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# The effect of drought stress on heterozygosity–fitness correlations in pedunculate oak (*Quercus robur*)

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• *Background and Aims* The interaction between forest fragmentation and predicted climate change may pose a serious threat to tree populations. In small and spatially isolated forest fragments, increased homozygosity may directly affect individual tree fitness through the expression of deleterious alleles. Climate change-induced drought stress may exacerbate these detrimental genetic consequences of forest fragmentation, as the fitness response to low levels of individual heterozygosity is generally thought to be stronger under environmental stress than under optimal conditions.
• *Methods* To test this hypothesis, a greenhouse experiment was performed in which various transpiration and growth traits of 6-month-old seedlings of *Quercus robur* differing in multilocus heterozygosity (MLH) were recorded for 3 months under a well-watered and a drought stress treatment. Heterozygosity–fitness correlations (HFC) were examined by correlating the recorded traits of individual seedlings to their MLH and by studying their response to drought stress.
• *Key Results* Weak, but significant, effects of MLH on several fitness traits were obtained, which were stronger for transpiration variables than for the recorded growth traits. High atmospheric stress (measured as vapour pressure deficit) influenced the strength of the HFCs of the transpiration variables, whereas only a limited effect of the irrigation treatment on the HFCs was observed.
• *Conclusions* Under ongoing climate change, increased atmospheric stress in the future may strengthen the negative fitness responses of trees to low MLH. This indicates the necessity to maximize individual multilocus heterozygosity in forest tree breeding programmes.

**Key words:** Climate change, drought stress, forest fragmentation, greenhouse experiment, growth traits, heterozygosity–fitness correlations, pedunculate oak, *Quercus robur*, transpiration.

## INTRODUCTION

Forests are essential to life on earth as they provide a multitude of ecosystem services, including climate mitigation, water regulation and biomass production (Lindenmayer and Franklin, 2002; Thompson *et al.*, 2009). Over recent decades the functioning and sustainability of forests have been increasingly challenged by various anthropogenic threats (Simberloff, 1999; Millennium Ecosystem Assessment, 2005). One of the most important threats is climate change (Noss, 2001; Lindner *et al.*, 2010). For Europe, climate projections predict increasing temperatures and irregular precipitation patterns during summer, which will increase the number and intensity of drought events (Stocker *et al.*, 2013). During such events, soil water shortage can be expected to induce the closure of stomata, which may directly damage leaf tissue of trees through the inhibition of leaf cooling (Bréda *et al.*, 2006). At more severe levels of drought stress, water transfer within the xylem may be irreversibly disrupted through vessel embolism (cavitation), which may result in losses of roots or twigs. Next to cavitation, tree mortality after long-term drought may also be caused by carbon starvation. This process induces the depletion of carbon resources during drought stress, as stomatal closure will lower carbon assimilation such that it is insufficient to supply the required amounts of carbon (McDowell *et al.*, 2008). Drought-induced physiological disorders will not only affect biomass production in the short term, but may also increase the susceptibility of trees to secondary stresses such as frost and fungal and insect attacks, which may affect tree health and eventually lead to tree death (Bréda *et al.*, 2006; Lindner *et al.*, 2010).

Next to climate change, the loss and fragmentation of forests through land-use changes poses a second major worldwide threat to forest sustainability (Hamrick, 2004; Millennium Ecosystem Assessment, 2005). Forest fragmentation results in small and spatially isolated forest fragments in which increased random genetic drift and inbreeding may erode genetic diversity of the tree populations (Jump and Peñuelas, 2006; Vranckx *et al.*, 2012). Genetic diversity is crucial, however, for the maintenance of vital and productive forests (Jump *et al.*, 2009), since population genetic variation provides the raw material for evolution, allowing adaptation of forest trees to environmental changes (Willi *et al.*, 2006). Moreover, increased homozygosity resulting

from inbreeding may directly affect individual tree fitness, through the expression of deleterious alleles that influence morphological, physiological and life-history traits. The relationships between heterozygosity and fitness traits are generally known as heterozygosity–fitness correlations (HFCs), and are more likely to occur in the small, non-random mating populations that typically occur in fragmented habitats (David, 1998; Hansson and Westerberg, 2002; Szulkin *et al.*, 2010). Quantifying HFCs may give insight into the short-term fitness of tree populations (Hedrick and Kalinowski, 2000; Reed and Frankham, 2003) and is also relevant to forest management and forest tree breeding programmes designed to maximize biomass production (Aravanopoulos and Zsuffa, 1998; Alig *et al.*, 2003).

The detrimental genetic consequences of forest fragmentation may be exacerbated by climate change-induced drought stress because the fitness response of tree species to low levels of heterozygosity is generally thought to be more pronounced under environmental stress than under optimal conditions (Armbruster and Reed, 2005). However, the effects of environmental stress on HFCs are not always clear (Keller and Waller, 2002), and beyond a certain level of stress HFCs may become less apparent (Audo and Diehl, 1995; David, 1998). Whereas the relationships between heterozygosity and fitness traits have been examined frequently in conifers (e.g. Mitton *et al.*, 1981; Ledig *et al.*, 1983; Bush *et al.*, 1987; Savolainen and Hedrick, 1995), similar studies are rare for broadleaved species (Aravanopoulos and Zsuffa, 1998). Furthermore, we are not aware of any study that has focused on the potentially detrimental interaction between climate change and habitat fragmentation by studying the effects of drought stress on HFCs.

Here we investigated HFCs and their response to drought stress in the economically important broadleaved tree species pedunculate oak (*Quercus robur*). We selected different transpiration variables and various growth traits as fitness variables (Van Hees, 1997; David, 1998; Bréda *et al.*, 2006). First, growth is an important fitness component, especially in indeterminate growers such as trees, which are characterized by size-dependent fecundity (David, 1998). Rapid early growth and strong biomass production will increase the competitive ability of seedlings, through which they may outcompete neighbouring seedlings when competition for light and resources is strong (King, 1981; Bush *et al.*, 1987; Scotti-Saintagne *et al.*, 2004). Furthermore, since the crown size of many tree species is strongly correlated with stem diameter (Hemery *et al.*, 2005), larger oak trees may also have greater crown areas for flowering and acorn production (Greenberg, 2000). Second, transpiration variables, such as stomatal conductance, water potential and the water content of seedlings, may give an indication of the water status of a plant and may influence the physiological processes that determine carbon fixation and growth (Bréda *et al.*, 2006). Low soil water content and high atmospheric evaporative demand may decrease the leaf water potential of oak seedlings and induce stomatal closure (Fort *et al.*, 1997; Cavender-Bares and Bazzaz, 2000). This may reduce the rate of stomatal conductance, limiting water fluxes at the cost of reduced photosynthesis and biomass production (Bréda *et al.*, 2006). Furthermore, although transpiration efficiency (ratio of biomass production to transpiration) generally increases during drought stress, Donovan and Ehleringer (1991) and Cavender-Bares and Bazzaz (2000) have suggested that this increase in transpiration efficiency may be lower during seedling establishment, when it is accompanied by increased seedling growth.

