Methods in Ecology and Evolution

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Article type : Research Article

Handling editor: Dr Satu Ramula

A new approach to study local adaptation in long-lived woody species: virtual transplant experiments.

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Running headline:

New methods to study long-term local adaptation

Abstract

1. Despite the importance of local adaptation and the extended literature that has addressed it, there are few methods available to explore local adaptation across large temporal scales. However, long-term patterns are likely to be essential to understanding adaptation in long-lived species, such as trees.

2. Here, we propose a methodology named 'virtual transplant experiment' (VTE), which uses long-term climatic variability to explore local adaptation to climate in natural tree populations. VTEs evaluate the historical response of populations to their local climate and to climates representative of conditions in other populations. We tested our methodology using simulated data and applied it in two case studies on: (i) *Pinus nigra* populations at the edge of the species distribution, where previous research has suggested strong climate adaptation, and (ii) *Fagus sylvatica* mesic populations, where parallel experiments showed no adaptation to macroclimate.

3. VTE results from simulated and real-world data matched our expectations, suggesting that the method accurately identified the patterns of local adaptation to climate in tree populations. VTEs consistently discriminated locally adapted populations in synthetic data with a known degree of local adaptation. As expected, *P. nigra* populations showed adaptation to local climate in the VTE, while *F. sylvatica* populations showed no overall local advantage.

4. Our method provides a new way to test for local adaptation over time scales encompassing the complete lifespan of trees. VTEs can complement current methods to study local adaptation by adding the ability to explore the long-term response to local climate in natural populations. The advantages and limitations of the different approaches to studying local adaptation stress the importance of combining multiple approaches to test for local adaptation in long-lived organisms.

Keywords: Common garden experiments, Dendrochronology, Local adaptation, Reciprocal transplant experiments, Tree adaptation, Tree-rings, Virtual transplant experiments.

Título: *Una nueva aproximación al estudio de la adaptación local en organismos longevos: trasplantes virtuales.*

Resumen*:*

1. A pesar de la importancia que tiene el grado de adaptación local y la extensa literatura que ha abordado el tema, aún carecemos de metodologías para estudiar la adaptación local a largo plazo. Sin embargo, es muy probable que estos patrones de adaptación sean esenciales para entender cómo especies longevas, como la mayoría de especies forestales, responden y se adaptan a su hábitat.

2. En este trabajo proponemos una nueva metodología, denominada trasplante virtual (VTE), que utiliza la variabilidad climática a largo plazo para explorar la adaptación local al clima en poblaciones forestales. VTE evalúa la respuesta del crecimiento de cada población bajo sus propias condiciones más frecuentes, así como las más frecuentes para las otras poblaciones estudiadas a lo largo de un amplio rango de definiciones climáticas. Examinamos nuestra metodología usando simulaciones con un nivel de adaptación local conocido y dos casos de estudio: (i) poblaciones de Pinus nigra en el límite de su distribución, cuyos resultados previos sugieren fuerte adaptación climática, y (ii) poblaciones mésicas de Fagus sylvatica, que han mostrado reducida adaptación al macroclima.

3. Los resultados fueron consecuentes con nuestras expectativas, sugiriendo que VTE identifica correctamente patrones de adaptación local al clima en poblaciones forestales. Las poblaciones sintéticas fueron correctamente clasificadas en función de su nivel de adaptación local y ambos casos reales coincidieron con los resultados previos disponibles.

4. En este artículo ofrecemos una nueva forma de investigar patrones de adaptación local a una escala temporal que es más consecuente con la esperanza de vida media de las especies forestales. VTE tiene la capacidad de explorar la adaptación a largo plazo, un aspecto poco reconocido en las aproximaciones actuales, pero tiene también limitaciones propias. En consecuencia, combinar múltiples métodos es probablemente nuestra mejor opción para entender los mecanismos de adaptación local en organismos longevos.

Introduction

Questions about the frequency and degree of local adaptation have been a central part of ecology for almost all its history. From the early provenance tests (reviewed by Langlet, 1971) to the most recent advances on the genetic structure of adaptation (Savolainen *et al.*, 2013), ecologists have studied the mechanisms, scale and environmental drivers of adaptation. The recent and rapid changes in climate have further increased the interest in understanding adaptation at broader spatial and temporal scales, as the degree of adaptation to local conditions influences how populations respond to environmental change (Valladares *et al.*, 2014). However, while genetic methods addressing adaptation have advanced rapidly, there has been little recent development of field approaches to study local adaptation (Langlet, 1971; Kawecki & Ebert, 2004), and all current approaches are based either on reciprocal transplant or common garden experiments (see Figure 1). Both approaches, however, have important limitations when applied to long-lived organisms, and thus may fail to fully capture their local adaptation patterns.

Reciprocal transplant experiments involve translocating individuals between populations to compare their performance under 'home' and 'away' conditions. They test local adaptation in a broad sense, including most potential drivers of adaptation (Kawecki & Ebert, 2004) and allow an unambiguous test of local adaptation. However, reciprocal transplants do have certain drawbacks and may not always be feasible for practical, ethical, or legal reasons. For instance, introducing plant material into new areas may help to spread pests, diseases or invasive genotypes (Kawecki & Ebert, 2004; Genton et al., 2005). Common garden experiments, where individuals from different populations are cultivated under common conditions to observe their genetic differences, remove the risk of introducing foreign material into ecosystems but these experiments often lack realism, as organisms do not live in their natural conditions. When common gardens are performed under natural conditions (e.g. in forests), they still frequently grown plants in regular grids, which may fail to capture effects of microhabitat heterogeneity and usually excludes intra and interspecific competition.

