tang K, Thornton KR, Stoneking M. 2007.** A New Approach for Using Genome
977 Scans to Detect Recent Positive Selection in the Human Genome. *PLoS biology*

978 5:e171

979 **Tarasov A, Vilella AJ, Cuppen E, Nijman IJ, Prins P. 2017.** Genome analysis
980 Sambamba: fast processing of NGS alignment formats. *Bioinformatics* **31**: 2032–
981 2034.

982 **Teixeira C, Filippo C De, Key FM, Andre AM. 2014.** ScienceDirect Advantageous
983 diversity maintained by balancing selection in humans. *Current opinion in genetics*
984 *& development* **29**: 45–51.

985 **The international brachypodium Consortium. 2010.** Genome sequencing and
986 analysis of the model grass *Brachypodium distachyon*. *Nature* **463**: 763–8.

987 **Tyler AL, Lee SJ, Young ND, Deulio GA, Benavente E, Reagon M, Sysopha J,**
988 **Baldini RM, Troia A, Hazen SP, et al. 2016.** Population structure in the model
989 grass *Brachypodium distachyon* is highly correlated with flowering differences
990 across broad geographic areas. *The Plant Genome* **9**:1–55.

991 **Wadgyamar SM, Lowry DB, Gould BA, Byron CN, Mactavish RM, Anderson JT.**
992 **2017.** Identifying targets and agents of selection: innovative methods to evaluate
993 the processes that contribute to local adaptation. *Methods in Ecology and Evolution*
994 **8**: 738–749.

995 **Wallace JS, Batchelor CH. 1997.** Managing water resources for crop production.
996 *Philosophical transactions of the Royal Society of London. Series B, Biological*
997 *sciences* **352**: 937–947.

998 **Wu Q, Han T, Chen X, Chen J, Zou Y, Li Z, Xu Y. 2017.** Long-term balancing
999 selection contributes to adaptation in *Arabidopsis* and its relatives. *Genome*
1000 *biology* **18**: 217.

1001 **Wyand RA, Brown K. 2003.** Genetic and forma specialis diversity in *Blumeria*
1002 *graminis* of cereals and its implications for host-pathogen co-evolution. *Molecular*
1003 *plant pathology* **4**: 187–198.

1004 **Zhong Y, Li Y, Huang K, Cheng Z. 2015.** Species-specific duplications of NBS-
1005 encoding genes in Chinese chestnut (*Castanea mollissima*). *Scientific reports* **5**:
1006 16638.

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1011 **Figures**

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1013 **Figure 1: Schematic representation of the regimes of selection analyzed in this** 1014 **study**

1015 A) Cross-population tests: in case of positive selection on a new mutation, SNPs
1016 linked to the advantageous mutation will also rise in frequency, leading to extended
1017 haplotypes in the vicinity of the selected mutation. Cross-population tests detect
1018 haplotypes that are positively selected in one population by estimating the length of
1019 haplotypes around each allele at a core SNP, then comparing these lengths between
1020 populations. Such tests are designed to identify hard-sweep, i.e. region where one
1021 haplotype reached near fixation B) Within-population tests: these scans measure
1022 haplotype length at a focal locus and compare it to the average length of haplotypes
1023 along the genome in a given population. They allow recovering regions with
1024 haplotypes longer than average (top 1% outliers) and can identify loci under selection
1025 independently of the number of haplotypes or of the completeness of the sweep C)
1026 Coalescence approach: balancing selection can maintain shared polymorphisms
1027 across populations due the habitat heterogeneity or negative frequency-dependent
1028 selection. Associated to old alleles, long-term balancing selection can be detected by
1029 calculating time to most recent common ancestor (TMRCA), which should increase at
1030 loci maintained by selection over long period of time.

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1032 **Figure 2: Geographical origin and genetic diversity of the 44 accessions**

1033 A) Geographical origin of the 44 accessions used in the study. The asterisk indicates
1034 the origin of the reference accession Bd21 B) Structure plot (K=2) displaying the
1035 limited level of admixture between the eastern and western population. Each bar
1036 represents the genetic data of one accession. An accession presents some admixture
1037 when the bar displays different colors. The height of the bar is proportional to the
1038 admixture level C) Nucleotide diversity (π in 5 kb windows) within the eastern and
1039 western populations D) LD decay in the eastern and western populations.

1040

1041 **Figure 3: Density of genes under selection**

1042 A) Number of genes per high-confidence region for the high-confidence gene sets B)
1043 Circular representation of gene density along chromosomes (i) density of genes under
1044 positive selection in the eastern population (DiploS/HIC-Rsb and DiploS/HIC-Hscan
1045 combined), scaled from 0 to 20 genes per 100 kb (ii) density of genes under positive

1046 selection in the western population (DiploS/HIC-Rsb and DiploS/HIC-Hscan
1047 combined), scaled from 0 to 20 genes per 100 kb (iii) overall gene density, scaled
1048 from 0 to 40 genes per 100 kb.

1049

1050 **Figure 4: QTL involved in resistance to rust**

1051 A) Rsb signals observed in QTL_{rust-3} (left panel) and QTL_{rust-4} (right panel). The
1052 dashed line indicates the 1% $-\log_{10}(\text{Rsb P-values})$ outlier threshold. Note that in order
1053 to reduce the size of the figure, points with $-\log_{10}(\text{Rsb P-values})$ values <1 are not
1054 displayed. Grey boxes display the position of regions detected by diploS/HIC. Red
1055 bars display the position of genes detected by the Rsb test B) Haplotype bifurcation
1056 diagram in QTL sub-regions displaying strong of selection. Left panel: zoom in on
1057 chromosome 3; right panel: zoom in on chromosome 4. The diagrams visualize the
1058 breakdown of LD at increasing distances from the selected focal SNP, displayed by a
1059 vertical black line. Each horizontal line represents a haplotype. Each accession is
1060 represented by two lines, one for each haplotype. Horizontal blue lines are merged
1061 when two accessions share the same haplotype. The thickness of the line is therefore
1062 correlated to the number of accessions sharing the same haplotype.

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1065 **Tables**

1066 **Table 1: Significant GO terms associated to each high-confidence gene set**

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Table 1: significant GO term associated to each test of selection

Test	Significant GO process	Pvalue
TMRCA	Phosphorylation	3.13E-08
DiploS/HIC-Rsb East	Response to stress including: - defense response	5.00E-04 9.4E-04
DiploS/HIC-Rsb West	Amide transport	5.00E-03
DiploS/HIC-Hscan East	Response to cadmium ion Small molecule metabolic process	6.6E-04 3.8E-03
DiploS/HIC-Hscan West	Pyruvate metabolic process Carbohydrate derivative metabolic process Phospholipid metabolic process	3.80E-03 3.80E-03 3.40E-03

A **B** **C**

A **B** **C** **D**

A **B**

A **B****West** **East**

Chromosome 3

Chromosome 4