

Field heritability of a plant adaptation to fire in heterogeneous landscapes

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Abstract

The strong association observed between fire regimes and variation in plant adaptations to fire suggests a rapid response to fire as an agent of selection. It also suggests that fire-related traits are heritable, a precondition for evolutionary change. One example is serotiny, the accumulation of seeds in unopened fruits or cones until the next fire, an important strategy for plant population persistence in fire-prone ecosystems. Here, we evaluate the potential of this trait to respond to natural selection in its natural setting. For this, we use a SNP marker approach to estimate genetic variance and heritability of serotiny directly in the field for two Mediterranean pine species. Study populations were large and heterogeneous in climatic conditions and fire regime. We first estimated the realized relatedness among trees from genotypes, and then partitioned the phenotypic variance in serotiny using Bayesian animal models that incorporated environmental predictors. As expected, field heritability was smaller (around 0.10 for both species) than previous estimates under common garden conditions (0.20). An estimate on a subset of stands with more homogeneous environmental conditions was not different from that in the complete set of stands, suggesting that our models correctly captured the environmental variation at the spatial scale of the study. Our results highlight the importance of measuring quantitative genetic parameters in natural populations, where environmental heterogeneity is a critical aspect. The heritability of serotiny, although not high, combined with high phenotypic variance within populations, confirms the potential of this fire-related trait for evolutionary change in the wild.

Keywords: cone serotiny, *Pinus halepensis*, *Pinus pinaster*, SNP-based relatedness

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Introduction

Evidence for the role of wildfires as a strong selective pressure on many plants has been accumulating in recent years. Studies have shown the adaptive nature of a number of traits that plants possess to deal with natural fires, including both fire survival traits and traits that enhance

postfire recruitment (Pausas *et al.* 2004a; Keeley *et al.* 2011; Pausas 2015). One line of evidence is strong within-species variation in these fire-adaptive traits that is consistent with different fire regimes at different parts of the species distribution. A paradigmatic example of a post-fire recruiting trait is serotiny, that is the accumulation of seeds within unopened fruits or cones (a canopy seed bank) until the heat of a fire opens them and allows dispersal (Lamont *et al.* 1991). Several studies document the prevalence of higher levels of serotiny in tree populations that live under frequent crown fires compared with areas where such fires are rare, particularly in *Pinus* species (reviewed in Hernández-Serrano *et al.* 2013). Because serotiny allows postfire regeneration, it is a key trait for the persistence of populations in fire-prone ecosystems, especially in nonresprouting species.

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A strong association between fire regime and traits suggests a rapid response to fire as an agent of selection and that therefore a significant part of the phenotypic variance has an additive genetic basis; that is, the traits are heritable, a precondition for evolutionary change. A few studies in common garden experiments in serotiny have indeed found significant narrow-sense heritability, h^2 (Rudolph *et al.* 1959; Hernández-Serrano *et al.* 2014). Glasshouse and common garden studies of heritability allow for fine control of local environments and genetic crosses. However, estimates of heritability measured under controlled environmental conditions can be systematically higher compared with natural environments (Roff & Simons 1997; Conner *et al.* 2003; Winn 2004). This is usually attributed to higher environmental variability in the field, as well as decreased expression of additive variance, leading in both cases to reduced h^2 estimates. In addition, glasshouse and garden studies typically include individuals that would not survive in the field, and this artificially increases progeny survival potentially biasing heritability estimates. A difference between studies in field and controlled environments would be particularly expected for complex traits, such as serotiny, a trait that involves both morphological and developmental variation. The genetic control of such a trait is in turn likely to be complex and influenced by many genes across the genome (Yang *et al.* 2010; Parchman *et al.* 2012; Budde *et al.* 2014), and reduced by multiple environmental factors (Kruuk & Hadfield 2007).