Both common garden and reciprocal transplant approaches are labour intensive and expensive to maintain, often forcing researchers to use a reduced number of populations or carry out short-term experiments. Even experiments specifically focused on long-term adaptation in trees rarely last more than a few decades (*e.g.* Wright, 2007; McLane *et al.*, 2011; Bennington *et al.,* 2012), which may be insufficient to capture meaningful adaptation patterns in long-lived species. Short-term experiments are also unlikely to include extreme climatic events that may be important for plant adaptation (Parmesan *et al.*, 2000, Merlin *et al.* 2018). In fact, results from long-term reciprocal transplant experiments do suggest that short-term studies could produce biased results. In the Wright (2007) and Bennington *et al.* (2012) experiments, significant local adaptation emerged only after c. 20 years, and earlier analysis would have failed to detect it. It is difficult to overstate the contribution of reciprocal transplant and common garden experiments to understanding local adaptation across life forms and ecosystems (Hereford, 2009; Leimu & Fischer, 2008). However, it is important to continue to develop new methodological approaches that address their weaknesses and allow researchers to explicitly test for long-term local adaptation under natural conditions (Carpenter, 1996; Symstad *et al.*, 2003).

Another major challenge in local adaptation studies is singling out and quantifying the importance of individual drivers of adaptation. For this, studies often correlate local advantage with ecological (or geographical) distances between populations (e.g. Kreyling *et al.*, 2014). However, separating the effects of correlated drivers is likely to require a large sample of populations, which may be difficult to achieve with a reciprocal transplant design. Common garden experiments can directly test and quantify the effect of individual drivers of local adaptation. However, this can be challenging if the drivers of adaptation are difficult to simulate or if they are highly stochastic (Nuismer & Gandon, 2008). Additionally, it has been suggested that certain provenances can happen to be better adapted to experimental conditions, which can confound or obscure local adaptation patterns in both reciprocal transplant and common garden experiments (Bischoff *et al.*, 2006). New, complementary approaches are therefore needed to test for the drivers of local adaptation across many populations and to help disentangle the importance of different environmental factors (Wang *et al.*, 2010, Blanquart *et al.*, 2013, Klisz *et al.*, 2019; Figure 1).

We propose a new method to explore long-term, local adaptation to climate in trees using dendrochronological methods. Tree-ring analyses are particularly suited to testing for local adaptation, as they naturally record long-term, high-resolution time-series of tree performance, measured as radial growth, and have been consistently shown to capture trees' responses to climate (Schweingruber, 1996; Amoroso *et al.*, 2017). We developed a methodology to do this and tested it, first using synthetic growth time-series with a known degree of local adaptation. We then applied the method to two case studies where we expected contrasting patterns of local adaptation: (i) a set of high-elevation *Pinus nigra* populations at the southwestern limit of the species range, where we expect strong adaptation to local climate, and (ii) a set of *Fagus sylvatica* populations in the center of the species range where we expect weak climate adaptation.

Material and methods

V*irtual Transplant Experiments* (VTEs) infer local adaptation from tree-ring time-series by studying the growth response of a population under its 'home' (i.e. the mean local climate) and 'away' conditions (i.e. the mean climate in a different location, see Figure 1). VTEs therefore take advantage of the natural year-to-year variability in climate at each population's origins and the increased likelihood that long climate records include extreme conditions, representative of climate in distant locations. VTEs do not require that both populations share environmental conditions on any given year but that, across the climatic record of each site, there are years where climatic conditions in one location were similar to those most frequently experienced in the second one, and *vice versa* (Figure 1). The result is a metric similar to the crossed comparison or response curve analyses classically used in local adaptation studies (see Kawecki & Ebert 2004; Blanquart *et al.*, 2013; Figure 1). However, the test of local adaptation can be derived without physically transplanting the trees, hence 'virtual transplants'.

To perform a VTE, we start with a standardized chronology of each studied population, coupled with an annual record of the relevant climate variables. As in other local adaptation approaches, the climatic variables to be tested should be carefully selected and disclosed, since this will affect the interpretation of the results. We, then define local climate conditions for each population and climate variable. Local climate is specified as a quantile range around the median climate, e.g. we could define local climate as all conditions falling between the 40 and 60% quantiles. Finally, we compare the performance of each population under its own local climate conditions and under the local climate conditions of each of the other studied populations. VTEs are an iterative approach, that repeat this analysis for a range of e quantiles (from very broad to very narrow definitions of the local climate). The result is a mean local advantage curve; see Figure S1 for a step-by-step workflow and Annex 1 for a commented workflow example showing how to implement VTEs R code. The mean local advantage for each quantile range and population is calculated as the difference between the mean performance of the ‘local’ individuals under ‘local climate’, and the mean performance of the ‘foreign’ individuals under that same ‘local climate’. Consistent local advantage, i.e. positive mean local advantage across a wide range of definitions, would provide strong evidence for local adaptation.

We introduced a multi-threshold approach to deal with one of the main challenges in VTEs, i.e. defining the range of conditions that constitute "local" or "home" climate. Since a ‘mean climate’ has to be calculated using a range of climatic values, VTE results are likely to be sensitive to the range chosen to define the local climate, as it will determine the number of years classed as having a home or away climate. We dealt with this with a similar approach to that used in ecosystem multifunctionality research. Since multifunctionality metrics can be strongly affected by the threshold used to score whether functions are delivered or not, Byrnes *et al.* (2014) proposed to calculate multifunctionality across a wide range of thresholds. For VTEs, we used intervals based on quantiles to define the local climate, which ranged from very narrow definitions (only years in the 0.49-0.51 quantile range constitute the local climate) to very broad ones (all values in the 0.05-0.95 quantile range are considered local climate).