HFCs are likely to emerge in *Q. robur* since forest stands of this tree species are small in many parts of Western Europe due to past deforestation and fragmented forest ownership (Wiersum *et al.*, 2005). Moreover, recent research has revealed variation in individual multilocus heterozygosity within small oak stands in northern Belgium, despite high heterozygosity levels at the population scale (Vranckx *et al.*, 2014). Therefore, it can be hypothesized (1) that this within-stand variation in individual multilocus heterozygosity may be correlated to the variation in transpiration and growth traits of the seedlings; and (2) that these HFCs are stronger under stress conditions. To test these hypotheses, we quantified multilocus heterozygosity based on nine neutral microsatellite loci in 150 seedlings originating from three populations (50 seedlings per population). A greenhouse experiment was performed in which seedlings of *Q. robur* that differed in multilocus heterozygosity were grown under standardized environmental conditions. Various transpiration and growth traits of 6-month-old seedlings were recorded for 3 months under both a well-watered and a drought stress treatment.

## MATERIALS AND METHODS

### *Study species*

Pedunculate oak (*Quercus robur*) is a keystone tree species of many European forest ecosystems, with a large natural range extending from southern Scandinavia to sub-Mediterranean Europe, and eastwards to the Ural Mountains (Bary-Lenger and Nebout, 1993). This monoecious, wind-pollinated tree species occurs on a wide range of soils, and displays a medium degree of drought tolerance, which may be attributed to its deep rooting system and the maintenance of high rates of stomatal conductance during moderate levels of drought stress (Epron and Dreyer, 1993). Compared with its closest congener, *Quercus petraea*, *Q. robur* prefers to grow on neutral soils characterized by good water-holding capacity or soils with a permanent water table within reach of the root system (Bary-Lenger and Nebout, 1993). Flower fertilization is followed by rapid development of acorns, which are dispersed during autumn by gravity, small rodents and birds. Although acorns can germinate and establish under a closed canopy, forest canopy openings are required for further seedling growth and development (Bary-Lenger and Nebout, 1993).

### *Seed collection and experimental set-up*

Acorns of *Q. robur* were collected in the autumn of 2011 in three small (<4 ha) monospecific pedunculate oak stands in the centre of Flanders (northern Belgium). These forest stands had been studied previously (Vranckx *et al.*, 2014) and showed high heterozygosity values at the population level, which were consistent with what was found in other population genetic studies of *Q. robur* (Mariette *et al.*, 2002; Hampe *et al.*, 2010). The maximum distance between these stands was less than 15 km, and all were located in a matrix of forest stands composed of other tree species and/or agricultural land (Table 1). Pollen exchange among the three forest stands (Vos, Keffers and

TABLE 1. *Characteristics of the three pedunculate oak (*Q. robur*) stands where acorns were collected*

| Site | Latitude (N) | Longitude (E) | Area (ha) | Population size | Density (per ha) | Plot size (ha) | Isolation (m) |
|---|---|---|---|---|---|---|---|
| Keffers | 50°50′26″ | 4°42′00ì | 3·04 | 328 | 118 | 0·49 | 400 |
| Vos | 50°49′27″ | 4°39′33ì | 3·97 | 682 | 195 | 0·24 | 175 |
| Chartreuse | 50°54′45″ | 4°46′25ì | 0·43 | 32 | 74 | 0·43 | 135 |

Chartreuse) was rather limited, as the minimum geographical distance between stands (~3400 m) was much greater than the average pollen dispersal distances ($\delta_p = 130–210$ m) for the three studied stands, based on the neighbourhood model implemented in the program NM+ (Chybicki and Burczyk, 2010). A circular plot containing 35 adult trees was established in the centre of each *Q. robur* stand, in which five seed traps (1·5 $m^2$ each) were randomly located. At the end of September, ten acorns from each seed trap were collected and stored at 5 °C for 4 weeks. Cold storage increased both the percentage and synchronization of seed germination (Manzanera *et al.*, 1993). After cold storage, 50 *Q. robur* seeds per forest stand were weighed and sown in the centre of 3 L open-bottom pots filled with commercial soil (20 % organic and 10 % dry matter, pH ~ 6, electrical conductivity = 750 μS $cm^{-1}$, 14:16:18 N:P:K 1 kg $m^{-3}$). The pots were randomly placed in a greenhouse under controlled climatic conditions with a 12/12 h day/night light regime, and kept at field capacity using an automatic drip irrigation system. The irrigation water contained a Peters professional nutrient mixture (20:20:20 N:P:K, including trace elements; Everris International, Geldermalsen, the Netherlands). High germination percentages were obtained (>95 %), with most of the seeds germinating in week 4 after sowing.

### *Microsatellite analyses*

At the end of the growth phase (April 2012) all seedlings were genotyped. A single leaf was taken from each seedling and stored in silica prior to DNA extraction. Dried leaf samples (200 mg) were ground before DNA extraction with a Nucleospin Plant II kit (Macherey-Nagel). We selected ten simple sequence repeat (SSR) loci that had been developed for *Q. petraea* (QpZAG9, QpZAG108, QpZAG46, QpZAG15, QpZAG110, QpZAG104; Steinkellner *et al.*, 1997), *Quercus macrocarpa* (MSQ4, MSQ13, MSQ16; Dow *et al.*, 1995; Dow and Ashley, 1996) and *Q. robur* (QrZAG112; Kampfer *et al.*, 1998). Polymerase chain reaction amplifications were carried out using a Multiplex PCR Master Mix kit (Qiagen) with the thermocycler programme of 15 min at 94 °C followed by 30 cycles of 45 s at 94 °C, 45 s at 50 °C and 45 s at 72 °C and final extension at 72 °C for 10 min. The amplified fragments were analysed with an ABI 3500 genetic analyser (Applied Biosystems, Foster City, CA, USA) and GeneMapper software (version 4·1). The microsatellite data were first analysed using the software Micro-Checker version 2·2·3 (Van Oosterhout *et al.*, 2004) to check for possible genotyping errors, including null alleles, stutter peaks and large allele drop-out. We observed a consistent high null allele frequency at one locus (MSQ16), which was removed from further analyses. Neutrality of the microsatellites was checked with the Ewens–Watterson homozygosity test of neutrality (Manly, 1985) using Popgene version 1·32 (100 000 permutations; Yeh *et al.*, 1999). This test compares observed allele frequencies with those expected under mutation–drift equilibrium, and is therefore useful for detecting deviations from neutrality due to selection or demographic changes. We found no significant departures from neutrality for any loci; however, the observed *F* (sum of squares of allele frequencies) for locus QpZAG104 was close to the lower limit of the 95 % confidence interval (Supplementary Data Table S1).

Three different metrics were calculated to detect HFCs in the genotyped oak seedlings. First, multilocus heterozygosity (MLH) was measured in each seedling as the percentage of microsatellite loci for which an individual was heterozygous, corrected for non-scored loci (Slate and Pemberton, 2002; Chapman *et al.*, 2009). A second microsatellite-based metric that has been proposed for the study of HFCs is mean $d^2$, which is the squared difference (in repeat units) between the two alleles at a locus, averaged over all the microsatellite loci for which an individual was examined (Coulson *et al.*, 1998; Hedrick *et al.*, 2001; Slate and Pemberton, 2002). Mean $d^2$ was calculated as follows:

$$\bar{d}^2 = \frac{1}{N}\sum_{i=1}^{N}(n_{i1} - n_{i2})^2$$

where $n_{i1}$ and $n_{i2}$ are the lengths in repeat units of the two alleles at the *i*th locus and *N* is the number of scored loci examined in an individual. Finally, we also calculated the standardized mean $d^2$ ($sMd^2$), which limits the influence of highly polymorphic loci on the metric. Therefore, $d^2$ was standardized with its locus-specific variance (Pemberton *et al.*, 1999). By studying the above genetic metrics, we actually investigated different ecological processes. MLH has been considered to better detect recent inbreeding events than $d^2$, whereas $d^2$ may also contain information about historical events in an individual's ancestry, such as the influence of population admixture (hybrid vigour, high $d^2$) (Coulson *et al.*, 1998; Pemberton *et al.*, 1999). To assess the relationship between the three tested genetic measures (MLH, $d^2$ and $sMd^2$), Spearman rank correlation coefficients ($r_s$) were calculated. All abbreviations and variables are listed in the Appendix.

