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Title: Microgeographic adaptation and the effect of pollen flow on the adaptive potential of a temperate tree species.

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Summary

- In species with long-distance dispersal capacities and inhabiting a large ecological niche, local selection and gene flow are expected to be major evolutionary forces affecting the genetic adaptation of natural populations. Yet, in species such as trees, evidence of microgeographic adaptation and the quantitative assessment of the impact of gene flow on adaptive genetic variation are still limited.
- Here, we used extensive genetic and phenotypic data from European beech seedlings collected along an elevation gradient, and grown in a common garden, to study the signature of selection on the divergence of eleven potentially adaptive traits, and to assess the role of gene flow in resupplying adaptive genetic variation.
- We found a significant signal of adaptive differentiation among plots separated by less than one kilometre, with selection acting on growth and phenological traits. Consistent with theoretical expectations, our results suggest that pollen dispersal contributes to increase genetic diversity for these locally differentiated traits.
- Our results thus highlight that local selection is an important evolutionary force in natural tree populations and suggest that management interventions to facilitate movement of gametes along short ecological gradients would boost genetic diversity of individual tree populations, and enhance their adaptive potential to rapidly changing environments.

Key words: Adaptive divergence; Climate change; Common garden ; Elevation gradient; *Fagus sylvatica*; Genetic diversity; Natural selection; Pollen dispersal

Introduction

Recent interest in microgeographic adaptation, *i.e.* adaptation at spatial scales compatible with substantial amounts of gene dispersal, suggests we must reconsider the scale at which evolution occurs (Richardson *et al.*, 2014). At this fine spatial scale, a major question that remains to be answered is whether gene flow is constraining or facilitating local adaptation. Too much gene flow would overwhelm the effects of natural selection that drive adaptive genetic divergence among populations (Lenormand, 2002) and decrease the adaptation of populations to their local environment (migration load). Conversely, gene flow can also resupply the genetic diversity eroded by genetic drift and selection, and thus facilitate future evolutionary responses (Pease *et al.*, 1989; Barton, 2001; Lenormand, 2002; Bridle *et al.*, 2010; Polechova & Barton, 2015). In many taxa, and especially long-lived organisms, gene flow is expected to be the main process maintaining the standing genetic variation within natural populations (Barton, 2001; Le Corre & Kremer, 2003; Bridle *et al.*, 2010). In the particular case of environments varying through both space and time, gene flow could also spread alleles pre-adapted to future environmental conditions (Davis & Shaw, 2001; Aitken *et al.*, 2008; Kremer *et al.*, 2012; Fitzpatrick & Reid, 2019). Although theoretical developments on this topic have been rich and provided interesting hypotheses to test in nature, empirical studies on the role and importance of gene flow in adaptation are still lacking (but see Fitzpatrick *et al.* 2015, 2016).

Increasing evidence of microgeographic adaptation in nature provides strong support for the idea that gene flow rarely prevents the emergence of local adaptation or adaptive divergence (Anderson *et al.* 2015; Eckert *et al.* 2015; Fitzpatrick *et al.* 2015; Moody *et al.* 2015; Peterson *et al.* 2016; for a review see Richardson *et al.* 2014). This evidence strengthens the conclusions of studies at larger spatial scales, showing that geographic or genetic distances among populations are not correlated with the level of local adaptation or adaptive genetic divergence (McKay & Latta, 2002; Leimu & Fischer, 2008; Hereford, 2009). These results suggest that selective pressures can be strong enough to counterbalance the effects of gene flow, and to shape genetic variation at a fine spatial scale. However, evidence of microgeographic adaptation is mainly reported for certain taxa (*e.g.* fish, Fitzpatrick *et al.* 2015; Moody *et al.* 2015; and annual or perennial herbs Anderson *et al.* 2015; Peterson *et al.* 2016), while others, such as temperate tree species, are underrepresented. In trees, there is a long tradition of large-scale adaptation studies, through provenance trials, following a quantitative genetic approach (Savolainen *et al.*, 2007; Alberto *et al.*, 2013). The development of genome-wide data and statistical methods has considerably changed this research field, and evidence of adaptations at regional and landscape scales are now flourishing (*e.g.* Csillery *et al.* 2014; Pluess *et al.* 2016). Yet, the adaptive response of phenotypic traits at the local scale is still poorly understood (but see Brousseau *et al.* 2013; Eckert *et al.* 2015). Methodological limitations that previously hampered the robust testing of local phenotypic adaptation have been largely overcome (Ovaskainen *et al.*, 2011), permitting more accurate assessments of the scale at which phenotypic adaptations occur.

Empirical evidence of beneficial effects of gene flow on adaptive potential in nature are still rare, and are often assessed in laboratory settings (*e.g.* Swindell & Bouzat 2006). Some experimental studies on inbred plant populations have demonstrated the beneficial effect of gene flow on the reduction of homozygosity and reintroduction of variation for fixed deleterious alleles (*e.g.* Costa e Silva *et al.* 2014; Bontrager & Angert 2019). However, for outbred species, the role of gene flow on the maintenance of genetic diversity has been difficult to demonstrate, especially when relying on natural crosses. This effect of gene flow is thought to be particularly important when the populations exchanging genes are strongly genetically differentiated (Pease *et al.*, 1989; Barton, 2001). Long-distance dispersal events could therefore play a major role in resupplying the genetic variation of populations and favoring the spread of beneficial alleles (Savolainen *et al.*, 2007; Kremer *et al.*, 2012). In wind-pollinated species (anemophilous), these long-distance dispersal events are common, with mean pollen dispersal distances that can easily reach hundreds of meters, with rare events of spread over tens of kilometres in some species (Austerlitz *et al.*, 2004; Petit & Hampe, 2006; Ashley, 2010; Kremer *et al.*, 2012). Pollen dispersal may thus be highly efficient for spreading beneficial alleles between populations (Kremer *et al.*, 2012). Even though a pollen grain brings half as many immigrating alleles as does a seed, gene flow by pollen or seed is expected to have similar consequences on population's adaptation and migration load (Lopez *et al.*, 2008).

Wind-pollinated tree species are therefore good study systems to look for empirical evidence of the beneficial effects of long-distance gene flow on adaptive capacities in nature.

However, to our knowledge, only two studies have looked for such evidence. In *Pinus contorta*, Yeaman & Jarvis (2006) showed a positive correlation between the level of genetic variance within populations for growth and the spatial heterogeneity of selection. This pattern is consistent with the theoretical expectation that gene flow among differentiated populations is an important source of genetic variation. Along a latitudinal gradient of genetically differentiated populations of *Pinus sylvestris*, Nilsson (1995) found that offspring harvested after one episode of reproduction showed a shift in average genetic values as compared to their mothers. This pattern is expected in the case of directional gene flow among locally differentiated populations (Figure 1). This effect of directional pollen dispersal on offspring's average genetic values for adaptive traits could be particularly beneficial if the environmental conditions of the recipient population change toward the one of the donor population (Davis & Shaw, 2001; Aitken *et al.*, 2008).

The aim of the present study is to investigate the effects of natural selection and long-distance gene flow on the adaptive potential of a major temperate tree species, the European beech (*Fagus sylvatica* L.). This anemophilous species can disperse pollen at long-distance (Piotti *et al.*, 2011), a feature thought to explain the low level of neutral genetic differentiation among populations observed from the local to the European scale (Comps *et al.*, 2001; Buiteveld *et al.*, 2007). Several phenotypic traits show significant genetic differentiation among beech provenances at the European scale (Robson *et al.*, 2018), in particular budburst date (Gomory & Paule, 2011; Kramer *et al.*, 2017), height (Rose *et al.*, 2009) and, to a lesser extent, wood anatomy, hydraulic traits and physiological foliar traits (Knutzen *et al.*, 2015; Hajek *et al.*, 2016; Stojnic *et al.*, 2018). These patterns are usually interpreted as a long-term response to selection, conferring better adaptation of beech populations to local climate and, most importantly, to summer drought. Our study aimed to test whether such patterns of phenotypic adaptation also exist at a fine spatial scale.

Here, we analysed the phenotypic and genotypic data of $\sim$ 2,300 beech seedlings from 60 maternal families grown in a common garden. These open-pollinated families were collected from three natural beech plots, spreading along an elevation gradient at fine spatial scale ($\sim$ 1.5 km in-between the most distant plots) at the southern, warm margin of this species' distribution. We focused on 11 potentially adaptive phenotypic traits, for which we previously found a significant genetic component in phenotypic variation (Gauzere *et al.*, 2016a). The first question investigated was: (1) is there genetic divergence across elevations for the quantitative traits of interest? We expected trees from the low-elevation, warmer and drier plot to have evolved traits favouring adaptation to drought, and trees from the high-elevation, colder plot to have evolved traits favouring adaptation to low temperatures. Our second question was (2) is there a signature of local selection in the differentiation pattern of the trait studied. We tested this by comparing traits differentiation to neutral differentiation shown by microsatellite markers. Our final question was: (3) does gene flow contribute to the additive variance of traits under selection at the local scale? To that purpose, we used parentage analysis to identify the offspring likely originating from local or distant pollen immigration events, and we tested how long-distance pollen dispersal affected the genetic values of offspring after one episode of reproduction.

Material and methods

Species, sampling plots and experimental design

Fagus sylvatica L. (European beech) is a monoecious, anemophilous and predominantly outcrossed tree species (Merzeau *et al.*, 1994; Wang, 2003). It is a shade-tolerant species requiring well drained and moderately deep soils and relatively high humidity rates (Breda *et al.*, 2006; Jump *et al.*, 2006). Its distribution ranges from the northern Mediterranean regions to the south of Scandinavia. On Mont-Ventoux, a mountain in the southeast of France, beech forests are located at the southern limit of their ecological range (Figure 2), in a mountainous Mediterranean ecosystem. On the northern face of Mont-Ventoux, the beech forest ranges almost continuously from 750 to 1,700 m a.s.l.. This steep elevation gradient provides almost linear variation in mean temperature and humidity with elevation (Davi *et al.*, 2011). Three plots were defined along this climatic gradient, at 1020 m (N1), 1140 m (N2) and 1340 m (N4) a.s.l (Table S1), over a total distance of about 1.5 km. A previous study showed that these plots have high pollen immigration rates (on average 53.3 %), moderate distances of pollen dispersal (on average 45.7 m), and non-negligible long-distance pollen dispersal events (Gauzere *et al.*, 2013b).

In August 2009, 20 open-pollinated families were sampled in each of the three plots (60 maternal progenies in total). Mother-trees were chosen for their high fertility and random location within the plot. In April 2010, the seeds were germinated and a subset of 46.4 seedlings on average per family were randomly planted in 25 blocks in a common garden experiment (with 1.8 seedlings per family in each block) at the State nursery of Aix-en-Provence (43 ° 30'N 5 ° 24'E). All seedlings were planted in independent pots of 1.2 L with sand substrate, fertilizer and regularly irrigated. The experiment ran for 3 years (from April 2010 to September 2013). Details on the seed collection, germination protocol and progeny test design can be found in Gauzere *et al.* (2016a).