The main response variable used in a VTE is a time-series of standardized tree-ring widths. Standardization minimizes confounding geometrical (bigger trees producing smaller rings due to increasing total tree volume) and ecological signals (e.g. a pest outbreak creating a 10-year growth decrease, which is unrelated to climate), while maximizing high-frequency climatic signals (see e.g. Speer, 2010). In our case studies, standardization was carried out using a 50-year flexible spline and the resulting, dimensionless, growth series were corrected for autocorrelation and averaged using a bi-weight, robust mean to obtain a residual chronology per site, as is common in dendrochronological studies (e.g. Speer, 2010). The choice of the standardization method will depend on the characteristics of the population, the goal of the study, and the species considered. There is abundant information on how to conduct the standardization in the dendrochronological literature (Cook, 1985;

Schweingruber, 1996; Speer, 2010). Nonetheless, we tested multiple commonly-used standardization methods (Figure S2), and found little-to-no influence of the detrending method on the VTE results.

Theoretical test with simulated data

To confirm that our VTE method is able to correctly identify local adaptation, we first tested it using simulated data. We built a synthetic dataset parameterized with data from a previous study (Manzanedo *et al.* 2018). This site was independent of the two study cases explained below, to avoid redundancy. We generated time-series of radial growth for two locations, A and B, using a formula (Eqn. 1), inspired by the linear aggregate model for tree growth described in Cook (1985) and widely used in dendrochronology. Using this synthetic dataset allows us to ensure that the generated growth series have a known level of local adaptation, given by the parameter 'c', and therefore to test if our method can correctly identify populations with known levels of local adaptation

$$TRI_i = a + b \cdot P_i + (1 - z_i) \cdot c + \varepsilon \qquad \text{[Eqn.1]}$$

Where: 'TRI_i' is the tree-ring index for year *i,* 'a' is the minimum annual growth required for tree survival, '$b*P_i$' is the relationship between climate (P, e.g. total annual precipitation or mean annual temperature) and growth (slope is 'b', i.e. the extent to which growth responds to climate overall). '$(1-z_i)*c$' is the local adaptation term, i.e. the extent to which growth declines with increasing distance from the optimum local climate: 'z_i' is distance in climatic conditions for year i compared to the optimum climate and 'c' defines the local adaptation strength, i.e. the speed of decline in growth with distance from the optimum. 'ε' is the measurement error (see also Figure S3).

This equation implicitly assumes: (i) adaptation to the long-term average climate (not to extremes), (ii) a linear decrease in performance with increasing distance from the local optimum climate, and (iii) that growth has to be positive, i.e. no missing rings. We used these simple working assumptions for our first test but we also explored how other shapes of climate response may affect the observed pattern (Fig 2, Figure S4). Mean local advantage curves (Figure 2b,d,f,h,j; Figure 3b,d,; Figure 4b; Figure 5b,d) should be interpreted as follows: a flat local advantage curve implies a constant growth difference between local and foreign provenances across local climate definitions. This does not imply that trees are unresponsive to climate rather that we can detect local adaptation regardless of the local climate definition. Values of mean local advantage consistently close to zero indicate that both provenances respond very similar to both climates (*i.e.* little local adaptation), positive values indicate that local provenances perform better under local conditions and negative that foreign provenances outperform local ones under their local climate.

We saw that a linear decrease in performance with increasing distance from the climatic optimum (Figure 2a) created a flat and constant local advantage regardless of how narrowly or broadly the local climate was defined (Figure 2b). However, when tree performance declined non-linearly with distance from the optimum local climate (Figure 2c,e) (e.g. because of plasticity), the resulting mean local advantage curve showed a decrease in the signal of local advantage with wider definitions of the local climate (Figure 2d,f). This is because at very broad climate definitions growth is similarly low under many local climates as it is under away climates. We then simulated an extreme case where local adaptation is a binary response (Figure 2g), i.e. performance is equally high under a wide range of local conditions but is strongly reduced outside that range. This would result in a flat local advantage curve for narrow definitions of the local climate, because performance does not change with distance from the optimum over this range, but an increasing decline in performance as the local climate definition is widened because unsuitable conditions are then included in the local climate (Figure 2h). It is also possible that the optimum performance does not coincide with the long-term mean of the climate variable, creating a skewed response curve (Figure 2i). In this case, increasing the width of the local climate definition initially increases local advantage by including the actual optimum, but further widening the local climate definition will decrease local advantage as more unsuitable climatic conditions are included, resulting in a hump-shaped mean local advantage curve (Figure 2j). It is clear that the shape of the local advantage curve may provide useful insights on the nature and drivers of local adaptation.

To realistically estimate the parameters in Eqn. 1, we used an independent tree-ring dataset from a previous study (Manzanedo *et al.*, 2018). This study showed that precipitation affected growth of a *Quercus cerris* population in central Italy. *Q. cerris* had a minimum annual growth (intercept, 'a') of 0.82 and a 0.0002 mm^{-1} increase in growth with increasing precipitation (slope, 'b'). We first approximated '*c*' using a property of standardized time-series that requires the long-term mean to equal 1. '*c*' was then kept constant in subsequent simulations. We generated growth time-series for 30 trees per population by sampling values from a normal distribution with sd = 0.2 around the population mean, similar to that reported in Manzanedo *et al.* (2018). Eqn.1 was used solely to generate the synthetic data to test the theoretical soundness of our method, and not to model growth in any of our case studies, as the assumptions for Eqn.1 do not necessarily hold true for natural populations. For example, Eqn.1 does not include a missing ring term. Including missing rings in our theoretical test would require a deep understanding of how missing rings occur that we do not think is currently available. This, however, should not affect our methodology. In fact, our case studies contained missing rings, which we treated like years with zero growth, which did not affect the application of our method. Attempting to include a missing ring term in Eqn.1 would introduce further uncertainty in this theoretical model.

