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ORIGINAL ARTICLE

Using partial genotyping to estimate the genetic and maternal determinants of adaptive traits in a progeny trial of *Fagus sylvatica*

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Abstract Understanding the determinants of phenotypic variation is critical to evaluate the ability of traits to evolve in a changing environment. In trees, the genetic component of the phenotypic variance is most often estimated based on maternal progeny tests. However, the lack of knowledge about the paternal relatedness hampers the accurate estimation of additive genetic and maternal effects. Here, we investigate how different methods accounting for paternal relatedness allow the estimation of heritability and maternal determinants of adaptive traits in a natural population of *Fagus sylvatica L.*, presenting non-random mating. Twelve potentially adaptive functional traits were measured in 60 maternal families in a nursery. We genotyped a subset of offspring and of all the potentially reproductive adults in the population at 13 microsatellite markers to infer paternal relationships and to estimate average relatedness within and between maternal families. This relatedness information was then used in family and animal models to estimate the components of phenotypic variance. All the studied traits displayed significant genetic variance and moderate heritability. Maternal effects were detected for the diameter increment, stem volume and bud burst. Comparison of family and animal models showed that unbalanced mating system led to only slight departures from maternal family assumptions in the progeny trial. However, neglecting the significant maternal effects led to an overestimation of the heritability. Overall, we highlighted the usefulness of relatedness pattern analyses using polymorphic molecular markers to accurately analyse tree sibling designs.

Keywords *Fagus sylvatica* · Functional traits · Heritability · Maternal effects · Pollen pool structure

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Introduction

Monitoring adaptive genetic diversity is of main importance to evaluate the short-term evolutionary potential of natural populations and provide relevant conservation or management guidelines (Hansen et al. 2012; Lefèvre et al. 2014). Among the proxies of the evolutionary potential of a population, the additive genetic variance (V_A) measures the trait variation directly available for selection. Classically, V_A is standardised by the total phenotypic variance (V_P) to derive an evolutionary parameter that is comparable across traits, populations and species, called heritability (h^2; Falconer and MacKay 1996). Evaluating the proportion of phenotypic variance due to environmental or genetic effects for key functional traits is also of main importance to inform mechanistic and species distribution models (Benito Garzon et al. 2011; Oney et al. 2013) and, more generally, trait

databases. However, for tree species, our knowledge about the determinants of functional trait variation is still limited (e.g. provenance tests have long focused on traits with agronomic value: growth, wood quality traits). Notably, while maternal effects on the expression of seedling's traits are frequently reported (mostly through seed quality in plants; Roach and Wulff 1987, Johnsen et al. 1995 and Brousseau et al. 2013) the relative importance of these effects on phenotypic determinism is rarely quantified. Yet, this environmental maternal determinism can play a key role on the short-term evolutionary response of natural populations in changing environments (as a substitute to plastic response; Kuijper and Hoyle 2015).

While studies in animal natural populations highlighted the interest of taking advantage of heterogeneous relatedness relationships to investigate complex trait determinism (e.g. dominance or maternal effects; Kruuk and Hadfield 2007), heterogeneous relatedness relationships are still commonly viewed as nuisance parameters in plant studies. Indeed, the classical methodology in tree quantitative genetics consists in sampling open-pollinated (OP) maternal families and using the so-called family model, which relies on the assumption of homogeneous relatedness relationships (i.e. families are only constituted of half-sibs, HS or full-sibs, FS; Gauzere et al. 2013b). This classical mixed-effects model framework prevents the easy and accurate estimation of maternal or non-additive parameters. In that case, the proper way to separate V_A from other variance components is to use (i) bi-parental crosses designs for species for which matings can be easily controlled (e.g. Hodge et al. 1996 in eucalyptus species) or (ii) seed characteristics (e.g. average seed weight per family) or mother habitat description as a covariate in quantitative genetic models (Scotti et al. 2010; Brousseau et al. 2013). However, this latter approach (ii) does not allow to evaluate the actual proportion of phenotypic variance explained by environmental maternal effects.

In addition to the difficulty to estimate maternal or non-additive variance components, the departure from the family assumption can also compromise the accurate estimation of V_A and h^2. Even if this assumption was proven valid in several seed orchard trials (Hodge et al. 1996; Gaspar et al. 2009; Hansen and Nielsen 2010), its relevance in tree natural populations is questionable. Indeed, tree natural populations are known to present assortative mating due to limited gene flow or flowering asynchrony, mixed-mating systems (both selfing and outcrossing) and unequal fecundities (departures listed in Gauzere et al. 2013b). The resulting non-negligible cryptic genetic structure within natural populations is expected to lead to an overestimation of V_A and h^2 (individuals are more related than HS; Gaspar et al. 2009, Scotti et al. 2010, Gauzere et al. 2013b) and to strongly affect the population adaptive potential. One of the most widely used method to account for these departures into quantitative genetic estimates consists in adjusting the family model estimates according to the average relatedness coefficient within families (ρ_w) expected from the empirical knowledge about the mating system of the species (Squillace 1974; Gauzere et al. 2013b).

The easy development of polymorphic molecular markers (such as microsatellite markers) for non-model species now offers the opportunity to refine the relatedness information used in quantitative genetic models. Molecular markers can be used to reconstruct genealogical relationships, through parentage analyses or pedigree-reconstruction methods (Marshall et al. 1998; Fernandez and Toro 2006), or to estimate genetic relatedness coefficients (e.g. Loiselle et al. 1995). The relatedness information can then inform an individual-based mixed model, called the "animal" model (Henderson 1984). In progeny trials, relatedness estimates can also be easily used to analyse the pollen pool structure and estimate mating system parameters (e.g. selfing rate, male reproductive success; Hansen and Nielsen 2010, Vidal et al. 2015). Yet, population and quantitative genetic studies are often dissociated, while population genetic analyses can be informative about (i) the potential departures from HS assumptions that can be encountered in the estimation of V_A and h^2 and (ii) the scale at which the evolutionary potential of a population should be defined.

Despite these recent advances, quantitative genetic studies in the wild are still more developed in animal than in plant species. Indeed, the genotyping of the whole progeny trial and their potential parents represents a strong experimental constraint in plant quantitative genetics (because of the large trial and population sizes and high immigration rates; Stinchcombe 2014). Yet, few studies have investigated the cost-effectiveness ratio of an increased genotyping effort to gain accuracy in variance or breeding value estimates (but see El-Kassaby et al. 2011). Recently, Gauzere et al. (2016) showed that genotyping a subset of offspring per family within a progeny trial at few polymorphic markers allows the reduction of the biases on V_A and h^2 in presence of common departures from HS assumptions. This study proposed to optimize the use of the genotypic information by constructing a hybrid relatedness matrix including the reconstructed pairwise genealogical relationships for the genotyped offspring and average marker-based relatedness coefficients for the non-genotyped offspring. A goal of this study is to apply this new approach for the first time on a real data set.