An alternative to measuring genetic variance under controlled conditions is to use the genetic similarity among individuals directly in the field to calculate how much of the phenotypic covariance among individuals is under heritable genetic control (Ritland 1996). Estimating quantitative genetic parameters directly in field populations is possible thanks to the current availability of large numbers of molecular markers that can be used to estimate relatedness among individuals, even when no pedigree information is available. The precision of early attempts with a few markers, usually microsatellites, was limited (Ritland & Ritland 1996; Andrew *et al.* 2005; Castellanos *et al.* 2011), but the now-established genomic tools allow for the use of hundreds or thousands of SNP markers (single nucleotide polymorphisms) to increase accuracy. Assessing genomic relatedness with large marker panels transformed the fields of animal and plant breeding; modern methods in these fields rely on genomic relatedness estimates (along with or instead of pedigrees) to obtain more accurate estimates of trait heritability and genomic prediction (Meuwissen *et al.* 2001; Hayes *et al.* 2010). This multi-marker approach is also gaining weight in human genetic studies, where dense data sets are revealing that the genetic architecture of many traits might be best

explained if many loci are taken into account and not just a few highly correlated with the traits of interest (reviewed in Vinkhuyzen *et al.* 2013).

The multi-SNP approach provides a direct and quantitative measure of identity-by-state (IBS), and as such an estimate of the realized genetic similarity among individuals, as opposed to the expected values of identity-by-descent (IBD) given by a pedigree (Powell *et al.* 2010). The IBS approach does not require a known base population and relies on the alleles that are identical in pairs of individuals at each specific locus. This approach thus provides an estimate of realized genomic relatedness that uses the information contained in all molecular markers, as opposed to inferring the most likely relationship between individual pairs. Certainly, measuring realized genome sharing using SNPs also poses several problems, mostly related to how well the marker sample represents the similarity of two genomes (see Speed & Balding 2015 for a recent review). But with a good genomic representation and in spite of additional limitations inherent to field studies, such as low sample sizes and complex environmental variation, genomic relatedness estimation has been used with success in field populations to estimate genetic variance (Robinson *et al.* 2013; Béréons *et al.* 2014). IBS measures can be particularly useful for long-lived, outcrossing plants growing in the field, whose individuals in overlapping generations are probably linked by complex relationships hard to depict in a pedigree. With the careful incorporation of sources of environmental variation in the models (Kruuk & Hadfield 2007; Støper *et al.* 2012; Villemereuil *et al.* 2013), estimating quantitative genetic parameters directly in field populations has the potential of providing a realistic estimate of how traits can respond to selection in the natural environment where they actually are subject to it.

In this study, we estimate the heritability of cone serotiny, an important trait for postfire recruitment, in wild populations of two widespread Mediterranean pines (*Pinus halepensis* and *Pinus pinaster*). The study populations are exposed to variable fire regimes across space. We aimed to estimate the potential of serotiny to respond to natural selection by fire in the species' natural setting. Taking advantage of the heterogeneity within our *P. halepensis* study population and the large sample size, we also assess the robustness of our field-based approach by subsampling the data.

Materials and methods

Study species

The study was performed in two widespread pine species, *Pinus halepensis* Mill. and *Pinus pinaster* Aiton.

P. halepensis is a drought-tolerant pine that occurs all around the Mediterranean coast, while *P. pinaster* occurs in the western part of the Mediterranean Basin and the Atlantic regions of France, Spain and Portugal. In our study area in eastern Spain, *P. halepensis* dominates on basic (carbonated) soils, while *P. pinaster* grows on noncarbonated soils. Both species thrive in ecosystems subject to frequent crown fires that often kill most individuals (Pausas *et al.* 2004b). They lack the ability to resprout, but several of their traits have been interpreted as adaptations to recurrent crown fires, such as a precocious development with early flowering, the retention of dead branches, and high levels of serotiny (Keeley & Zedler 1998; Tapias *et al.* 2004). These characteristics define the pine syndrome of post-fire seeder or fire embracer (Pausas 2015). In our study area, these two species live predominantly in crown-fire ecosystems (i.e. with fire intervals shorter than the lifespan of the pine tree), but they also occur in more productive zones where trees grow taller and crown fires are rare; in such regions, fires were probably not absent in the past, but rare or limited to understory fires. Therefore, variability in traits defining their recruitment strategies (e.g. the serotiny level) is expected through the distribution area of these pines.