Case study #1: climate-limited populations at the edge of their distribution

In our first case study, we explored the local adaptation patterns of five *P. nigra* relict populations from the higher elevations of the Baetic and Rif ranges of southern Spain and northern Morocco, respectively. These isolated and genetically diverse populations are at the southwestern limit of the species' distribution and have been shown to be strongly limited by climate, particularly aridity (see further information and locations on the study sites in Camarero *et al.*, 2013). We used growth data from 84 trees (128 cores), which were collected, crossdated, and measured following standard dendrochronological methods (Schweingruber, 1996; Camarero *et al.*, 2013).

We obtained 1901-2009 time-series of total annual precipitation and mean annual temperature for each site from the Climate Research Unit repository, CRU TS3.1. We chose to use interpolated CRU data for both case studies for three main reasons: (1) to have a consistent climate data source for all the populations, regardless of the meteorological station coverage available for each country or region, (2) for most of our populations we had no plot-level meteorological data that would be more reliable than that calculated by CRU, and (3) in this first test of our method, we focus on local adaptation to macroclimate variables, which are likely to be well measured in the CRU data. We calculated the mean annual aridity index (AI) following the De Martonne equation, $AI = P(T+10)^{-1/2}$. We chose AI as a relevant adaptation variable due to its proven correlation with tree growth in the sampling locations (Camarero *et al.*, 2013). We then calculated average growth indices for all possible combinations of home and away conditions across the gradient of local climate definitions, as described in the methods below, from very narrow (0.49-0.51% quantiles) to very broad (0.05-0.95% quantiles).

Case study #2: mesic populations of a widespread tree species

In our second case study, we focus on populations of a widespread tree species, located in the central part of the species distribution. These populations are expected to be less likely to show strong climate adaptation, as biotic interactions such as competition or plant-pathogen interactions may influence growth more than climate (Ettinger *et al.*, 2011). This was also suggested by parallel common garden experiments using the same provenances, which suggested local adaptation to microhabitat but not to macroclimate (unpublished data). The samples were collected from areas with flat (Hainich, Leipzig, Gorovei), or hilly (Freiburg, Valea Neagra, Suceava) topography. We also avoided sampling areas with unusually high slopes or exposed terrain within the sampling sites. We sampled 180 dominant *F. sylvatica* trees (360 cores) in six populations from two European countries (three in Germany, three in Romania). Sample preparation and analysis followed the methodologies described for case study #1. However, due to the large geographical and climatic distance between populations and the low overlap in climate between countries, we carried out VTE tests only within countries. We calculated adaptation to mean annual aridity, as in case study #1, but also included total annual precipitation and mean annual temperature as individual variables, as they have been suggested to influence *F. sylvatica* growth (e.g. Rozas, 2001; Cavin & Jump, 2016). We focus on these relatively crude, yet commonly used (discussed in Chakraborty *et al.*, 2015), measures of climate for this initial demonstration of our method, so that we could use the same climate variables across our different case studies. Further VTEs should consider using more precise climate variables, such as climate conditions during particular seasons.

Results

Theoretical test with simulated data

Our methodology correctly identified local adaptation in simulated tree-ring time-series (Figure 3a,b). Populations simulated to be adapted to their local optimum climate had crossed reaction norms (i.e., both populations were the best performers under their local climate conditions), which are the key signature of local adaptation (*sensu* Kawecki & Ebert, 2004). The pattern of mean local advantage (or lack of thereof) was consistent for all definitions of local climate (Figure 3b,d) and we never found crossing reaction norms for populations that had a local adaptation term equal to zero (Figure 2c,d).

Our method was robust to changing the model parameters used to generate the tree-ring series (Figure S5). VTE results were qualitatively robust to changes in parameter values of between one and three orders of magnitude (Figure S5). Similarly, changes in the shape of the mean home advantage curve resulted in different climate response curves, following our hypothesized relationships, but we were always able to recover the signal of local adaptation with the VTEs (Figure 2, Figure S4).

Case study #1

Consistent with our hypothesis and previous results, we found that four out of five *P. nigra* populations (F, M, G, T) showed adaptation to their local climate across most local climate definitions (Figure 4, Table 1), although F showed no local advantage under some local definitions (e.g. the 25-75% quantile interval shown in Figure 4a). In general, consistently locally adapted populations occurred in intermediate to wetter sites (M, G, T), while populations at the dry end of our gradient showed symptoms of maladaptation (A). In the maladapted populations, foreign provenances outperformed local ones and local performance only improved when conditions became less arid than the typical climate at these locations. Interestingly, for two populations there was evidence for local advantage only when the local climate was defined using intermediate quantile values and evidence for local disadvantage if the local climate was defined very narrowly (M, G). This is compatible with an asymmetric response curve and might suggest adaptation to extreme climates (Figure 2).

Case study #2

We observed no clear advantage for most *F. sylvatica* populations under local climatic conditions except for the Leipzig (L) population, which had a slightly higher performance under its own climate (Figure 5, Figure S6). However, Leipzig trees grew better under all conditions, which suggests they are not locally adapted but that they likely experience better habitat quality in their local area or that the Leipzig population has higher fitness than the others. Interestingly, we found different degrees of climate sensitivity per country. German populations seemed to respond more to changes in local precipitation values and were almost unresponsive to changes in mean annual temperature, while populations from Romania showed the opposite pattern (Figure S6, Table 2). Overall, local populations failed to consistently outperform foreign ones, under their local conditions, showing no local advantage regardless of the local climate definition or even showing local disadvantage (local populations are outcompeted under their local conditions). These findings are consistent with our hypothesis and previous experimental results, suggesting that mesic *F. sylvatica* populations are not strongly adapted to their local climates.