Fagus sylvatica L. is one of the main forest tree species in Europe. Despite its ecological and economic importance, our knowledge about the determinants of its phenotypic variation is still limited (but see Kramer 2004, Bontemps et al. 2016). *F. sylvatica* is a monoecious tree species, predominantly outcrossed and anemophilous (Merzeau et al. 1994). However, Gauzere et al. (2013a)

showed a wide variability of the mating system between trees, with selfing rates varying from 0 to 48 % and the effective number of pollen donors varying from 2 to 364 among mother-trees. Thus, we can expect paternal relatednesses to be non-negligible within and between *F. sylvatica* maternal families. Here, we thus investigated how to take advantage of paternal relatedness information, now partially reachable thanks to molecular markers, to increase our knowledge about the genetic, maternal and environmental determinism of *F. sylvatica* traits. To that purpose, we sampled 60 open-pollinated families from a continuous population of *F. sylvatica* on the Mont-Ventoux, a mountain in the south-east of France culminating at 1911 m a.s.l., and genotyped a subset of the phenotyped offspring at 13 microsatellite markers. To sum up, our objectives were threefold: (i) to investigate the pollen pool structure within a *F. sylvatica* population and thus identify potential departures from HS assumptions in our progeny trial, (ii) to compare different methods to estimate h^2 based on family and animal models previously tested with simulations (Gauzere et al. 2016) and (iii) to investigate the genetic and maternal determinants for different potentially adaptive traits.

Material and methods

Field sampling and half-sib trial

On the north-side of the Mont-Ventoux (44 ° 11' N; 17 ° 5' E), a *F. sylvatica* population ranges almost continuously from 750 to 1700 m in elevation. Along this elevational gradient, we defined three plots within which we achieved the exhaustive mapping and genotyping of the reproductive individuals (Gauzere et al. 2013a). This sampling design is relevant to perform paternity recovery analysis as we know that most of the mating events occur within the maternal neighbourhood (average pollen dispersal distance δ = [35; 63] m; Gauzere et al. 2013a). These plots were chosen at elevations N1: 1020 m (dimension: 1.30 ha); N2: 1140 m (2.20 ha); N4: 1340 m (0.80 ha) and extend over ∼1.5 km (see Gauzere et al. 2013a for more details on the studied plots).

The progeny trial used in this study included a total of 60 open-pollinated families. In August 2009, 20 highly fertile and randomly distributed trees were chosen as mother-trees in each plot. We collected an average of 344 seeds per mother-tree (min = 202, max = 733) directly from the canopy. All the seeds were dried to a humidity rate of 8 % and a sample of 100 seeds per family was randomly chosen to measure the average seed weight (g). Seeds were then rehydrated and conserved at +4 ° C during 10 weeks to break dormancy and initiate germination. In April 2010, a total of 5475 seedlings were successfully germinated (91 seedlings per family on average; Online Supplementary Material A) and were transfered in a common garden at the State nursery of Aix-Les-Milles (43 ° 30'N; 5 ° 24'E). In the common garden, all seedlings were planted in independent pots of 1.2 L with sand substrate and fertilizer, arranged in 50 complete-blocks, each block including about two seedlings per family. The seedlings grew 3 years (from April 2010 to September 2013) in the common garden.

Microsatellite genotyping

For this study, we used here the genotype dataset published in Gauzere et al. (2013a) completed with 710 additional genotyped seedlings and 189 additional genotyped adults (17 adults added for N1; 2 adults added for N2; 170 adults added for N4). A total of 2088 offspring (35 offspring per family on average; 38 % of the trial; Online Supplementary Material A) were genotyped at 13 microsatellite markers (see Gauzere et al. 2013a for genotyping details). All the potentially reproductive adults within each plot (690 in total) were genotyped at the same markers (Gauzere et al. 2013a). The mother identities were confirmed for the genotyped offspring.

Paternity analysis

The genetic dataset was first used to reconstruct the "one-generation" paternal pedigree for all the genotyped offspring and to assess the realised male reproductive success (also called male fertility) of each potentially reproductive tree within each plot. We used the likelihood-based software CERVUS version 3.0 (Marshall et al. 1998). Using Mendelian probabilities of inheritance, this method tests whether (H1) a given potential father is the true father against the hypothesis (H2) that a random missing adult is the true father. For each seedling, the most-likely male (M1) is compared to the second most-likely (M2) using the criterion Δ, computed as the difference of Mendelian log-probabilities of paternity between M1 and M2. The father M1 is assigned if Δ is higher than a critical value Δ_c. Δ_c was determined by simulating 10,000 offspring and considering (i) the global allelic frequencies calculated from the three plots, (ii) 0 % of typing error, (iii) 100 % sampling of candidate fathers , (iv) a confidence level of 95 % and (v) allowing selfing events. For each offspring, paternity was assigned to M1 if (1) more than six loci with non-missing data matched between the offspring and M1, (2) Δ of the pair "offspring-father" was higher than Δ_c and (3) the potential father belonged to the same plot as the mother. Indeed, as the plots are not isolated, the potential fathers contributing to median- and long-distance mating events are more numerous than the contributors at short-distance (within the plots). Therefore, it is much more unlikely to be able to retrieve the true father outside the plots, with large

expected type I error rates (i.e. the wrong tree is assigned while the true father was not sampled).

These "relaxed" hypothesis were chosen to favour the paternity assignations within the plots, despite the risk of type I errors. As the pedigree-based quantitative genetic methods were shown to be highly robust to pedigree errors (up to 20 %; Charmantier and Réale 2005, Gauzere et al. 2016), we thus did not expect these assumptions to bias h^2 estimates. Moreover, the use of more realistic assumptions, as for instance considering non-null typing error and that a subset of the fathers has been sampled, would have increased the type II errors (i.e. an offspring is unassigned while its father was sampled; Oddou-Muratorio et al. 2003) and thus decreased the performance of the quantitative genetic methods used thereafter.

Relatedness estimates

The genetic dataset was also used to estimate paternal relatedness coefficients within and between maternal families. First, knowing the maternal genotype for each seedling, the haplotype corresponding to the paternal contribution was extracted from its diploid genotype. To deal with ambiguous characterization of the paternal alleles (occuring when the offspring is heterozygous and the parents share an allele; Online Supplementary Material B), the paternal contributions were described as diploid genotypes: at each locus, if the paternal contribution can be unambiguously deduced, the paternal allele was doubled and thus the reconstructed genotype was homozygous; in ambiguous cases the two possible alleles of the father were recorded and thus the paternal genotype was heterozygous (see Online Supplementary Material B).