Field localities and phenotypic data collection

Eight field localities of *P. halepensis* and three of *P. pinaster* in eastern Spain were included in this study. All stands are the result of natural recruitment and show a range of tree ages and sizes. The paired distance between stands varies between 14 and 182 km in *P. halepensis* and 27 and 96 km in *P. pinaster*. Five of *P. halepensis* and two of *P. pinaster* stands grow in dry low-productivity habitats at low altitude and close to the coast; the remaining are located inland in cooler and wetter regions (see Table S1, Supporting information for stand details). Fire recurrence also varies between the coast and the inland, as coastal stands have been historically subjected to a high frequency of crown fires, while inland areas rarely burn in crown fires (Verdú & Pausas 2007). Patterns of variation in serotiny at the same localities were described in a previous study by Hernández-Serrano *et al.* (2013), who showed a higher average prevalence of serotiny in stands with high fire frequency compared with those with sporadic crown fires. Most trees in all stands tend to have some serotinous cones, but the level of serotiny can vary strongly among individuals. The differences between fire regimes thus lie in the frequency distribution of the proportion of serotinous (closed) cones in each tree across stands. The association of serotiny with fire in these species occurs

in spite of high within-stand variation in the trait; if the variance in the proportion of serotinous cones per tree is partitioned in our current data set, the component due to variation within the stands is higher (57% for *P. halepensis* and 54% for *P. pinaster*) than among them (43% and 46%, respectively; see also Hernández-Serrano *et al.* 2013).

For phenotypic characterization, we used the data from Hernández-Serrano *et al.* (2013), who selected and georeferenced 40–67 individual trees in each stand, for a total of 367 trees for *P. halepensis* and 194 trees for *P. pinaster* (Table 1). To reduce the variation in tree age across stands, small trees with diameters <10 cm were avoided. At this size, trees in the study species are already reproducing, but cone numbers tend to be low and thus provide a poor assessment of serotiny. Serotiny was estimated for each tree by counting the number of serotinous and nonserotinous (open or partially open) cones, and defined as the proportion of serotinous cones in relation to the total number of cones per tree. That is, while serotiny is a binary trait at cone level, the degree of serotiny varies continuously across trees. Leaf tissue of each individual was collected and dried in silica gel for DNA extraction.

SNP genotyping

Individual trees were genotyped at 251 SNP loci, using species-specific SNP assays. This is a modest set of markers that would be limited for pedigree reconstruction, but that is useful for relatedness estimation (see Discussion). In *P. halepensis*, genotyping was conducted with an Illumina SNP array developed from the transcriptome, designed to include known genes with adaptive roles in forest trees (Pinosio *et al.* 2014), and used to study patterns of spatial genetic structure in this species (Budde 2014b). The two individuals used by Pinosio and colleagues for transcriptome sequencing and SNP discovery were selected from two of the localities that vary in their levels of serotiny. For *P. pinaster*, the genotyping SNP assay was designed based on a subsample of the 1536-plex SNP array for this species by Chancerel *et al.* (2011) and some additional SNPs from Grivet *et al.* (2011); see details in Budde *et al.* (2014). These polymorphisms include a wide selection of candidate genes associated with adaptation to climate, stress response, wood properties, phenology and growth in pines (Budde *et al.* 2014; Jaramillo-Correa *et al.* 2015). SNP data were retrieved from Budde *et al.* (2014) for *P. pinaster* and from Budde (2014a) for *P. halepensis*. Genotypes were used to estimate heritability as explained below. Individual trees and loci with >10% missing data were removed from the analysis.

