

# Power and limits of selection genome scans on temporal data from a selfing population

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1 Tracking genetic changes of populations through time allows a more direct study of the evolutionary processes acting on the population than a single contemporary sample. Several statistical methods have been developed to characterize the demography and selection from temporal population genetic data. However, these methods are usually developed under the assumption of outcrossing reproduction and might not be applicable when there is substantial selfing in the population. Here, we focus on a method to detect loci under selection based on a genome scan of temporal differentiation, adapting it to the particularities of selfing populations. Selfing reduces the effective recombination rate and can extend hitch-hiking effects to the whole genome, erasing any local signal of selection on a genome scan. Therefore, selfing is expected to reduce the power of the [method test](#). By means of simulations, we evaluate the performance of the method under scenarios of adaptation from new mutations or standing variation at different rates of selfing. We find that the detection of loci under selection in predominantly selfing populations remains challenging even with the adapted method. Still, selective sweeps from standing variation on predominantly selfing populations can leave some signal of selection around the selected site thanks to historical recombination before the sweep. Under this scenario, ancestral advantageous alleles at low frequency leave the strongest local signal, while new advantageous mutations leave no local footprint of the sweep.

29 [selective sweep](#) | [neutrality test](#) | [temporal differentiation](#) | [Medicago truncatula](#)

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## 39 Introduction

40 Several evolutionary processes (such as migration, selection or drift) can change the genetic make-up of populations through time. Thus, patterns of genetic diversity can inform us about the evolutionary history of the populations (see Pool *et al.* 2010, for a review). However, observing the genetic diversity changes through time (instead of at a single time point) can provide more precise information about the evolutionary processes in

48 action.

49 Since the beginning of the 20th century, researchers have used repeated observations of hereditary characters in the same populations (e.g. color patters in *Diabrotica soror*, Kellogg and Bell 1904) (e.g. color patterns in *Diabrotica soror*, Kellogg and Bell 1904) or subfossil records (e.g. banding patterns in *Cepaea* snails, Diver 1929) to study evolution. An emblematic example is the time-series data on the frequency of the *medionigra* phenotype in a population of the moth *Callimorpha dominula*, which inspired the discussion on the prevalence of selection over drift between Fisher and Wright (Fisher and Ford 1947; Wright 1948) and has continued to offer insight into the evolutionary process through recent re-analyses (e.g. Foll *et al.* 2014, and references therein).

64 The technological advances in molecular genetics have allowed these temporal studies to switch from Mendelian characters to polytene chromosomes (e.g. Dobzhansky 1943), isozymes (e.g. Yamazaki 1971) and, eventually, to high throughput DNA sequencing (e.g. Frachon *et al.* 2017). Indeed, molecular genetics has opened the door to the study of short-generation-time microorganisms (Biek *et al.* 2015), ancient samples (e.g. subfossil samples, museum and herbaria specimens; Leonardi *et al.* 2017) and experimental populations (Schlötterer *et al.* 2015), allowing for an increasing availability of temporal population genetic data.

76 Temporal population genetic data [allows allow](#) to study the change of allele frequencies through time. In the absence of migration, mutation and selection, these changes are the product of genetic drift. As such, they can be used to estimate the effective population size,  $N_e$ , either with moment based (e.g. Krimbas and Tsakas 1971; Nei and Tajima 1981; Waples 1989) or likelihood-based approaches (e.g. Anderson *et al.* 2000; Williamson and Slatkin 1999). If a sample from the source of migration is available, it is also possible to co-estimate migration and drift with an extension of the likelihood method (Wang and Whitlock 2003). These methods all assume short timescales and low mutation rates, so that no new mutations arrive during the studied period. However, for temporal data over larger scales in which mutations can no longer be neglected, it is also

possible to co-estimate substitution rate with effective population size (Drummond *et al.* 2002; Rambaut 2000). Finally, like for migration or mutation rates, it is also possible to make inferences about selection. Two strategies can be followed. First, three or more temporal samples may be used to separate the random component (drift) from the systematic component (selection) in the changes of allele frequencies (e.g. Bollback *et al.* 2008; Buffalo and Coop 2019; Feder *et al.* 2014). Alternatively, if only two temporal samples are available, loci under selection may be detected by an outlier approach (e.g. Goldringer and Bataillon 2004, described in more detail below).

Most of the statistical methods in population genetics, including those mentioned above, have been developed for outcrossing populations. Methods specifically adapted to selfing populations are scarce (Hartfield *et al.* 2017). Nevertheless, many plants reproduce through selfing or partial selfing (see Whitehead *et al.* 2018, for a recent overview), including an important proportion of the species considered in temporal monitoring programs (e.g. supplementary text S1). Selfing, by increasing homozygosity and linkage disequilibrium, shapes the genetic diversity of **population populations** in a particular way (Golding and Strobeck 1980; Vitalis and Couvet 2001). Notably, it generates repeated multi-locus genotypes that can **persists-persist** over several generations due to the lack of effective recombination (Jullien *et al.* 2019). The dynamics of adaptation is also impacted by selfing, with a general reduction in the efficacy of selection (e.g. Burgarella *et al.* 2015). In the light of these drastic effects, there is a need for development or adaptation of methods to take into account different mating systems.

Even if we can adapt methods to release the assumption of random mating, distinguishing selection from demography in highly selfing species may be problematic because of the reduced effective recombination due to self-fertilization. Selective sweeps in these populations could involve a genome-wide hitch-hiking effect, which prevents any difference in genetic diversity between the neutral and adaptive regions. If that were the case, even methods adapted to selfing would fail to detect regions under selection, which questions the relevance of temporal genome scans in predominantly selfing populations. On the other hand, a temporal genome scan on a highly selfing *Arabidopsis thaliana* population (**selfing rate**,  $\sigma \approx 0.94$ ) revealed several **outliers-outlier** regions with compelling evidence for the action of selection on them (Frachon *et al.* 2017). Therefore, in the planning of future research, there is a need to understand under which circumstances selfing imposes a limit for the detection of loci under selection.

In this work, we introduce several modifications to the temporal genome scan approach proposed by Goldringer and Bataillon (2004) to take into account partial self-fertilization. Then, by means of simulated

data, we evaluate the performance of **that-this** method for the estimation of the effective population size (the first step of this genome scan) and the detection of regions under selection under different scenarios of adaptation (from new mutations or from standing variation) and selfing. Our results highlight the importance of taking into account the mating system in the analysis of population genetic data. They also highlight a threshold beyond which loci under selection **can-cannot** be detected for highly selfing populations. We applied the approach to a population of the predominantly selfing species *Medicago truncatula* and re-discuss some of the results from Frachon *et al.* (2017) temporal genome scan on *Arabidopsis thaliana*.

## Materials and Methods

**Overview of the Genome Scan Method.** In a single isolated population, allele frequencies change through time under the action of selection, **that-which** acts upon specific loci, and genetic drift, **that-which** acts upon the whole genome. In order to identify loci subjected to selection, we use a procedure inspired from the test for homogeneity of differentiation across loci by Goldringer and Bataillon (2004). The principle is that, in the case of complete neutrality across loci, all sampled markers should provide estimates of genetic differentiation drawn from the same distribution. Assuming a single isolated population, this distribution depends on the strength of genetic drift, that is, on the length of the period,  $\tau$  in number of generations, and on the effective population size,  $N_e$  (see Table S1 for a summary of notation). On the other hand, if some of the studied polymorphisms are under selection or linked to selected variants, we expect some heterogeneity in the distribution of differentiation values, because directional selection induces larger values of **of-genetic differentiation** than expected under the neutral case. The approach we describe uses the expected distribution of temporal differentiation to identify those polymorphisms showing outlier values compared to a neutral expectation. In Frachon *et al.* (2017), we already made some modifications (to account for the uncertainty of the initial allele frequency) to the approach proposed by Goldringer and Bataillon (2004) for the standard (random-mating or haploid) case. Here, we present further modifications for a more general case including partial self-fertilization.

**Estimation of effective population size.** The estimated magnitude of drift between two time samples is used as a null model in this temporal genome scan method. Temporal differentiation can be measured by estimating the  $F_{ST}$  with the analysis of variance approach proposed by Weir and Cockerham (1984). Weir and Cockerham's (1984) analysis of variance partitions variance within individuals, among individuals within population and between populations, allowing to account for

204 the correlation of allele identity within individuals due  
 205 to selfing in the  $F_{ST}$  estimate. Temporal  $F_{ST}$  can be used  
 206 to estimate  $N_e$  as  $\hat{N}_e = \frac{\tau(1-\hat{F}_{ST})}{4\hat{F}_{ST}}$  (Frachon *et al.* 2017;  
 207 Skoglund *et al.* 2014).

208 **Building null distribution of drift.** In order to test the  
 209 homogeneity between the focal-locus  $l$  and genome-  
 210 wide differentiation, the null distribution for single lo-  
 211 cus  $F_{ST}^l$  is ~~build~~ built through simulations of drift. ~~For~~  
 212 ~~these simulations~~, Each of these simulations consists  
 213 of the following steps: 1) draw initial allele frequency  
 214  $\pi_0$  of the locus (conditional on data), 2) simulate allele  
 215 frequency change for  $\tau$  generations (based on  $\hat{N}_e$ ), 3)  
 216 simulate samples by sampling genotypes (genotype  
 217 frequencies based on  $\hat{F}_{IS}$ ) and 4) calculate  $F_{ST}^*$  for the  
 218 simulated sample. The proportion of  $F_{ST}^*$  equal or  
 219 larger than the observed  $F_{ST}^l$  provides an estimate of  
 220 the  $p$ -value for the test. A detailed description of these  
 221 steps follows.