These paternal genotypes were then used to estimate pairwise paternal relatednesses among each pair of seedlings k and k', $\widetilde{f}_{k,k'}$, using the software SPAGEDI (Hardy and Vekemans 2002) and the kinship coefficient of Loiselle et al. (1995). The allelic frequencies of the 690 adults, considered as a single population, were used as the reference allelic frequencies. The genetic relatednesses of pairs of seedlings within the same family were then computed as : $\widetilde{\rho}_{k,k'} = 0.25 + \widetilde{f}_{k,k'}$, and for pairs of individuals from different families : $\widetilde{\rho}_{k,k'} = \widetilde{f}_{k,k'}$. Average within- and between-family genetic relatednesses ($\widetilde{\rho}_w$ and $\widetilde{\rho}_b$, respectively) were calculated as:

$$\widetilde{\rho}_{w,j} = \sum_{k \neq k' \in family(j)} \frac{\widetilde{\rho}_{k,k'}}{n_j(n_j-1)/2} \qquad \widetilde{\rho_w} = \frac{\sum_j \widetilde{\rho}_{w,j}}{n}$$

$$\widetilde{\rho}_{b,jj'} = \sum_{k \in j} \sum_{k' \in j'} \frac{\widetilde{\rho}_{k,k'}}{n_j n'_j} \qquad \widetilde{\rho}_b = \frac{\sum_j \widetilde{\rho}_{b,jj'}}{n(n-1)/2}$$

with n_j and $n_{j'}$the sample size of the genotyped offspring of family j and j' and n the number of families.

Average genetic relatedness within and between families were analysed for each plot and relatively to the pairwise distances between the sampled mother-trees (following Robledo-Arnuncio et al. 2007). These marker-based coefficients were also used in the quantitative genetic models (see below), and, in this case, negative $\widetilde{\rho}_{w,j}$ and $\widetilde{\rho}_{b,jj'}$ were replaced by zero (following Gay et al. 2013).

Phenotypic traits

The traits presented below, and for which we estimated h^2, were measured on half of the trial (25 blocks, 2783 seedlings). These traits, related to growth, morphology, physiology and phenology, were chosen because of their demonstrated effects on plant fitness.

Growth traits —In trees, growth traits are often used as predictors for survival (Bigler and Bugmann 2004). Here, we measured height (ΔH) and diameter (ΔD) increment between august 2010 and november 2011. Assuming the stem to be a cylinder, we estimated a proxy of the aboveground stem volume as: $V_{stem} = h_{2011} \times \pi \times (d_{2011}/2)^2$ (in cm^3), where h_{2011} and d_{2011} were respectively the height and diameter measured in November 2011.

Phenological traits —Phenological traits involved in leaf development, such as bud burst and leaf senescence for deciduous species, are main determinants of the length of the growing season and thus the primary tree productivity (Churkina et al. 2005). The timing of bud burst was monitored weekly from April to May in 2011 and 2012. Five stages were used to follow the bud burst dynamic: (1) buds are dormant or swelling (equivalent to the stage 00 in the BBCH scale); (2) bud scales are broken (BBCH 07); (3) at least 15 % of the leaves are emerging (BBCH 09); (4) at least 50 % of the leaves are emerging (BBCH 15) and (5) leaves are spread out but have not reached their mature sizes (BBCH 19; see Online Supplementary Material C). The timing of leaf senescence was monitored weekly from October to November in autumn 2011. Three stages were used to follow senescence dynamics : (1) leaves have not fallen and are not coloured; (2) at least 10 % of the leaves are coloured or have fallen and (3) at least 50 % of the leaves are coloured or have fallen (see Online Supplementary Material D). For each survey, phenology was always monitored by the same two groups of observers.

We focused on two critical stages of bud burst and leaf senescence dynamics: for bud burst development, stage 3 (BBCH 9) corresponds to the most sensitive stage to frost damages; for leaf senescence, we focused on stage 2 as indicative of the end of vegetation period. We used a linear interpolation to estimate the date of passage from stage 2 to 3, $t_{b2\rightarrow 3}$, for bud burst phenology and the date of passage from

stage 1 to 2, $t_{s1\to2}$, for leaf senescence. These dates were traduced in sum of forcing units required to achieve bud burst and leaf senescence, as the sum of degree days (i.e. sum of $T^\circ > 5^\circ C$) since February 27th (approximate date of bud dormancy break). We computed the duration of the vegetative season in 2011 as the lag between the dates of bud burst and leaf senescence: $VD = t_{s1\to2} - t_{b2\to3}$. Note that the dates of bud burst in spring 2012 presented a larger spread than in 2011 since in winter 2011–2012 many buds had frozen. Damages due to frost were recorded in June 2012 by visually estimating the percentage of empty buds per seedling after bud burst. Only the subset of seedlings with less than 25 % of buds damaged was analysed in the second year of monitoring (Table 1). At the end of summer 2011, three light-exposed leaves on the stem of each seedling were collected to measure morphological and physiological traits.

Morphological traits —Leaf mass area (LMA) is not only linked to the plant photosynthetic capacity but also to the dessication tolerance, both affecting the vegetative and reproductive biomass and therefore fitness (Dudley 1996). We first measured the fresh leaf area (LA in cm^2) with a planimeter. The leaves were then dried at 60 °C during about 3 days to finally record the leaf dry mass (LM in mg). The leaf mass area was calculated as $LMA = \frac{LM}{LA}$ (in mg/cm^2).

Physiological traits —Photosynthetic capacity can be evaluated through net CO_2 assimilation (A), which partly depends on the amount of nitogen per unit of leaf mass area (the RubisCO constitutes 20 to 30 % of the leaf nitrogen) and the stomatal conductance for water vapour (g_w). Intrinsic water use efficiency (WUE), defined as the ratio of A to g_w, represents the compromise between carbon gain and water loss. As A, g_w and WUE are hardly measurable on a large number of individuals, leaf carbon isotope composition ($\delta^{13}C$) is often used as a surrogate of WUE (Farquhar et al. 1989) to conduct quantitative genetic analysis (e.g. Marguerit et al. 2014). Note that $\delta^{13}C$ of bulk leaf material is an integrated measure of WUE, partially reflecting the carbon used for leaf growth and partly carbon assimilated shortly (days) before the harvest (Brendel 2001). For $\delta^{13}C$ and leaf nitrogen analyses, the leaves of only a subset of 1034 individuals, representative of all plots, families and blocks, were dried and ground in a ball mill. A subsample of 1 ± 0.1 mg was weighed into tin capsules. Leaf nitrogen content was measured with a continuous flow elemental analyser (Carlo Erba NA 1500; CE Instruments, Rodano, Italy) and the carbon isotope composition with a coupled isotope ratio mass spectrometer (Thermo-Finnigan; Delta S, Bremen, Germany). $\delta^{13}C$ was calculated according to the