Table 1 Sample sizes, environmental and phenotypic variability in *Pinus halepensis* and *P. pinaster* study populations. Mean and range (maximum and minimum) of tree-level values are shown for elevation above sea level, mean annual temperature (T), total annual precipitation (P) and the percentage of serotinous cones. The five coastal stands of *P. halepensis* are a subset of the eight total stands

Dataset	N stands/trees	Elevation (m)	T (°C)	P (mm)	Serotiny (%)
<i>Pinus halepensis</i>					
All stands	8/367	619 (121–941)	14.4 (12.9–17.1)	562 (468.3–704.5)	44.0 (2.6–96.8)
Coastal stands	5/238	471 (121–775)	15.0 (13.6–17.1)	597 (509.3–704.5)	53.1 (5.9–96.8)
<i>Pinus pinaster</i>					
All stands	3/194	707 (402–901)	13.9 (13.0–15.4)	543 (464.6–586.0)	36.1 (0–100)

SNP-based relatedness and heritability

Pairwise relatedness between all pairs of individuals was estimated based on their SNP genotypes. To give more weight to shared rare alleles, genotype scores are centred as follows (VanRaden 2008). Genotypes are coded in a matrix **M** according to whether they are homozygote (–1), heterozygote (0) or the other homozygote (1) at each locus. **M** has *n* rows for the number of individuals, and *m* columns for the number of loci. A second matrix **P** is built so that each column *i* is $2(p_i - 0.5)$, where p_i is the frequency of the minor allele for locus *i*. The subtraction of **P** from **M** to get **Z** sets the mean values of allele effects to zero. **Z** is then used to calculate **G**, the relatedness (similarity) matrix.

Several methods have been developed to estimate **G**; we used the realized relatedness method of VanRaden (2008) and Astle & Balding (2009) as implemented in the *kin* function of package *synbreed* in R (Wimmer *et al.* 2012; see also Appendix S1, Supporting information). In this approach, $\mathbf{G} = \mathbf{ZDZ}'$ where **D** is diagonal and given by $D_{ii} = 1/m[2\sum p_i(1-p_i)]$. This method weights markers by the reciprocals of their expected variance. We obtained very similar results when using another measure of relatedness by VanRaden (2008), $\mathbf{G} = \mathbf{ZZ}' / 2\sum p_i(1-p_i)$, where markers are weighted by the sum of the expected variances (results not shown). These methods rescale estimates of relatedness considering the current sample as the base population, and relatedness values can be seen as a measure of excess allele sharing compared with unrelated individuals. Negative values are thus common and correspond to individuals sharing fewer alleles than expected given the sample.

We use a linear mixed ‘animal model’ approach to include the variance explained by shared SNPs when explaining the phenotypic variance in serotiny (Wilson *et al.* 2010). The univariate models were as follows:

$$y = \mathbf{X}\beta + \mathbf{Z}_1a + \mathbf{Z}_2s + e$$

where *y* is the vector of serotiny values, β is the vector of fixed effects with incidence matrix **X** linking it with the

individual records, **Z**₁ and **Z**₂ are incidence matrices for the random effects *a* (the additive genetic effects) and *s* (the stand of origin), and *e* is a vector of residual errors. The variance–covariance structure of random factor *a* in the model is defined by \mathbf{GV}_a , where **G** is the relatedness between tree pairs as explained above, and **V**_a is the additive variance to be estimated. Two fixed effects were included to account for environmental variability among trees: mean annual temperature (°C; which reflects the variability due to distance from the coast and altitude), and the fire recurrence history (low or high frequency of crown fires). A third fixed effect, the diameter of the tree, was included to remove potential effects of tree age on our measures of serotiny (although models were unaffected by the inclusion of this variable). Overall, stem diameter was not correlated with serotiny (only very weakly in three out of the eleven stands) and is not expected to bias heritability estimates for this trait. In addition to the additive genetic effects, the stand of origin of each tree was also included as a categorical random factor to account for spatial proximity and other unmeasured local environmental effects that could be confounded with genetic variation. All these factors were chosen because they all contributed to some extent to explain variance in serotiny in preliminary analyses. To test for the effect of not including the spatial and environmental predictors in the models, we also ran a ‘naïve’ version of each model that included only the relatedness. We ran Bayesian models using package *MCMCglmm* for R (Hadfield 2010). Each analysis was iterated long enough to obtain 2000 independent chains (see Table S2 and Appendix S2, Supporting information for model details, scripts and prior selection).