222 Goldringer and Bataillon (2004) considered the ob-  
 223 served allele frequency in the sample as the initial al-  
 224 lele frequency in the population,  $\pi_0$  (from this point  
 225 subscript 0 indicates values at time  $t = 0$ ). This ap-  
 226 proach ignores the uncertainty due to sampling. In-  
 227 stead, in Frachon *et al.* (2017), we improved this step by  
 228 assuming that allele counts observed in a sample of  $n_0$   
 229 diploid individuals come from a binomial distribution  
 230  $B(2n_0, \pi_0)$ , where  $\pi_0$  is the (unknown) allele frequency  
 231 in the population. Using Bayes inversion formula and  
 232 assuming a uniform prior for the allele frequency, this  
 233 allows to sample from the posterior probability distri-  
 234 bution with  $Beta(k_0 + 1, 2n_0 - k_0 + 1)$ , where  $k_0$  is the  
 235 observed count of the reference allele in the sample.  
 236 However, this assumes that allele copies within an in-  
 237 dividual are independent samples from the population.  
 238 In (partially) selfing populations, gene copies are not  
 239 independent samples, but individuals are. Genotype  
 240 counts observed in the simulated sample of  $n_0$  indi-  
 241 viduals can be modelled as coming from a multinomial  
 242 distribution  $Mult(n_0, \gamma_0)$ , where  $\gamma_0$  are the geno-  
 243 type frequencies in the population. Similarly to Fra-  
 244 chon *et al.* (2017), assuming the same prior probability  
 245 for the three genotype frequencies, we sample genotype  
 246 frequencies in the population from the posterior proba-  
 247 bility distribution with ~~Dir( $K_0, 1$ )Dir( $K_0 + 1$ )~~, where  $K_0$   
 248 is the observed genotype counts in the sample of the fo-  
 249 cal locus at time  $t = 0$ .

250 Allele frequencies  $\pi_t$  at subsequent generations ( $t \in$   
 251  $[1, \tau]$ ) were simulated following a binomial distribu-  
 252 tion as  $\pi_t \sim B(2\hat{N}_e, \pi_{t-1}) / 2\hat{N}_e$ , where  $\hat{N}_e$  is the genome  
 253 wide estimate of the effective population size and  $\pi_0$  is  
 254 determined by  $\gamma_0$ . Simulated genotype counts in sam-  
 255 ple at time  $t = \tau$ ,  $K_\tau^*$ , were taken from a multinomial  
 256 distribution,  $K_\tau^* \sim Mult(n_\tau, \gamma_\tau)$ , where  $n_\tau$  is the sam-  
 257 ple size (in number of diploid individuals) at time  $t = \tau$   
 258 and  $\gamma_\tau$ :

$$\gamma_{AA,\tau} = \pi_\tau^2 + \hat{F}_{IS}(1 - \pi_\tau)\pi_\tau$$

$$\gamma_{Aa,\tau} = 2(1 - \pi_\tau)\pi_\tau(1 - \hat{F}_{IS})$$

$$\gamma_{aa,\tau} = (1 - \pi_\tau)^2 + \hat{F}_{IS}(1 - \pi_\tau)\pi_\tau$$

are the genotype frequencies in the populations ~~in as a~~  
 function of the allele frequency  $\pi_\tau$  and inbreeding co-  
 efficient  $F_{IS}$  (Haldane 1924), ~~assuming constant selfing~~  
 rate and using the Weir and Cockerham's (1984) multi-  
 locus inbreeding coefficient estimate ~~.~~

~~For each simulation, we estimated the genetic~~  
 differentiation  $F_{ST}^*$ . ~~The proportion of  $F_{ST}^*$  equal or~~  
 larger than the observed  $F_{ST}^l$  provides an estimate of  
 the  $p$ -value for the test from both temporal samples.

Preliminary results revealed that filtering loci accord-  
 ing to minor allele frequency (MAF) was required to  
 assure a uniform distribution of  $p$ -values from neu-  
 tral sites (Fig. S1). Distribution of  $p$ -values is impor-  
 tant for studies at the genomic scale where thousands  
 of loci are tested (see François *et al.* 2016, for a re-  
 view). In these studies, a false discovery rate (FDR) is  
 estimated to control for multiple testing and this FDR  
 estimation assumes the uniformity of  $p$ -values under  
 the null model (Storey 2002). The criterion to filter  
 loci was to have a minimum global MAF: loci with  
 $\min\left(\frac{p_0 + p_\tau}{2}, 1 - \frac{p_0 + p_\tau}{2}\right) < 0.05$  were removed from the  
 dataset. Thus, loci to be tested were chosen based on  
 their genetic diversity, a bias that has to be taken into ac-  
 count in the test. This was done by discarding drift sim-  
 ulations that produced  $\min\left(\frac{p_0^* + p_\tau^*}{2}, 1 - \frac{p_0^* + p_\tau^*}{2}\right) < 0.05$ ,  
 where  $p_i^*$  is the frequency of the reference allele in the  
 sample at time  $t$  in the simulation.

**Simulations.** We produced simulated data using the  
 individual-based forward population genetics simula-  
 tor SLiM 1.8 (Messer 2013). We considered a single iso-  
 lated population sampled twice, at the beginning and  
 at the end of a time interval of  $\tau$  generations. The  
 population size  $N = 500$  (number of diploid individ-  
 uals) and the selfing rate  $\sigma$  were constant through time,  
 and the effective population size was  $N_e = \frac{(2-\sigma)N}{2}$  un-  
 der neutrality (Li 1955; Pollak 1987). The genome was  
 composed by two linkage groups of size  $2.5 \times 10^8$  base  
 pairs. The neutral mutation rate per base pair was  
 $\mu = 10^{-8}$  and the recombination rate between base pairs  
 was  $r = 10^{-8}$ . The recombination rate between the two  
 linkage groups was 0.5.

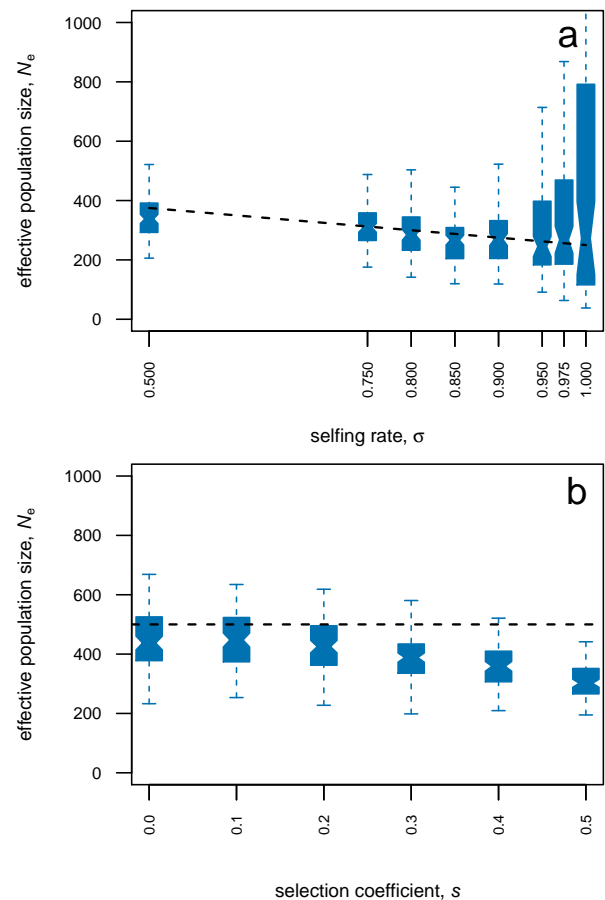
In order to start our simulations with a population  
 at mutation-drift equilibrium, SLiM was run for  $20N_e$   
 generations. After that period of neutral evolution, two  
 different selective scenarios were simulated: adapta-  
 tion from new mutations or from standing variation.  
 In the first case a new advantageous dominant muta-  
 tion was introduced at  $t = 0$  with a selection coefficient  
 $s$  at a random position in the genome. In the case of  
 selection on standing variation, a random neutral poly-  
 morphic site was chosen at  $t = 0$  and a selection coeffi-  
 cient  $s$  was assigned randomly to one of the two alleles,

312 which also **becomes** became dominant. The initial conditions of the simulations of selection on standing variation are, therefore, variable. The initial frequency of the allele under selection is known to affect the signal of the sweep: an advantageous allele starting at high frequency is expected to leave a lower signal than one starting at a lower frequency (Berg and Coop 2015; Innan and Kim 2004). This will likely affect the power of our test. We therefore studied the effect of the initial frequency of the advantageous allele in scenarios of adaptation from standing variation with additional simulations, where the site under selection was randomly chosen among those sites with the required allele frequency. The frequency of an allele is expected to correlate with its age (Kimura and Ohta 1973), and older alleles can accumulate more mutations in their neighborhood and recombine with the background variation. **That, which** could favour the presence of a local signal of selection (Fig. S2). Because ancestral alleles are older than derived alleles, we also studied the effect of the nature of the allele in the set of simulations **focusing on** of adaptation from standing variation.

334 In order to ease comparisons across scenarios, the advantageous allele was also set as dominant. Indeed, in outcrossing populations adapting from new mutation, advantageous dominant alleles are expected to spread faster than recessive ones, an effect known as Haldane's sieve (Haldane 1927). However, this effect is reduced or absent in predominantly selfing populations (Charlesworth 1992; Ronfort and Glémin 2013) and populations adapting from standing variation (Orr and Betancourt 2001).

344 Simulated populations were sampled at generations  $t = 0$  and  $t = \tau$ , with sample sizes  $n_0 = n_\tau = 50$  diploid individuals. Data for 10,000 polymorphic loci were taken randomly from all polymorphic sites in the sample, except for the locus under selection that was always included in the data.

350 Different scenarios were considered by exploring values of selfing rate  $\sigma \in [0, 0.5, 0.75, 0.8, 0.85, 0.9, 0.95, 0.99, 1]$ , selection coefficient  $s \in [0, 0.1, 0.2, 0.3, 0.4, 0.5]$ , duration of period of selection  $\tau \in [5, 10, 25, 50, 100, 200]$  (in generations), type of selection (neutral, new mutation or standing variation) and, in the case of selection from standing variation, whether the locus was chosen randomly among all loci or among the loci with the required initial frequency ( $\pi_0 \in [0.1, 0.5, 0.9]$ ) and nature (ancestral or derived) of the allele becoming advantageous. The combinations of parameter values were chosen to highlight the patterns studied in this work by creating strong selective sweeps. For each scenario, 100 simulation replicates were performed. Replicates in which the advantageous allele was lost were discarded and replaced by additional replicates.