Table 1 Description and descriptive statistics for the traits measured

Abbreviation	Trait	Category	N_w	Unit	mean	minimum	maximum	CV
ΔH	Total height increase	Growth	2403	no unit	1.474	0.096	31.083	0.824
ΔD	Total diameter increase	Growth	2403	no unit	1.089	0.084	3.585	0.425
V_{stem}	Stem volume	Growth	2406	cm^3	15.785	0.122	148.494	0.832
$t_{b2\to3(2011)}$	Bud burst date in 2011	Phenology	2605	Degree days	251.047	155.099	407.657	–
$t_{b2\to3(2012)}$	Bud burst date in 2012	Phenology	1854	Degree days	341.238	154.360	640.004	–
$t_{s1\to2}$	Senescence date in 2011	Phenology	2412	Degree days	3171.505	2997.012	3250.858	–
VD_{2011}	Vegetation season duration in 2011	Phenology	2412	Degree days	2921.650	2651.925	3095.759	–
LM	Dry leaf mass	Morphology	2518	g	0.184	0.008	0.648	0.449
LA	Leaf area	Morphology	2518	cm^2	31.570	3.850	85.860	0.388
LMA	Leaf mass area	Morphology	2518	$g\ m^{-2}$	57.463	4.668	150.979	0.166
$\%N$	Nitrogen content in leaves	Physiology	1038	%	2.138	1.340	3.040	–
$\delta^{13}C$	Water use efficiency	Physiology	1038	‰	−29.105	−33.450	−25.760	–

With N_w the number of phenotyped individuals and CV the coefficient of variation. For $t_{b2\to3(2012)}$, N_w represents the amount of individuals phenotyped with less than 25 % of the buds damaged by frost

international standard (Vienna Pee Dee Belemnite, VPDB) using the following equation:

$$\delta^{13}C = \frac{R_{sa} - R_{sd}}{R_{sd}} \times 1000$$

where R_{sa} and R_{sd} are the isotopic ratios $^{13}C/^{12}C$ of the sample and the standard, respectively. The precision of spectrometric analysis (standard deviation of $\delta^{13}C$) was assessed with internal laboratory reference material with a matrix close to the measured samples (oak leaves, n = 16, SD = 0.05 ‰) and precision among the different runs ranged from 0.08 to 0.13 ‰.

For growth and morphological traits, we calculated the global intra-population coefficient of variability, CV, as:

$$CV = \frac{\sigma(P)}{\mu(P)}$$

This CV coefficient was not calculated for the other traits since they do not have natural zero, which make mean-scaling approaches meaningless (Hansen et al. 2011; Brendel 2014).

Heritability estimates

For each trait P, we estimated h^2 using four methods exploiting different information about paternal relatednesses (methods described in Gauzere et al. 2016). We chose to consider together the 60 maternal families sampled from the three different plots, and thus neglect the genetic differentiation between plots, to optimise the use of the available genetic information (notably the $\widetilde{\rho}_{b,jj'}$).

Family model —First, we used the classical family model to estimate variance components. This mixed model considers the mother identity (Fam_j) as a random effect with variance V_F. For each seedling k of the family j, the phenotypic value P is given by:

$$P_{p,m,n,j,k} = \mu + P_h + B_m + M_p + Fam_j + \epsilon_{p,m,n,j,k} \quad (1)$$

with $\epsilon_{p,m,n,j,k}$ a residual random effect with variance V_R. All the other terms are fixed effects, with μ the mean phenotype, P_h the plot effect, B_m the block effect and M_p the observer effect (only included for the analysis of the phenological traits).

This model was analysed using the "lme" function of the R software. For each trait, we tested whether the random family effect was significant, comparing hierarchical models with and without random family component with the "anova" procedure. Three traits were transformed to match the assumption of homoscedasticity of the residuals: ΔH with a logarithm transformation, LM and LA with a square-root transformation.

Family and residual variance components (V_F and V_R) were used to derive classical narrow-sense h^2 estimates, called h^2_{Fam}, assuming true half-sib families, and corrected h^2 estimates, called $h^2_{doubleadj}$, accounting for average genetic relatedness estimated with the genetic markers $\widetilde{\rho}_w$ and $\widetilde{\rho}_b$ (Squillace 1974; Gauzere et al. 2016):

$$h^2_{Fam} = \frac{4V_F}{V_F + V_R} \quad (2)$$

$$h^2_{doubleadj} = \frac{V_F}{(\widetilde{\rho}_w - \widetilde{\rho}_b)V_R + \widetilde{\rho}_w V_F} \quad (3)$$

The confidence intervals for h^2_{Fam} and $h^2_{doubleadj}$ were derived using the delta method (see Online Supplementary Material E).

Animal model —Second, we used the animal model to account for the available pairwise relatedness information. This model considers the same fixed effects as the family model but includes a random additive genetic effect $a_{j,k}$ for seedling k in family j:

$$\begin{aligned} &P_{p,m,n,j,k} = \mu + P_h + B_m + E_n + M_p + a_{j,k} + \epsilon_{p,m,n,j,k} \\ &\{a_{j,k}\} \sim N(0, A \times V_A) \\ &\{\epsilon_{p,m,n,j,k}\} \sim N(0, I_{de} \times V_R) \end{aligned} \quad (4)$$

where the variance-covariance matrix of the additive genetic effects, G, is given by $G = A \times V_A$, with A the genetic relatedness matrix and V_A the additive genetic variance; the variance-covariance matrix of the residual effects is given by $I_{de} \times V_R$, with I_{de} the identity matrix and V_R the residual variance. The heritability was estimated as:

$$h^2 = \frac{V_A}{V_R + V_A} \quad (5)$$

We also used an animal model accounting for a maternal random effect:

$$P_{p,m,n,j,k} = \mu + P_h + B_m + E_n + M_p + a_{j,k} + m_j + \epsilon_{p,m,n,j,k} \quad (6)$$

with m_j the maternal effect associated to family j. As for the ϵ, all m_j were assumed independent, with variance V_M. Using this model, h^2 and h^2_{mat} were estimated as:

$$h^2 = \frac{V_A}{V_R + V_M + V_A} \quad (7)$$

$$h^2_{mat} = \frac{V_M}{V_R + V_M + V_A} \quad (8)$$

Note that in the following, the model without maternal effect (eq. 4) will be refered as a "non-informed" model and the model specifying maternal effect (eq. 6) as an "informed" model.