Serotiny was modelled as a binary trait at the cone level for each tree, that is the number of closed cones with respect to the total number of recorded cones per tree, assuming a probit link function and a binomial error distribution. For the prior distribution of variance components, we used parameter expanded priors following the χ^2 distribution with one degree of freedom, and the residual variance component fixed to one.

Narrow-sense heritability (h^2) was estimated as the proportion of the total phenotypic variance assigned to the individual (i.e. to the additive genetic variance, V_a):

$$h^2 = \frac{V_a}{V_a + V_s + V_r + V_1}$$

where V_s is the variance explained by the stand of origin, V_r the residual variance, and $V_1 = 1$, which is the variance implicit to the probit link model. Because binary data do not provide enough information for the estimation of the residual variance, V_r was fixed to 1.

We first ran all analyses for the complete set of individuals (eight stands in *P. halepensis* and three in *P. pinaster*). Pooling stands together ensures a higher sample size for the models and is possible because the study region harbours a homogenous gene pool for both *Pinus* species, with no genetic structure among the stands (see Appendix S4, Supporting information for an analysis of intrapopulation genetic structure in *P. halepensis*, and Budde *et al.* (2014) for *P. pinaster*).

Then, to assess a potential effect of the across-stand environmental variability included in the sample on the h^2 estimates, we also ran the models for the subset of five stands of *P. halepensis* that are located in the coastal area, where the frequency of crown fires and serotiny are higher. The overall environmental variance in this subset of stands is lower compared with the complete data set (Table 1, Table S1 in Supporting information). We did not run the same analysis with the inland populations because the low sample sizes prevented the models from running properly.

Results

The estimates of pairwise relatedness varied markedly, from low negative values (even within stand) to closely related trees (Table S1, Supporting information), as expected for pine species with high levels of gene flow but some restricted seed dispersal (González-Martínez *et al.* 2006; Juez *et al.* 2014). The average pairwise relatedness was close to zero, also as expected (mean = -0.003 , range = -0.409 to 0.905 for *P. halepensis* and mean = -0.005 , range = -0.304 to 0.884 for *P. pinaster*; see histograms in Fig. S1 of Supporting information). Consistent with the low genetic structure across stands detected with the microsatellites (see Appendix S4, Supporting information), the estimated pairwise relatedness values were only poorly correlated with the pairwise geographic distances between trees ($r = -0.12$, $N = 67\,161$ pairs for *P. halepensis* and $r = -0.17$, $N = 18\,721$ for *P. pinaster*). The absence of population genetic structure together with high phenotypic variation provides ideal conditions to estimate heritability in the field.

Estimated heritability values (h^2) for serotiny in the two pine species were 10% and 12%, respectively (Fig. 1, Table 2). In *P. halepensis*, values for h^2 were similar between the two spatial groups (all stands and coastal stands only) and their credible intervals showed considerable overlap (Table 2).

In all cases, deviance information criterion (DIC) values for the naïve models were larger than for the complete model (Table S2, Supporting information), indicating a better fit for the latter. The naïve models included only the relatedness among individuals and no environmental or spatial predictors and showed estimated h^2 values substantially higher than our final estimates (Fig. 1, Table 2).

Discussion

Our field estimates of heritability in two Mediterranean pine species, combined with previous evidence for selection pressures due to fire on cone serotiny and high phenotypic variance within populations, confirm the potential of this trait for evolutionary change in its natural setting. Serotiny is a key fire-related trait in *Pinus*, and evidence from different sources corroborates that it can be under strong selection by fire (Keeley & Zedler 1998; Schwiik & Ackerly 2001; Pausas 2015). In both *Pinus halepensis* and *P. pinaster*, serotiny is higher in areas where the trees are exposed to high crown-fire frequency compared with regions with low frequency of these fires (Hernández-Serrano *et al.* 2013).