### *Treatment phase*

After 6 months at field capacity (growth phase), 100 of the 150 seedlings were exposed to the treatment phase (8 May 2012) and the 50 remaining seedlings were harvested for initial biomass estimation. The 100 seedlings subjected to the treatment phase were selected in such a way that individuals with high and low MLH values originated equally from all three forest stands. Subsequently, these seedlings were assigned to two irrigation treatments such that MLH levels and forest stands were uniformly represented within and between treatments. These drought

stress regimes were established based on relative extractable soil water (REW):

$$\mathrm{REW} = \frac{\theta_V - \theta_{WP}}{\theta_{FC} - \theta_{WP}}$$

and measurements of the actual volumetric moisture content ($\theta_V$) with a TRIME TDR FM3 sensor (IMKO, Ettlingen, Germany). The volumetric soil water contents at field capacity [$\theta_{FC}$, suction pressure ($pF$) = 2·0] and at wilting point ($\theta_{WP}$, $pF$ = 4·2) were measured and calculated for the applied commercial soil (40·8 and 5·8 %, respectively). The normal water treatment consisted of 50 seedlings subjected to a relative extractable soil water above 0·80 ($\theta_V$ = 33·8 %), which greatly exceeds the general water deficit threshold for trees (REW = 0·30–0·40; Granier *et al.*, 2007). To determine the effect of severe drought stress, the remaining seedlings were irrigated up to a relative extractable soil water content of 0·10 ($\theta_V$ = 9·3 %). This value corresponds to the estimates (REW = 0·05–0·2) recorded by Bréda *et al.* (2006) in European forests during the severe summer drought of 2003. Target weights for watering were calculated for all seedlings based on relative extractable soil water and the relationship between pot weight and soil moisture content. This relationship was regularly checked (weeks 1, 4 and 10) and adjusted for changes in plant weight. Seedlings were watered three times a week up to their target weight and pots were weighed before and after irrigation. To prevent soil evaporation and percolation, the surface and bottom of the pots were covered with aluminium and plastic foil respectively. Five control pots without seedlings were weighed to correct for soil evaporation. During the entire experiment, air temperature ($T_a$), relative air humidity (RH) and photosynthetically active radiation (PAR) were measured every 5 min. PAR was converted from W $m^{-2}$ to the more usual µmol $m^{-2}$ $s^{-1}$, based on the conversion factors of McCree (1972). Mean daytime values of $T_a$, PAR and vapour pressure deficit (VPD, calculated using $T_a$ and RH) are given in Table 2. Pots were fully randomized every week to reduce position effects within the greenhouse. Sulphur vaporization and preventive spraying with acaricides (Floramite, Nissorun) were successfully applied for the control of powdery mildew (*Microsphaera alphitoides*) and red spider mite (*Tetranychus urticae*).

TABLE 2. *Mean values and their standard errors (in parentheses) for air temperature ($T_a$), VPD and PAR during the treatment phase and during the measurements of $g_s$ and ψ in* Q. robur

| Time | Plant variable | $T_a$ (°C) | VPD (kPa) | PAR (µmol $m^{-2}$ $s^{-1}$) |
|---|---|---|---|---|
| Treatment period | | | | |
| Entire day | | 22·8 (0·1) | 1·30 (0·017) | 359·3 (29·9) |
| Morning | | 20·7 (0·1) | 0·90 (0·019) | 285·2 (39·1) |
| Midday | | 24·7 (0·2) | 1·67 (0·028) | 619·6 (50·6) |
| Day 35 | | | | |
| Morning | $g_s$ | 20·7 (1·2) | 0·81 (0·211) | 237·8 (62·1) |
| Midday | $g_s$ | 23·0 (0·5) | 1·31 (0·097) | 432·2 (109·5) |
| Day 49, midday | $g_s$ | 25·6 (0·4) | 1·88 (0·094) | 511·1 (155·9) |
| Day 65, midday | $g_s$ | 22·6 (0·8) | 1·61 (0·108) | 868·9 (159·2) |
| Day 92, midday | $\psi_{md}$ | 21·8 (0·3) | 0·97 (0·047) | 145·8 (12·9) |
| Day 93, predawn | $\psi_{pd}$ | 17·9 (0·1) | 0·19 (0·004) | 0 |

*Measurements*

At the start of the treatment phase, we recorded the following morphological variables: number of leaves ($L_n$) and branches ($B_n$), stem length ($S_L$), stem diameter at base ($S_{D,base}$) and top ($S_{D,top}$), branch length ($B_L$) and diameter at the base ($B_{D,base}$) and top of the branches ($B_{D,top}$). These measurements were repeated every 3 weeks and at the end of the treatment phase. Total woody volume ($V_{tot}$, stem + branches) was derived using Smalian's formula (West, 2009):

$$V_{tot} = \frac{A_{base} + A_{top}}{2} S_L$$

where $A_{base}$ and $A_{top}$ are the areas at the base and top of the stem or branches, respectively. On days 35, 49 and 65, leaf stomatal conductivity ($g_s$) was measured on two randomly selected top leaves of all seedlings using a steady-state SC-1 leaf porometer (Degacon Devices, Pullman, WA, USA). These measurements were performed in two rounds between 0900–1200 h and 1230–1530 h local time on day 35, whereas on the other days $g_s$ was measured only once (between 1230 and 1530 h), since climatic conditions were not homogeneous during the mornings of days 49 and 65. Midday ($\psi_{md}$) and predawn ($\psi_{pd}$) leaf water potentials were determined on day 92 (1230–1500 h) and 93 (0300–0530 h) respectively, using a Scholander pressure chamber (model 615, PMS instruments, Albany, USA). In total, we examined $\psi_{md}$ and $\psi_{pd}$ for 60 seedlings, in each of which two top leaves were randomly selected. These seedlings were characterized by low (≤0·67) and high (≥0·89) levels of MLH. The water potential range ($\Delta\psi$) per seedling was calculated as $\psi_{pd} - \psi_{md}$. Climatic conditions during measurements of leaf stomatal conductivity and water potential are given in Table 2.