The influence of maternal effects was tested comparing the hierarchical models (4) and (6) with a log-likelihood ratio test (Wilson et al. 2010). For traits with significant maternal effects, we tested if the average phenotypic values

were correlated to the average weight of the seeds measured at the family level.

Animal models were used considering two different relatedness matrices A: (a) the one-generation pedigree information (HS or FS) available for the pairs of assigned individuals and half-sibs assumption for other pairs of offspring (method "HSFS"; El-Kassaby et al. 2011; Gauzere et al. 2016) and (b) hybrid relatedness information, combining the one-generation pedigree information for the pairs of assigned individuals (i.e. genotyped individuals for which we retrieved the father) and the average relatedness information within and between each family estimated from the genotyped individuals ($\widetilde{\rho}_{w,j}$ and $\widetilde{\rho}_{b,jj'}$) for the pairs of unassigned or ungenotyped individuals (instead of $\rho_{w,j}$= 0.25 and $\rho_{b,jj'}$ = 0) (method "MH"; see Online Supplementary Material F; Gauzere et al. 2016). Gauzere et al. (2016) showed that the "MH" method provided more reliable h^2 estimates than the "HSFS" method if progenies were sampled from populations with related adults, else they performed equally. Analyses of the animal models were performed with the software AsReml v3.0.5 (Gilmour et al. 2006). Confidence intervals for h^2 were provided by AsReml.

Results

Male reproductive success and variation of relatednesses

Among the 2783 phenotyped individuals, the father was retrieved for 658 of them; the one-generation pedigree was thus known for 24 % of the phenotyped individuals (and 46 % of the genotyped individuals; Online Supplementary Material A). Based on the whole genotyped seedlings in the trial, we found that male reproductive success was highly unbalanced, with 60 % of the adults with null estimated fertility, many individuals with low fertility and few individuals with high fertility (see Online Supplementary Material G). The average selfing rate was 6.4 %.

Average paternity relatedness estimated from all the genotyped individuals showed a weak departure from half-sib assumptions, with $\widetilde{\rho}_w$ = 0.276 and $\widetilde{\rho}_b$ = 0.001 (Fig. 1). Paternal relatednesses tended to increase more $\widetilde{\rho}_w$ than $\widetilde{\rho}_b$ (deviation of 0.026 and 0.001, respectively). Plot N1 had the highest $\widetilde{\rho}_{w,j}$ and the most variable $\widetilde{\rho}_{b,jj'}$ and plot N2 the most variable $\widetilde{\rho}_{w,j}$ (Fig. 1). At the scale of Mont-Ventoux, one could expect maternal pollen pools within each plot to be more related than maternal pollen pools from different plot. However, the $\widetilde{\rho}_{b,jj'}$ estimated between families from different plots ("between-plots" boxplot in Fig. 1) were only slightly lower than $\widetilde{\rho}_{b,jj'}$ estimated between families within the same plot, and values covered the same range than the $\widetilde{\rho}_{b,jj'}$ estimated within plot N1. The low $\widetilde{\rho}_{b,jj'}$-values may be explained by the spatial genetic structure of the pollen cloud. Indeed, $\widetilde{\rho}_{b,jj'}$ rapidly decreased with the distance among mother-trees: above a threshold included in [50; 100] m, corresponding to the range of the average pollen dispersal distance (δ = 46 m; Gauzere et al. 2013a), relatednesses among maternal pollen pools were almost unsensitive to distance (Fig. 2).

Heritability estimates

The low genetic structure within and among plots supported our choice to estimate genetic parameters from all the 60 families together. Overall, as the plots are non-genetically isolated units in a largest population, estimating one global genetic variability parameter provides a more integrative measure of the adaptive potential of the *F. sylvatica* population. Overall, the traits measured in the common garden

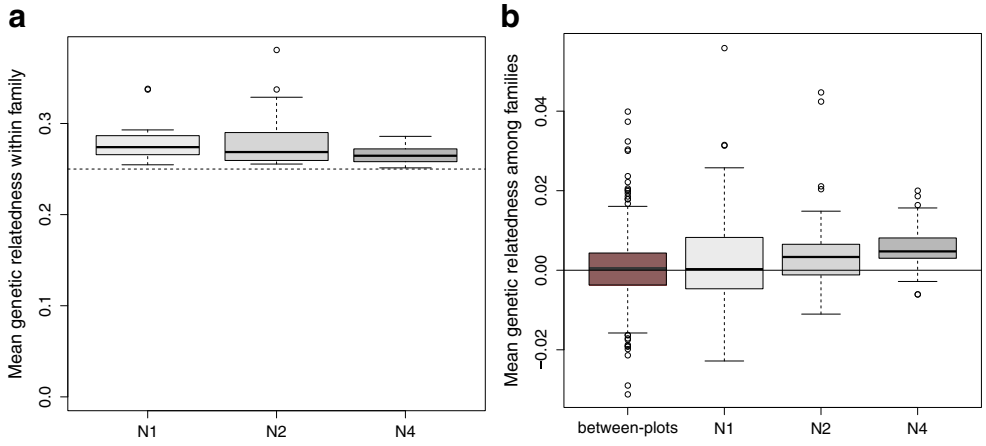

Fig. 1 **a** Variation of average genetic relatedness within families, $\widetilde{\rho}_{w,j}$, within-plots. The dashed line represents the relatedness value of 0.25 expected under half-sib assumptions. **b** Variation of average genetic relatedness between families, $\widetilde{\rho}_{b,jj'}$, within- and between-plots. The line represents the relatedness value of 0.0 expected under half-sib assumptions. In both graphs, the thick line in the boxplots represents the median of the values and the bounds of the whiskers of the first and third quartiles

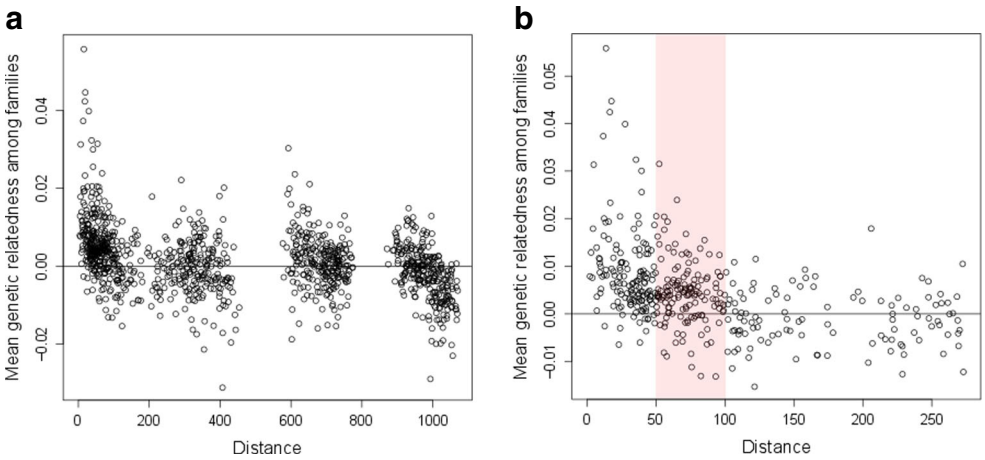

Fig. 2 Relationship between the mean pairwise genetic relatedness and the distance separating each pair of mother-trees. **a** Over the whole distance range, with four clouds corresponding respectively to within-plot, N1–N2, N2–N4 and N1–N4 mother-trees. **b** A within-plot zoom allows to define a threshold between 50 and 100 m (indicated by the coloured band) above which the mean genetic relatedness among families no more depends on the pairwise distances among mother-trees