Heritability estimates for cone serotiny are around 10% for both pine species and show that a significant amount of additive genetic variance in this trait can be exposed to natural selection, even though a large part of the phenotypic variance (the remaining 90%) is caused by environmental effects or nonadditive genetic sources. A potential source of environmental effects, that is the presence of maternal effects, was not considered here because it is hardly expected for a trait expressed late in the life of a plant. The field h^2 estimate for serotiny in *P. halepensis* is therefore lower than the previous estimate of 20% obtained in a common garden (Hernández-Serrano *et al.* 2014). The 95% credible interval (CI) of the common garden estimate (9–40%) only marginally included the field estimate (10% with 7–14% CI) and had much broader credible intervals (i.e. lower precision) than the new field estimate. The lower h^2 detected in field conditions agrees with previous evidence of higher h^2 values for plant traits under relatively uniform garden conditions (Conner *et al.* 2003; Winn 2004; Carlon *et al.* 2011 for a study in corals). The difference is particularly unsurprising in our study, because wild *P. halepensis* and *P. pinaster* populations

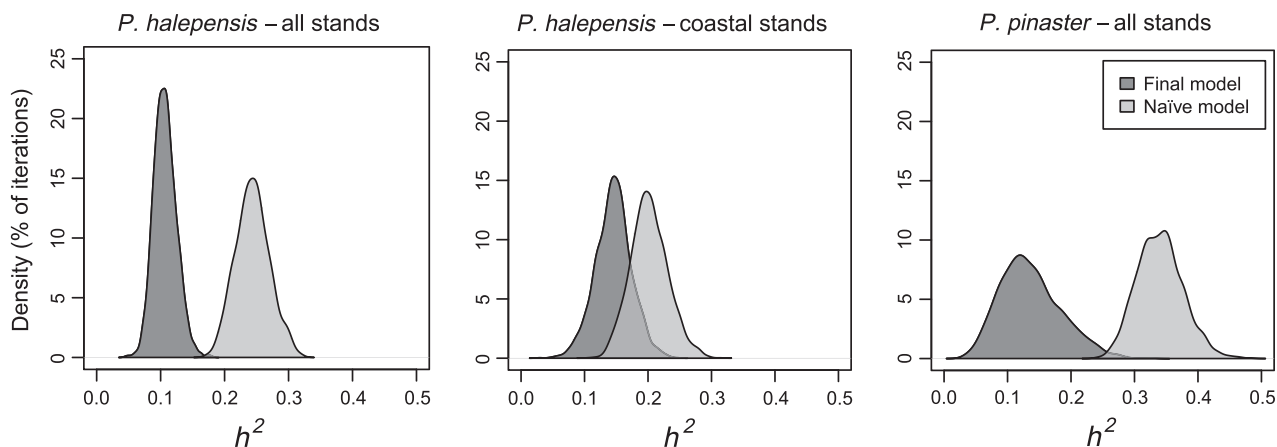


Fig. 1 Distribution of heritability (h^2) estimates for cone serotiny in *Pinus halepensis* and *P. pinaster*. The plots show the density of the posterior distribution of h^2 estimates based on MCMC chains (with effective size = 2000 iterations). Estimates reported in the main text and Table 2 correspond to the mode of each distribution. 'Naïve' models (in light grey) did not include any of the environmental and spatial predictors included in the final models.

Table 2 Estimates of heritability (h^2) with corresponding 95% credibility intervals (CI) for cone serotiny in wild populations of *Pinus halepensis* and *P. pinaster*. 'Naïve' models did not include spatial or environmental predictors. The mean and standard errors of variance components are shown for the final models (V_A = additive variance, V_S = variance due to stand of origin)

Dataset	Naïve model		Final model			
	h^2	CI for h^2	V_A	V_S	h^2	CI for h^2
<i>P. halepensis</i>						
All stands	0.25	0.19–0.30	0.26 ± 0.001	0.14 ± 0.004	0.10	0.07–0.14
Coastal stands	0.19	0.15–0.26	0.40 ± 0.001	0.40 ± 0.01	0.14	0.09–0.20
<i>Pinus pinaster</i>						
All stands	0.33	0.27–0.42	0.61 ± 0.002	2.39 ± 0.04	0.12	0.05–0.23

extend over heterogeneous landscapes, and large environmental variation is expected.