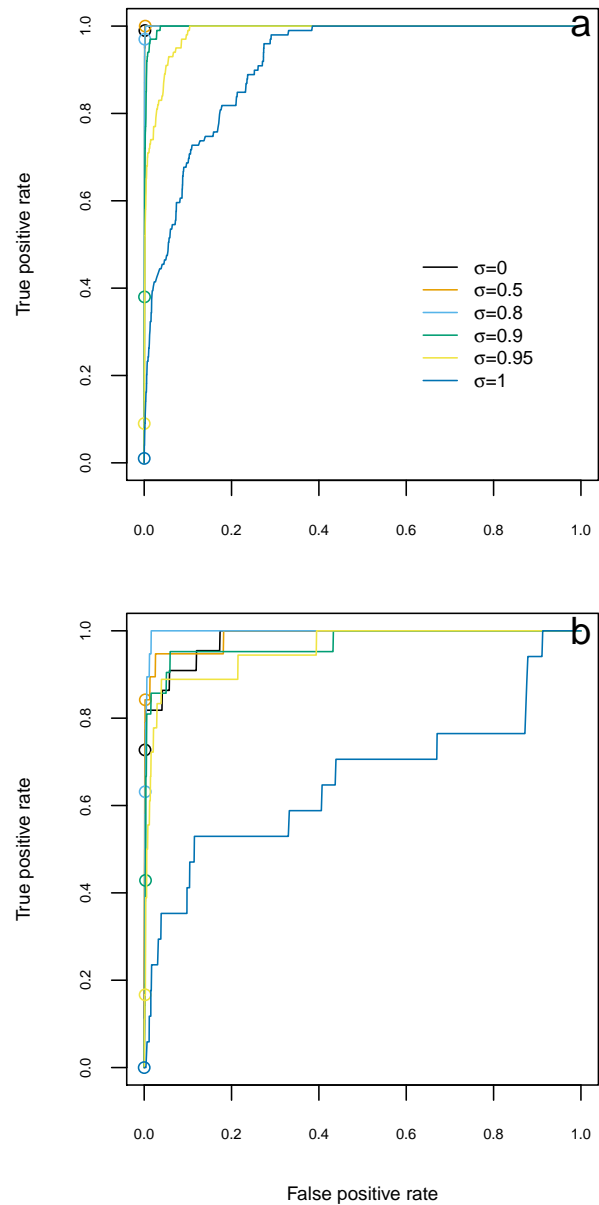
**Fig. 1. Effective population size estimates from temporal differentiation.** Estimates of  $N_e$  from  $F_{ST}$  were obtained from simulated data of populations of  $N = 500$  diploid individuals, sampled twice with  $\tau = 25$  generations between samples and selfing rate  $\sigma$ . **(a)** Neutral selfing population. **(b)** Outcrossing population under selection: a new advantageous mutation appears at generation  $t = 0$  with coefficient of selection  $s$ . Samples are made of 50 diploid individuals genotyped at 10,000 biallelic markers (including the locus under selection), but only loci with a global MAF over 0.05 are used in the estimate (see details in Materials and Methods). Boxplot for estimates from 100 simulation replicates. Dashed line marks true effective population size,  $N_e$ , in panel **(a)** and census size,  $N$ , in panel **(b)**.

**Analysis of simulated data.** For each simulation replicate, data were analysed as described in the two previous sections. In addition, the effective population size was also estimated from  $F_C$  (following Waples 1989) for comparison. For a given scenario, the true positive rate was estimated from the test results at loci under selection. The false positive rate was estimated from the test results at the neutral loci on the linkage group that does not include the locus under selection. Neutral loci on the linkage group with the locus under selection were used to characterize the footprint of selection due to hitch-hiking by estimating the positive rate as a function of the distance to the locus under selection. In order to quantify the variance of the selection signal, loci were bootstrapped and the 95% quantile interval for the proportion of positive tests was calculated. Positive tests were defined with an arbitrary threshold of  $p$ -value  $< 0.001$ . In order to control for multiple testing, FDR estimates were quantified with  $q$ -values for each locus, following Storey (2002), with the *qvalue* R pack-

387 age (Storey *et al.* 2019). Manhattan and QQ plots were  
388 generated with *qqman* R package (Turner 2017).

389 As explained in the previous section, the genetic diver-  
390 sity around the selected locus could influence the out-  
391 lier test in a scenario of adaptation from standing vari-  
392 ation in a predominantly selfing species (Fig. S2). ~~In~~  
393 ~~such scenarios, we therefore~~ Therefore, we examined  
394 the structure of genetic diversity at the beginning of the  
395 sweep in those scenarios. This allowed us to charac-  
396 terize the effects of historical recombination previous  
397 to the outset of the selective sweep. For each simu-  
398 lated population, the haplotypes, defined as the whole  
399 linkage group under selection, were classified in two  
400 groups: one group for haplotypes carrying the advan-  
401 tageous allele and one group for haplotypes with the  
402 neutral allele. Genetic diversity (measured as the aver-  
403 age expected heterozygosity per bp) within the haplo-  
404 types carrying the advantageous allele and differentia-  
405 tion between the two groups of haplotypes (measured  
406 as the  $F_{ST}$ ) were calculated ~~on~~ in 3,000 bp windows at  
407 increasing distance from the locus under selection.

408 **Real data application.** *Medicago truncatula* is an annual,  
409 predominantly selfing species (Siol *et al.* 2008) of the  
410 legume family (Fabaceae), found around the Mediter-  
411 ranean Basin. We conducted a temporal survey in  
412 a population located in Cape Corsica (42° 58.406' ~~North,~~  
413 ~~9° 22,015' 22,015'~~ East, 362 m.a.s.l.). Samples  
414 of around 100 pods were collected in 1987 and 2009  
415 along three transects running across the population,  
416 with at least one meter distance between each pod col-  
417 lected in order to avoid over-sampling the progeny of  
418 a single individual. The seeds were stored in a cold  
419 room between collection year and 2011, when plants  
420 were replicated from seeds in standardized greenhouse  
421 conditions. Using these samples, we collected leaf ma-  
422 terial from 64 plants from 1987 and 96 plants from 2009  
423 grown in greenhouse for DNA extraction. Between  
424 100 mg and 200 mg of young leaves were ground in  
425 liquid nitrogen in a Qiagen Retsch Tissue Lyser (Qia-  
426 gen N. V., Hilden, Germany) during 2 × 1 min at 30 Hz.  
427 The fine powder was mixed with 600 µl of pre-heated  
428 lysis buffer consisting of 100 mM Tris-HCl pH 8.0,  
429 20 mM EDTA pH 8.0, 1.4 M NaCl, 2 % (w/v) CTAB,  
430 1 % (w/v) PVP40 and 1 % (w/v) sodium bisulphite plus  
431 8 µl of 10 mg/ml RNase per sample added extempo-  
432 raneously. After incubation for 20 min at 65 °C un-  
433 der medium shaking, 600 µl of chloroform were added  
434 and mixed with a vortex mixer. Each sample was cen-  
435 trifuged for 15 min at 10,000 g and 10 °C and the upper  
436 phase was transferred into a new tube and then mixed  
437 with 60 µl of 3M-3 M sodium acetate and 600 µl of  
438 cold isopropanol. DNA was precipitated by another  
439 centrifugation (30 min, 15,000 g, 4 °C), rinsed with  
440 300 µl of 70 % cold ethanol, centrifuged again (10 min,  
441 15,000 g, 4 °C), dried for 10 min at room tempera-  
442 ture and resuspended in 100 µl of sterile deionized wa-



**Fig. 2. Power and false positive rate of the genome scan for increasing decision thresholds.** Receiver operating characteristic curve is estimated from 100 replicates of simulated data of one population of  $N = 500$  diploid individuals, sampled twice with  $\tau = 25$  generations between samples, selection coefficient  $s = 0.5$  and selfing rate  $\sigma$ . (a) Selection on new mutation. (b) Selection on standing variation. Circles mark the values for positive threshold of  $p$ -value  $> 0.001$ , which is the threshold used in Fig. 3.

443 ter. The genotyping was performed using two SNP  
444 chips specifically developed for *M. truncatula* (Loridon  
445 *et al.* 2013). Out of the 1920 SNPs, 137 were lo-  
446 cated in genes encoding flowering time, 721 in other  
447 candidate genes, in particular genes involved in sym-  
448 biosis, and 1062 at random positions of the genome.  
449 The 1920 SNPs were widespread across all eight link-  
450 age groups (Table S2). The genotyping assays were  
451 achieved at GenoToul (Genomic Platform in Toulouse,  
452 France) and at the BioMedical Genomics Center (Min-  
453 neapolis, University of Minnesota, USA) using Golden-  
454 Gate Assay (Fan *et al.* 2006, 2003) and respectively Illu-

455 mina's VeraCode technology (Lin *et al.* 2009) or Bead  
456 Array technology. Data generated from the BeadX-  
457 pressTM reader (384SNP SNP × 480 DNA) or BeadAr-  
458 ray Reader (1536SNP SNP × 192 DNA) were analyzed  
459 as detailed in Loridon *et al.* (2013).  
460 These data were analysed using the temporal genome  
461 scan described in this paper, providing estimates of  
462 the effective population size (from  $F_{ST}$ ) and the self-  
463 ing rate (from  $F_{IS}$ ) of the population. Confidence  
464 intervals (CI) for those estimates were obtained  
465 by an approximate bootstrap procedure over loci  
466 (DiCiccio and Efron 1992). For loci with a global MAF  
467 higher than 0.05, we obtained a  $p$ -value for the test of  
468 homogeneity. In order to control for multiple testing,  
469 FDR estimates were quantified with  $q$ -values for each  
470 locus, following Storey (2002).

## 471 Results

472 **Accuracy of  $N_e$  estimates.** In neutral scenarios, esti-  
473 mates of effective population size derived from  $F_{ST}$  per-  
474 formed reasonably well, decreasing in the presence of  
475 partial selfing following the  $N_e$  theoretical expectation  
476 (Fig. 1a). Selfing also caused an increase of error in  
477 the estimation of  $N_e$ . In the presence of selection, esti-  
478 mates of effective population size decreased with the  
479 strength of the selective coefficient of the causal muta-  
480 tion (Fig. 1b). As expected, effective population size esti-  
481 mates from  $F_C$  showed a clear bias in the presence of  
482 partial self-fertilization while the bias was negligible in  
483 estimates from  $F_{ST}$  (Fig. S3a).