All the potentially reproductive adults within the three plots, including the mother-trees, and an average of 23.9 offspring per family were genotyped at 13 microsatellites markers (690 adults and 1437 offspring in total; see Gauzere *et al.* 2013b for the genotyping details).

Phenotypic measurements

A set of 11 different phenotypic traits were measured on the offspring grown in the common garden. These traits, already introduced in Gauzere *et al.* (2016a), are presented briefly below (see also Table 1). Height and diameter growths were measured between August 2010 and November 2011 (ΔH, ΔD). For the whole trial, phenological events were also recorded: the date of budburst in 2011 and 2012 ($t_{b2\rightarrow3(2011)}$, $t_{b2\rightarrow3(2012)}$), the date of leaf senescence in 2011 ($t_{s1\rightarrow2(2011)}$) and the duration of the growing season in 2011 ($VD_{2011} = t_{s1\rightarrow2(2011)} - t_{b2\rightarrow3(2011)}$). At the end of the growing season in 2011, three light-exposed leaves were collected on the stem of each seedling to measure morphological and physiological traits. On fresh leaves, we first measured the leaf area (LA), and put them to dry at 60 ° C to then measure the leaf dry mass (LM) of each seedling. The leaf mass area, related to the photosynthetic capacity and stomatal conductance of the plant (Reich *et al.*, 2003), was calculated as $LMA = \frac{LM}{LA}$. Finally, for a subset of 1031 individuals (evenly sampled across families), we measured the leaf carbon isotope composition ($\delta^{13}C$), as a surrogate for water use efficiency (Farquhar *et al.*, 1989). The nitrogen content in the leaves (*%N*) was also estimated for this subset of individuals, as leaf nitrogen density is linked to the photosynthetic capacity of leaves (Kattge *et al.*, 2009). Note that three of these traits were transformed before analyses to limit departure from linear model assumptions: ΔH with a logarithm transformation, and *LM* and *LA* with a square-root transformation (following Gauzere *et al.* 2016a).

Test for population genetic differentiation

To assess the genetic differentiation of quantitative traits among plots we ran a specific linear mixed model (called "animal model") that uses relatedness information from a pedigree to dissociate the contribution of genetic, population and environmental effects on the total phenotypic variance of traits. Thus, we analysed each trait Y measured in common garden using the following model, and focused particularly on the significance of the fixed plot effect P:

$$Y_{i,f,p,k} = \mu + P_i + B_f + M_p + a_k + \varepsilon_{i,f,p,k} \tag{1}$$

with, as fixed effects, μ the intercept, P_i the effect of the plot of origin, B_f the effect of the block and M_p the observer effect (only included for the analysis of the phenological traits). The random terms are the additive genetic values of the individuals k, $\{a_k\} \sim N(0,\ AV_A)$ with A the matrix of pairwise relatedness derived from a pedigree, and V_A is the additive genetic variance, and the residuals, $\{\varepsilon_{i,f,p,k}\} \sim N(0,\ I_{de}V_R)$ with I_{de} the identity matrix and V_R the residual variance.

The (co)variance structure of the additive genetic effects was defined using a one-generation pedigree reconstructed in a previous study (Gauzere *et al.*, 2016a). Here, we did not model maternal effects because of the lack of power to accurately dissociate maternal and additive genetic effects (Gauzere *et al.*, 2016b). Model (1) was fitted in AsReml-R (Gilmour *et al.*, 2006). Using similar model, Gauzere *et al.* (2016a) previously showed that all traits presented moderate narrow-sense heritability, with h^2 ranging from 0.12 to 0.35.

Signature of natural selection accounting for genetic drift

We used the method developed by Ovaskainen *et al.* (2011) to detect signatures of natural selection in trait differentiation among populations. This method requires genotypic and the phenotypic data, that are first analysed under a neutral model that assumes that the genes coding for the trait are as divergent as the neutral markers ($Q_{st} = F_{st}$ case). This first model thus considers the population mean genetic additive values as normally distributed, with the covariance between pairs of population means being proportional to the pairwise average coancestry between these populations (*i.e.* assuming neutrality for the trait):

$$\mathbf{A}^{\mathbf{P}} \sim N(0, 2V_A \times \theta^{\mathbf{P}}) \tag{2}$$

with $\mathbf{A}^{\mathbf{P}}$ the vector of the population-level effects , V_A the additive genetic variance of the trait, $\theta^{\mathbf{P}}$ the matrix of pairwise population-level coancestry coefficients. Note that the $\theta^{\mathbf{P}}$ matrix provides an estimation of Fst.

The method then considers an animal model for each quantitative trait Y, that decomposes the additive value into a population and an individual effect. The population-level additive effect is determined by the mean genetic additive values in the parent population and the individual-level additive effects a_k are defined the same way as in model (1). The presence of selection on the trait is tested with the S statistics, which evaluates whether the realized pattern of population mean genetic additive values is likely under the neutral model (eq. 2).

To apply this method to our dataset, we first used the genotyping of the adult trees and the admixture F-model for neutral divergence among populations implemented in the R-package RAFM, to estimate $\theta^{\mathbf{P}}$ and the index of neutral genetic differentiation, Fst (Karhunen & Ovaskainen, 2012). We then analysed the traits measured in the common garden using the same fixed effects as in model eq.(1) and the one-generation pedigree using the R-package DRIFTSEL, to estimate V_A, $\mathbf{A}^P$ and S (Karhunen *et al.*, 2013). These Bayesian analyses were repeated three times per trait to ensure convergence of the MCMC chains. We provide the average posterior S estimates (the chains provided very similar S values; Table S2). We also measured a low autocorrelation within the chains, indicating that the space of the parameter values is efficiently explored.

Immigrant characterization based on molecular markers

Categorical assignments of paternity

We first tried to identify the father of the genotyped seedlings to characterize their immigration status. To that purpose, we used the likelihood-based method of categorical paternity assignment implemented in CERVUS (Marshall *et al.*, 1998) with allelic frequencies estimated from the genotypes of all adult trees. All the reproductive adults from the three sampled plots were considered as candidate fathers, but only fathers within the mother's plot were assigned (see below). We considered 0 % typing error, 100 % sampling of candidate fathers, a confidence level of 95 %, and allowed selfing (following Gauzere *et al.* 2016a). These choices were made to favour assignments to genotyped fathers, despite the risk of type I errors (*i.e.* a wrong tree is assigned while the true father is not sampled), knowing that quantitative genetic methods are robust to pedigree errors (Charmantier & Réale, 2005; Gauzere *et al.*, 2016b). We successfully assigned the paternity for 45.8 % of the genotyped offspring. We considered that individuals assigned to a father located within their mother's plot come from "local" pollen pool, while individuals with no compatible father were considered "immigrant". Non-categorised individuals include individuals genotyped at less than 6 markers, offspring not assigned because of compatibility with multiple fathers, and offspring assigned to a father outside their mother's plot. Indeed, given the large number of trees potentially contributing to median- and long-distance mating events, we considered that our ability to retrieve the true father outside the mother's plot was low and presented large expected type I error rate.

Probability of long-distance pollination

To refine the "immigrant" status, we also used the whole genetic dataset and a non-categorical paternity analysis to assess the likelihood for each genotyped seedlings to belong to long-distance immigrant pollen pool. To that purpose, we estimated the Mendelian transition probabilities (T) that each offspring has the observed genotype g_0 while originating from crossfertilization of female j with an ungenotyped male from a population with the allelic frequencies BAF: $T(g_0|g_j,BAF)$. We estimated this transition probability using (1) the global allelic frequencies estimated from a larger-scale study on the whole north-face of the Mont-Ventoux (AF_{global}; Lander *et al.* 2011), and (2) the local allelic frequencies estimated from the genotyping of all the reproductive trees in the three study plots (AF_{local}). This allowed us to compute the probabilities that each offspring has the observed genotype g_0 given that it originates from long-distance and local pollination event, $T(g_0|g_j,AF_{global})$ and $T(g_0|g_j,AF_{local})$ respectively. From these two probabilities, we derived for each offspring the LOD-score of long-distance immigration vs. local immigration:

$$v_{g_0} = log\left(\frac{T(g_0|g_j,AF_{global})}{T(g_0|g_j,AF_{local})}\right) \tag{3}$$

$v_{g_0} > 0$ indicating that offspring g_0 is more likely originating from a long-distance pollination event and $v_{g_0} < 0$ that it is more likely originating from a short-distance pollination event. These transitions probabilities were estimated using the MEMMi model (Gauzere *et al.*, 2013b).

Impact of pollen immigration on quantitative traits

We explicitly tested whether *(i)* offspring originating from distant fathers presented more genetic variance for quantitative traits than offspring originating from local fathers, which is expected in the case of migration from genetically differentiated populations in an island model, and whether *(ii)* offspring originating from distant fathers had shifted average genetic values for quantitative traits as compared to the ones originating from local fathers, which is expected in the case of directional pollen immigration from genetically differentiated populations along the gradient (Figure 1). In both cases *(i)* and *(ii)*, pollen dispersal would result in increasing genetic variance and adaptive capacities. We ran two different models depending on how the immigration status was characterized (categorical or quantitative variable).

First, for each phenotypic trait Y, we looked for differences in the mean and variance of additive genetic values (a_k) between "local" and "immigrant" groups, using the following animal model:

$$Y_{i,f,p,k} = \mu + P_i + B_f + M_p + a_k + \varepsilon_{i,f,p,k} \tag{4}$$

with the same fixed effects than in model (1), but here the (co)variance structure of the additive genetic effects depend on the immigration classes, with l = "local" and m = "immigrant": $\begin{bmatrix} V_{Al} & 0 \\ 0 & V_{Am} \end{bmatrix}$. Note that the effect of "local" and "immigrant" categories on the average trait values was tested and found to be never significant. This fixed effect is thus absent in the final model. From model (4), we thus estimated two variance components: V_{Al} and V_{Am}.

We then performed a random regression model to investigate the effect of long-distance pollen dispersal, ν, on the variation in additive genetic variance. Random regression models are commonly used in evolutionary biology and breeding to allow additive genetic effects (or any random effect) to vary with a covariate (*e.g.* environment, age; Nussey *et al.* 2008). This model defines a random intercept and slope at the additive genetic level, as deviation from the mean linear effect of ν on Y, to model the variation of V_A with ν as: $V_A(\nu) = V_{A\ intercept} + \nu^2.V_{A\ slope} + 2.\nu.cov_A(intercept, slope)$. The effects of ν on V_A was tested using the following models:

$$Y_{i,f,p,k} = \mu + P_i + B_f + M_p + \alpha\nu_k + a_k + \varepsilon_{i,f,p,k} \tag{5}$$

$$Y_{i,f,p,k} = \mu + P_i + B_f + M_p + \alpha\nu_k + \tilde{a}_k + \varepsilon_{i,f,p,k} \tag{6}$$

where $\tilde{a}_k = a_k + \beta_k\nu_k$ describes the random intercept and slope of ν on Y at the additive genetic level in model (6). In both models (5) and (6) the term $\alpha\nu_k$ is the fixed effect of the LOD-score of long-distance vs. local migration on the trait.