The estimation of field heritability is a different approach from the more traditional common garden estimation in that it controls for environmental variance statistically instead of directly. By doing that, the field approach could be less precise (although it was not the case in this study), but allows for the estimation of h^2 of traits that are difficult or impossible to measure in a common garden experiment. Variance components and therefore the heritability of a trait are population-specific parameters, and thus, the estimation of these parameters directly in the field is expected to provide a more realistic view of how the traits can respond to selection. The two *Pinus* populations studied here provide a good system for this approach, because the sampled stands are part of large, interconnected populations with extensive gene flow. In addition, phenotypes and levels of pairwise relatedness are variable within populations, providing enough statistical power for the estimation of variance components (see Table S1, Supporting information). The

populations also encompass high among-stand variation in environmental conditions, including spatial variation in fire regime. The models used to estimate h^2 must therefore include the potential sources of environmental variance on the traits to distinguish them from additive genetic variance (Lee *et al.* 2010; Stopher *et al.* 2012). Failing to do so can result in artificially high estimates of genetic variance, particularly if close relatives also tend to share the same environment (i.e. within a stand). Our models account for fire recurrence, mean annual temperature, tree size and stand locality; the exclusion of these factors did indeed inflate estimates of h^2 (even doubling them; 'naïve models' in Table 2) and confirmed that environmental variables and phenotypes covary to some extent in our study populations (Table S2, Supporting information). Other unmeasured environmental variation could also affect phenotypes in these populations. For instance, soil fertility has been suggested as a potential driver for serotiny (Keeley *et al.* 2011; Keeley 2012; Clarke *et al.* 2013); however, this has never been confirmed in pines, and the variability in soils within

populations is small in our study system (*P. halepensis* stands are all on calcareous soils and *P. pinaster* grows on noncarbonated soils). It is known that seed predation can contribute to shape cone characteristics in pines, including *P. halepensis* (Mezquida & Benkman 2005); however, there is no evidence of spatial variation in seed predators within our population, and it is likely that small variations in predator density, or other unknown biotic factors, are already captured by the environmental and fire parameters considered (e.g. temperature and fire regime covary with important spatial variation such as distance from the coast and altitude). The fact that we obtained similar h^2 values for a coastal subset of *P. halepensis* stands growing under more homogeneous conditions suggests that the models are capturing the most relevant across-stand environmental variables.

Environment-driven variation is therefore an important component of the total phenotypic variance in serotiny, and this is probably the case for many other adaptive traits. If the importance of environmental factors varies in space, the sampled spatial scale could affect heritability estimates, as has been shown for other plant traits (e.g. Andrew *et al.* 2005). Our results at two different spatial scales (coastal stands only versus all stands, Table 2) showed an increase in V_A but not in h^2 . At even larger scales, it is possible that h^2 could vary as a result of changing environments. The approach of measuring h^2 directly in the field thus needs to take spatial scale into consideration and is in turn a useful method for assessing the natural variation in h^2 across wild populations of a species. Variation in h^2 is a crucial aspect for understanding trait evolution in wild populations (Charmantier & Garant 2005), yet little explored in plants (see e.g. Carr & Fenster 1994).

It is remarkable that serotiny, a fire-adaptive trait that is related to fitness (because it directly affects recruitment after a wildfire), is quite variable within populations. As is the case for many fitness characters, serotiny is a complex trait, potentially with a highly polygenic determination as suggested for *P. contorta* (Parchman *et al.* 2012) and *P. pinaster* (Budde *et al.* 2014). In these two species, multiple genetic regions are associated with cone serotiny, directly or through correlations with other traits that are also adaptations to fire and covary with serotiny (Budde *et al.* 2014). This complex genetic architecture, in combination with the high levels of gene flow that characterize pine populations and the environmentally driven variation detected in this study, could help explain the high natural trait variability found among and within stands. Despite these factors, selection by fire appears to be strong enough to maintain high differences in phenotype frequencies associated with fire regime. In fact, *P. halepensis* and *P. pinaster* present, among the species studied to

date, some of the strongest relationships between serotiny and fire regime (see review in Hernández-Serrano *et al.* 2013).