484 **Footprint of selection.** The temporal genome scan dis-  
485 criminated well between selected and neutral (on a sep-  
486 arate linkage group) sites in most scenarios (Fig. 2). Yet,  
487 in the case of selection on new mutations, extreme self-  
488 ing rate decreased the performance of the test (Fig. 2a).  
489 In scenarios of selection on standing variation the per-  
490 formance of the test was lower (Fig. 2b). In addition, the  
491 effect of selfing was more complex in the case of selec-  
492 tion on standing variation. Moderate levels of selfing  
493 seemed to improve the discrimination capacity while  
494 the performance for high levels of selfing was similar  
495 to the one for outcrossing simulations. Only complete  
496 selfing reduced dramatically the discrimination capac-  
497 ity of the method.

498 These results, however, only consider the causal poly-  
499 morphisms and completely independent neutral vari-  
500 ants, ignoring linked sites that could have been sub-  
501 ject to hitch-hiking. In practice, the signal for the de-  
502 tection of regions under selection comes mainly from  
503 those linked sites, that which can create a local excess  
504 of outlier loci testing positive around the site under se-  
505 lection (even if the later is not in the data set). We there-  
506 fore examined the footprint of selection at increasing  
507 distance from the advantageous allele, within a linkage  
508 group. As expected, the highest probability of positive  
509 test was at the locus under selection; then probability

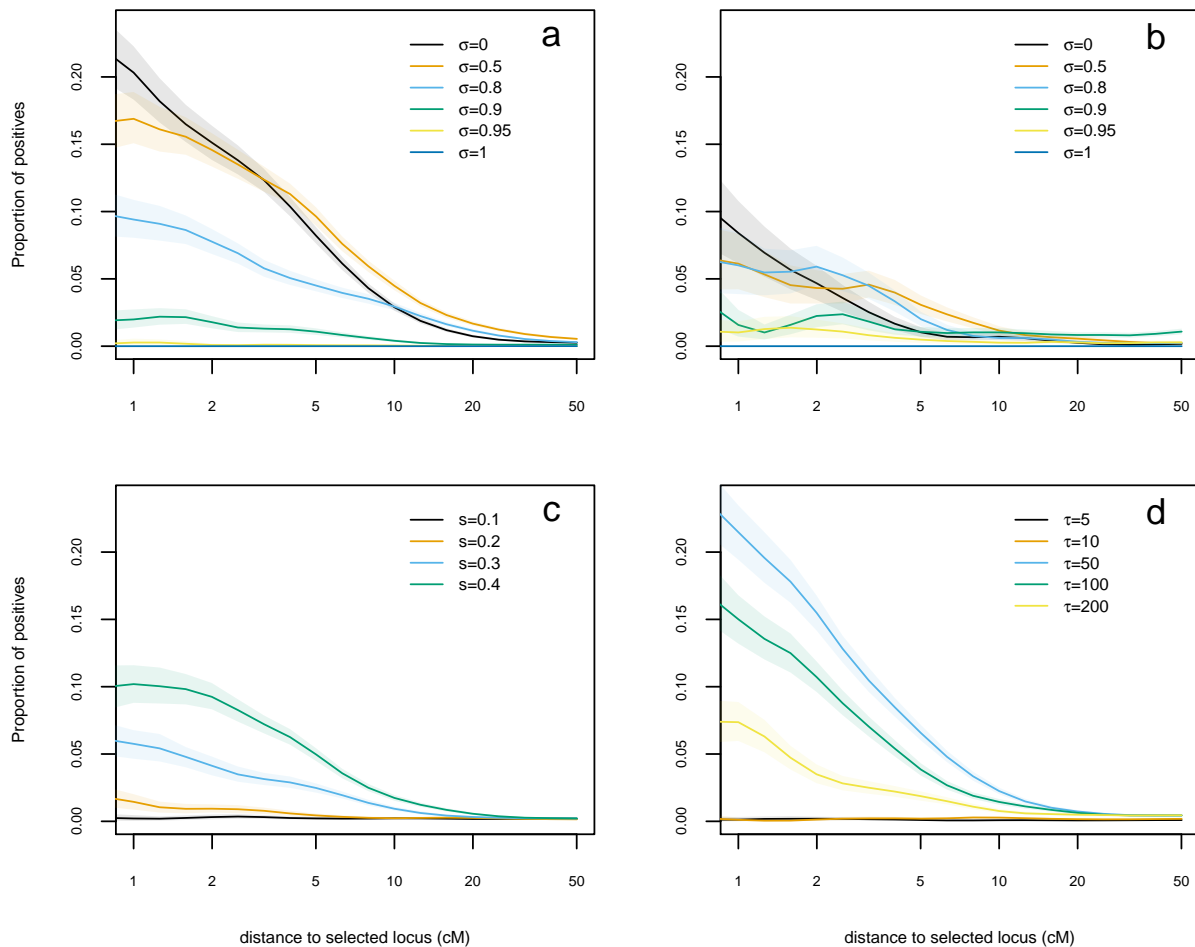
decreased with distance and reached very low values,  
similar to those for neutral loci on a separate linkage  
group (Fig. 3).

The distance at which the hitch-hiking effect-signal  
disappears depends on the scenario, being smaller  
for selection on scenarios of standing variation  
and scenarios with high selfing rate, and larger  
for scenarios on new mutation and scenarios of  
intermediate selfing rate. congruent with results  
by Hartfield and Bataillon (2020). Selfing rate increases  
distance at which there is a hitch-hiking effect  
(Fig. S5; Hartfield and Bataillon 2020). However, this  
did not translate into a signal from  $F_{ST}$  at larger  
distances, but to a reduction in power.

Under a scenario of adaptation from new mutations,  
the overall strength of the signal decreased with in-  
creasing selfing rate (Fig. 3a) and decreasing the selec-  
tion coefficient (Fig. 3c). The time sample interval also  
influenced the strength of the signal, with intermediate  
values being more favourable for the detection of out-  
lier loci (Fig. 3d).

The results for the signal of hitch-hiking mirror those of  
the power to detect the causal site. In the case of selec-  
tion on standing variation, there was an overall reduc-  
tion of the signal compared to scenarios of selection on  
new mutation (Fig. 3a,b) except for highly selfing popu-  
lations ( $\sigma \geq 0.95$ ). Nevertheless, as in the scenario of  
adaptation from new mutations, predominantly selfing  
populations had a weaker signal of selection than out-  
crossing populations. However, the strength of the sig-  
nal for population-populations with intermediate levels  
of selfing ( $\sigma = 0.5$ ,  $\sigma = 0.8$ ) was similar or even higher  
than outcrossing populations (Fig. 3b).

The initial frequency of the advantageous allele and  
whether it is ancestral or derived also influenced the  
strength of the signal in predominantly selfing popu-  
lations ( $\sigma = 0.95$ , Fig. 4). As expected, the lower the  
initial frequency, the stronger was the signal of selec-  
tion, but this was modulated by the nature of the al-  
lele. With low initial frequencies, selection on the an-  
cestral allele left a stronger signal. Symmetrically, the  
signal was stronger for derived alleles if the initial fre-  
quency was high. To understand these results, we ex-  
amined the genetic diversity and the differentiation be-  
tween the group of haplotypes carrying the advanta-  
geous mutation and the group not carrying it at the out-  
set of the sweep. We found that this genetic differentia-  
tion decreases with the distance from the selected site,  
with higher differentiation for scenarios with stronger  
signal of selection (i.e. when the advantageous allele  
is ancestral with low initial frequency). Besides, ances-  
tral alleles were associated to more diverse local haplo-  
types than derived alleles at the same frequency. These  
results show that not-no further recombination during  
the sweep is required to generate a local signal of selec-  
tion.



**Fig. 3. Selection footprint on the selected chromosome under different scenarios.** Proportion of positive tests in function of distance to the locus under selection. Estimates obtained from 100 replicates of simulated data of one population of  $N = 500$  diploid individuals, sampled twice with  $\tau$  generations between samples and selection coefficient  $s$  and selfing rate  $\sigma$ . (a) Adaptation from new mutation,  $\tau = 25$ ,  $s = 0.5$ . (b) Adaptation from standing variation,  $\tau = 25$ ,  $s = 0.5$ . (c) Adaptation from new mutation,  $\tau = 25$ ,  $\sigma = 0$ . (d) Adaptation from new mutation,  $s = 0.5$ ,  $\sigma = 0$ . Distance measured in centimorgans (cM). Coloured areas mark the 95% bootstrap interval.

566 *Medicago truncatula*. From the 1920 SNPs geno- 587  
 567 typed, 1224 were polymorphic in the sample and 588  
 568 987 had a global MAF higher than 0.05. Effective 589  
 569 Genetic differentiation between time samples was 590  
 570 large,  $\hat{F}_{ST} = 0.207$  (95 % CI of 0.197 to 0.217) and so was 591  
 571 the inbreeding coefficient,  $\hat{F}_{IS} = 0.972$  (95 % CI of 0.969 592  
 572 to 0.974). From those values, effective population size 593  
 573 was estimated at  $\hat{N}_e = 42$  (from  $\hat{F}_{ST} = 0.207$  95 % CI of 39 594  
 574 to 44) and selfing rate at  $\hat{\sigma} = 0.986$  (from  $\hat{F}_{IS} = 0.972$  95 % 595  
 575 CI of 0.984 to 0.987). The test of homogeneity did not 596  
 576 identify any SNP as a strong candidate for being under 597  
 577 selection: the lowest  $p$ -value was 0.04 with a corre- 598  
 578 sponding  $q$ -value of 0.48 (Fig. 5 and Table S2).