presented a large phenotypic variation ($CV_{mean} \in [0.17; 0.8]$; Table 1). The growth traits, notably V_{stem} and ΔH, presented a larger phenotypic variation ($CV = 0.69$ on average) than morphological traits ($CV = 0.33$ on average). For all traits, the inclusion of a random family component improved the models, indicating that V_A and h^2 were significantly different from zero (results not shown). Significant maternal effects were found for three traits: two growth traits, ΔD and V_{stem} ($V_M = 0.17$, p value $= 3.8e^{-05}$ and $V_M = 0.13$, p value $= 2.3e^{-05}$, respectively), and one phenological trait, $t_{b2\rightarrow3(2011)}$ ($V_M = 0.09$, p value $= 0.02$; Table 2). No maternal effect was detected the second year of bud burst monitoring ($t_{b2\rightarrow3(2012)}$). Among these three traits, only the mean phenotypic values of V_{stem} were significantly correlated to the family average of seed weight (see Online Supplementary Material H). This phenotypic correlation was positive, indicating that families with larger seed weight also had the larger vegetative production at the seedling stage after 2 years of growth.

For traits unaffected or slightly affected by maternal effects, the different statistical methods produced similar h^2 estimates. Even the confidence intervals were quite similar between the family models (h^2_{Fam} and $h^2_{doubleadj}$) and the animal models (h^2_{HSFS} and h^2_{MH}; Fig. 3). Much larger differences between the family and animal methods were found for the traits affected by maternal effects. The family and hybrid methods presented a difference of 0.69 for $h^2_{\Delta D}$, 0.58 for $h^2_{V_{stem}}$ and 0.37 for $h^2_{tb2\rightarrow3(2011)}$ (Fig. 4). Overall, heritability estimates obtained with the family method (eq. 2) tended to be higher and to have larger confidence intervals than the estimates derived from the animal methods. The two methods using the animal model, i.e. the methods using the incomplete pedigree (method HSFS) or hybrid relatedness information (method MH), provided comparable h^2 estimates, the HSFS method tending to provide sightly higher h^2 values than the MH method.

Focusing on hybrid method estimates, i.e. the more robust method to family departures according to previous simulation work (Gauzere et al. 2016), we showed that trait heritabilities were low to moderate (global mean = 0.22). The height increment presented the highest heritability with $h^2_{\Delta H} = 0.35$ and the stem volume the lowest heritabilty with $h^2_{V_{stem}} = 0.13$. For the traits displaying maternal effects, the proportion of maternal variance was similar to the proportion of additive genetic variance (Table 2), highlighting the non-negligible contribution of the maternal effects in the family effects. For the traits presenting significant maternal effects, h^2 were lower ($\text{mean}_{h^2 mat} = 0.14$) and the estimates were less accurate (larger confidence intervals) than for the other traits (Figs. 3 and 4).

Table 2 Proportion of additive genetic and maternal effects explaining the total phenotypic variance (h^2 and h^2_{mat}, respectively) of each trait using the "Hybrid" method

Trait	h^2	p val(Mat)	h^2_{mat}
ΔH_{ln}	0.35	0.14	–
ΔD	0.17	3.8^{-05}	0.17
V_{stem}	0.14	2.3^{-05}	0.13
$t_{b2\rightarrow3(2011)}$	0.12	0.02	0.09
$t_{b2\rightarrow3(2012)}$	0.15	1	–
$t_{s1\rightarrow2}$	0.20	0.36	
VD_{2011}	0.26	0.22	–
$LM_{\surd}$	0.26	1	–
$LA_{\surd}$	0.21	1	–
LMA	0.20	0.28	–
$\%N$	0.30	0.59	–
$\delta^{13}C$	0.26	0.76	–

h^2_{mat} was only estimated for the traits presenting significant maternal effects (i.e. p val(Mat) < 0.05). $_{ln}$ and $_{\surd}$ indicate that a transformation has been applied to the original variable

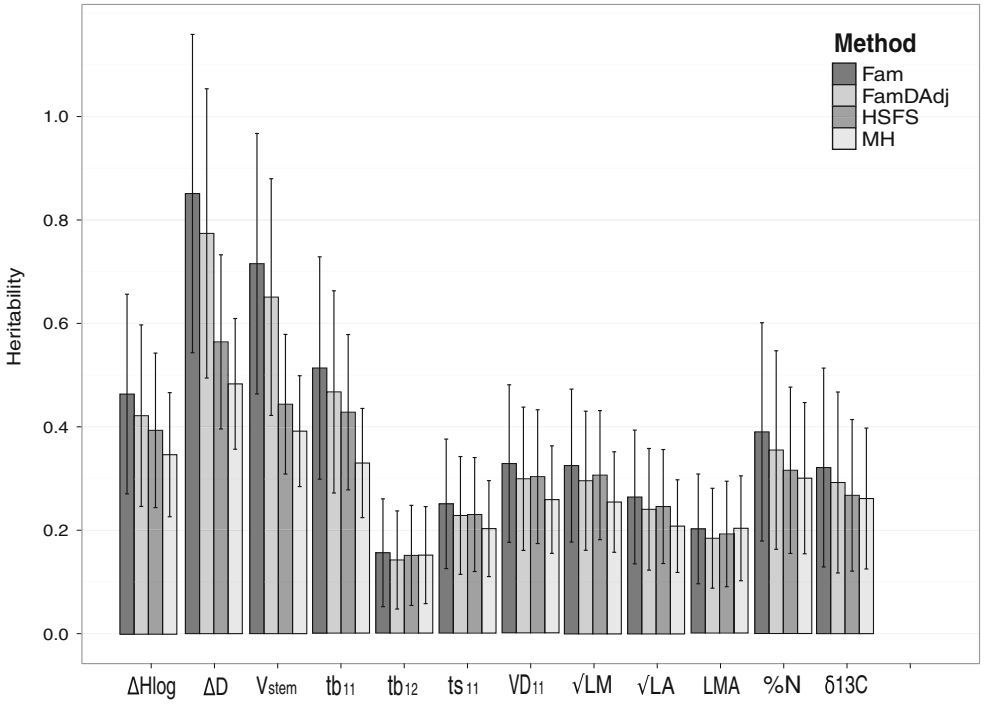

Fig. 3 Comparison of different methods to estimate heritability for the 12 studied traits (trait code in Table 1). The "Fam" and "FamDAdj" methods use the family model (models 1, eq. 2 and eq. 3; also called non-informed model). The "HSFS" and "MH" methods use the animal model (model 4 without maternal effects, eq. 7). The "FamDAdj" accounts for the $\widetilde{\rho}_w$ and $\widetilde{\rho}_b$. The "HSFS" method uses the incomplete pedigree information available, and the "MH" method uses hybrid relatedness information, mixing pedigree and average marker-based coefficients. Whiskers around the estimates represent the 95 % confidence intervals