At day 100 of the treatment phase (16 August 2012) all seedlings were harvested and measurements of fresh ($W_F$) and dry weight ($W_D$, 48 h at 85 °C) of the woody parts (stem + branches), leaves, roots and whole plants were performed. We calculated for each seedling above-ground biomass ($W_{AG}$) and water content (WC) as follows:

$$\mathrm{WC} = 100\left(1 - \frac{W_D}{W_F}\right)$$

*Calculated variables*

To estimate the initial biomass of the seedlings followed during the treatment phase, we established linear regression models between biomass data ($W_F$, $W_D$ and $W_{AG}$) and morphological input variables [ln($V_{tot}$), ln($S_L$), ln($L_n$) and $B_n$], which were obtained from the 50 seedlings harvested at the start of the treatment phase. The models with the highest $R^2_{adj}$ (0·84–0·97) were selected for initial biomass estimation (Supplementary Data Table S2). A good model fit was also confirmed by Mallow's $C_p$ selection criterion, as the $C_p$ value was equal to the number of regressors in the chosen models (Gagné and Dayton, 2002). The relative growth rates (RGRs) of various morphological and biomass variables were calculated as:

$$\mathrm{RGR}_X = \frac{\ln(X_2) - \ln(X_1)}{t_2 - t_1}$$

where $X_1$ and $X_2$ are the values of the studied variables at times $t_1$ and $t_2$ respectively (Evans, 1972). Daily transpiration rate (TR) was determined by weighing the pots before and after watering and correcting for soil evaporation (blank pots) and leaf loss. We also accounted for differences in seedling size by dividing the transpiration rate by the $\ln(V_{tot})$ of the seedlings. Transpiration efficiency (TE) was calculated as the ratio between the amount of dry biomass produced during the treatment phase and the total amount of water consumed during this period. We also estimated biomass water productivity (WP), which can be defined as the total dry biomass produced per unit of water transpired, normalized for atmospheric conditions (Steduto, 2003; Steduto *et al.*, 2007). Many studies have shown that WP is approximately constant for a given species, regardless of the growth conditions (irrigation treatment), after the variation in atmospheric conditions is normalized (Steduto, 2003; Steduto *et al.*, 2007). To calculate WP we first normalized cumulative transpiration (CT) for daily mean VPD measured between 0800 and 1800 h on day $j$ ($VPD_j$)

$$CT_i = \sum_{j=1}^{i=100} \frac{TR_j}{VPD_j}$$

Secondly, we constructed a linear regression model that included irrigation treatment, the cumulative transpiration normalized for atmospheric conditions ($CT_i$), MLH and irrigation treatments as input variables, and total dry biomass production as dependent variable. Main effects and interaction terms that were not significant ($P > 0{\cdot}05$), were excluded from this model using a backward selection procedure. Finally, biomass water productivity was estimated as the regression coefficient of the final regression model with $CT_i$ and irrigation treatment as input variables and dry biomass production as dependent variable (Steduto *et al.*, 2007).

### *Statistical analyses*

We used linear regression models to examine the effects of irrigation treatment (factor), multilocus heterozygosity (covariate) and their interaction term on the response variables measured and calculated during the treatment phase. To test for variation caused by environmental differences between the three seed collection sites, the forest stand in which the acorns were collected was included in the model as a fixed factor. Forest stand was not included as a random effect in our model, since the number of independent clusters within forest stands was too limited (three stands) to properly estimate its standard deviation. We also accounted for the effect of seed size on the measured response variables by including log seed weight in the initial model. Non-significant stand and seed size effects were excluded from the initial models (Supplementary Data Table S3), such that well-fitting final models (highest $R^2_{adj}$) were obtained. For all tested models, $R^2_{adj}$ values and their significance levels were calculated as a measure of goodness of fit, and partial $R^2$ coefficients ($R^2_p$) were obtained for each fixed effect to compare them between the different final models. To examine the effect of time of measurement, repeated measures ANOVAs with between-subject factors forest stand, irrigation regime and MLH were applied to transpiration rates corrected for seedling size (within-subject effect = irrigation event, 39 levels) and stomatal conductance day 35 (within-subject effect = time of measurement, 2 levels). If the assumption of sphericity was not met (significant Mauchly's test), the Huyn–Feldt statistic was used for within-subject tests. All statistical analyses were performed using SPSS software (SPSS 20·0; SPSS, Chicago, IL).

## RESULTS

A total of 146 of the 150 studied seedlings (97·3 %) were successfully genotyped for all nine microsatellite loci. The markers were highly polymorphic, with an average number of 17·7 ± 2·01 (s.e.) alleles per locus. Multilocus heterozygosity ranged between 0·44 and 1, whereas $d^2$ varied between 9·3 and 751·7 and $sMd^2$ between $4{\cdot}3 \times 10^{-5}$ and $8{\cdot}0 \times 10^{-3}$. The three variables were significantly correlated with each other. Multilocus heterozygosity was more strongly correlated to standardized mean $sMd^2$ ($r_s = 0{\cdot}39$, $P < 0{\cdot}0001$) than to mean $d^2$ ($r_s = 0{\cdot}27$, $P = 0{\cdot}006$). In addition, the statistical power of $d^2$ and $sMd^2$ to detect significant HFCs was much more limited [lower $R^2_p$ values (<0·1–2·1 %) compared with MLH (3–11 %)] (cf. Coltman and Slate, 2003; Slate and Pemberton, 2002; Chapman *et al.*, 2009). Therefore, we only report the outcome of the HFC tests based on MLH.

### *Water use and transpiration*

Almost all transpiration and water use variables differed strongly ($P < 0{\cdot}05$) between the two irrigation treatments (Table 3A), with significantly higher estimates for seedlings subjected to normal water conditions compared with drought-stressed seedlings (Figs 1 and 2). Changes in $g_s$ during the day also differed significantly between the two irrigation treatments (significant time × stress effect, $P < 0{\cdot}05$). For drought-stressed seedlings, $g_s$ decreased by 9·6 %, from 67·7 ± 5·8 (s.e.) mmol $m^{-2}$ $s^{-1}$ in the morning of day 35 to 61·3 ± 5·2 mmol $m^{-2}$ $s^{-1}$ at midday, whereas $g_s$ of well-watered seedlings significantly increased ($P < 0{\cdot}05$) by 32·5 %, from 337·6 ± 28·5 to 447·1 ± 44·3 mmol $m^{-2}s^{-1}$ at midday. More importantly, the results of the linear regression models indicated that $g_s$ and water content were significantly correlated ($P < 0{\cdot}05$) with the MLH of the seedlings, whereas $\psi_{md}$ and $\Delta\psi$ showed marginally significant relationships ($0{\cdot}05 \leq P < 0{\cdot}1$) (Table 3A). For all of these variables, estimates increased with increasing MLH (Fig. 2), except for $\Delta\psi$, for which the opposite relationship was observed in the drought-stressed seedlings (Fig. 2D). Although significant HFCs were found, only a small proportion of the variance ($R^2_p = 4$–11 %) could be explained by MLH (Table 3A). Marginally significant interaction terms ($0{\cdot}05 \leq P < 0{\cdot}1$) between irrigation treatment and MLH were obtained for measures of $g_s$ on day 49 and for $\Delta\psi$ (Table 3A). These interactions are visualized in the non-parallel regression lines in Fig. 2A, D, where estimates of $g_s$ on day 49 and $\Delta\psi$ were more strongly correlated with MLH under water stress than under normal water conditions. The effects of forest stand and seed weight on the transpiration variables were limited. A few variables showed significant differences between the three seed collection sites (Table 3), but no effects of seed weight were found.