## 579 Discussion

580 **Estimating effective population size under selfing and** 603  
 581 **selection.** It is well known that self-fertilization reduces 604  
 582 the effective size of populations. Our results show that 605  
 583 Weir and Cockerham's (1984)  $F_{ST}$  allows to measure the 606  
 584 amount of drift between two temporal samples, even 607  
 585 in the presence of selfing. However, the precision of 608  
 586 the estimates diminishes with the rate of selfing. We 609

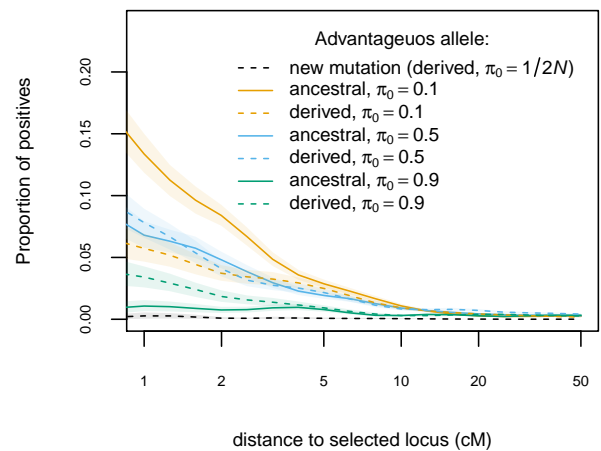
hypothesize that it is the consequence of the effect of re-  
 duced effective recombination in selfing, which reduces  
 the number of loci with independent evolutionary histo-  
 ries. This effect is most extreme for completely selfing  
 populations, where the whole genome behaves as a  
 single locus, reducing dramatically the information  
 available in the data. In order to test this hypothesis,  
 we estimated  $N_e$  for a similar set of simulations under  
 an unrealistic model where each locus was simulated  
 independently (supplementary text S2). On those sim-  
 ulations, the precision of  $N_e$  estimates was no longer  
 reduced by selfing (Fig. S3b).

An alternative and common way to estimate  $N_e$  from  
 temporal data uses the standardized variance in allele  
 frequencies ( $F_C$ , Nei and Tajima 1981). This was the  
 approach originally proposed for the temporal genome  
 scan by Goldringer and Bataillon (2004). However, es-  
 timates from  $F_C$  suffer from a bias (Fig. S3) because this  
 approach assumes that the  $2n$  gene copies (in a sample  
 of  $n$  diploid individuals) are independent draws from  
 the population gene pool, and uses a binomial distribu-  
 tion to model it (Waples 1989). However, in the case of  
 partially or predominantly selfing populations, the two

610 gene copies within an individual are not independent  
 611 samples from the population, which explains the bias  
 612 of the estimate. Using Weir and Cockerham's (1984)  
 613  $F_{ST}$  estimate corrects for this bias.

614 Strong selection also reduces the effective population  
 615 size by reducing the number of breeding individu-  
 616 als or increasing the variance of reproductive success  
 617 among individuals (Robertson 1961; Santiago and Ca-  
 618 ballero 1995). This is reflected in the estimation of  $N_e$ ,  
 619 which decreases with the strength of the selective co-  
 620 efficient of the mutation under selection in our simula-  
 621 tions (Fig. 1b). Note that, in our case, this effect is not  
 622 only driven by the local increase of temporal genetic  
 623 differentiation around the site under selection. The  
 624 strong selection considered in our simulations increases  
 625  $F_{ST}$  genome-wide, even in regions unlinked to the site  
 626 under selection (e.g. distance of about 50 centimorgan  
 627 or larger; Fig. S5). The combined effect of selfing (i.e. in-  
 628 creased linkage disequilibrium) and selection produced  
 629 very strong drift compared to a neutrally evolving pop-  
 630 ulation with the same census size (Fig. S5).

631 The effective population size estimated in Cape Cor-  
 632 sica *M. truncatula* was extremely low, in agreement  
 633 with the results from microsatellites data on the same  
 634 population ( $\hat{N}_e = 20$ , Jullien *et al.* 2019). However, such  
 635 small  $N_e$  estimates might reflect other processes in  
 636 addition to drift (e.g. immigration, Jullien *et al.* 2019).  
 637 ~~Indeed, the distribution of  $p$ -values from the genome~~  
 638 ~~scan shows a departure from the expected distribution~~  
 639 ~~which could indicate an inappropriate null model~~  
 640 ~~(Fig. S7a). This deficit of low  $p$ -values means that~~  
 641 ~~the outlier test is too conservative because the null~~  
 642 ~~distribution of . For instance, gene flow into the~~  
 643 ~~studied population can increase temporal  $F_{ST}$  is wider~~  
 644 ~~than the distribution observed genomewide. The~~  
 645 ~~QQ plots for simulated populations show that, as the~~  
 646 ~~selfing rate increases, the test progressively shifts from~~  
 647 ~~delivering an excess of low  $p$ -values to delivering a~~  
 648 ~~deficit of low  $p$ -values (Fig. S7b), as observed for *M.*~~  
 649 ~~*truncatula*. This implies that the test is not appropriate~~  
 650 ~~for such high levels of selfing ( $\sigma > 0.99$ ), where the~~  
 651 ~~whole genome evolves as a single locus, but the~~  
 652 ~~method can perform well with slightly lower selfing~~  
 653 ~~rates ( $\sigma = 0.95$ ). In addition, the assumption that~~  
 654 ~~temporal data collected from the same geographical~~  
 655 ~~location belong to a single continuous population is~~  
 656 ~~likely to be wrong in many cases, even more so for~~  
 657 ~~predominantly selfing populations, where the spatial~~  
 658 ~~structure is usually very strong. Jullien (2019) proposes~~  
 659 ~~to address this issue by performing demographic~~  
 660 ~~inference using approximate Bayesian computation~~  
 661 ~~(Csilléry *et al.* 2010) that can potentially discriminate~~  
 662 ~~isolated from admixed populations, and jointly~~  
 663 ~~estimate drift, migration and selfing parameters and~~  
 664 ~~bias  $N_e$  estimates (Jullien *et al.* 2019). The effects of the~~  
 665 ~~violation of model assumptions are discussed further~~  
 666 ~~below.~~

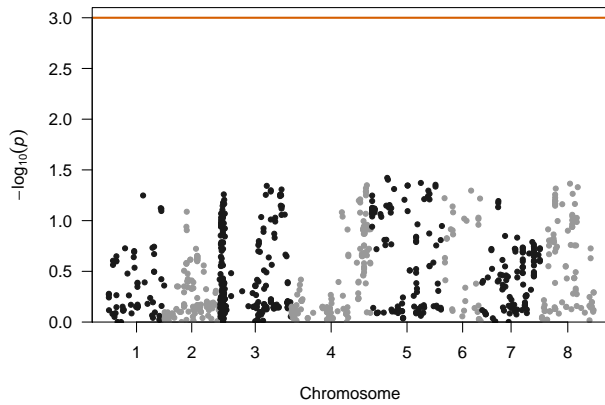


**Fig. 4. Influence of derived/ancestral state and initial frequency on selection footprint.** Proportion of positive test in function of distance to the locus under selection. Estimates obtained from 100 replicates of simulated data of one population of  $N = 500$  diploid individuals, sampled twice with  $\tau = 25$  generations between samples and selection coefficient  $s = 0.5$ . Adaptation on standing variation, selfing rate  $\sigma = 0.95$ .

In our simulations we have considered that individual genotypes are available. However, in many temporal experiments, sequencing is performed for pools of individuals (Pool-seq; e.g. Franks *et al.* 2016) to reduce the costs. Estimating effective population sizes remains possible, using Pool-seq adapted  $F_{ST}$  and  $F_C$  estimates (Hivert *et al.* 2018; Jónás *et al.* 2016). However, since individual information is lost, these estimates cannot take into account the within individual allele identity correlation due to selfing. When studying a selfing species, we therefore recommend working with individuals genotypes. If Pool-seq is necessary due to budget limitations, we advise to follow a similar procedure as in Frachon *et al.* (2017) and reproduce seeds by selfing for one or more generations so that sequenced individuals are completely homozygous and can be treated as effectively haploid samples. However, this approach is likely to only give good results with predominantly selfing species (with a small proportion of heterozygous loci to be removed) and divides the amount of data by two.

**Limits imposed by selfing to temporal genome scans of selection.** Our results show that the search of for regions under selection with temporal  $F_{ST}$  genome scans in predominantly selfing populations can be challenging. In the case of complete selfing, the whole genome behaves as single locus and selection, if present, affects the whole genome. In partially selfing populations, the strength of the signal left by selection depends on whether the advantageous allele had the time to recombine with different genetic backgrounds. In a population with a selfing rate as high as  $\sigma = 0.95$ , outcrossing events are unlikely to occur during the sweep of a new beneficial mutation and produce no effective recombination if they happen between close relatives sharing the same homozygous genotype. As a results, like com-





**Fig. 5. Genome-scan for selection based on temporal differentiation in cape Corsica *Medicago truncatula* population.**  $-\log_{10}(p\text{-value})$  of the simulation-based test of the null hypothesis that the locus-specific differentiation measured at each SNP is only due to genetic drift. Only SNP markers with  $\text{MAF} > 0.05$  and known position at the reference genome are shown. Red line marks  $p\text{-value} = 0.001$ , the significance threshold used for figures 3 and 4.

pected to be old). With Being older, such allele has had more time for mutations to accumulate on both lineages, creating diversity that is unique to the haplotypes under selection. This will lead to more sites with a significant allele frequency change around the causal mutation (e.g. Fig. S2b). In the case of selection on an allele starting at high frequency, the situation is reversed. The lowest signal is for selection on high frequency ancestral alleles that not only will have a small change on allele frequency but also carry diversity common to the whole population. Finally, low frequency ancestral alleles might have the potential to create the strongest signal, but they are scarce in an equilibrium population (Fig. S6) so they might not be the most it is unlikely they represent a frequent case of sweep from standing variation.

Although temporal genome scans in predominantly selfing species are able to reveal the footprint of selection on standing variation, how prevalent is adaptation from pre-existing variation in selfing populations remains an open question. In the short-term, selection is most likely acting on standing variation (Barrett and Schluter 2008), but there are ~~not no~~ specific predictions for selfing populations. Theoretical models predict an overall limitation of adaptation in selfers compared to outcrossers (Hartfield and Glémin 2016). However, selfers can hold high levels of cryptic genetic variation for polygenic traits, due to negative linkage disequilibrium. This diversity may allow a selfing population to adapt to changing conditions as quickly as outcrossing populations (Clo *et al.* 2019). Indeed, there is some evidence of selective sweeps in selfing species (Bonhomme *et al.* 2015; Huber *et al.* 2014). In agreement with this, the temporal genome scan in a predominantly selfing population of *A. thaliana* from a previous study showed footprints of selection (Frachon *et al.* 2017). Such positive results could have been favoured by the relatively large genetic variation of this population (Baron *et al.* 2015; Frachon *et al.* 2017). Based on the simulation results we present here, we can assume that this population has been adapting from standing variation over a short period of time (eight generations) rather than from many new mutations occurring during (or shortly before) the studied period.