Discussion

Our methodology performed well in simulated and real datasets, consistently detecting local adaptation patterns when they were expected. These results highlight the potential contribution of tree-ring research to the study of local adaptation in long-lived woody species and under more natural conditions. The main application of our method would be for woody species with marked secondary growth. However, it could also be potentially implemented in other organisms with annual increment marks, such as long-live herbaceous species with annual growth rings (e.g. Roeder *et al.*, 2017), or shell growth rings, as in sclerochronological studies.

Advantages of VTEs

VTEs are easy and fast to implement. They do not require expensive experimental set-ups, plant production, transportation, or maintenance and can thus be used to study many populations across a large spatial scale. Unlike traditional approaches, VTEs can potentially assess local adaptation using data on climate-growth responses across the whole lifetime of a tree (Bischoff *et al.*, 2006; Kreyling *et al.*, 2014), offering a more integrated picture of local adaptation in long-lived organisms. It may also be possible in the future to use them to disentangle the relative importance of local adaptation to mean versus infrequent extreme climate conditions (Wright, 2007; Bennington *et al.*, 2012). Lastly, by using naturally occurring and adult trees, a VTE avoids handling or planting biases that could obscure adaptation in other experimental approaches (discussed in Kawecki & Ebert, 2004).

The VTE method can easily be expanded to address other facets of tree performance by using other tree-ring or wood anatomy metrics. For instance: earlywood and latewood widths or densities can provide information on intra-annual components of growth (e.g. Uggla *et al.*, 2001); wood isotopic composition can inform us about photosynthesis and stomatal conductance rates (McCarroll & Loader, 2004); and blue intensity or maximum wood density can be used to infer summer/autumn stress conditions (Campbell *et al.*, 2007). Local adaptation patterns from different dendrochronological traits should be carefully interpreted. Contradictory patterns between multiple traits, although not expected, could occur and interpreting the different responses would require information on the functional significance of the different traits and perhaps approaches that combine results from multiple traits to explore multiple dimensions of plant function (e.g. Pérez-Ramos *et al.* 2019).

Both in our simulation and in the study cases, we did not consider carry-over or time-lagged effects. By eliminating year-to-year autocorrelation when building our chronologies, we are likely to have removed any carry-over effects. However, VTEs could be used to investigate the importance of carryover effects, for example by defining the local climate based on conditions in previous years, considering droughts occurring at a particular time during the growing season, or by comparing VTEs with chronologies with different levels of autocorrelation removed from them. Age-related effects are also of major importance in dendrochronological science (e.g. Nehrbass- Ahles *et al.*, 2014). However, we do not expect age to be an influential factor in VTEs because (i) standardization should eliminate most (if not all) of the age-related differences between our trees, and (ii) VTEs decouple growth from calendar years (by relating it to home and away climatic conditions) and, thus, any age-related differences should be randomized between groups and climate definitions.

Testing for long-term local adaptation with VTEs requires a discussion on what factors constitute "local" climatic conditions and how broad or narrow that definition should be. Using a multi-threshold approach, rather than focusing on a single range of conditions, allows a broad-range of local climate definitions to be tested. This method is, firstly, more robust as it avoids choosing an anomalous local climate definition that could cause misleading results, and secondly, it provides more information, as the shape of the multi-threshold, local advantage response curve may also be informative of the processes driving local advantage (see Figure 2). For example, two of the populations (M, G) in case study #1 showed curve shapes compatible with an asymmetric response to climate, which might suggest that local adaptation to extreme events may be more important than adaptation to the long-term mean climate (Gutschick & BassiriRad, 2003). Asymmetric local adaptation can influence how tree populations respond to climate change (Petit & Hampe, 2006) and how effective climate change adaptation strategies will be in preserving particular populations. Strategies such as assisted migration or planting provenance selection (see Sgró *et al.*, 2011; or Aitken *et al.*, 2008) typically assume adaptation to the long-term mean climate, which may be a naïve approach for many populations (see e.g. Bucharova, 2017). VTEs can contribute to an improvement of climate change adaptation strategies by identifying populations that fit with the assumption of adaptation to the long-term mean climate and those that do not fit the assumption and which may be more strongly adapted to extremes or infrequent climate events.

Limitations of VTEs

There are also some limitations of VTEs that need to be considered. VTEs can only identify local adaptation to particular environmental factors and therefore their success depends on a good knowledge of the potential drivers of adaptation. This means that while VTEs can explore a hypothetical adaptation mechanism better than reciprocal transplants, failure to detect local advantage in VTEs may not necessarily indicate a lack of local adaptation; it may simply mean that the relevant environmental variables were not considered. This stresses the importance of carefully choosing the environmental variables and considering other important factors when interpreting VTEs. We would recommend that future VTEs carefully explore the role of different climatic factors in affecting tree growth and carefully justify their use of variables. In this case we used simple annual measures of climate to make it easier to compare across case studies but future VTEs could consider testing for adaptation to summer vs. winter temperatures for instance.

In addition, biotic drivers of adaptation are not easily captured by VTEs. Biotic interactions, such as herbivore attack (see e.g. Schweingruber 1996), clearly influence tree growth and local adaptation but the lack of long-term data on biotic factors, e.g. variation in herbivore abundance over time at a given site, mean it is challenging to incorporate them in VTEs. Local adaptation to specialist, site specific enemies or mutualists cannot be assessed with VTEs. However, promising developments in reconstructing biotic factors (e.g. Klopcic *et al.*, 2010) may allow them to be incorporated in VTEs in the future, in order to address long-term local adaptation to biotic factors.

VTE tests are also limited to comparing populations in which there is some overlap in environmental conditions. This may pose a challenge to the study of scattered, discontinuous populations. Future ways to address this may include using intermediate populations to provide indirect information on the similarities between target populations, or using pairwise comparisons between subsets of populations (Blanquart *et al.*, 2013).