TABLE 3. *Results of linear regression models performed to examine the effect of irrigation regime, MLH and their interaction on (A) transpiration and (B) growth trait variables for Q. robur*

| | | Correlation model | | Irrigation | | MLH | | Irrigation × MLH | | Forest stand | | Log seed weight | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Variable | $F_{model}$ | $R^2_{adj}$ | $F$ | $R^2_p$ | $F$ | $R^2_p$ | $F$ | $R^2_p$ | $F$ | $R^2_p$ | $F$ | $R^2_p$ |
| | | | | | | (A) Transpiration | | | | | | | |
| Stomatal conductance | log $g_s$ day 35 a.m. | 43·03*** | 0·68 | 10·27** | 0·10 | 4·76** | 0·05 | 0·36 | <0·01 | 4·66** | 0·09 | | |
| | log $g_s$ day 35 p.m. | 73·79*** | 0·69 | 10·02** | 0·10 | 7·13** | 0·07 | 0·26 | <0·01 | | | | |
| | log $g_s$ day 49 p.m. | 110·81*** | 0·85 | 35·55*** | 0·27 | 9·06** | 0·09 | 3·20(*) | 0·03 | 5·57** | 0·11 | | |
| | log $g_s$ day 65 p.m. | 121·81*** | 0·86 | 16·43*** | 0·15 | 11·14** | 0·11 | 0·17 | <0·01 | 2·75(*) | 0·06 | | |
| Leaf water potential | $\psi_{md}$ | 40·35*** | 0·68 | 14·01*** | 0·21 | 2·81(*) | 0·05 | 1·81 | 0·03 | | | | |
| | $\psi_{pd}$ | 58·93*** | 0·75 | 5·87** | 0·10 | 0·10 | <0·01 | 0·34 | <0·01 | | | | |
| Water potential range | $\Delta\psi$ | 9·52*** | 0·31 | 8·61** | 0·14 | 3·14(*) | 0·06 | 3·93(*) | 0·07 | | | | |
| Total transpiration, corrected | TR | 124·84*** | 0·80 | 9·74** | 0·10 | 1·33 | 0·01 | 0·25 | <0·01 | | | | |
| Water content | WC | 61·47*** | 0·66 | 6·10** | 0·06 | 4·11** | 0·04 | < 0·01 | <0·01 | | | | |
| | | | | | | (B) Growth traits | | | | | | | |
| RGR (Evans, 1972) | $RGR_{diameter}$ | 69·40*** | 0·74 | 20·06*** | 0·18 | 3·67(*) | 0·04 | 2·17 | 0·02 | | | 2·75 | 0·03 |
| | $RGR_{length}$ | 3·58** | 0·08 | 0·03 | <0·01 | 2·95(*) | 0·03 | 0·45 | <0·01 | | | | |
| | $RGR_{woody\ volume}$ | 46·26*** | 0·66 | 15·21*** | 0·14 | 4·53** | 0·05 | 2·22 | 0·02 | | | 2·25 | 0·02 |
| | $RGR_{dry\ biomass}$ | 64·86*** | 0·67 | 7·55** | 0·08 | 3·23(*) | 0·03 | 0·04 | <0·01 | | | | |
| | $RGR_{fresh\ biomass}$ | 102·28*** | 0·76 | 14·10*** | 0·13 | 2·78(*) | 0·03 | 0·28 | <0·01 | | | | |
| | $RGR_{above\ ground}$ | 18·28*** | 0·48 | 8·47** | 0·09 | 2·51 | 0·03 | 1·76 | 0·02 | 5·31** | 0·11 | | |
| | $RGR_{roots}$ | 20·80*** | 0·56 | 2·93(*) | 0·03 | 0·12 | < 0·01 | 0·05 | <0·01 | 6·55** | 0·13 | 4·03** | 0·04 |
| Transpiration efficiency | TE | 2·41(*) | 0·06 | 0·47 | <0·01 | 0·05 | <0·01 | 0·41 | <0·01 | | | 8·51** | 0·09 |

To account for differences between forests and seed sizes, forest stand and log seed weight were included in the analysis as fixed effects. Non-significant stand and seed size effects were excluded from the final models, such that well-fitting models (highest $R^2_{adj}$) were obtained. $F$-statistics, $R^2_p$ coefficients and significance levels for main effects and interactions are presented for the final models (after model reduction).

(*)$0{\cdot}05 \leq P < 0{\cdot}1$; **$0{\cdot}001 \leq P < 0{\cdot}05$; ***$P < 0{\cdot}001$.

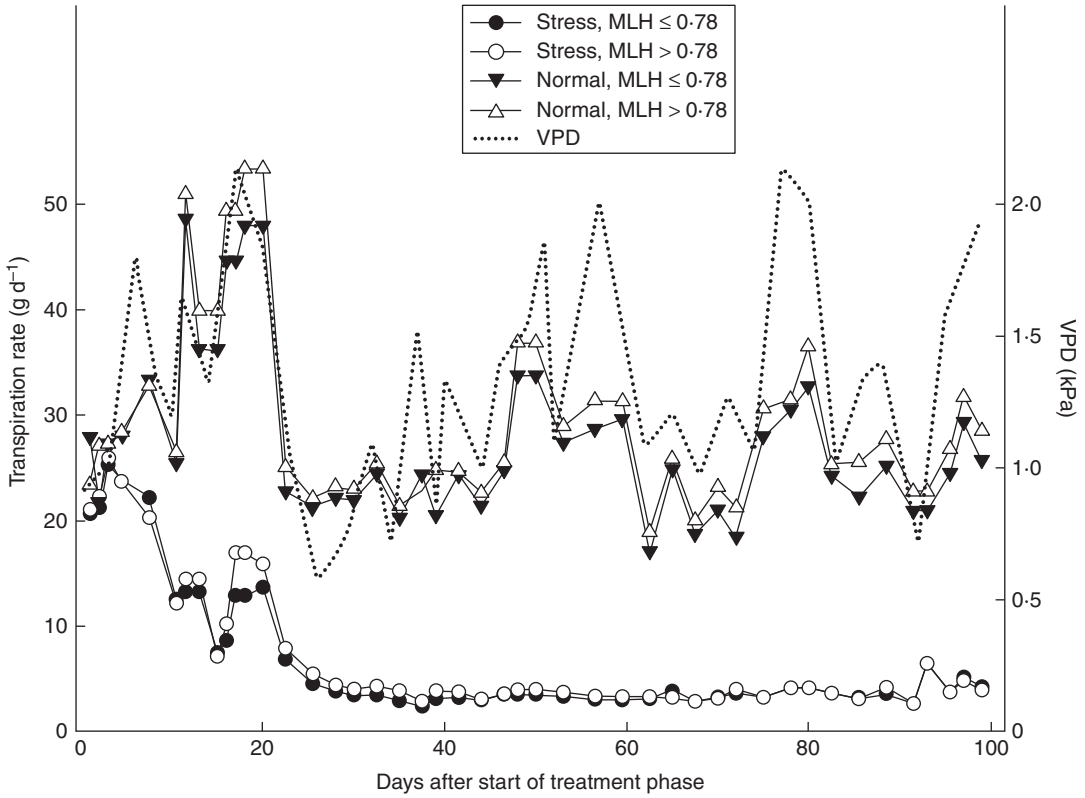


FIG. 1. Daily transpiration rates of *Q. robur* corrected for seedling size, under drought stress and normal water conditions (see key). Seedlings were divided into two groups with MLH ≤0·78 and >0·78.

The repeated measures ANOVA model used to examine the effect of time on TR indicated a highly significant time and time × irrigation effect (both $P < 0{\cdot}001$) (Fig. 1). The mean TR of drought-stressed *Q. robur* seedlings declined strongly from 25·6 ± 1·5 g (s.e.) on day 5 to 4·4 ± 0·5 g on day 28, after which TR stabilized. Seedlings under normal water conditions showed daily TRs that were highly correlated ($r_s = 0{\cdot}45$, $P < 0{\cdot}05$) to atmospheric demand (VPD), with a slightly but not significantly higher TR when MLH was >0·78 compared with seedlings with MLH ≤0·78 (Fig. 1).