From the model (6), we estimated the variances of the random intercept and slope, and the covariance between them. Note that for each individual k, we only have one value of ν and Y, but the model estimates these (co)variance components by comparing phenotypic similarities and the effects of ν on Y between relatives.

The significance of the qualitative and quantitative effects of pollen immigration on V_A were tested by comparing the two hierarchical models (1) and (4), and the two models (5) and (6), with a log-likelihood ratio test. We performed these analyses with AsReml-R, considering only the information about maternal relatednesses, as we (often) ignore the father's identity of "immigrant" individuals.

Relatedness and *Nep* estimates

We used the genetic dataset to estimate the average paternal relatedness ($\bar{\rho_p}$) and effective number of pollen donors ($Nep = 1/\bar{\rho_p}$) in the "local" and "immigrant" pollen clouds. We first extracted the haplotype corresponding to the paternal contribution to each diploid genotype following Gauzere *et al.* (2016a). These paternal genotypes were then used to estimate the pairwise paternal relatednesses among each pair of offspring k and k' ($\rho_{k,k'}$) using the kinship coefficient of Loiselle *et al.* (1995) implemented in SPAGEDI (Hardy & Vekemans, 2002), and the local allelic frequencies (AF_{local}). These coefficients were estimated removing all the selfed individuals to only compare the outcrossed pollen pools.

Results

Neutral genetic differentiation among plots and pollen immigration

The admixture F-model of Karhunen & Ovaskainen (2012) estimated a posterior Fst value of 0.026 [0.024; 0.029], consistent with previous estimates of neutral genetic differentiation among populations measured with a comparable method but from 51 populations over a much wider area of Mont-Ventoux ($Fst = 0.026$; Lander *et al.* 2011). The coancestry estimates within plots (diagonal elements of the θ^P matrix) indicated that plot N1 tended to have lower effective population size than plot N2 and N4 (Table 2). Plots N1 and N2 exhibited slightly higher between-plots coancestry coefficient, suggesting that the two closest plots exchanged more gene flow ($\theta_{N1N2} > \theta_{N1N4} \sim \theta_{N2N4}$). Overall, the coancestry estimates were low (maximum $\theta_{N1} = 0.057$; Table 2) indicating that effective population sizes were globally large. This result is consistent with the large effective number of pollen donors Nep estimated for these sites using the same dataset (Table S1; Gauzere *et al.* 2013b).

Using paternity assignment, we categorized n = 658 offspring as "local" (45.5 %) and n = 492 as "immigrant" (34 %). A total of 295 (20 %) offspring were outside of these two categories (*i.e.* ambiguous origin). Note that this "immigrant" status includes offspring with non-sampled fathers which could be located either at a short- or long-distance to the mother-tree's plot. We checked that the LOD-score of long-distance vs. local immigration was indeed higher on average for the "immigrant" than "local" individuals, with $\overline{v}$ = -0.33 and 0.18 for local and immigrant offspring respectively (*p-value* < 0.001 ANOVA test; Figure S1).

The analysis of the pairwise coefficient of paternal relatedness showed that within the immigrant pollen pool fathers were significantly less related than within the local pollen pool, with $\overline{\rho}_p$ = 0.011 and $\overline{\rho}_p$ = 0.007 within the local and immigrant pollen pools respectively (*p-value* < 0.001 ANOVA test). Consequently, we estimated a higher effective number of pollen donors (Nep) in the immigrant than in the local pollen pool (Nep = 26.4 and 31.3 for local and immigrant pollen pools respectively).

Patterns of genetic trait differentiation and test for selection

Significant genetic differentiation across elevations was found for ΔH_{log}, ΔD, $t_{b2\rightarrow3(2011)}$ and $t_{b2\rightarrow3(2012)}$, while marginal differentiation was found for $t_{s1\rightarrow2(2011)}$ (as revealed by P effect in model (1); Figure 3). Using the method of Karhunen & Ovaskainen (2012), we detected a signal of natural selection for three of these four traits: ΔH_{log}, ΔD and $t_{b2\rightarrow3(2011)}$ (*i.e.* $S >$ 0.90; Figure 3). For growth traits, plots at intermediate and high elevations presented the highest height and diameter increments ($\Delta H_{logN2} = 0.88$, $\Delta H_{logN4} = 1.03$ and $\Delta D_{N2} = 0.82$, $\Delta D_{N4} = 0.72$), while the site at the lowest elevation showed the lowest growth ($\Delta H_{logN1} = 0.56$ and $\Delta D_{N1} = 0.50$; Figure 3). For the phenological traits, buds flushed earlier for plots from high elevations as compared to low elevation plots ($t_{b2\rightarrow3(2011)N1} = 101.9$; $t_{b2\rightarrow3(2011)N2} = 100.3$; $t_{b2\rightarrow3(2011)N4} = 99.8$; Figure 3). Interestingly, contrary to growth and phenological traits, morphological and physiological traits (except LMA) tended to be slightly less differentiated then expected under neutral processes (with $S \in [0.24; 0.39]$), with a strong conservation of the genetic values between plots (*e.g.* for the leaf mass $LM_{\sqrt{N1}} = 0.44$, $LM_{\sqrt{N2}} = 0.45$, $LM_{\sqrt{N4}} = 0.44$; Figure 3).

Effect of pollen immigration on within-plot genetic variation

Using the categorical assignment and the model (4), we only estimated marginally significant difference in V_A between "local" and "immigrant" individuals for $t_{b2\rightarrow3(2012)}$ (p_{value} = 0.088; for all the other traits $p_{value} > 0.1$). In that case, we predicted higher V_A within the "immigrant" than the "local" group ($V_{A_{immig}}$ = 16.57 and $V_{A_{local}}$ = 3.16; Table 3). Using the random regression model (6), we found significant variation in additive genetic variance with the likelihood of provenance from long-distance pollen pool for ΔH_{log} (p_{value} = 0.005), and a marginally significant effect for $t_{b2\rightarrow3(2012)}$ (p_{value} = 0.078; for all the other traits $p_{value} > 0.1$; Table 3). The estimated variance-covariance terms showed increasing V_A with higher likelihood of long-distance pollination event for both ΔH_{log} and $t_{b2\rightarrow3(2012)}$ (Figure 4). Nevertheless, for these traits, the fixed effect of ν was not significant, which indicated no global trend in the variation of breeding values with the likelihood that an offspring originates from long-distance pollen dispersal. Interestingly, these two different analyses highlighted the same trend towards a higher genetic variance for offspring originating from long-distance dispersal than from local dispersal.

Discussion

The scale of phenotypic adaptation

This study provides evidence that divergent selection can act on phenotypic traits at fine spatial scale in temperate tree species. The study beech plots showed important dispersal capacities, with pollen dispersal that can easily reach 100 m, a fat-tailed pollen dispersal kernel and high rates of immigration (Gauzere *et al.*, 2013b; Oddou-Muratorio *et al.*, 2018), similarly to other anemophilous tree species (see Ashley 2010; Kremer *et al.* 2012 for reviews). Since dispersal distances overlap the spatial scale of the selective environment, our results provide strong evidence that adaptive genetic differentiation can occur despite high gene flow (see Fitzpatrick *et al.* 2015; Peterson *et al.* 2016 for recent evidence in other taxa). This also suggests that selective pressures on seedling growth (in diameter and height) and on budburst timing were strong enough to counteract the homogenizing effect of gene flow on structuring the genetic variation at the scale of a few hundred meters up to one kilometre. We found that the seedlings derived from the plots at high elevation, inhabiting the lowest temperature conditions, flushed earlier and had a higher height and diameter growth in the common garden than the plot at low elevation. For the budburst date, this pattern of genetic differentiation is opposed to the pattern of phenotypic variation measured *in situ*, where seedlings from plots at low elevations flushed earlier than plots at high elevations (Davi *et al.* 2011). Beech populations originating from higher longitude or elevation have also been shown to be genetically earlier in provenance tests (Teissier du Cros *et al.*, 1988; Gomory & Paule, 2011; Kramer *et al.*, 2017), suggesting that these populations evolved phenological traits promoting a longer growing season length. In forestry, growth is considered a good approximation of the performance of trees in a given condition (*e.g.* Rehfeldt *et al.* 1999, 2002). *In situ* and *ex situ*, the highest diameter growth values were measured at intermediate to high elevations (Cailleret & Davi 2011 and present study), suggesting that the genetic and environmental influences on growth traits are in the same direction. At this southern edge of the ecological conditions tolerated by beech (Figure 2), the reduced allocation to stem growth at the low elevation plot is likely an adaptive response to drought, which has previously been described by comparing marginal vs central beech populations (Rose *et al.*, 2009). These studies, including ours, shed light on the phenotypic traits under selection and underlying adaptation at different spatial scales. However, they do not provide a direct assessment of the strength of natural selection or the ecological bases of selection, nor do they quantify phenotypic mismatch and maladaptation, which would require selection analyses (MacColl, 2011).

The weak adaptive divergence of morphological and physiological traits as compared to growth and phenological traits along ecological gradients remains an open question. Meta-analyses highlighted that growth and phenological traits are the most genetically differentiated phenotypic traits at large spatial scale in temperate and boreal tree species (Savolainen *et al.*, 2007; Alberto *et al.*, 2013). Here, our study suggests that this conclusion also holds at a narrower spatial scale. The existing literature also tend to indicate that morphological and physiological traits generally exhibit a lower fraction of genetically controlled phenotypic differentiation, and a higher within-population genetic variation than other traits (Hajek *et al.*, 2016). A putative explanation is that latitudinal or elevation gradients do not fully capture the spatial scale of variation for drought stress, even if they capture a strong variation in temperature.

In particular in our study, the important micro-heterogeneity in soil water capacity on Mont-Ventoux (Nourtier *et al.*, 2013) may generate micro-local differentiation of morphological and physiological traits, blurring the signal of divergent selection for morphological and physiological traits at a larger spatial scale (Cubry *et al.* unpublished). Another possible explanation is that morphological and physiological traits could be under stabilizing selection (*e.g.* Lamy *et al.* 2011). Unfortunately, the power to detect the signal of stabilizing selection is low for species with low neutral genetic differentiation, such as trees (Savolainen *et al.*, 2007), and patterns of weak quantitative trait differentiation are difficult to interpret (Whitlock, 2008). With the development of genomic resources, this question of microgeographic adaptation is now mainly addressed using genome-wide association studies (*e.g.* Eckert *et al.* 2015). However, associations between genotype, phenotype and environment often provide weaker evidence of adaptive differentiation at the phenotypic levels (Le Corre & Kremer, 2012), likely because of the complex multi-locus determinism of the traits underlying phenotypic adaptation. Using a more precise characterisation of ecological gradients, *e.g.* by considering the spatial-scale of water availability, and new methods to compare the similarity of habitats and phenotypes offer promising prospects to better understand the spatial scale of phenotypic adaptation and the ecological drivers of adaptive divergence (Karhunen *et al.*, 2014; Csillery *et al.*, 2020).