The use of SNP genotypes for relatedness estimation is gaining popularity in ecological studies; this is because they are abundant and ubiquitous in the genome and easy to obtain, even for nonmodel species, thanks to new genotyping approaches. A few published tests on the required numbers of markers suggest that studies using randomly selected SNPs from across the genome will need a large number of markers to get accurate genomic relatedness matrices and genetic variance estimates (Stanton-Geddes *et al.* 2013; Béréanos *et al.* 2014). We use here a modest number of SNPs (251 in each population); however, we have evidence suggesting that this number of SNPs is correctly capturing the relatedness structure among our studied individuals. For instance, using a larger panel of SNP genotypes for 150 *P. pinaster* trees (with unknown serotiny levels, from Jaramillo-Correa *et al.* 2015), we found that relatedness matrices calculated with 250 SNPs are highly correlated with matrices built using around 1480 SNPs ($r = 0.79 \pm 0.006$; $N = 100$ repetitions, Appendix S3, Supporting information). This larger panel contained SNPs developed by Chancerel *et al.* (2013) and chosen by Jaramillo-Correa *et al.* (2015) for being putatively neutral, while in our case, SNPs were discovered in the transcriptome of individuals with contrasted fire phenotypes in the case of *P. halepensis*, and from known functional genes potentially related to climate adaptation in the case of *P. pinaster*. SNPs in our study are therefore within functional genes, including some associated with the studied trait (see Budde *et al.* 2014), and are thus more likely to depict the inheritance of serotiny than the same or even a larger number of 'random' genomic SNPs. Consistently, our data appear to correctly describe the relatedness structure in the study populations, and the obtained results for h^2 estimates are in accordance with expectations based on previous estimates in a common garden and the anticipated reduction due to environmental heterogeneity when moving to a natural setting.

With a higher number of informative markers, it is likely that the precision of our h^2 estimate could increase, but meanwhile, our study is the first to estimate field-based h^2 for a fire-adaptive trait and provides insight into the natural genetic-based variation of important plant adaptations in a region where fire regimes change rapidly. Field estimates seem more useful to study evolutionary potential of wild populations compared with otherwise useful common gardens. In this particular case, heritabilities of around 10% are not large and suggest that potential for evolutionary change in response to fire could be limited despite high

standing phenotypic variation. It is thus possible that natural pine populations are not capable of quickly adapting to the abrupt changes in fire regime currently taking place in the Mediterranean Basin (Pausas & Fernández-Muñoz 2012).

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Data accessibility

- 1 Serotiny phenotypes and locality descriptors, and the genomic relatedness matrix (G matrix) for *Pinus halepensis* and *P. pinaster* are available from the Dryad depository: doi:10.5061/dryad.r6fr1.
- 2 Genotypes are available from the Dryad depository as follows:
 - i SNP and SSR genotypes for *Pinus halepensis* (Budde 2014a): Dryad doi: 10.5061/dryad.n193d.
 - ii SNP genotypes for *P. pinaster* (Budde *et al.* 2013): Dryad doi:10.5061/dryad.1p2s5/2.

- 3 R code for analyses is provided in Table S2 and Appendix S3, both as online Supporting Information.
- 4 Sampling locations are included in Table S1 in online Supporting Information.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Estimation of genomic relatedness.

Appendix S2 Estimation of heritability in *MCMCglmm*.

Appendix S3 Regional Genetic Structure and figure with results of STRUCTURE analysis for *Pinus halepensis*.

Appendix S4 Comparison of relatedness estimates calculated with a large SNP data set.

Fig. S1 Histograms of pairwise relatedness values for three data sets.

Table S1 Location, characteristics and paired genomic relatedness values of the study stands of *Pinus halepensis* and *P. pinaster*.

Table S2 Heritability estimates (h^2) for cone serotiny in *Pinus halepensis* and *P. pinaster*, for complete, "naïve" and null models.