An integrative approach

All approaches to local adaptation have advantages and disadvantages (Figure 1). Integrative approaches are therefore likely to be the best option to assess local adaptation in natural forests. This will require ingenuity, but the different strengths and weaknesses of the various methods inspire optimism that synergistic solutions can provide a much more complete picture. For example, a VTE could be used to easily and rapidly screen large portions of a species range to identify candidate populations that seem to show local adaptation. This could offer an overview of the local adaptation landscape that can then be used to inform immediate forest management decisions. The candidate populations can also be tested using reciprocal transplant and common garden experiments, to confirm local adaptation and identify the key factors driving it.

Understanding and modelling local adaptation in long-lived species is a challenging task. Here we propose a new approach to address this question, which expands the tools available and will complement existing approaches to give a more complete and accurate picture of local adaptation in long-lived organisms.

Author's contributions:

RDM conceived the idea and designed the methodology; RDM and EA analysed the data and wrote the text and figures with input from all authors; RMNC, EA, and MF coordinated the fieldwork. All authors contributed constructive comments and approved the submitted version of this manuscript.

Acknowledgements

We thank Floris Schenk, Michael Scherer-Lorenzen, and Filippo Bussotti for assisting in the dendrochronological tree core sampling procedure, and the *Ispettorato Generale del Corpo Forestalle dello Stato* for sampling authorizations. Fieldwork was supported by the FunDivEurope project, European Union Seventh Framework Programme (FP7/2007-2013 grant no. 265171). RDM is currently supported by the Swiss National Foundation (SNF) through the Early Post.doc Mobility Scheme. We thank Fernando Valladares, Raquel Benavides, Santiago Soliveres, and Neil Pederson for helpful comments and fruitful discussions over earlier versions of the manuscript and the VTE method, as well as to two anonymous reviewers for their valuable contribution to improve this manuscript. The authors declare that there is no conflict of interest regarding the publication of this article.

Data Accessibility:

All the tree-ring data used in this paper is publicly available via dryad data repository, with doi:10.5061/dryad.f79j433; and has been contributed to the International Tree Ring Data Bank (https://www.ncdc.noaa.gov/data-access/paleoclimatology-data/datasets/tree-ring), with site codes: GERM224, GERM225, ROMA011, ROMA012, SPAI78, SPAI079, SPAI080, SPAIN081, and MORC024.

References

Aitken, S.N., Yeaman, S., Holliday, S., Wang, T., & McLane. C. (2008). Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications, 1.1,* 95–111.

Amoroso, M.M., Daniels, L.D., Baker, P.J. & Camarero, J.J. (2017) Introduction. In M.M. Amoroso, L.D. Daniels, P.J. Baker & J.J. Camarero (Eds.), Dendroecology: Tree-ring analyses applied to ecological studies. Cham: Springer International Publishing.

Bennington, C.C., Fetcher., N., Vavrek, M.C., Shaver, G.R., Cummings, K.J., & McGraw, J.B. (2012). Home site advantage in two long-lived arctic plant species: results from two 30-year reciprocal transplant studies. *Journal of Ecology, 100,* 841–851.

Bischoff, A., Crémieux, L., Smilauerova, M., Lawson, C.S., Mortimer, S.R., Dolezal, J., … & Müller-Schärer, H. (2006). Detecting local adaptation in widespread grassland species – the importance of scale and local plant community. *Journal of Ecology, 94,* 1130–1142.

Blanquart, F., Kaltz, O., Nuismer, S.L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters, 16,* 1195–1205.

Bucharova, A. (2017). Assisted migration within species range ignores biotic interactions and lacks evidence. *Restoration Ecology, 25,* 14–18.

Byrnes, J.E.K., Gamfeldt, L., Isbell, F., Lefcheck, J.S., Griffin, J.N., Hector, A., … & Duffy. J.E. (2014). Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. *Methods in Ecology and Evolution, 5,* 111–124.

Camarero, J.J., Manzanedo, R.D., Sanchez-Salguero, R., & Navarro-Cerrillo, R.M. (2013). Growth response to climate and drought change along an aridity gradient in the southernmost *Pinus nigra* relict forests. *Annals of Forest Science, 70,* 769–780.

Campbell, R., McCarroll, D., Loader, N.J., Grudd, H., Robertson, I., & Jalkanen, R. (2007). Blue intensity in *Pinus sylvestris* tree-rings: developing a new palaeoclimate proxy. *The Holocene, 17,* 821–828.

Carpenter, S.R. (1996). Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology, 77,* 677–680.

Cavin, L. & Jump, A.S. (2017). Highest drought sensitivity and lowest resistance to growth suppression are found in the range core of the tree *Fagus sylvatica* L. not the equatorial range edge. *Global Change Biology, 23,* 362–379.

Chakraborty, D., Wang, T., Andre, K., Konnert, M., Lexer, M.J., Matulla, C., & Schüler S. (2015). Selecting Populations for Non-Analogous Climate Conditions Using Universal Response Functions: The Case of Douglas-Fir in Central Europe. *PLoS ONE, 10,* e0136357.

Cook, E.R. (1985). A time series approach to tree-ring standardization. PhD thesis, University of Arizona.

Ettinger, A.K., Ford, K.R., & HilleRisLambers, J. (2011). Climate determines upper, but not lower, altitudinal range limits of Pacific Northwest conifers. *Ecology, 92,* 1323–1331.

Genton, B.J., Kotanen, P.M., Cheptou, P.O., Adolphe, C., & Shykoff, J.A. (2005). Enemy release but no evolutionary loss of defence in a plant invasion: an inter-continental reciprocal transplant experiment. *Oecologia, 146,* 404–414.