Effects of pollen dispersal on quantitative trait variation at a local scale

For populations locally adapted and experiencing strong selective pressures that vary through space, theory suggests that moderate gene flow can maintain quantitative genetic variation within these populations (Barton, 2001; Le Corre & Kremer, 2003; Bridle *et al.*, 2010). Although adaptive genetic differentiation is widespread, only a few studies have provided evidence that gene flow is an important process to maintain the high levels of genetic variation usually detected in nature (*e.g.* Yeaman & Jarvis 2006). Here, consistent with this theoretical expectation, we detected an effect of pollen dispersal on increased genetic variance for two locally differentiated traits, *i.e.* height growth and budburst date. However, we likely lacked power to detect this effect for the third heritable trait under selection, diameter growth. Because female flowers are receptive before the emission of local pollen (protogyny) and lower populations flower earlier than higher populations, we hypothesized a directional pollen flow across elevations in Gauzere *et al.* (2013a), which would have resulted in shifted genetic values between immigrant and local individuals (Figure 1; Nilsson 1995). Our results did not support this expectation.

We used two different approaches to characterize the pollen immigration status. Both analyses suggested that immigrant pollen brings more genetic variance for locally differentiated traits than local pollen. These effects were quantitatively high, with genetic variance for immigrants more than twice as high as for local offspring. However, this result had large standard errors around the estimates, and the significance changed depending on how the immigration status was characterized. We checked that "immigrants" individuals originated from more diverse fathers (higher Nep), and had paternal genotypes more similar to global than local allelic frequencies (higher ν). Nevertheless, our immigrant status still likely suffers some inaccuracy. In particular, the low neutral genetic differentiation among the study beech plots ($Fst = 0.026$) and, overall, on the northern face of the Mont-Ventoux (Lander *et al.*, 2011), may reduce our ability to retrieve the origin of a pollen grain using molecular markers. Moreover, the low levels of quantitative genetic differentiation measured along the elevation gradient suggest that the expected effects of pollen flow on genetic values after one episode of reproduction is necessarily moderate. Here, this effect may thus be difficult to detect, even knowing the true immigration status of all offspring.

The approach used here provides a direct quantification of the impact of contemporary pollen immigration on the genetic variance of quantitative traits. Although using molecular markers to distinguish migrants and local offspring could have been done on numerous existing datasets, to our knowledge no study before ours had reiterated the approach first used by Nilsson (1995). However, we cannot estimate the immigration load associated with this increase in genetic variance for the locally differentiated traits. Although this genetic load is expected to be beneficial under rapidly changing environmental conditions (Yeaman & Jarvis, 2006; Savolainen *et al.*, 2007; Kremer *et al.*, 2012), this remains to be tested. Applying this approach to other species and populations may improve our ability to both understand and quantify the impact of gene flow on the adaptive potential of quantitative traits under natural conditions. This effect should be more detectable for populations departing from migration-selection equilibrium, such as isolated (or marginal) populations, or transplanted plant populations (see Fitzpatrick *et al.* 2015, 2016 for examples in guppies).

The present results about trait differentiation and genetic variance of migrants and non-migrants extend previous studies about pollen dispersal kernels and migration rates (Gauzere *et al.*, 2013a), trait heritabilities (Gauzere *et al.*, 2016a) and variance of fecundities (Oddou-Muratorio *et al.*, 2018). Pulling all effects together suggests building a predictive mechanistic model to both (i) check whether the observed levels of genetic variance in migrant pollen are consistent with the amounts of migrant genetic variance that would be predicted mechanistically and (ii) further investigate the adaptive dynamics at the scale of few generations (following *e.g.* Kuparinen *et al.* 2010). This work is beyond the scope of the present study because it still requires a map of beech density over the whole northern face of Mont-Ventoux and an extrapolation model to propose a spatially explicit distribution of adaptive diversity. Point (ii) would require information about the variation in selection gradients or adaptive landscapes with elevation. Unfortunately, empirical estimates of selection need specific approaches in natural populations of long-lived species and, hence, remain scarce in forest trees (but see Bontemps *et al.* 2017).

Gene flow in the context of climate change

One of the most documented consequences of climate change on tree ecosystem functioning is probably the impact of climate warming on the advancement of spring phenology (Menzel *et al.*, 2006; Fu *et al.*, 2015). Understanding the adaptive or maladaptive value of the response of phenology to climate has become a key focus of evolutionary studies (Tansey *et al.* 2017; Gauzere *et al.* in press). An underrated issue is that these phenological changes are also likely to affect gene flow among populations, and efficiency of long-distance pollen dispersal. For many temperate tree species, we now have strong evidence that climate warming reduces spring phenology synchrony among individuals experiencing the same environmental conditions (Zohner *et al.*, 2018), but also leads to more uniform spring phenology across climatic gradients (Vitasse *et al.*, 2018). The evolutionary consequences of pollen dispersal may therefore change with increasing climate warming. In this study, we detected an effect of local selection and pollen dispersal on the adaptive potential of traits that are known to affect dispersal or reproduction in anemophilous tree species (tree height and phenology; Klinkhamer *et al.* 1997; Gauzere *et al.* 2013a; Oddou-Muratorio *et al.* 2018). In particular, long-distance pollen dispersal events are only effective if the donor and recipient trees have synchronous phenology (Aitken *et al.*, 2008). Our results thus suggest that refining the models of phenotypic adaptation by incorporating the feedbacks between traits under divergent selection and dispersal-reproductive capacities may be important to thoroughly understand the evolutionary consequences of gene flow in plant species (*e.g.* Soularue & Kremer 2012).

With the concern that populations could be maladaptated and lag behind with increasing climate change, new conservation and management strategies are needed to mitigate these effects. Assisted gene flow, *i.e.* the managed movement of individuals or gametes between populations within species ranges, is one of the tools proposed to help populations adapt to new climatic conditions (Aitken & Whitlock, 2013; Aitken & Bemmels, 2016). However, assisted gene flow among distant populations, at different latitudes, may have unexpected consequences for the response of individuals or new hybrids, because of outbreeding depression (Aitken & Whitlock, 2013), but also because other environmental cues than temperature can govern physiological responses in trees (see for instance the role of photoperiod in bud development; Way & Montgomery 2015). Here, our results suggest some effect of local pollen dispersal on increasing genetic variation for locally differentiated traits after solely one episode of reproduction. Therefore, programs considering the movements of gametes along short ecological gradients could be efficient to boost genetic diversity and introduce genetic innovation within natural or managed tree populations. Our results also encourage the development of evolution-oriented forest management and silviculture, whereby thinning could be done to favour the spread of long-distance gene flow (*e.g.* by reducing local densities; Lefèvre *et al.* 2014). Nonetheless, contrarily to assisted gene flow, natural gene flow can also lead to increased maladaptation through migration load (Aitken & Whitlock, 2013).

Conclusion

By combining population genetics and quantitative genetics, we provided a first comprehensive analysis of the effects of gene flow on the adaptive potential of a tree species in nature. We found that gene flow among trees at different elevations did not prevent adaptive differentiation among plots separated by less than one kilometre, and that divergent selection at this fine spatial scale acted mainly on growth and phenological traits, likely as an adaptation to drought and temperature variation. For two genetically differentiated traits, our analyses suggested an effect of pollen dispersal on increasing genetic diversity after one episode of reproduction. However, our power to detect and estimate these effects was probably quite low. Our results thus suggest that local selection is an important evolutionary force in natural tree populations and that, under rapidly changing environmental conditions, gene flow can increase the adaptive capacities of natural populations.

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Author contributions

J.G., E.K.K. and S.O.M. conceived and designed the study. J.G collected the genetic and phenotypic data, performed the analyses, wrote the first draft of the text and contributed the revisions. S.O.M. conceived and coordinated the beech experiment. J.G., H.D., O.B., E.K.K. and S.O.M. discussed and interpreted the findings. E.K.K., H.D., O.B. and S.O.M. contributed to the analyses and the revisions of preliminary drafts.

Data archiving statement

Data available upon request from the authors

References

Aitken SN , Bemmels JB. 2016. Time to get moving: assisted gene flow of forest trees. *Evolutionary Applications*, **9**: 271–290.

Aitken SN , Whitlock MC. 2013. Assisted gene flow to facilitate local adaptation to climate change. *Annual Review of Ecology, Evolution and Systematics*, **44**: 367–388.

Aitken SN, Yeaman S, Holliday JA, Wang T , Curtis-McLane S. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications*, **1**: 95–111.

Alberto FJ, Aitken SN, Alia R, Gonzalez-Martinez SC, Hanninen H, Kremer A, Lefevre F, Lenormand T, Yeaman S, Whetten R *et al.* 2013. Potential for evolutionary responses to climate change evidence from tree populations. *Global Change Biology*, **19**: 1645–1661.

Anderson JT, Perera N, Chowdhury B , Mitchell-Olds T. 2015. Microgeographic Patterns of Genetic Divergence and Adaptation across Environmental Gradients in *Boechera stricta* (Brassicaceae). *American Naturalist*, **186**: S60–S73.

Ashley MV. 2010. Plant parentage, pollination, and dispersal: How DNA microsatellites have altered the landscape. *Critical Reviews in Plant Sciences*, **29**: 148–161.

Austerlitz F, Dick C, Dutech C, Klein E, Oddou-Muratorio S, Smouse P , Sork V. 2004. Using genetic markers to estimate the pollen dispersal curve. *Molecular Ecology*, **13**: 937–954.

Barton. 2001. *Integrating Ecology and Evolution in a Spatial Context: Adaptation at the edge of a species range*. Silvertown, J. and Antonovics, J. (Eds), pp. 365-392. Blackwell Science, New York.

Bontemps A, Davi H, Lefevre F, Rozenberg P , Oddou-Muratorio S. 2017. How do functional traits syndromes covary with growth and reproductive performance in a water-stressed population of *Fagus sylvatica*? *Oikos*, **126**: 1472–1483.

Bontrager M , Angert AL. 2019. Gene flow improves fitness at a range edge under climate change. *Evolution Letters*, **3**: 55–68.

Breda N, Huc R, Granier A , Dreyer E. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science*, **63**: 625–644.

Bridle JR, Polechova J, Kawata M , Butlin RK. 2010. Why is adaptation prevented at ecological margins? New insights from individual-based simulations. *Ecology Letters*, **13**: 485–494.

Brousseau L, Bonal D, Cigna J , Scotti I. 2013. Highly local environmental variability promotes intrapopulation divergence of quantitative traits: an example from tropical rain forest trees. *Annals of Botany*, **112**: 1169–1179.

Buiteveld J, Vendramin GG, Leonardi S, Kamer K , Geburek T. 2007. Genetic diversity and differentiation in European beech (*Fagus sylvatica* L.) stands varying in management history. *Forest Ecology and Management*, **247**: 98–106.

Cailleret M , Davi H. 2011. Effects of climate on diameter growth of co-occurring Fagus sylvatica and Abies alba along an altitudinal gradient. *Trees*, **25**: 265–276.

Charmantier A , Réale D. 2005. How do misassigned paternities affect the estimation of heritability in the wild? *Molecular Ecology*, **14**: 2839–2850.

Comps B, Gomory D, Letouzey J, Thiebaut B , Petit R. 2001. Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. *Genetics*, **157**: 389–397.