### *Growth rate and biomass production*

Seedling growth was strongly affected by water availability of the soil. First, we observed a highly significant ($P < 0{\cdot}001$) positive relationship between dry biomass production and the total amount of water transpired during the treatment phase (Fig. 3). Second, most growth traits were influenced by the irrigation regime, with significantly higher RGRs ($P < 0{\cdot}05$) under normal water conditions than under drought stress (Table 3B). Efficiency in producing a certain amount of dry biomass per unit of water transpired (TE) was 23 % higher in drought-stressed seedlings [5·01 ± 0·32 (s.e.)] than in well watered seedlings (4·07 ± 0·23). However, this difference between irrigation treatments was not significant ($P > 0{\cdot}05$), and TE was also not associated with the MLH of a seedling (Table 3B). Similar results were obtained for biomass WP, since the regression coefficients of the linear regression model between $CT_i$ and dry biomass production were not significantly ($P > 0{\cdot}05$) influenced by the other input variables of the model (irrigation treatment and MLH). This was indicated by the non-significant interaction terms ($CT_i$ × irrigation treatment and $CT_i$ × MLH), which were removed, together with the non-significant main effect MLH, from the linear regression model.

A significant effect of MLH ($P < 0{\cdot}05$) on growth variables was obtained when the RGR of woody volume was tested as trait (Table 3B). Under drought stress, mean values of $RGR_{woody\ volume}$ were 7·5 times larger in seedlings of the highest MLH class (MLH = 1) compared with seedlings with the lowest MLH values (MLH = 0·44) (Fig. 4A). The RGR of stem diameter and RGRs of dry and fresh biomass showed a weak ($R^2_p = 3$–$5$ %), marginally significant ($0{\cdot}05 \leq P < 0{\cdot}1$), positive relationship with MLH (Table 3B, Fig. 4B). In contrast to some of the transpiration variables, no significant interaction terms between MLH and irrigation treatment were found for any growth trait (Table 3B, Fig. 4). Finally, most of the growth traits were not significantly influenced by the forest stand in which the acorns were collected or by the weight of the acorns. Furthermore, the weight of the acorns was not significantly correlated with the MLH of the acorns ($r_s = 0{\cdot}12$; $P = 0{\cdot}23$).

## DISCUSSION

Our results showed weak but significant effects of MLH on several transpiration and growth traits in the temperate broad-leaved tree species pedunculate oak (*Q. robur*). Since selectively

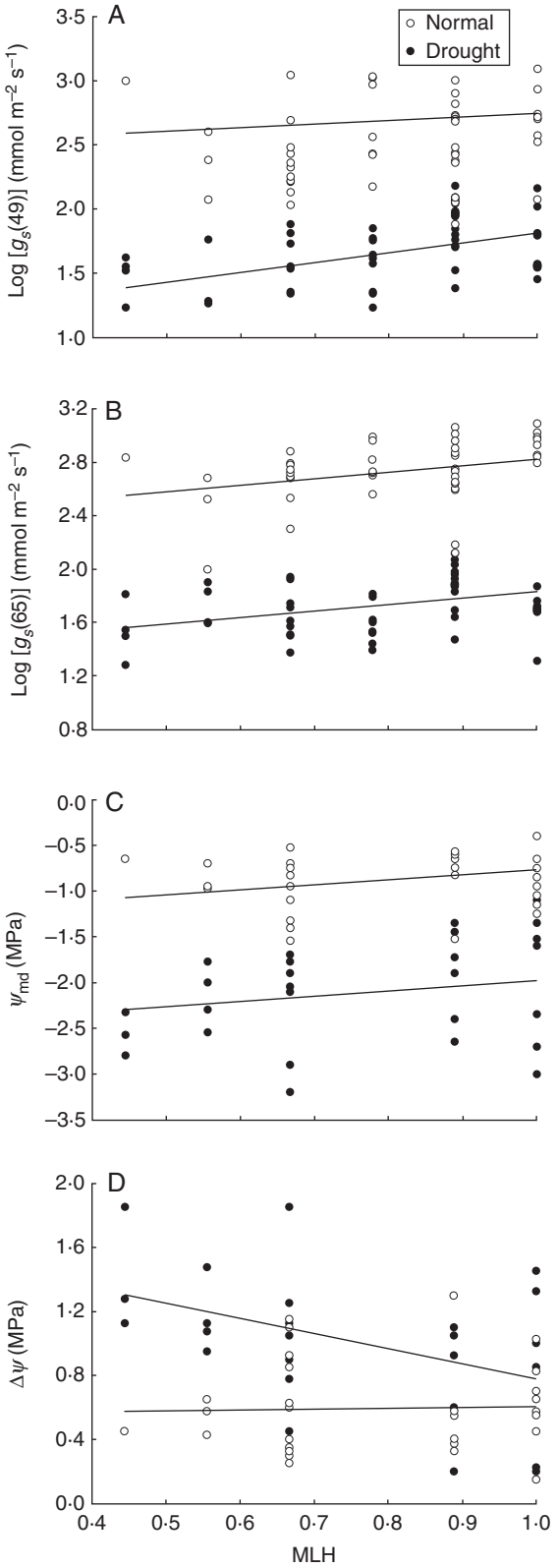


FIG. 2. Relationships between MLH of *Q. robur* and the following transpiration variables: $g_s$ at (A) day 49 and (B) day 65, (C) $\psi_m$ and (D) $\Delta\psi$. HFCs were compared between seedlings subjected to normal water conditions and drought-stressed seedlings (see key). The plotted regression lines are based on estimates of the significant fixed effects of the linear regression models (Table 3).

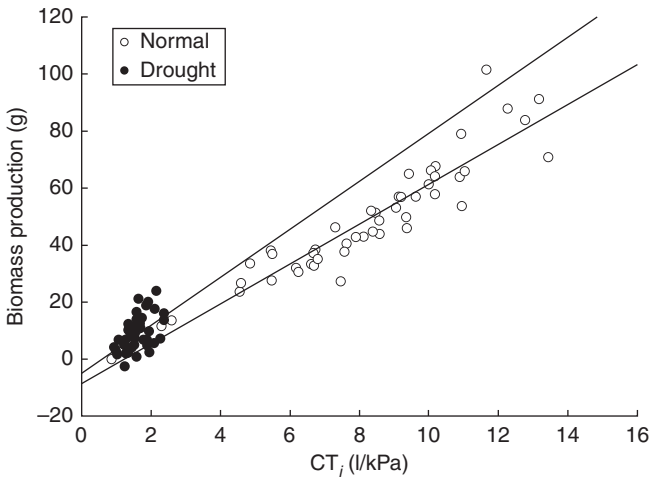


FIG. 3. Biomass production of *Q. robur* as a function of cumulative transpiration standardized for VPD ($CT_i$), compared between seedlings under normal water conditions and drought-stressed seedlings (see key). Regression lines are plotted for each irrigation treatment and are based on the coefficients of the linear regression model.