The absence of evidence for selection in the *M. truncatula* dataset is manifest. It is reasonable to attribute this results to the extreme selfing rate estimate ( $\hat{\sigma} = 0.986$ ) in this population, for which our simulation approach gives little hope to detect any signal of selection. Indeed, the same selfing lineages are observed throughout the study period of 22 generations (Julien *et al.* 2019), which further suggest that almost no recombination occurs in the population. If selection, either on new or pre-existing mutations, has occurred, it has changed the frequencies of multilocus genotypes without leaving ~~different~~ any local signal along the genome. This would have ~~reduced~~

703 pletely selfing populations, the selective sweep will reduce genetic diversity on the whole genome and will leave no local signal of selection.

706 On the contrary, when adaptation proceeds through standing variation, historical recombination can put an allele on different genetic backgrounds before it becomes advantageous and sweeps. In that case, the sweeping haplotypes are only similar among them and different from the haplotypes in the rest of the population around the site under selection. As the distance to the selected site increases, the diversity of the sweeping haplotypes raises and the differentiation with the other haplotypes segregating in the population decreases due to the action of historical recombination (Fig. S4). These differences along the genome create the local signal of selection, even if no further (effective) recombination occurs during the sweep.

720 Selection on standing variation is usually associated to a weak signal of selection (i.e. a soft sweep, Hermisson and Pennings 2005). Our results indeed confirm this expectation for outcrossing populations (compare Fig. 3a,b for  $\sigma = 0$ ). Interestingly, for predominantly selfing populations, the situation is reversed. Yet, the strength of the local signal of selection, and therefore the power of the outlier test depends not only on the initial frequency of the advantageous allele, but also on its genetic background. Consider an advantageous derived allele at low frequency. Because of its low frequency, it is likely a young allele (Fig. S6) and few mutations will have accumulated around it. The selected haplotypes will therefore display low diversity and little differentiation from the deleterious haplotypes (Fig. S4). Thus, we expect that only few mutations will show a significant change in allele frequencies around the site under selection (e.g. Fig. S2a). On the other hand, if the ancestral allele (at low frequency) becomes advantageous, it will be likely to be on a very old lineage (since a high frequency derived allele is ex-

dramatically dramatically reduced the effective population size, which is consistent with our extremely low estimate  $\hat{N}_e = 42$ . An examination of the distribution of  $p$ -values from the genome scan shows a departure from the expected distribution which could indicate an inappropriate null model (Fig. S7a). This deficit of low  $p$ -values means that the outlier test is too conservative because the null distribution of temporal  $F_{ST}$  is wider than the distribution observed genomewide. However, the QQ plots for simulated populations show that, as the selfing rate increases, the test progressively shifts from delivering an excess of low  $p$ -values to delivering a deficit of low  $p$ -values (Fig. S7b), as observed for *M. truncatula*. The present analysis cannot be conclusive on any of these hypotheses about the presence of selection and the observed diversity changes might be better explained by a demographic model including can be the consequence of demographic processes, such as genetic exchanges with neighbouring populations, as discussed by Jullien *et al.* (2019).

**Model assumptions.** The method and simulations presented here are based on simple models with one unstructured population, constant parameters through time and a single selective event. These models are useful to highlight the effects of inbreeding and to point out to some solutions for the problems posed by the presence of inbreeding. Real populations are complex systems and other demographic and selective processes are in action and they have consequences for the dynamics of the selective sweeps and the performance of the statistical methods.

The assumption that temporal data collected from the same geographical location belong to a single continuous population is probably wrong in many cases, even more so for predominantly selfing populations, where the spatial structure is usually very strong (Bonnin *et al.* 2001). Estimates of effective population size from temporal samples have been shown to be affected by population structure, often leading to underestimation (Gilbert and Whitlock 2015; Ryman *et al.* 2014). Such bias can make the tests conservative, aggravating the problem of detecting loci under selection. One way to address this issue is to perform demographic inference under more complex scenarios to be able to discriminate isolated from admixed populations, and jointly estimate drift, migration and selfing parameters (e.g. using approximate Bayesian computation as in Jullien *et al.* 2019). Note, however, that some scenarios might enhance the signal of selection. Detection of sweeps from standing variation could be easier in populations with a larger historical effective size than in populations that had always been at a smaller constant size, because they benefit from higher historical recombination.

The presence of multiple loci under selection is also an important factor to consider, because strong

selective interference is expected under selfing (Hartfield *et al.* 2017). Of particular interest is background selection which reduces the effective population size, an effect exacerbated by selfing (Nordborg 1997; Roze 2016). In addition to the reduction in diversity, purifying selection could also mimic the temporal signal of a selective sweep. Neutral alleles that are linked to less deleterious backgrounds can quickly rise to high frequencies (Cvijović *et al.* 2018). Recently, Johri *et al.* (2020) proposed using approximate Bayesian computation for joint inference of demography and purifying selection, which could lead to more appropriate null models for the detection of loci involved in adaptation. Further research is needed in this direction, it is unclear at this point the implications for temporal genome scans and partially selfing populations.

Future developments should not focus only on improving the inference of the null model. The classification of loci as neutral or into different selection categories can benefit from other information than just the genetic differentiation. For instance, reduction of genetic diversity around the locus under selection (e.g. Fig. S4) can add information about the presence and origin of the selection. Supervised machine learning methods have been shown to perform well and are promising tools for this type of task (reviewed by Schrider and Kern 2018). While these methods might improve the classification of loci under scenarios where there is some local footprint of selection, predominantly selfing population will remain a challenge as long as selective sweeps produce genome-wide hitch-hiking.

**Conclusions.** Identifying regions under selection with a temporal genome scan can fail for several reasons. First, timing is paramount. A sample at the beginning and at the end of the selective sweep would be ideal for detection of detecting selection. However, the start and duration of the sweep and the sampling times cannot be synchronized except for some experimental evolution studies. Frequency of the advantageous allele at the beginning of the sweep can also reduce the chances of capturing any signal. On top of that, selfing presents itself as a strong additional difficulty for this task, reducing the efficacy of selection (reduced  $N_e$ ) and extending hitch-hiking effects that blur the distinction between neutral and selected regions. Nevertheless, scenarios of adaptation from standing variation can leave some signal of selection in highly selfing populations. Curiously, adaptation from new mutation under the same highly selfing scenarios leave leaves no signal, reversing the general expectation for the footprint of selection from “hard” and “soft” sweeps. Chance plays an important role in the success of a genome scan. Therefore, researchers should focus on the factors that can be controlled, such as the use of adapted methods to

910 selfing species, to increase the probability of a positive 985  
911 outcome. 986  
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912 **ADDITIONAL INFORMATION** 989  
990  
913 **Author contributions.** Miguel Navascués, Laurène Gay, Joëlle Ronfort and Re- 991  
914 naud Vitalis conceived and designed the research. MN and RV developed the 992  
915 temporal  $F_{ST}$  scan for partially selfing populations and the code implementing it. 993  
916 Arnaud Becheher and MN evaluated the performance of the method. Karine Lori- 994  
917 don, LG and JR produced the *Medicago truncatula* data set. MN wrote the article 995  
918 with the help of all authors that critically reviewed and approved the text. 996  
997  
919 **Conflict of interest disclosure.** The authors of this article declare that they have 998  
920 no financial conflict of interest with the content of this article. [MN, JR and RV are](#) 999  
921 [recommenders from PCI Evolutionary Biology.](#) 1000  
1001  
922 **Data availability.** The method described in this work is implemented in DriftTest, a 1002  
923 C program available at Zenodo (Navascués and Vitalis 2020). An R script (R Core) 1003  
924 Team 2018) to reproduce the simulations and analyses in this work is available at 1004  
925 Zenodo (Becheher *et al.* 2018). SNP data for *Medicago truncatula* is available at 1005  
926 Data INRAE (Gay 2020). 1006  
927  
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929 INRA metaprogramme "Adaptation of Agriculture and Forests to Climate Change" 1009  
(ACCAF). 1010  
1011  
1012  
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# Supplementary

## Information

### S1: Predominantly selfing species in project Baseline

Project Baseline will track the evolution of natural populations from more than 60 plant species in the next 50 years through seed collection ([www.baselineseedbank.org](http://www.baselineseedbank.org), Etterson *et al.* 2016). Many of the species included are capable of self-fertilization and at least six of them reproduce predominantly by selfing: *Bromus diandrus* (Kon and Blacklow 1990), *Bromus tectorum* (Novak *et al.* 1991), *Elymus canadensis* (Sanders and Hamrick 1980), *Impatiens pallida* (Schemske 1978), *Stipa pulchra* (Larson *et al.* 2001) and *Triodanis biflora* (Goodwillie and Stewart 2013).

### S2: Simulation of independent loci

Additional simulations were performed to show the effect in the estimation of  $N_e$  of non-independent sample of gene copies within individuals (i.e.  $F_{IS}$ ) without the effect of linkage disequilibrium due to selfing. The simulation approach is very similar to the simple drift model used to build null distribution of the test described in the main text. The main difference is that initial allele frequency is set as a fixed parameter. Simulations are used to generate temporal data at 10,000 biallelic loci per pseudo-observed dataset. Initial allele frequencies ( $\pi_0$ ) are fixed to 0.5 for all loci. Allele frequency  $\pi$  in generation  $t$  were simulated with a binomial distribution as  $\pi_t \sim B(2N_e, \pi_{t-1})/2N_e$ , where  $N_e$  is the effective population size in number diploid individuals, for generations  $t \in [1, \tau]$ . Thus, each locus is simulated independently from each other, ignoring the linkage among them that should have occurred due to selfing. Genotype counts in samples,  $K_t^*$ , at time  $t = 0$  and  $t = \tau$  are taken from a multinomial distribution,  $K_t^* \sim \text{Mult}(n_t, \gamma_t)$ , where  $n_t$  is the sample size (in number of diploid individuals) at time  $t$  and  $\gamma_t$ :

$$\gamma_{AA,t} = \pi_t^2 + F_{IS}(1 - \pi_t)\pi_t$$

$$\gamma_{Aa,t} = 2(1 - \pi_t)\pi_t(1 - F_{IS})$$

$$\gamma_{aa,t} = (1 - \pi_t)^2 + F_{IS}(1 - \pi_t)\pi_t$$

are the genotype frequencies in the populations in function of the inbreeding coefficient  $F_{IS}$ , which is determined by the selfing rate  $F_{IS} = \sigma / (2 - \sigma)$ . Simulations were performed with parameters values for  $N_e = 500$  diploid individuals,  $\tau = 25$  generations,  $n_0 = n_{25} = 50$  diploid individuals and selfing rate,  $\sigma$ , had values of 0, 0.5, 0.75, 0.8, 0.85, 0.9, 0.925, 0.95, 0.975 or 1. Results are presented in Fig. S3.