Teissier du Cros E, Thiebaut B , Duval H. 1988. Variability in beech - budding, height growth and tree form. *Annals of Forest Science*, **45**: 383–398.

Csillery K, Lalaguee H, Vendramin GG, Gonzalez-Martinez SC, Fady B , Oddou-Muratorio S. 2014. Detecting short spatial scale local adaptation and epistatic selection in climate-related candidate genes in European beech (Fagus sylvatica) populations. *Molecular Ecology*, **23**: 4696–4708.

Csillery K, Ovaskainen O, Sperisen C, Buchmann N, Widmer A , Gugerli F. 2020. Adaptation to local climate in multi-trait space: evidence from silver fir (*Abies alba* mill.) populations across a heterogeneous environment. *Heredity*, **124**: 77–92.

Davi H.and Gillmann M, Ibanez T, Cailleret M, Bontemps A, Fady B , Lefèvre F. 2011. Diversity of leaf unfolding dynamics among tree species: New insights from a study along an altitudinal gradient. *Agricultural and Forest Meteorology*, **151**: 1504–1513.

Davis M , Shaw R. 2001. Range shifts and adaptive responses to Quaternary climate change. *Science*, **292**: 673–679.

Eckert AJ, Maloney PE, Vogler DR, Jensen CE, Mix AD , Neale DB. 2015. Local adaptation at fine spatial scales: an example from sugar pine (*Pinus lambertiana*, Pinaceae). *Tree Genetics & Genomes*, **11**: 42.

Farquhar G, Ehleringer J , Hubick K. 1989. Carbon Isotope Discrimination And Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**: 503–537.

Fitzpatrick SW, Gerberich JC, Angeloni LM, Bailey LL, Broder ED, Torres-Dowdall J, Handelsman CA, Lopez-Sepulcre A, Reznick DN, Ghalambor CK *et al.* 2016. Gene flow from an adaptively divergent source causes rescue through genetic and demographic factors in two wild populations of Trinidadian guppies. *Evolutionary Applications*, **9**: 879–891.

Fitzpatrick SW, Gerberich JC, Kronenberger JA, Angeloni LM , Funk WC. 2015. Locally adapted traits maintained in the face of high gene flow. *Ecology Letters*, **18**: 37–47.

Fitzpatrick SW , Reid BN. 2019. Does gene flow aggravate or alleviate maladaptation to environmental stress in small populations? *Evolutionary Applications*, **12**: 1402–1416.

Fu YH, Piao S, Vitasse Y, Zhao H, De Boeck HJ, Liu Q, Yang H, Weber U, Hanninen H , Janssens IA. 2015. Increased heat requirement for leaf flushing in temperate woody species over 1980-2012: effects of chilling, precipitation and insolation. *Global Change Biology*, **21**: 2687–2697.

Gauzere J, Klein E, Davi H, Brendel O , Oddou-Muratorio S. 2016a. Using partial genotyping to estimate the genetic and maternal determinants of adaptive traits in a progeny trial of *Fagus sylvatica*. *Tree Genetics and Genomes*, **12**: 115.

Gauzere J, Klein EK , Oddou-Muratorio S. 2013a. Ecological determinants of mating system within and between three *Fagus sylvatica* populations along an elevational gradient. *Molecular Ecology*, **22**: 5001–5015.

Gauzere J, Oddou-Muratorio S, Gay L , Klein E. 2016b. Partial genotyping at polymorphic markers can improve heritability estimates in sibling groups. *Molecular Ecology Resources*, **16**: 1340–1352.

Gauzere J, Oddou-Muratorio S, Pichot C, Lefèvre F , Klein E. 2013b. Biases in quantitative genetic analyses using open-pollinated progeny tests from natural tree populations. *Acta Botanica Gallica*, **160**: 227–238.

Gauzere J, Teuf B, Davi H, Chevin L, Caignard T, Delzon S, Ronce O , Chuine I. in press. Where is the optimum? predicting the variation of selection along climatic gradients and the adaptive value of plasticity. a case study on tree phenology. *Evolution Letters*.

Gilmour A, Gogel B, Cullis B , Thompson R. 2006. *Asreml User Guide Release 2.0*. Hemel Hempsted: VSN International Ltd.

Gomory D , Paule L. 2011. Trade-off between height growth and spring flushing in common beech (Fagus sylvatica L.). *Annals of Forest Science*, **68**: 975–984.

Hajek P, Kurjak D, von Wuehlisch G, Delzon S , Schuldt B. 2016. Intraspecific variation in wood anatomical, hydraulic, and foliar traits in ten European beech provenances differing in growth yield. *Frontiers in Plant Science*, **7**: 791.

Hardy O , Vekemans X. 2002. SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**: 618–620.

Hereford J. 2009. A Quantitative Survey of Local Adaptation and Fitness Trade-Offs. *American Naturalist*, **173**: 579–588.

Jump AS, Hunt JM, Martinez-Izquierdo JA , Penuelas J. 2006. Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. *Molecular Ecology*, **15**: 3469–3480.

Karhunen M, Merila J, Leinonen T, Cano JM , Ovaskainen O. 2013. driftsel: an R package for detecting signals of natural selection in quantitative traits. *Molecular Ecology Resources*, **13**: 746–754.

Karhunen M , Ovaskainen O. 2012. Estimating Population-Level Coancestry Coefficients by an Admixture F Model. *Genetics*, **192**: 609.

Karhunen M, Ovaskainen O, Herczeg G , Merila J. 2014. Bringing Habitat Information Into Statistical Tests Of Local Adaptation In Quantitative Traits: A Case Study Of Nine-Spined Sticklebacks. *Evolution*, **68**: 559–568.

Kattge J, Knorr W, Raddatz T , Wirth C. 2009. Quantifying photosynthetic capacity and its relationship to leaf nitrogen content for global-scale terrestrial biosphere models. *Global Change Biology*, **15**: 976–991.

Klinkhamer P, deJong T , Metz H. 1997. Sex and size in cosexual plants. *Trends in Ecology & Evolution*, **12**: 260–265.

Knutzen F, Meier IC , Leuschner C. 2015. Does reduced precipitation trigger physiological and morphological drought adaptations in European beech (*Fagus sylvatica* L.)? Comparing provenances across a precipitation gradient. *Tree Physiology*, **35**: 949–963.

Kramer K, Ducousso A, Gomory D, Hansen JK, Ionita L, Liesebach M, Lorent A, Schueler S, Sulkowska M, de Vries S *et al.* 2017. Chilling and forcing requirements for foliage bud burst of European beech (Fagus sylvatica L.) differ between provenances and are phenotypically plastic. *Agricultural and Forest Meteorology*, **234**: 172–181.

Kremer A, Ronce O, Robledo-Arnuncio JJ, Guillaume F, Bohrer G, Nathan R, Bridle JR, Gomulkiewicz R, Klein EK, Ritland K *et al.* 2012. Long-distance gene flow and adaptation of forest trees to rapid climate change. *Ecology Letters*, **15**: 378–392.

Kuparinen A, Savolainen O , Schurr FM. 2010. Increased mortality can promote evolutionary adaptation of forest trees to climate change. *Forest Ecology and Management*, **259**: 1003–1008.

Lamy JB, Bouffier L, Burlett R, Plomion C, Cochard H , Delzon S. 2011. Uniform Selection as a Primary Force Reducing Population Genetic Differentiation of Cavitation Resistance across a Species Range. *PlosOne*, **6**: e23476.

Lander TA, Oddou-Muratorio S, Prouillet-Leplat H , Klein EK. 2011. Reconstruction of a beech population bottleneck using archival demographic information and bayesian analysis of genetic data. *Molecular Ecology*, **20**: 5182–5196.

Le Corre V , Kremer A. 2003. Genetic variability at neutral markers, quantitative trait loci and trait in a subdivided population under selection. *Genetics*, **164**: 1205–1219.

Le Corre V , Kremer A. 2012. The genetic differentiation at quantitative trait loci under local adaptation. *Molecular Ecology*, **21**: 1548–1566.

Lefèvre F, Boivin T, Bontemps A, Courbet F, Davi H, Durand-Gillmann M, Fady B, Gauzere J, Gidoin C, Karam M *et al.* 2014. Considering evolutionary processes in adaptive forestry. *Annals of Forest Science*, **71**: 723–739.

Leimu R , Fischer M. 2008. A Meta-Analysis of Local Adaptation in Plants. *Plos One*, **3**: e4010.

Lenormand T. 2002. Gene flow and the limits to natural selection. *Trends in Ecology and Evolution*, **17**: 183–189.

Loiselle B, Sork VL, Nason JD , Graham C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (rubiaceae). *American Journal of Botany*, **82**: 1420–1425.

Lopez S, Rousset F, Shaw FH, Shaw RG , Ronce O. 2008. Migration load in plants: role of pollen and seed dispersal in heterogeneous landscapes. *Journal of Evolutionary Biology*, **21**: 294–309.

MacColl ADC. 2011. The ecological causes of evolution. *Trends in Ecology & Evolution*, **26**: 514–522.

Marshall T, Slate J, Kruuk L , Pemberton J. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**: 639–655.

McKay JK , Latta R. 2002. Adaptive population divergence: markers, QTL and traits. *Trends in Ecology and Evolution*, **17**: 285–291.

Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kuebler K, Bissolli P, Braslavska O, Briede A *et al.* 2006. European phenological response to climate change matches the warming pattern. *Global Change Biology*, **12**: 1969–1976.

Merzeau D, Comps B, Thiébaut B , Letouzey J. 1994. Estimation of fagus sylvatica mating system parameters in natural populations. *Annals of Forest Science*, **51**: 163–173.

Moody KN, Hunter SN, Childress MJ, Blob RW, Schoenfuss HL, Blum MJ , Ptacek MB. 2015. Local adaptation despite high gene flow in the waterfall-climbing Hawaiian goby, *Sicyopterus stimpsoni*. *Molecular Ecology*, **24**: 545–563.

Nilsson J. 1995. Genetic-Variation In The Natural Pollen Cloud Of Pinus-Sylvestris - A Study Based On Progeny Testing. *Scandinavian Journal of Forest Research*, **10**: 140–148.

Nourtier M, Chanzy A, Cailleret M, Yingge X, Huc R , Davi H. 2013. Transpiration of silver fir (*Abies alba* mill.) during and after drought in relation to soil properties in a mediterranean mountain area. *Annals of Forest Science*, **71**: 683–695.

Nussey DH, Wilson AJ, Morris A, Pemberton J, Clutton-Brock T , Kruuk LEB. 2008. Testing for genetic trade-offs between early- and late-life reproduction in a wild red deer population. *Proceedings of the Royal Society B-Biological Sciences*, **275**: 745–750.

Oddou-Muratorio S, Gauzere J, Bontemps A, Rey JF , Klein EK. 2018. Tree, sex and size: Ecological determinants of male vs. female fecundity in three *Fagus sylvatica* stands. *Molecular Ecology*, **27**: 3131–3145.

Ovaskainen O, Karhunen M, Zheng C, Arias JMC , Merila J. 2011. A new method to uncover signatures of divergent and stabilizing selection in quantitative traits. *Genetics*, **189**: 621–U729.