Discussion

Departure from random mating faintly affected h^2 estimates

Unexpectedly, the unbalanced mating system in the studied *F. sylvatica* population only moderately increased the overall average marker-based relatedness within ($\widetilde{\rho}_w$ = 0.276) and between families ($\widetilde{\rho}_b$ = 0.001) as compared to the assumptions of open-pollinated families (ρ_w = 0.25 and ρ_b = 0). As a consequence, for the nine studied traits not affected by maternal effects, the use of pedigree information in an animal model (methods "HSFS" and "MH") did not change h^2 estimates compared to the simpler family model. In that case, the similar performances of the methods supported previous simulation work that showed that average marker-based relatedness coefficients derived from microsatellite markers can be efficiently used to estimate h^2 (Gauzere et al. 2016). Another potential source of bias on h^2 estimates is the presence of bi-parental inbreeding in the natural population, i.e. relatedness among parents (Bush et al. 2011; Gauzere et al. 2013b). In that case, the "HSFS" method which uses only the one-generation pedigree is expected to be more biased than the methods using the average marker-based relatedness coefficients (e.g. "MH" method; Gauzere et al. 2016). The similar h^2 estimates obtained with these two methods thus suggested that relatedness among parents is low enough not to affect h^2 estimates and we do not expect inbreeding or inbreeding depression to significantly impact h^2 estimates based on our progeny population. In the literature, most inbreeding estimates for beech populations suggest that the levels of bi-parental inbreeding and selfing are negligible and thus support our assumption (Buiteveld et al. 2007; Chybicki et al. 2009). Overall, the observed departures from random mating (i.e. pollen dispersal restricted by distance, unbalanced male reproductive success among trees, selfing) are

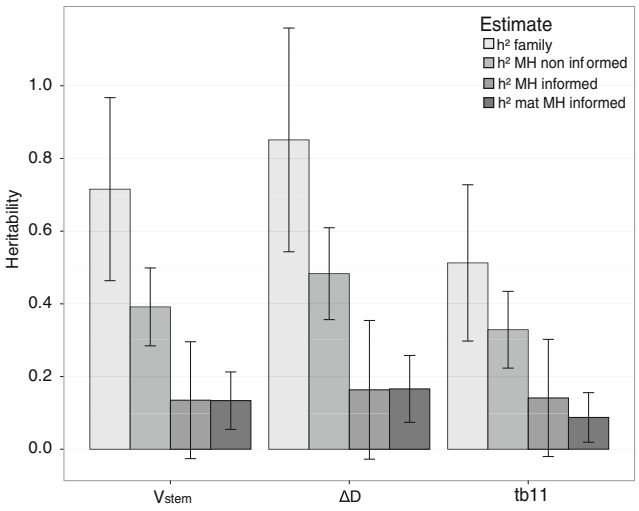

Fig. 4 Estimation of h^2 for the traits presenting significant maternal effects using the family method and the hybrid matrix method ("MH") with models where maternal effects were non-informed and informed. The estimation of h^2_{mat} using the informed MH method is also represented. The bars around the estimates represent the confidence intervals at 95 %

probably counterbalanced by the large effective population size (Ne) in Mont-Ventoux; Lander et al. (2011) estimated $Ne = 1.82 \times 10^3$ for the western region of Mont-Ventoux, where our study site is located. Thus, in large, outcrossed and weakly inbred natural populations, highly unequal male fertilities are not likely to result in significant departures from half-sib assumptions.

Isolation and decline of beech stands due to climate change are already observed in Mediterranean areas (Penuelas and Broada 2003). To our knowledge, increased selfing rate in small beech populations was never reported, but inbreeding levels were found to increase with isolation (Jump and Penuelas 2006). In these fragmented or isolated populations, inference of additive variances using family estimates may be strongly biased. Note however that departure from random mating is not expected to systematically affect h^2. In cases where family trials significantly departs from both $\rho_w = 0.25$ and $\rho_b = 0$, these departures can have balancing effects on the estimation of h^2: $\rho_w > 0.25$ leads to overestimate V_A and h^2, while $\rho_b > 0$ leads to underestimate V_A and h^2 (Squillace 1974; Gauzere et al. 2016). In these situations, neglecting ρ_b and correcting h^2 according to ρ_w only will lead to underestimate h^2 (e.g. considering $h^2 = \frac{1/\rho_w \times V_F}{V_R + V_F}$; Gauzere et al. 2016). Only the double correction from both ρ_w and ρ_b increment can correctly account for both departures (Squillace 1974; Gauzere et al. 2016).

Maternal effects strongly affected h^2 estimates

In this study, neglecting maternal effects for some traits was the main source of variation in h^2 estimates using the classical family model. For the three traits presenting maternal effects, the family and animal methods produced largely divergent h^2 estimates, with an overestimation of h^2 when using family methods. Similarly, Hansen and Nielsen (2010) showed that in presence of moderate departure from $\rho_w = 0.25$, models using half-sib assumptions or pedigree information lead to the same h^2 estimates, except for traits presenting significant dominance effects. Our study confirms that in presence of maternal or non-additive genetic effects, accounting for paternal relatedness information at the individual level through an animal model can strongly improve h^2 estimate. Surprisingly, animal models accounting for maternal effects estimated h^2 with a reduced precision as compared to family or non-informed animal models. This is likely due to the use of insufficient paternal relatedness information (paternal relatednesses known for about 24 % of the trial) to properly disentangle additive from maternal inheritance. However, it is interesting to notice that even if the whole trial has been genotyped, the amount of father-related individuals (depending on the mating system of the population) would still limit an accurate estimation of variance components (Gauzere et al. 2016). Moreover, Gauzere et al. (2016) showed that insufficient paternal relatedness information led to significantly underestimated h^2 in such cases. Here, the true h^2 value for the traits presenting maternal effects is probably higher than the one estimated with the informed animal models. However, this underestimation does not completely explain why $V_M + V_A$ values estimated with the informed animal model were lower than the global V_A estimated with the family model. One possible explanation is that the use of incomplete pedigree information also conducted to underestimate V_M, as suggested by Morrissey et al. (2007), who showed that pedigree errors can downwardly bias V_M.