Gutschick, V.P., & BassiriRad, H. (2003). Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. *New Phytologist, 161,* 21–42.

Hereford, J. (2009). A quantitative survey of local adaptation and fitness trade-offs. *The American Naturalist, 173,* 579–588.

Kawecki, T.J. & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters, 7,* 1225–1241.

Klisz, M., Buras, A., Sass-Klaassen, U., Puchałka, R., Koprowski, M., & Ukalska, J. (2019). Limitations at the limit? Diminishing of genetic effects in Norway spruce provenance trials. *Frontiers in Plant Science, 10*, 306.

Klopcic, M., Jerina, K., & Boncina, A. (2010). Long-term changes of structure and tree species composition in Dinaric uneven-aged forests: are red deer an important factor? *European Journal of Forest Research, 129,* 277–288.

Kreyling, J., Buhk, C., Backhaus, S., Hallinger, M., Huber, G., Jentsch, A., ... & Beierkuhnlein, C. (2014). Local adaptations to frost in marginal and central populations of the dominant forest tree *Fagus sylvatica* L. as affected by temperature and extreme drought in common garden experiments. *Ecology and Evolution, 4,* 594–605.

Langlet, O. (1971). Two hundred years of genecology. *Taxon, 20,* 653–721.

Leimu, R. & Fischer, M. (2008). A meta-analysis of local adaptation in plants. *PLoS ONE, 3,* e4010.

Manzanedo, R.D., Ballesteros-Cánovas, J., Schenk, F., Stoffel, M., Fischer, M., & Allan, E. (2018). Increase in CO_2 concentration could alter the response of *Hedera helix* to climate change. *Ecology and Evolution,* DOI:10.1002/ece3.4388.

McCarroll, D. & Loader, N.J. (2004). Stable isotopes in tree-rings. *Quaternary Science Reviews, 23,* 771–801.

McLane, S.C., Daniels, L.D., & Aitken, S.N. (2011). Climate impacts on lodgepole pine (*Pinus contorta*) radial growth in a provenance experiment. *Forest Ecology and Management, 262,* 115–123.

Merlin, M., Duputié, A., & Chuine, I. (2018). Limited validation of forecasted northward range shift in ten European tree species from a common garden experiment. *Forest Ecology and Management, 410,* 144–156.

Nehrbass-Ahles, C., Babst, F., Klesse, S., Nötzli, M., Bouriaud, O., Neukom, R., Dobbertin, M., & Frank, D. (2014). The influence of sampling design on tree-ring-based quantification of forest growth. *Global Change Biology, 20(9),* 2867–2885.

Nuismer, S. & Gandon, S. (2008). Moving beyond common-garden and transplant designs: insight into the causes of local adaptation in species interactions. *The American Naturalist, 171,* 658–668.

Parmesan, C., Root, T.L., & Willig, M.R. (2000). Impacts of extreme weather and climate on terrestrial biota. *Bulletin of the American Meteorological Society, 81,* 443–450.

Perez-Ramos, I.M., Matías, L., Gómez-Aparicio, L., & Godoy, O. (2019). Functional traits and phenotypic plasticity modulate species coexistence across constrasting climatic conditions. *Nature Communications, 10,* 2555.

Petit, R.J., & Hampe, A. (2006). Some evolutionary consequences of being a tree. *Annual Reviews in Ecology Evolution and Systematics, 37,* 187–214.

Roeder, A., Schweingruber, F.H., Fischer, M., & Roscher, C. (2017). Growth ring analysis of multiple dicotyledonous herb species- A novel community-wide approach. *Basic and Applied Ecology, 21,* 23–33.

Rozas, V. (2001). Detecting the impact of climate and disturbances on tree-rings of *Fagus sylvatica* L. and *Quercus robur* L. in a lowland forest in Cantabria, Northern Spain. *Annals of Forest Sciences, 58,* 237–251.

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics, 14,* 807–820.

Schweingruber, F.H. (1996). *Tree-rings and environment dendroecology*. Berne, Switzerland: Paul Haupt.

Sgró, C., Lowe, A.J., Horrman, A.A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications, 4.2,* 326–337

Speer, J.H. (2010). *Fundamentals of tree-ring research.* Tucson, US. University of Arizona Press.

Symstad, A.J., Chapin III, F.S., Wall, D.H., Gross, K.L., Huenneke, L.F., Mittelbach, G.G., Peters, D.P.C., & Tilman, D. (2003). Long-term and large-scale perspectives on the relationship between biodiversity and ecosystem functioning. *BioScience, 53,* 89–98.

Uggla, C., Magel, E., Moritz, T., & Björn, S. (2001). Function and dynamics of auxin and carbohydrates during earlywood/latewood transition in Scots pine. *Plant Physiology, 125,* 2029–2039.

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M., Balaguer, L., … & Nicotra, A.B. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters, 17,* 1351–1364.

Wang, T., O'Neill, G.A., & Aitken, S.N. (2010). Integrating environmental and genetic effects to predict responses of tree populations to climate. *Ecological Applications, 20,* 153–163.

Wright, J.W. (2007). Local adaptation to serpentine soils in *Pinus ponderosa*. *Plant & Soil, 293,* 209–217.