Pease C, Lande R , Bull J. 1989. A Model Of Population-Growth, Dispersal And Evolution In A Changing Environment. *Ecology*, **70**: 1657–1664.

Peterson ML, Kay KM , Angert AL. 2016. The scale of local adaptation in *Mimulus guttatus*: comparing life history races, ecotypes, and populations. *New Phytologist*, **211**: 345–356.

Petit R , Hampe A. 2006. Some evolutionary consequences of being a tree. *Annual Review of Ecology Evolution and Systematics*, **37**: 187–214.

Piotti A, Leonardi S, Buiteveld J, Geburek T, Gerber S, Kramer K, Vettori C , Vendramin GG. 2011. Comparison of pollen gene flow among four European beech (*Fagus sylvatica* l.) populations characterized by different management regimes. *Heredity*, **108**: 322–331.

Pluess AR, Frank A, Heiri C, Lalaguee H, Vendramin GG , Oddou-Muratorio S. 2016. Genome-environment association study suggests local adaptation to climate at the regional scale in Fagus sylvatica. *New Phytologist*, **210**: 589–601.

Polechova J , Barton NH. 2015. Limits to adaptation along environmental gradients. *Proceedings of the National Academy of Sciences of the United States of America*, **112**: 6401–6406.

Rehfeldt G, Tchebakova N, Parfenova Y, Wykoff W, Kuzmina N , Milyutin L. 2002. Intraspecific responses to climate in *Pinus sylvestris*. *Global Change Biology*, **8**: 912–929.

Rehfeldt G, Ying C, Spittlehouse D , Hamilton D. 1999. Genetic responses to climate in *Pinus contorta*: Niche breadth, climate change, and reforestation. *Ecological Monographs*, **69**: 375–407.

Reich P, Wright I, Cavender-Bares J, Craine J, Oleksyn J, Westoby M , Walters M. 2003. The evolution of plant functional variation: Traits, spectra, and strategies. *International Journal of Plant Sciences*, **164**: S143–S164.

Richardson JL, Urban MC, Bolnick DI , Skelly DK. 2014. Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology & Evolution*, **29**: 165–176.

Robson TM, Garzon MB , Consortium BD. 2018. Phenotypic trait variation measured on European genetic trials of *Fagus sylvatica* L. *Scientific Data*, **5**: 180149.

Rose L, Leuschner C, Koeckemann B , Buschmann H. 2009. Are marginal beech (*Fagus sylvatica* L.) provenances a source for drought tolerant ecotypes? *European Journal of Forest Research*, **128**: 335–343.

Savolainen O, Pyhajarvi T , Knurr T. 2007. Gene flow and local adaptation in trees. *Annual Review of Ecology Evolution and Systematics*, **38**: 595–619.

Costa e Silva J, Potts BM , Lopez GA. 2014. Heterosis May Result in Selection Favouring the Products of Long-Distance Pollen Dispersal in Eucalyptus. *Plos One*, **9**: e93811.

Soularue JP , Kremer A. 2012. Assortative mating and gene flow generate clinal phenological variation in trees. *BMC Evolutionary Biology*, **12**: 79.

Stojnic S, Suchocka M, Benito-Garzon M, Torres-Ruiz JM, Cochard H, Bolte A, Cocozza C, Cvjetkovic B, de Luis M, Martinez-Vilalta J *et al.* 2018. Variation in xylem vulnerability to embolism in European beech from geographically marginal populations. *Tree Physiology*, **38**: 173–185.

Swindell W , Bouzat J. 2006. Gene flow and adaptive potential in *Drosophila melanogaster*. *Conservation Genetics*, **7**: 79–89.

Tansey CJ, Hadfield JD , Phillimore AB. 2017. Estimating the ability of plants to plastically track temperature-mediated shifts in the spring phenological optimum. *Global Change Biology*, **23**: 3321–3334.

Vitasse Y, Signarbieux C , Fu YH. 2018. Global warming leads to more uniform spring phenology across elevations. *PNAS*, **115**: 1004–1008.

Wang KS. 2003. Mating system in isolated stands of european beech (*Fagus sylvatica* l.). *Forest Genetics*, **10**: 159–164.

Way DA , Montgomery RA. 2015. Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant Cell and Environment*, **38**: 1725–1736.

Whitlock MC. 2008. Evolutionary inference from Q(ST). *Molecular Ecology*, **17**: 1885–1896.

Yeaman S , Jarvis A. 2006. Regional heterogeneity and gene flow maintain variance in a quantitative trait within populations of lodgepole pine. *Proceedings of the Royal Society B-Biological Sciences*, **273**: 1587–1593.

Zohner CM, Mo L , Renner SS. 2018. Global warming reduces leaf-out an flowering synchrony among individuals. *eLife*, **7**: e40214.

Supporting Information

Fig. S1 Distribution of the LOD-score of long-distance vs local immigration between 'immigrant' and 'local' groups.

Table S1 Location, climate and ecological characteristics of the study plots.

Table S2 Test for plot differentiation and signal of selection in quantitative traits.

Table S3 Differences in additive genetic variance between 'immigrant' and 'local' groups estimated from model eq. (4).

Table S4 Effect of pollen immigration on the variation in additive genetic variance estimated from model eq. (6).

Figure legends

Figure 1. Expected effects of random and directional gene flow between locally differentiated populations on individuals genetic values. (a) In the case of random gene flow, migrant gene flow (orange arrows) tends to homogenise the frequencies of the red, grey and blue genotypes in each environment. Hence, in the core population (*e.g.* grey habitat), individuals and gametes from migrant gene flow should exhibit more genetic variance for the traits under local selection ($v_1 > v_2$) than individuals and gametes from local gene flow (*i.e.* gene flow within population, represented by the green arrows), while the mean genetic value (dotted line) should be identical (assuming a gradual shift of the optimum). (b) In the case of directional gene flow (*e.g.* only the red genotypes migrate to the grey habitat), migrant gene flow should introduce individuals and gametes with shifted mean genetic values (dotted lines; $m_1 \neq m_2$), while the variance in genetic values between migrant and local gene flow should be similar (assuming stabilising selection is the same in each habitat).

Figure 2. Climatic space explored by the three study plots in comparison to the whole species range of *Fagus sylvatica*. This climatic space is represented as the variation of temperature and summer precipitation over beech distribution area, extracted for the SAFRAN database for the period 1958-2015 (collected on a 8 km^2 grid represented by grey dots). Each black triangle represents the average climate recorded since 2007 with HOBO weather stations for the plot N1 (1020 m), N2 (1140 m) and N4 (1340 m a.s.l.).

Figure 3. Level of trait adaptive divergence between the three plots located at 1020 m (N1), 1140 m (N2) and 1340 m (N4) a.s.l. for the growth, phenological, physiological and morphological traits measured on seedlings of *Fagus sylvatica*. Histograms represent the *a posteriori* distributions of additive genetic values estimated for each plot. The dotted lines represent the median of these distributions. We also provide the test for population differentiation (*P*) and signal of selection (*S*) for these quantitative traits. *S* coefficient close to 1 and 0 indicates signal of divergent and stabilizing selection respectively. With "***" $p < 0.001$, "**" $0.001 < p < 0.01$, "*" $0.01 < p < 0.05$, "." 0.05 < <p < 0.09, "ns" $p > 0.09$.

Figure 4. Change in additive genetic variance (V_A) with the LOD-score of long-distance im-

migration vs. local immigration (ν), estimated using model (6) in *Fagus sylvatica*. This relationship is represented only for the two traits, out of 11 tested, for which we found that this effect was significant or marginally significant: (left) the log-transformed height growth (ΔH_{log}) and (right) the budburst date in 2012 ($t_{b2\rightarrow3(2012)}$). The solid line represents the estimate, and the dashed lines the 95 % confidence intervals around this estimate. From the random regression model (6), we can estimate the change in V_A with a covariate x as: $V_A(x) = var(trait_{intercept} + trait_{slope}.x) = V_{A\ intercept} + x^2.V_{A\ slope} + 2.x.cov_A(intercept, slope)$, using the estimates provided in Table 3.

Table 1: Description and descriptive statistics for the traits measured on seedlings of *Fagus sylvatica*. With N_w the number of phenotyped individuals. DOY: day of the year.

Abbreviation	Trait	Category	N_w	Unit	Transformation
ΔH_{log}	Total height increase	Growth	2380	rate	log
ΔD	Total diameter increase	Growth	2380	rate	-
$t_{b2\rightarrow3(2011)}$	Budburst date in 2011	Phenology	2334	DOY	-
$t_{b2\rightarrow3(2012)}$	Budburst date in 2012	Phenology	1835	DOY	-
$t_{s1\rightarrow2}$	Senescence date in 2011	Phenology	2386	DOY	-
VD_{2011}	Vegetation season duration in 2011	Phenology	2193	DOY	-
$LM_{\surd}$	Dry leaf mass	Morphology	2491	g	square-root
$LA_{\surd}$	Leaf Area	Morphology	2491	cm^2	square-root
LMA	Leaf Mass Area	Morphology	2491	$g.m^{-2}$	-
$\%N$	Nitrogen content in leaves	Physiology	1031	%	-
$\delta^{13}C$	Water use efficiency	Physiology	1031	‰	-

Table 2: Matrix of the coancestry coefficients estimated by the admixture F-model of Karhunen & Ovaskainen (2012) for the three plots of *Fagus sylvatica*. The diagonal correspond to the within-plot coancestry coefficient, with its median value and confidence intervals (*i.e.* values of the estimates that bound 95 % of the posterior distribution). Lower diagonal elements are the median coancestry coefficients among-plots, with confidence intervals in brackets.

	N1	N2	N4
N1	0.057 [0.033; 0.085]		
N2	0.018 [0.005; 0.035]	0.029 [0.017; 0.045]	
N4	0.012 [0.00; 0.024]	0.013 [0.002; 0.023]	0.035 [0.02; 0.052]

Table 3: Effect of pollen immigration on the variation in additive genetic variance for traits measured in seedlings of *Fagus sylvatica*. This effect was found significant or marginally significant for two genetically differentiated traits: using model (4) for $t_{b2\rightarrow3(2012)}$ with $pvalue$ = 0.088; using model (6) for ΔH_{log} with $pvalue$ = 0.005 and for $t_{b2\rightarrow3(2012)}$ with $pvalue$ = 0.078. Model (4) estimates an additive genetic variance (V_A) for each "immigrant" and "local" group (with standard error in brackets). Model (6) estimates the additive genetic variance-covariance terms for the intercept and slope of the trait with the LOD-score of long-distance immigration vs. local immigration ν (with standard error in brackets). The diagonal elements thus provide $V_{A\ intercept}$ and $V_{A\ slope}$, and the off-diagonal element $cov_A(intercept, slope)$. See Supporting Information Tables S3 and S4 for the model outputs for all phenotypic traits.