Table S1. Notation.

symbol	meaning
$\gamma_t$	genotype frequencies in the population at time $t$
$\pi_t$	allele frequency (in the population) at generation $t$
$\sigma$	rate of reproduction by selfing in the population
$\tau$	time in generations between the two samples
AA, Aa, aa	the three possible genotypes of a bi-allelic locus
$F_{ST}^l$	observed differentiation statistic $F_{ST}$ at focal locus $l$
$K_t$	observed genotype counts in the sample at time $t$
$K_t^*$	simulated genotype counts in the sample at time $t$
$k_t$	observed count of the reference allele in sample at time $t$
$N$	census population size in number of diploid individuals
$N_e$	effective population size in number of diploid individuals
$n_t$	sample size (number of diploid individuals) at time $t$
$p_t$	frequency of the reference allele in the sample at generation $t$ ; $p_t = k_t/2n_t$
$p_t^*$	simulated frequency of the reference allele in the sample at generation $t$ ; $p_t^* = k_t^*/2n_t$
$s$	selection coefficient of the adaptive locus
$t$	time, measured in generations, with $t = 0$ for first sample and $t = \tau$ for last sample

Table S2. Genome scan results for *M. truncatula*.

This table is provided in a supplementary file.

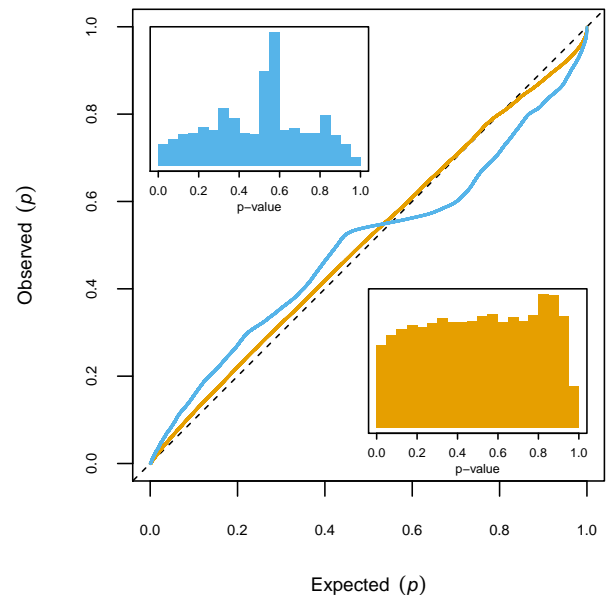
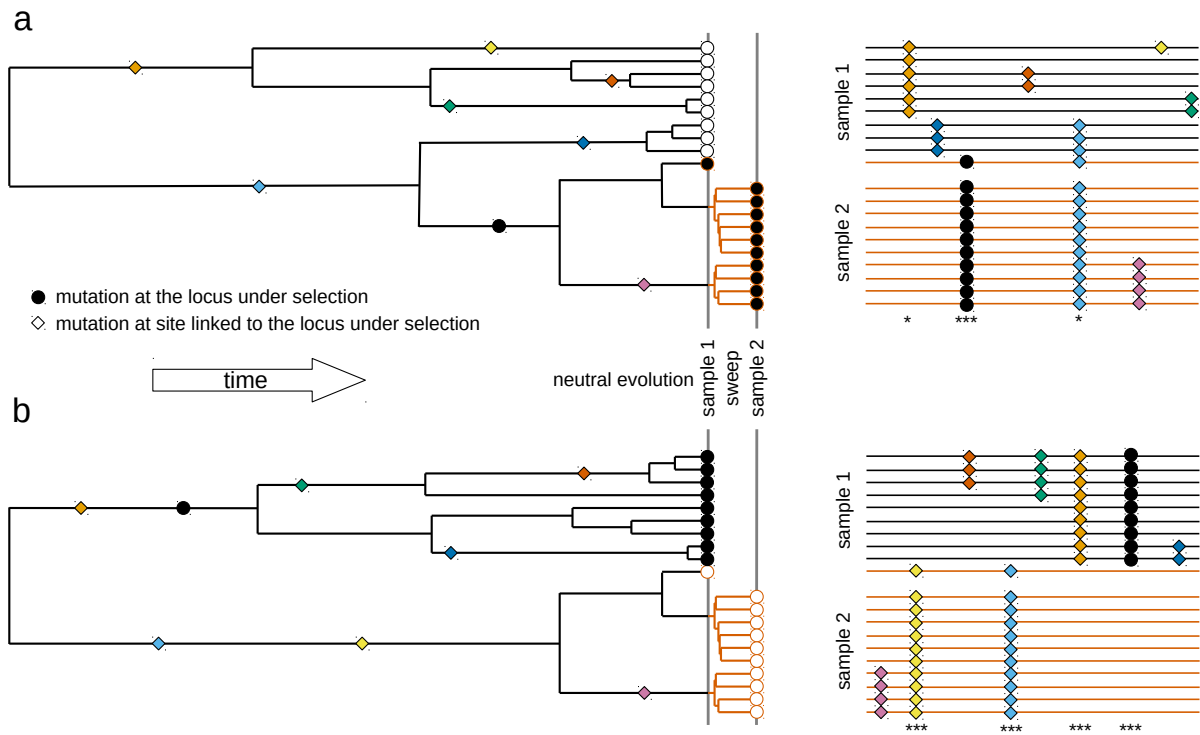
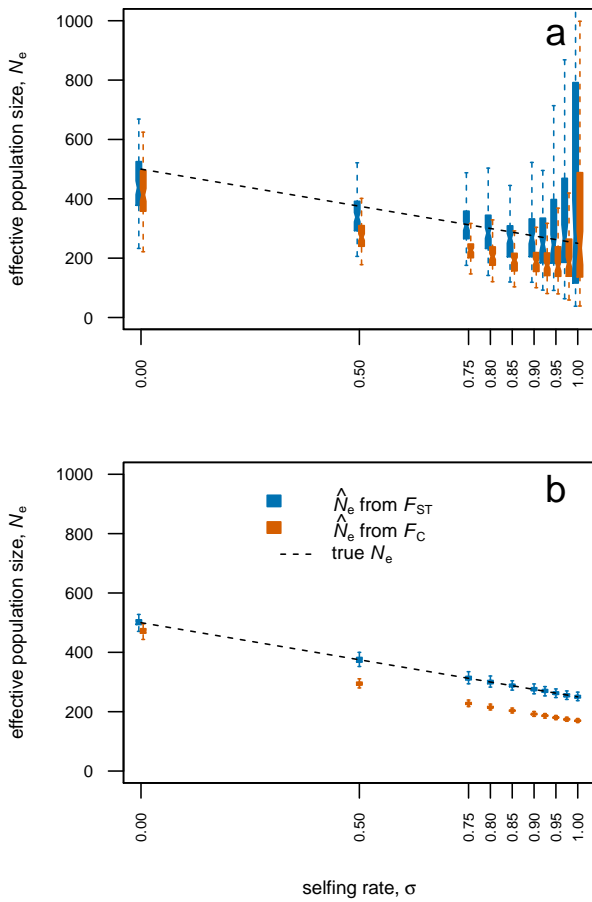


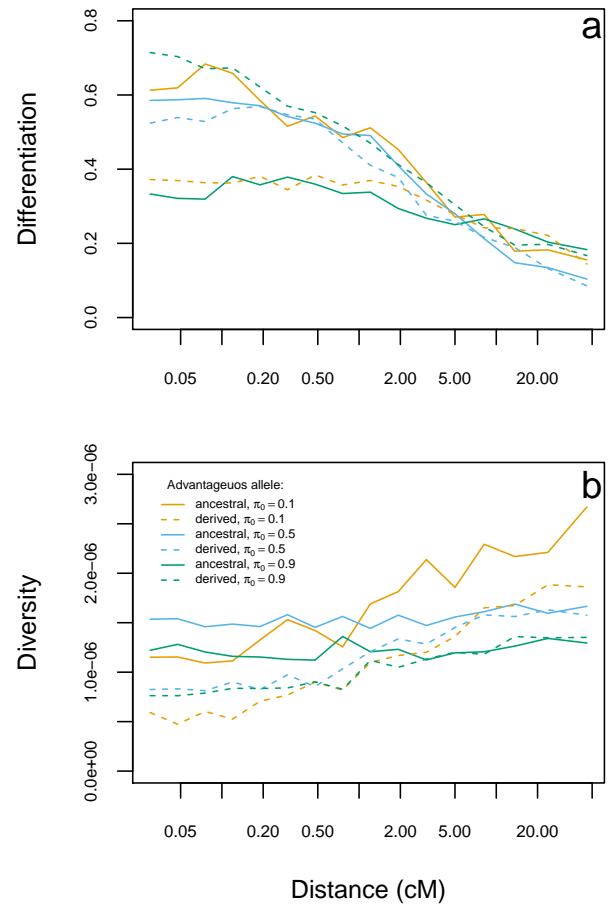
Fig. S1. Effect of a filter on minor allele frequency (MAF) on the distribution of  $p$ -values.  $p$ -values calculated from 100 simulation replicates of a neutral outcrossing population of  $N = 500$  diploid individuals, sampled twice with  $\tau = 25$  generations between samples. The blue histogram shows the distribution of  $p$ -value using all loci, with corresponding blue line in the QQ-plot. The orange histogram shows the distribution of  $p$ -value using loci with a minimum global allele frequency of 0.05, with corresponding orange line in the QQ-plot.