Unfortunately, the absence of complete pedigrees in tree natural populations is the rule rather than the exception, notably because of the high migration rates by pollen (Ashley 2010) leading to unknown paternity. Even if the bias produced by the use of incomplete relatedness information seems hard to quantify (it will depend on the mating system and the sampling design), understanding whether V_A, h^2 and V_M are underestimated or overestimated is central to accurately evaluate the adaptive potential of natural populations. Here, we highlighted that the comparison of several methods to estimate V_A and h^2 using different relatedness information is a first informative analysis when there is little knowledge about the potential departures from random mating or trait determinism.

Overview of the determinism of functional traits for beech

Heritabilities were significant but globally low (ranging from 0.13 to 0.35), suggesting low to moderate genetic control for the functional traits and the environmental conditions investigated in this study. Some theoretical and empirical literature shows that traits closely linked to fitness should present low h^2 because *(i)* the genetic variance of these traits should be eroded by selection and *(ii)* these traits should present a complex genetic architecture and thus capture more environmental variance (Merilä and Sheldon 1999). Surprisingly, in this study, we found no differences in the h^2 values among classes of functional traits: for instance, growth traits, expected to be highly related to fitness, presented on average $h^2 = 0.21$ and morphological traits $h^2 = 0.22$. Based on this evolutionary parameter, we cannot conclude about the genetic architecture or the form of selection applied on the different functional traits. An increasing amount of studies challenges the relevance of h^2 as a good proxy of the past or future evolutionary response of natural populations, notably because low h^2 can either be the result of low V_A, high V_R or both (Hansen et al. 2011). However, h^2 is still the most frequently reported evolutionary

parameter and it has the advantage to be applicable to any kind of trait, and in particular to quantitative traits without natural zero, such as most of the traits considered in this study.

Comparison of our h^2 estimates with other ex situ h^2 values reported in the literature is difficult because, first, these estimates greatly depend on the population studied and the environment in which the phenotypic and genetic variances are expressed (Charmantier and Garant 2005), and second, to our knowledge, only one study has already estimated the heritability of phenological and ecophysiological traits for beech populations in common garden conditions (Kramer 2004). Based on a mixture of progenies sampled in several European provenances, they estimated a narrow-sense heritability, h^2_{ns}, of 0.56 and 0.58 for 2 years of bud burst monitoring, and non-significant broad-sense heritabilities, h^2_{bs}, for LMA and $\%N$. h^2_{ns} were estimated assuming $V_F = 4 \times V_A$, and h^2_{bs} using a clonal test. Note that using the family method, we estimated a $h^2_{ns} = 0.51$ for the first year of bud burst monitoring, which was quite similar to the estimates of Kramer (2004). Overall, phenological traits are commonly assumed to be under strong genetic control in the literature (Howe et al. 2003). In our progeny test, the low h^2 estimates for the 2 years of bud burst phenology can be partly explained. Indeed, $h^2_{tb2\rightarrow3(2011)}$ was probably underestimated due to general methodological biases linked to the presence of maternal effects (as described above) and $h^2_{tb2\rightarrow3(2012)}$ was probably deflated due to the particular environmental conditions in autumn 2011, which increased the environmental variance ($V_{tb2\rightarrow3(2011)} = 1377$, $V_{tb2\rightarrow3(2012)} = 4675$).

We can also compare our common garden h^2_{MH} estimates with the in situ h^2 estimates for several functional traits in plot N1 produced by Bontemps et al. (2016) using the Ritland's method (Ritland 1996). We found equivalent h^2 estimates for $\delta^{13}C$ and LA (Bontemps et al. 2016; Online Supplementary Material I). For bud burst phenology, LM, LMA and $\%N$ the confidence intervals for the ex situ and in situ estimates did not overlap (Bontemps et al. 2016; Online Supplementary Material I). Overall, in situ h^2 estimates were higher than ex situ estimates. This trend has also been highlighted by Weigensberg and Roff (1996), contrary to the expectation that h^2 should be overestimated in controlled conditions due to the reduction of the environmental variability. Note also that ex situ h^2 were estimated based on juvenile traits, while in situ they were measured on adult traits. The lower heritability estimated for juveniles may be due to the fact that juveniles are more subject to non-additive (e.g. epigenetic) or maternal effects. Despite the difficulties to compare h^2 derived from different common garden experiments, some studies showed that for the same population, h^2 estimated in controlled conditions were comparable to h^2 estimated in natural conditions (Weigensberg and Roff 1996; St Juliana and Janzen 2007) and thus should provide relevant estimations of both the significance and the magnitude of h^2 in nature.

Finally, the maternal determinism on beech's traits had not been investigated yet to our knowledge. Here, maternal effects were detected for diameter growth and stem volume traits, as well as for the first year of bud burst monitoring. In the second year, this effect was no more detected, suggesting that maternal effects do not permanently affect the expression of the dates of bud burst in the studied species and population. The significant correlation between the average stem volume and seed weight suggested that maternal effects were also non-permanent effects on V_{stem} phenotypic variation (commonly in plants, seedlings with more ressource available in the seeds produced higher vegetation in the first years of growth; e.g. Baraloto et al. 2005). Thus, maternal effects observed in this study can be view as nuisance effects, i.e. effects conducting to wrongly evaluate the available variance for selection, if we look at the long-term evolvability. At the opposite, this proportion of variance explained by maternal effects can play an important role in the short-term evolutionary response of natural populations and affect predicted evolutionary trajectories, notably for tree species which present long lifetime and high demographic loss at juvenile stage (Petit and Hampe 2006). Overall, the maternal determinism of growth and phenological traits questions previous studies which investigated the genetic determinism and adaptive potential on seedlings for species within this wide phylogenetic family without considering maternal effects.

Conclusions

Combining population and quantitative genetics approaches, we showed here that average marker-based coefficients can be efficiently used to both investigate the genetic structure and mating characteristics of natural populations and to estimate heritability using the new hybrid relatedness matrix (Gauzere et al. 2016). Comparing different methods, our results confirmed that the methods using family assumptions can accurately estimate the available additive genetic variance of large wild outcrossed plant populations, except in presence of maternal effects. Now that paternal relatednesses can be partially reconstructed using molecular markers, the sample of maternal families with large relatedness variance became helpful to improve our knowledge about the complex determinism of functional traits.

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