Model 4

Trait	$V_{A_{immig}}$	$V_{A_{local}}$
$t_{b2\rightarrow3(2012)}$	16.57 (8.2)	3.16 (5.6)

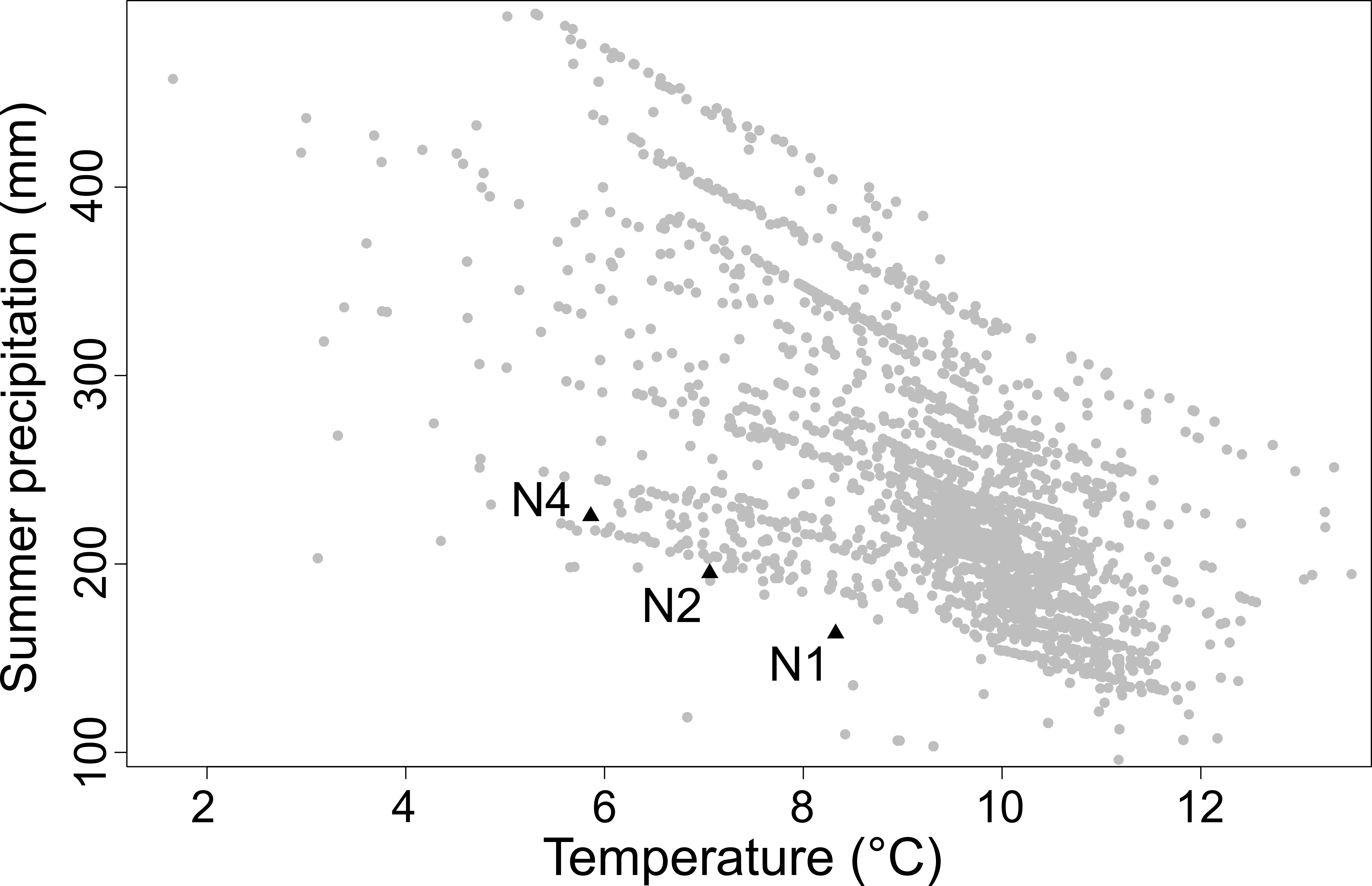
Model 6

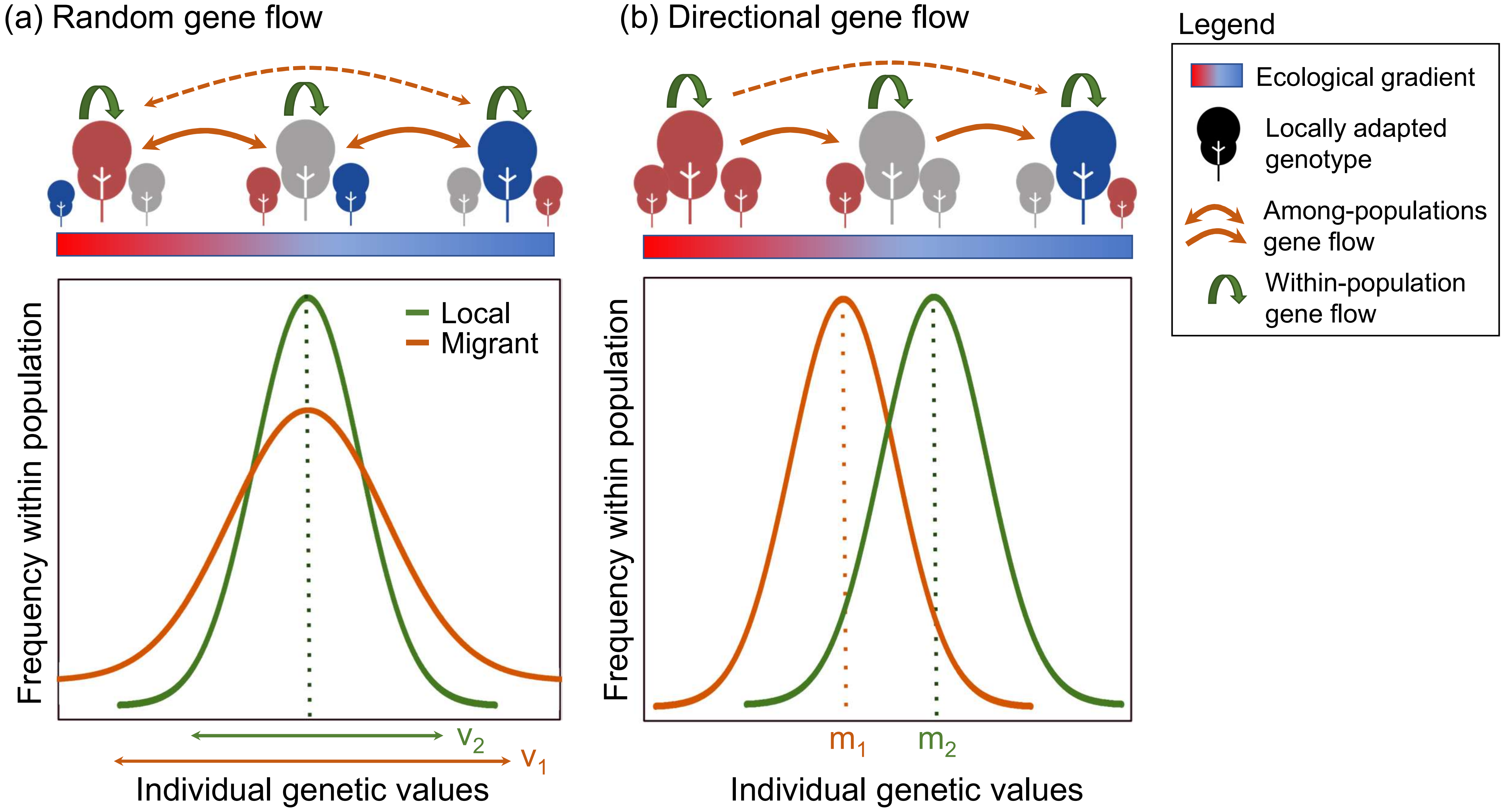
additive genetic effects for ΔH_{log}

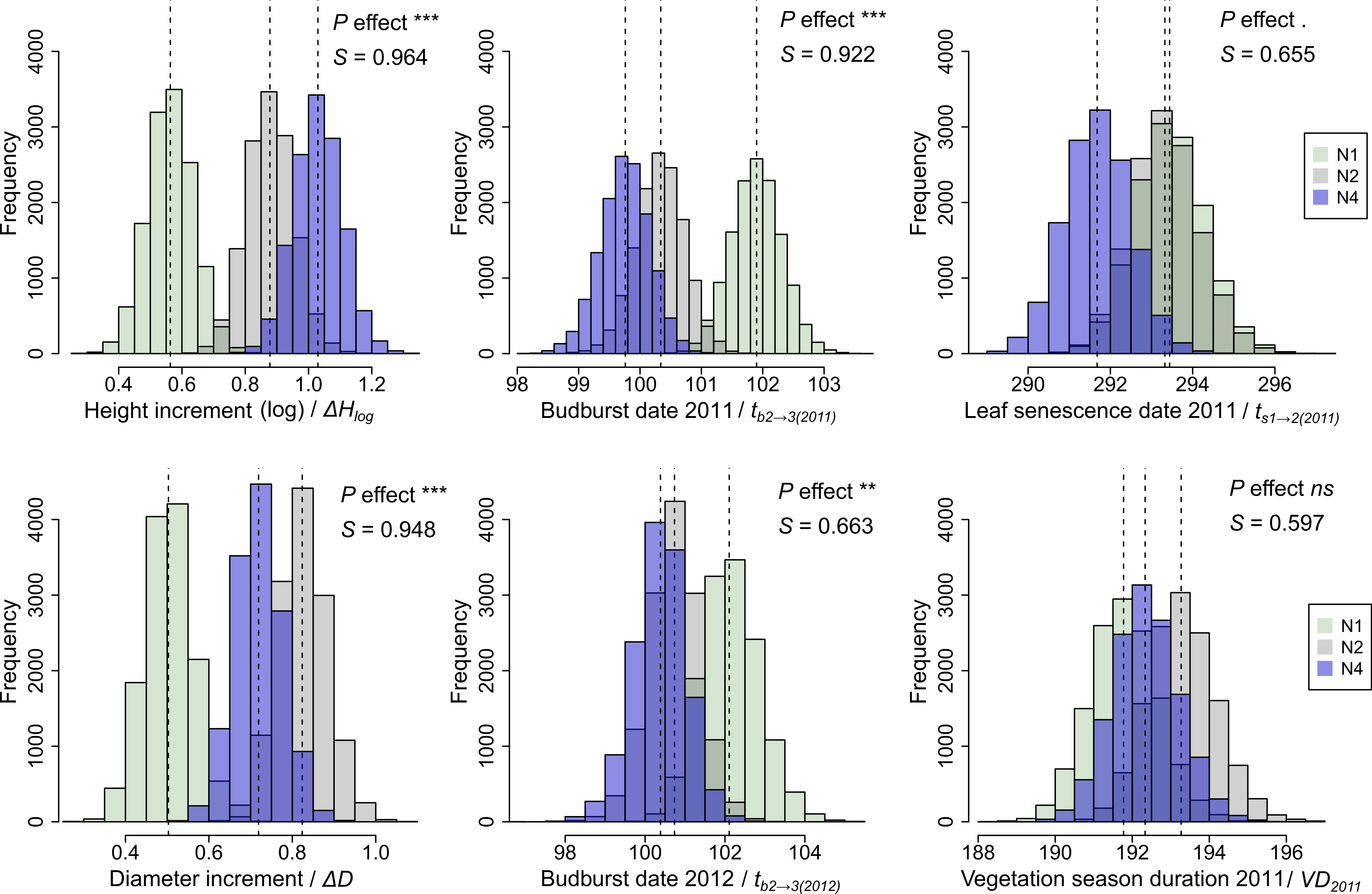
	$\Delta H_{log_{intercept}}$	$\Delta H_{log_{slope}}$
$\Delta H_{log_{intercept}}$	0.16 (0.041)	
$\Delta H_{log_{slope}}$	0.029 (0.010)	0.0092 (0.008)