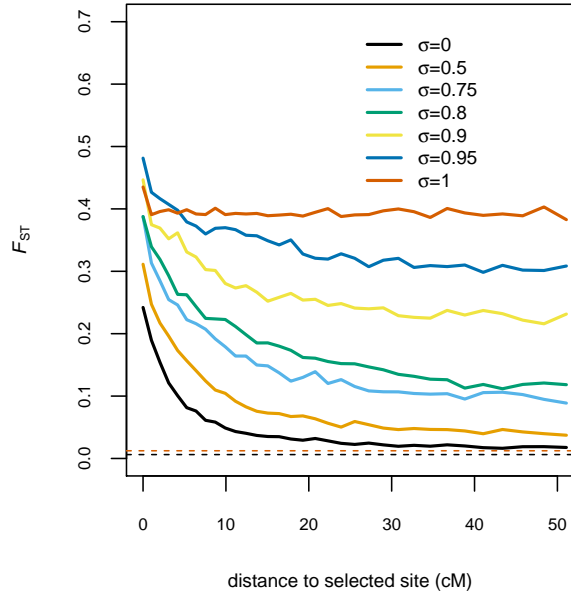
**Fig. S2. Schematic representation of the consequences of selection on standing variation in the temporal pattern of genetic diversity.** Gene genealogies with samples carrying the derived allele represented with a circle filled in black and samples carrying the ancestral allele represented with a circle filled in white. Mutations are represented as coloured squares over the genealogy at their time of mutation; the site under selection is represented with a circle filled in black. Lineages of the genealogy subject to positive selection are represented in red. Next to each genealogy a schematic representation of the sequence alignment of the samples is presented with each sequence represented as a line at the same height that the corresponding sample of the genealogy. Polymorphisms are represented with the corresponding coloured squares in the genealogy, indicating the sequences that carry the derived alleles. Sequences carrying the allele under positive selection are represented with a red line. Asterisks signal polymorphic sites showing large (\*) and extreme (\*\*\*) changes in allele frequency between the two temporal samples. This figure represents only a small region around the locus under selection that did not recombine through the period considered (hence can be represented as a single genealogy). **(a)** Gene genealogy of two temporal samples taken just before a low frequency derived allele becomes advantageous and after the sweep. The mutation is young, as expected for a low frequency derived allele (Fig. S6), so the lineages carrying the allele that will become advantageous had little time to accumulate mutations and become distinctive from other haplotypes in the population. Most of the alleles on the haplotypes that swept and are found in the second sample on high frequency were already at high frequency in the first sample. **(b)** Gene genealogy of two temporal samples taken just before a low frequency ancestral allele becomes advantageous and after the sweep. The mutation is old, as expected for a high frequency derived allele (Fig. S6); thus, the split between the lineages carrying the ancestral and derived allele is very old. Several mutations have accumulated in both lineages, making them very distinctive. Several alleles on the haplotypes that swept and are found in the second sample on high frequency were at low frequency in the first sample, creating a strong local signal of selection around the selected site.



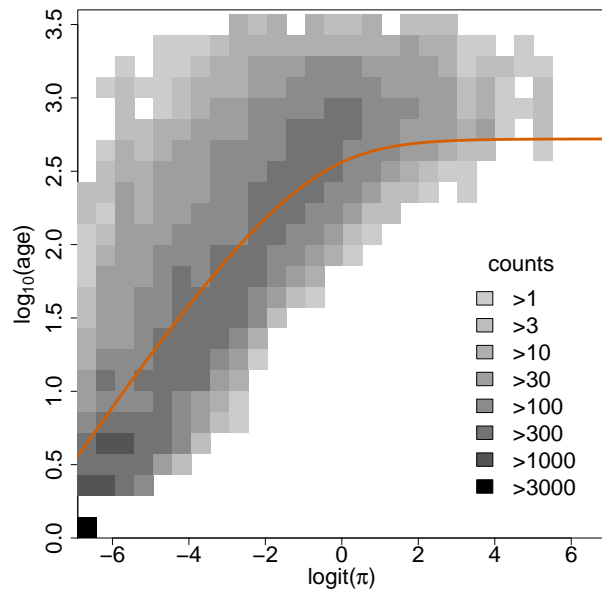
**Fig. S3. Effective population size estimates from temporal differentiation from  $F_C$  and  $F_{ST}$ .** Estimates obtained from simulated data of populations of  $N = 500$  diploid individuals, sampled twice with  $\tau = 25$  generations between samples and 100 estimates at independent simulation replicates. Dashed line marks true  $N_e = \frac{N(\sigma-2)}{2}$ . (a) Simulations performed with SLIM (Messer 2013) as described in the main text and presented partially in Fig. 1. (b) Simulations performed with a simple model of independent loci with initial allele frequency of 0.5, as described in supplementary text S2.



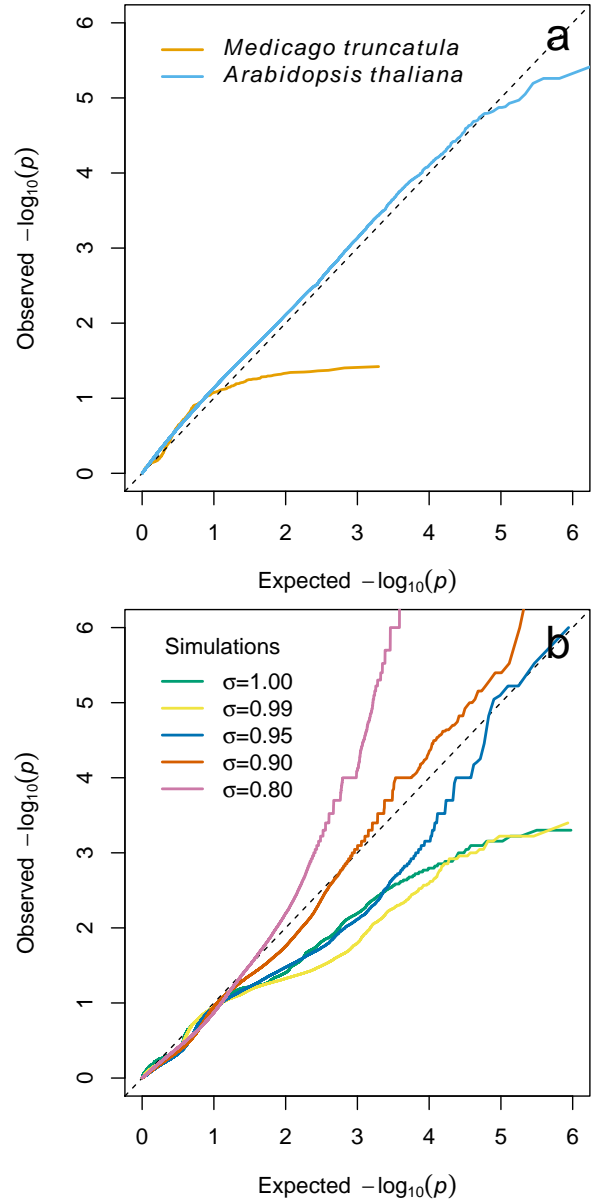
**Fig. S4. Effect of recombination occurring before the selective sweep on differentiation and diversity of the haplotypes carrying the advantageous allele.** Genetic patterns found in a simulated population of  $N = 500$  diploid individuals reproducing predominantly by selfing ( $\sigma = 0.95$ ) after  $20N_e$  generations. (a) Differentiation is measured as the  $F_{ST}$  between haplotypes carrying the advantageous allele and those carrying the alternative allele. (b) Diversity is measured as the average heterozygosity per bp. Both differentiation and diversity were calculated on 3-000 bp windows at different distances to the loci under selection. The mean from 100 simulation replicates is shown.



**Fig. S5. Genetic differentiation as a function of the distance to locus under selection.** Estimates of  $F_{ST}$  (mean across 100 simulation replicates) from polymorphisms in a window of size  $1000\text{Kb}-1000\text{Kb}$  ( $\approx 1\text{cM}$ ) at different distances to locus under selection. Simulation of populations of  $N = 500$  diploid individuals, sampled twice with  $\tau = 25$  generations between samples and different rates of selfing,  $\sigma$ . A new advantageous mutation appears at generation  $t = 0$  with coefficient of selection  $s = 0.5$ . Samples are made of 50 diploid individuals genotyped at 10,000 biallelic markers (including the locus under selection). **Boxplot for 100 estimates from simulation replicates.** For reference, expected  $F_{ST}$  values for  $\sigma = 0$  (black) and  $\sigma = 1$  (red) are shown with dashed lines.



**Fig. S6. Relationship between frequency ( $\pi$ ) and age of mutation.** Bidimensional histogram for the frequency and age (in generations) of all mutations in a simulated population of  $N = 500$  diploid individuals reproducing predominantly by selfing ( $\sigma = 0.95$ ) after  $20N_e$  generations. Theoretical expectation of age ( $\frac{-2\pi}{1-\pi} \ln(\pi) \frac{1}{2N_e}$ ) is shown with an orange line (Kimura and Ohta 1973).



**Fig. S7. Departure from expectations of the  $p$ -value distribution for *Medicago truncatula* genome scan.** (a) QQ plots for *Medicago truncatula* (this study) and *Arabidopsis thaliana* (Fig. S21 Frachon et al. 2017). For *M. truncatula*,  $p$ -values estimated for 987 SNP loci that were polymorphic in the sample with a global minor allele frequency higher than 0.05 (Table S2). Null model considered  $N_e = 42$  and  $\sigma = 0.99$ . (b) QQ plots for simulations under a scenario of adaptation from new mutations in a population of  $N = 500$  diploid individuals sampled twice with  $\tau = 25$  generations between samples and different rates of selfing,  $\sigma$ . A new advantageous mutation appears at generation  $t = 0$  with coefficient of selection  $s = 0.5$ . Samples are made of 50 diploid individuals genotyped at 10,000 biallelic markers (including the locus under selection). The plot consider the distribution of  $p$ -values from 100 simulation replicates.