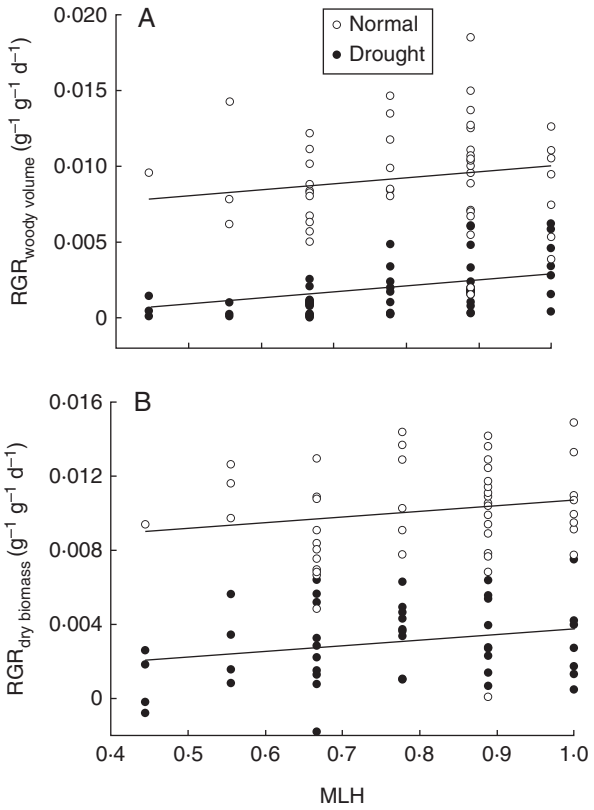


FIG. 4. Relationships between MLH of *Q. robur* and (A) RGR of woody volume and (B) RGR of dry biomass. HFCs were compared between seedlings subjected to normal water conditions and drought-stressed seedlings (see key). The plotted regression lines are based on estimates of the significant fixed effects of the linear regression models (Table 3).

neutral molecular markers were used to quantify genetic diversity, HFCs cannot readily be attributed to functional overdominance at the scored loci (the so-called direct effect hypothesis) (Queller *et al.*, 1993; Savolainen and Hedrick, 1995), but they would require small non-random mating population structures to emerge. In these populations, processes such as genetic drift, inbreeding and recent bottlenecks are more likely to occur (Young *et al.*, 1996), and consequently HFCs may arise as a result of associative overdominance, either at linked fitness loci (the local effect hypothesis) or at genome wide distributed loci (the general effect hypothesis; i.e. classical inbreeding depression) (Szulkin *et al.*, 2010). Although *Q. robur* is a wind-pollinated species with a highly outcrossing breeding system (selfing rate 2–5 %; Steinhoff, 1993), it has been shown that, in tree species with similar traits, biparental inbreeding may lower the number of heterozygous individuals in small, fragmented forest stands (Jump and Peñuelas, 2006; Vranckx *et al.*, 2012). Furthermore, recent research conducted in the same forest stands by Vranckx *et al.* (2014) has demonstrated less diverse pollen pools and increased correlated paternity in small stands. Ultimately, this may lead to stronger effects of biparental inbreeding in subsequent generations. In this study some variation in MLH was already detected for individual seedlings. However, at the population scale we found high heterozygosity levels, which were consistent with what was found in other population genetic studies on *Q. robur* (Mariette *et al.*, 2002; Hampe *et al.*, 2010).

### *Strength of HFCs in* Q. robur *seedlings*

The observed proportion of the variance in transpiration and growth traits that was explained by MLH was substantially larger than that reported in earlier HFC studies (0·07–3·3 %; Szulkin *et al.*, 2010). Furthermore, explained variances of $\leq$1 % for MLH have been reported to be common when microsatellite markers are used to quantify genetic diversity (Coltman and slate, 2003; Chapman *et al.*, 2009). The stronger HFCs that we obtained can be attributed to several factors. First, since most empirical HFC studies have been performed on animal species with separate sexes (Coltman and Slate, 2003; Chapman *et al.*, 2009; Szulkin *et al.*, 2010), significant publication bias is likely. Plant species can be expected to show stronger HFCs than animal species, which may be related to the sessile nature and often self-compatible breeding systems of plants. This makes them more prone to selfing and biparental inbreeding, especially when habitat fragmentation occurs (González-Varo *et al.*, 2012). Second, the choice of genetic metric may also influence the strength of the HFC (Slate and Pemberton, 2002; Chapman *et al.*, 2009). Our study indicated that MLH had a higher power to detect HFCs than the variables $d^2$ and $sMd^2$. This is consistent with the results of previous meta-analyses (Coltman and Slate, 2003; Chapman *et al.*, 2009) and may indicate the occurrence of recent inbreeding events rather than population admixture (Coulson *et al.*, 1998; Pemberton *et al.*, 1999). As already mentioned, $d^2$ may show stronger HFCs than MLH when population admixture strongly increases the number of highly heterozygous individuals and when fast-evolving molecular markers (mutation rate $>$0·001) are used (Goudet and Keller, 2002). Third, contrary to previous studies in conifers (Ledig *et al.*, 1983; Savolainen and Hedrick, 1995), we examined *Q. robur* seedlings under controlled common garden conditions, which increased the probability of detecting HFCs (David and Jarne, 1997; Keller and Waller, 2002). Heterozygosity–fitness correlations are expected to be stronger in seedlings compared with adult trees, as growth and survival are most strongly affected during the earliest life stages (David, 1998). Adult trees may cope better with stressful conditions such as drought stress as their roots may access deeper water sources, whereby higher rates of transpiration and photosynthesis are maintained (Bond, 2000; Cavender-Bares and Bazzaz, 2000). Furthermore, less fit homozygotes, still present in the seedling cohort, may be absent from older generations (Honnay *et al.*, 2008). Because of this gradual decrease in the number of homozygous individuals from seedlings to adult trees and the reduced environmental stress perceived by the adults, one would expect that under natural conditions HFCs would have been absent or undetectable in adult cohorts. However, Ziehe and Hattmer (2002) still found positive associations between heterozygosity level and diameter growth of adult trees in natural populations of the temperate broadleaved tree species *Fagus sylvatica*.

Perhaps the most important factor influencing the magnitude of HFCs is the fitness trait under study. In our study, transpiration and growth traits showed (marginally) significant relationships with MLH. HFCs were, however, stronger for transpiration variables ($R^2_p = 4–11$ %) than for growth traits ($R^2_p = 3–5$ %). When *Q. robur* seedlings are facing drought stress, stomatal conductance is directly reduced as a result of a highly efficient stomatal control mechanism. This allows the leaf water potential to remain above the critical threshold value at which cavitation damage occurs (Vivin *et al.*, 1993; Cochard *et al.*, 1996). Contrary to the growth traits, which were recorded over a longer period of time, the transpiration variables were measured at midday (1230–1530 h). Consequently, transpiration was strongly affected, as the seedlings were exposed to the highest possible values of VPD and high levels of PAR (Table 2). Similar midday depression of gas exchange has been reported frequently in oaks and many other tree species (Weber and Gates, 1990; Epron and Dreyer, 1993) and was confirmed in this study by the decreased midday stomatal conductance ($-$9·6 %) of the drought-stressed seedlings on day 35.

Another possible explanation for the stronger correlations between transpiration variables and MLH is that these HFCs could be the result of an association with a microsatellite near the coding region of the studied trait (the local effect hypothesis). Although theoretical papers often suggest that transpiration and growth traits are both typically controlled by numerous loci (David, 1998; Szulkin *et al.*, 2010), recent research has detected a relatively limited number of quantitative trait loci (QTLs) for growth rate and several transpiration traits in *Q. robur* (Scotti-Saintagne *et al.*, 2004; Brendel *et al.*, 2008; Gailing *et al.*, 2008). For example, in the study of Scotti-Saintagne *et al.* (2004) three QTLs for height growth were found which explained between 9·5 and 18·7 % of the mean variance. One of the problems of QTLs is that they may be specific to a given environment, growth stage or genetic background, because of which QTL consistency across studies is often low (Teulat *et al.*, 2001). Only in the study of Gailing *et al.* (2008) were some of the microsatellites used in our study located within QTLs for height growth and stomatal density. For height increment, two microsatellites (QpZag 104 and QpZag 46) were positioned within one QTL region

(LG2M), explaining 3·4 % of the variance in height growth, whereas for stomatal density three microsatellites (QpZag 104, QpZag 46 and QpZag 9) occurred within two QTLs (LG2F and LG7F), explaining a higher percentage (3·7 and 7·2 %, respectively) of the phenotypic variance (Gailing *et al.*, 2008). It has been shown that increased stomatal density may improve drought resistance, as stomata present at high density are often smaller and have small guard cells, which contributes to better control of transpiration (Roussel *et al.*, 2009).