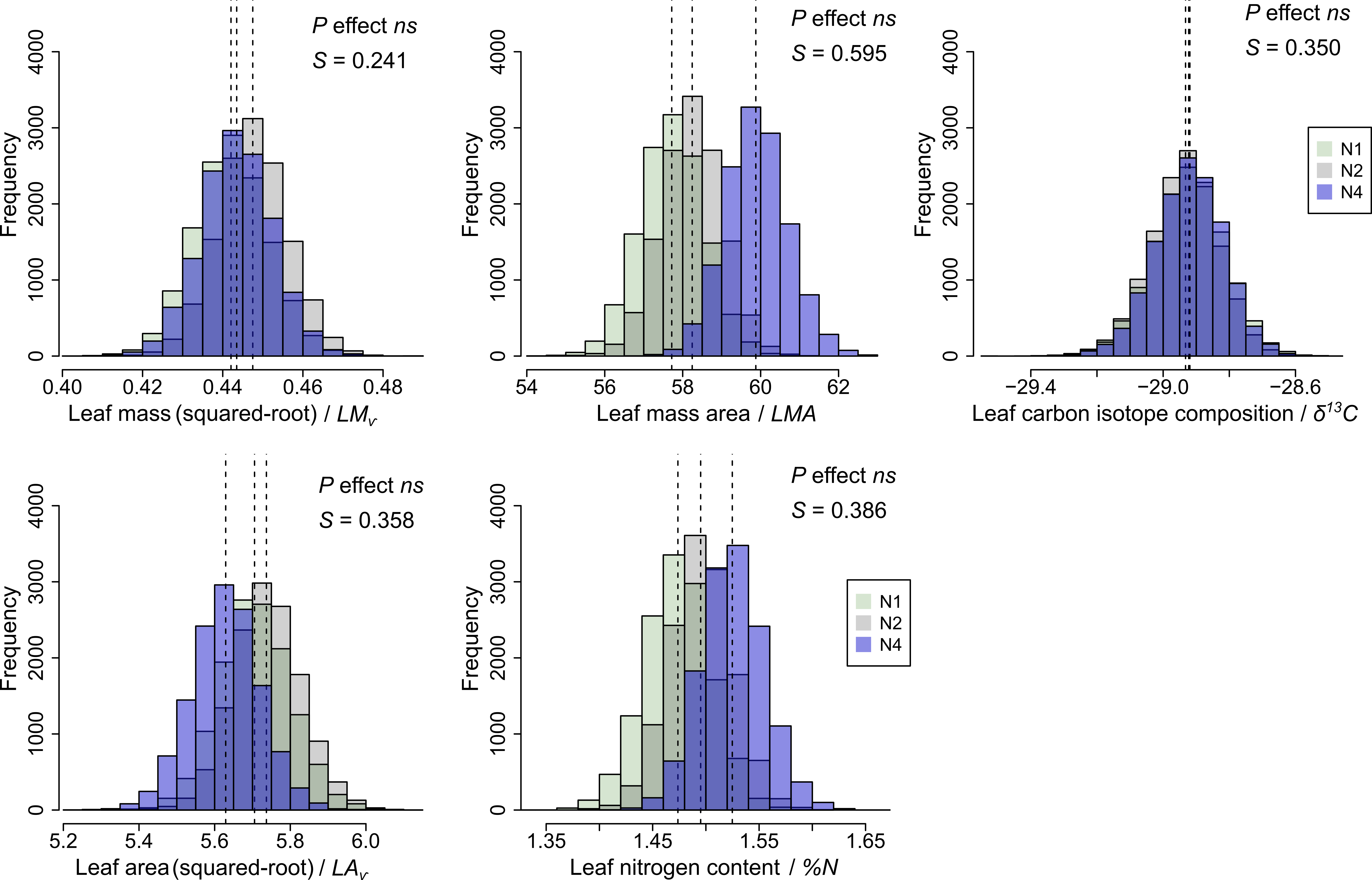
additive genetic effects for $t_{b2\rightarrow3(2012)}$

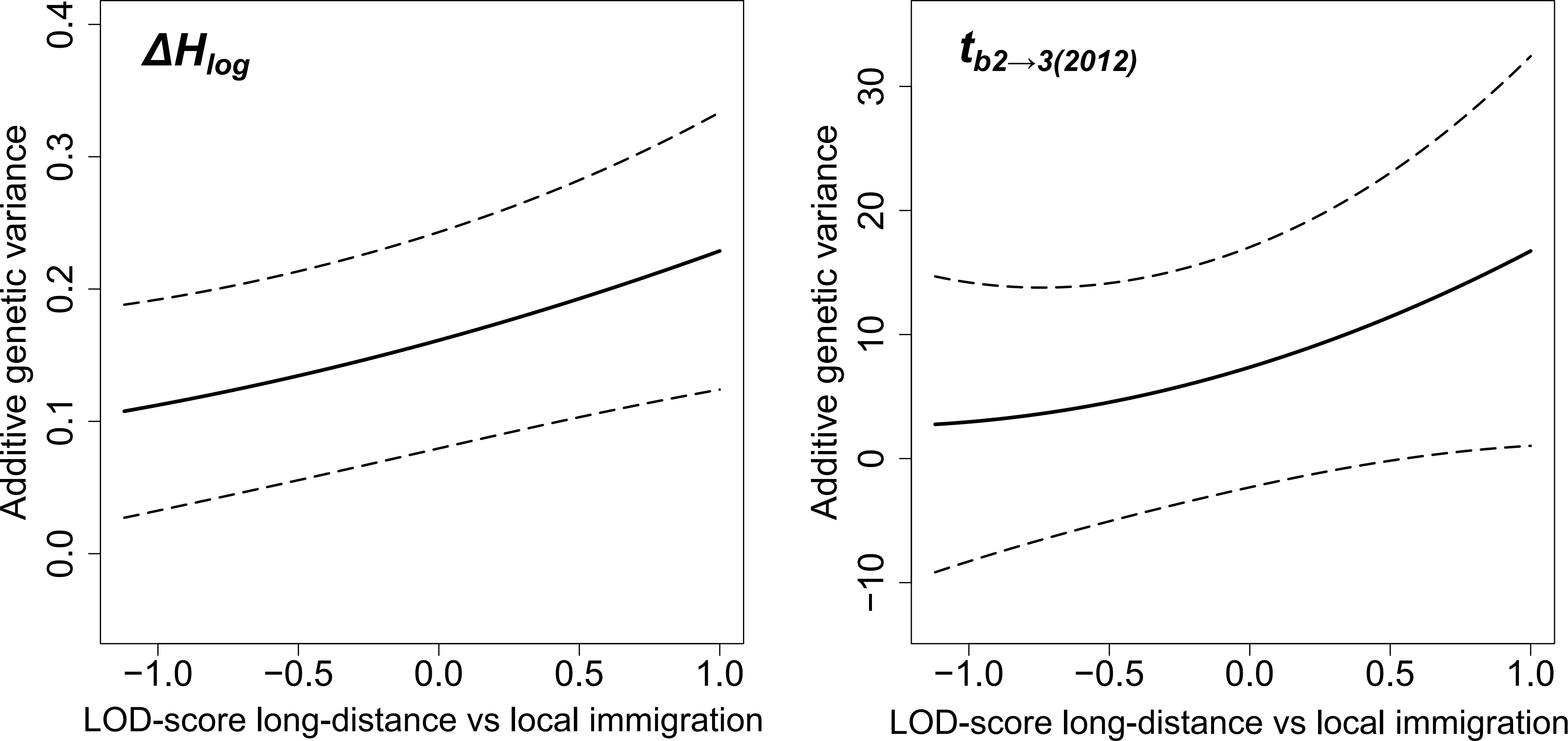
	$t_{b2\rightarrow3(2012)_{intercept}}$	$t_{b2\rightarrow3(2012)_{slope}}$
$t_{b2\rightarrow3(2012)_{intercept}}$	7.36 (4.8)	
$t_{b2\rightarrow3(2012)_{slope}}$	3.44 (2.12)	2.47 (2.18)


Summer precipitation (mm)
100
200
300
400
Temperature (°C)
2
4
6
8
10
12
N4
N2
N1


(a) Random gene flow
(b) Directional gene flow
Legend
Ecological gradient
Locally adapted genotype
Among-populations gene flow
Within-population gene flow
Local
Migrant
Frequency within population
Individual genetic values
v_2
v_1
m_1
m_2


P effect ***
S = 0.964
Frequency
0
1000
2000
3000
4000
0.4
0.6
0.8
1.0
1.2
Height increment (log) / ΔH_{log}
P effect ***
S = 0.922
Frequency
98
99
100
101
102
103
Budburst date 2011 / $t_{b2\rightarrow3(2011)}$
P effect .
S = 0.655
Frequency
290
292
294
296
Leaf senescence date 2011 / $t_{s1\rightarrow2(2011)}$
N1
N2
N4
P effect ***
S = 0.948
Frequency
0.4
0.6
0.8
1.0
Diameter increment / ΔD
P effect **
S = 0.663
Frequency
98
100
102
104
Budburst date 2012 / $t_{b2\rightarrow3(2012)}$
P effect ns
S = 0.597
Frequency
188
190
192
194
196
Vegetation season duration 2011/ VD_{2011}
N1
N2
N4


P effect ns
S = 0.241
Frequency
0
1000
2000
3000
4000
0.40
0.42
0.44
0.46
0.48
Leaf mass (squared-root) / LMv
P effect ns
S = 0.595
Frequency
54
56
58
60
62
Leaf mass area / LMA
P effect ns
S = 0.350
Frequency
−29.4
−29.0
−28.6
Leaf carbon isotope composition / δ13C
N1
N2
N4
P effect ns
S = 0.358
Frequency
5.2
5.4
5.6
5.8
6.0
Leaf area (squared-root) / LAv
P effect ns
S = 0.386
Frequency
1.35
1.45
1.55
1.65
Leaf nitrogen content / %N
N1
N2
N4


ΔH_{log}
$t_{b2\rightarrow 3(2012)}$
Additive genetic variance
0.0
0.1
0.2
0.3
0.4
−10
0
10
20
30
−1.0
−0.5
0.0
0.5
1.0
LOD-score long-distance vs local immigration
LOD-score long-distance vs local immigration