### *Interaction between drought stress and heterozygosity*

It has been claimed that HFCs are more pronounced under elevated environmental stress levels than under optimal conditions (Armbruster and Reed, 2005; Lesbarrères *et al.*, 2005), possibly exacerbating climate change effects in small, inbred tree populations. In our study, little evidence was found to support this hypothesis, as most of the examined fitness traits showed no significant interaction between irrigation treatment and MLH. However, for the water potential range, we found stronger HFCs in drought-stressed seedlings compared with well watered seedlings. Plants can recover from water deficits overnight through internal hydraulic redistribution, which removes water potential gradients among leaves and roots (Domec *et al.*, 2004; Bauerle *et al.*, 2008). This will alleviate plant water stress, as root function, cell turgor for growth and leaf water content are maintained (Nardini and Pitt, 1999). The variation in recovery rate of the water potential that was observed in the drought-stressed seedlings was influenced by the level of midday leaf water potential, which, in turn was affected by the individual MLH.

Next to the limited effect of the irrigation treatment, the strength of the HFCs may also have been influenced by the atmospheric stress level. Stomatal conductance was more correlated with MLH on days 49 and 65, which were both characterized by high VPD and PAR levels (Table 2), whereas on day 35 there were lower atmospheric stress levels and weaker HFCs (Table 3). Previous studies on tree transpiration have indicated not only that the physiological response of trees to drought stress depends on the water status of the soil, but also that the VPD and PAR level may also play a major role in transpiration and growth (Van Hees, 1997; Oren and Pataki, 2001; Zweifel *et al.*, 2005). The significant interaction between irrigation treatment and MLH that was observed on day 49 might be attributed to the combined effect of low soil moisture and high atmospheric stress (e.g. high VPD and PAR), which may have imposed stronger overall drought stress conditions on seedlings (Van Hees, 1997). This is in contrast with the findings of Mitton (1993) and Audo and Diehl (1995), who found stronger HFCs at moderate stress levels, whereas we demonstrated that higher stress levels (especially atmospheric stress) exacerbated the effects of low genetic diversity on tree transpiration.

### *Implications for future forest management under climate change*

The existence of HFCs in natural populations of pedunculate oak indicate that increased homozygosity could ultimately limit biomass production below the potential yield. We found that relative growth rates of biomass production and woody volume declined with increasing number of homozygous loci under both irrigation treatments. Considering ongoing climate change, the projected increase in temperature of 2·0–3·1 °C for Central Europe by 2100 (CMIP5 model, scenario RCP4·5, 25th–75th percentile), will raise the VPD of the air by 3–6 % $°C^{-1}$ (Kirschbaum, 2000; Stocker *et al.*, 2013), leading to greater exposure of trees to atmospheric drought stress. Since we have shown stronger relationships between transpiration rates and MLH under higher VPD levels and since biomass production was strongly correlated with total transpiration, one can expect lower biomass production in homozygous individuals in the future. Moreover, more frequent and severe drought events may also limit the water availability in the soil, which may worsen the effect of high levels of atmospheric stress (Kirschbaum, 2000; Zweifel *et al.*, 2005). In our study, however, the effect of limited soil water availability on the strength of the HFCs was only observed for measurements of stomatal conductance on day 49 (highest VPD), indicating a large tolerance of pedunculate oak seedlings to soil water stress (Van Hees, 1997; Bréda *et al.*, 2006). Furthermore, since the roots of adult trees may tap into deeper water sources, they will be even less susceptible to soil water stress than seedlings (Bond, 2000; Cavender-Bares and Bazzaz, 2000).

To narrow the gap between average and potential yields under current and future environmental conditions, individual MLH should be maximized in forest tree breeding programmes. In naturally regenerating forest stands, intense natural selection at the seedling stage may preserve high levels of MLH in older age classes (Bush and Smouse, 1991). However, natural regeneration of oak is often problematic due to factors such as low seed quantity and quality, strong predation and the lack of appropriate site conditions (Lorimer, 1992; Abrams, 2003). Moreover, in small fragmented forest stands, which are common in many parts of Western Europe, inbreeding may reduce the number of seedlings with high MLH. Extensive gene flow between stands may mitigate loss of genetic diversity. However, we previously showed that even in wind-pollinated species, such as oak, a reduction in tree density or population size may decrease local pollen diversity and increase correlated paternity (Vranckx *et al.*, 2014), which may lead to stronger effects of biparental inbreeding in subsequent generations. So, even under high pollen immigration rates from outside the stand, an important fraction of mating events will occur at short distances between neighbouring trees (Sork *et al.*, 2002; Breed *et al.*, 2013). Processes such as biparental inbreeding and reduced gene flow may be avoided by the maintenance of large continuous forest stands (Jump and Peñuelas, 2006; Vranckx *et al.*, 2012). Not only may this increase MLH, but it will also retain and enlarge the gene pool for adaptation, which is probably the best strategy to counter current and future environmental changes (Hamrick, 2004; Jump *et al.*, 2009).

## SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: results of the Ewens–Watterson homozygosity test of neutrality for all microsatellites. Table S2: linear regression models relating biomass data to morphological input variables. Table S3: results of the initial linear regression models performed to examine the effects of irrigation regime, multilocus heterozygosity and their interaction on transpiration and growth trait variables.

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## APPENDIX

### List of abbreviations and variables

| | |
|---|---|
| $A_{base}$ | area at base of stem or branches |
| $A_{top}$ | area at top of stem or branches |
| $B_{D,base}$ | branch diameter at base |
| $B_{D,top}$ | branch diameter at top |
| $B_L$ | branch length |
| $B_n$ | number of branches |
| CT | cumulative transpiration |
| $CT_i$ | cumulative transpiration standardized for vapour pressure deficit |
| $g_s$ | leaf stomatal conductivity |
| HFC | heterozygosity–fitness correlation |

| | |
|---|---|
| $L_n$ | number of leaves |
| MLH | multilocus heterozygosity |
| PAR | photosynthetically active radiation |
| $pF$ | suction pressure |
| REW | relative extractable soil water |
| RGR | relative growth rate |
| RH | relative humidity |
| $R^2_p$ | partial $R^2$ coefficient |
| $S_{D,base}$ | stem diameter at base |
| $S_{D,top}$ | stem diameter at top |
| $S_L$ | stem length |
| $T_a$ | air temperature |
| TE | transpiration efficiency |
| TR | transpiration rate |
| VPD | vapour pressure deficit |
| $VPD_j$ | VPD on day $j$ |
| $V_{tot}$ | total woody volume (stem + branches) |
| $W_{AG}$ | above-ground biomass |
| WC | water content |
| $W_D$ | dry weight |
| $W_F$ | fresh weight |
| WP | water productivity |
| $\Delta\psi$ | water potential range |
| $\theta_{FC}$ | volumetric soil water content at field capacity |
| $\theta_{WP}$ | volumetric soil water content at wilting point |
| $\theta_V$ | volumetric moisture content |
| $\psi_{md}$ | midday leaf water potential |
| $\psi_{pd}$ | predawn leaf water potential |