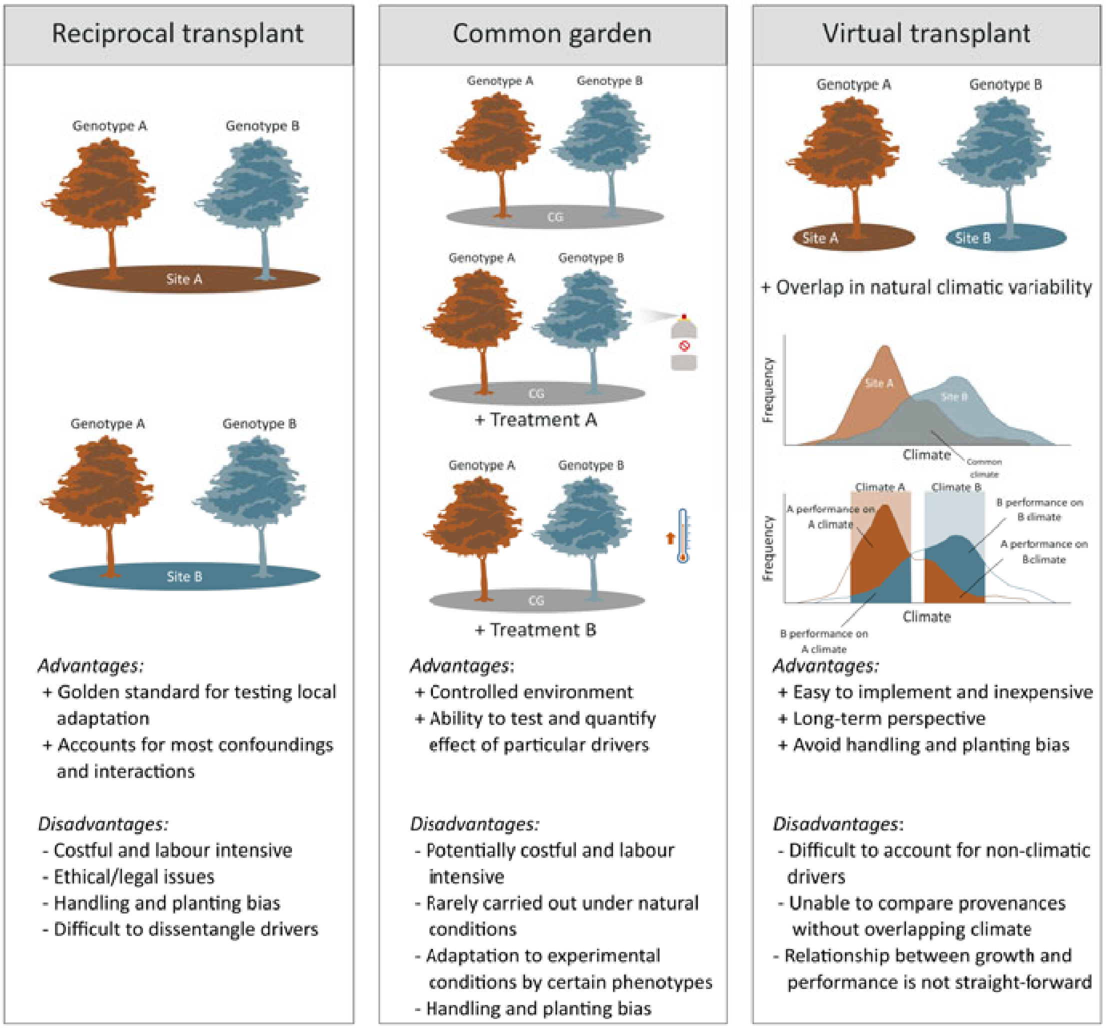
Tables

Table 1: *Mean local advantage for tree populations in Case Study #1 in regards to mean annual aridity (Aridity). Mean local advantage is calculated as the difference between the local performance and the average performance of all foreign provenances, for all the quantiles considered in Figure 4.*

COUNTRY	Population name	Population code	Aridity
SPAIN	Filabres	F	-0.02
	Almijara	A	+0.05
	María	M	+0.05
	Mágina	G	+0.01
	Tallasemptane	T	+0.02

Table 2: *Mean local advantage for tree populations in Case Study #2 in regards to total annual precipitation (TAP), mean annual temperature (MAT), and mean annual aridity (Aridity). Mean local advantage is calculated as the difference between the local performance and the average performance of all foreign provenances, for all the quantiles considered in Figure 5 and Figure S6.*

			CLIMATE VARIABLE TESTED		
COUNTRY	Population name	Population code	TAP (mm)	MAT (° C)	Aridity
GERMANY	Hainich	H	-0.04	-0.02	+0.04
	Leipzig	L	+0.09	+0.02	+0.07
	Freiburg	F	-0.08	+0.01	-0.03
ROMANIA	Gorovei	G	+0.01	-0.02	+0.00
	Suceava	S	-0.11	-0.02	-0.14
	Valea Neagra	V	+0.01	+0.04	-0.04



Advantages:
- \+ Golden standard for testing local adaptation
- \+ Accounts for most confoundings and interactions

Disadvantages:
- \- Costful and labour intensive
- \- Ethical/legal issues
- \- Handling and planting bias
- \- Difficult to dissentangle drivers

Advantages:
- \+ Controlled environment
- \+ Ability to test and quantify effect of particular drivers

Disadvantages:
- \- Potentially costful and labour intensive
- \- Rarely carried out under natural conditions
- \- Adaptation to experimental conditions by certain phenotypes
- \- Handling and planting bias

Advantages:
- \+ Easy to implement and inexpensive
- \+ Long-term perspective
- \+ Avoid handling and planting bias

Disadvantages:
- \- Difficult to account for non-climatic drivers
- \- Unable to compare provenances without overlapping climate
- \- Relationship between growth and performance is not straight-forward

Figure 1 | Advantages and disadvantages in classic and novel approaches to testing for local adaptation in tree populations. Local adaptation between two populations, A and B, can be assessed by reciprocally transplanting them (‘reciprocal transplants’) or by growing them under common environmental conditions (‘common gardens’). In this paper we propose a third approach that uses tree-ring growth and the natural overlap in long-term climate between populations to infer local adaptation (‘virtual transplant experiments’ VTEs).

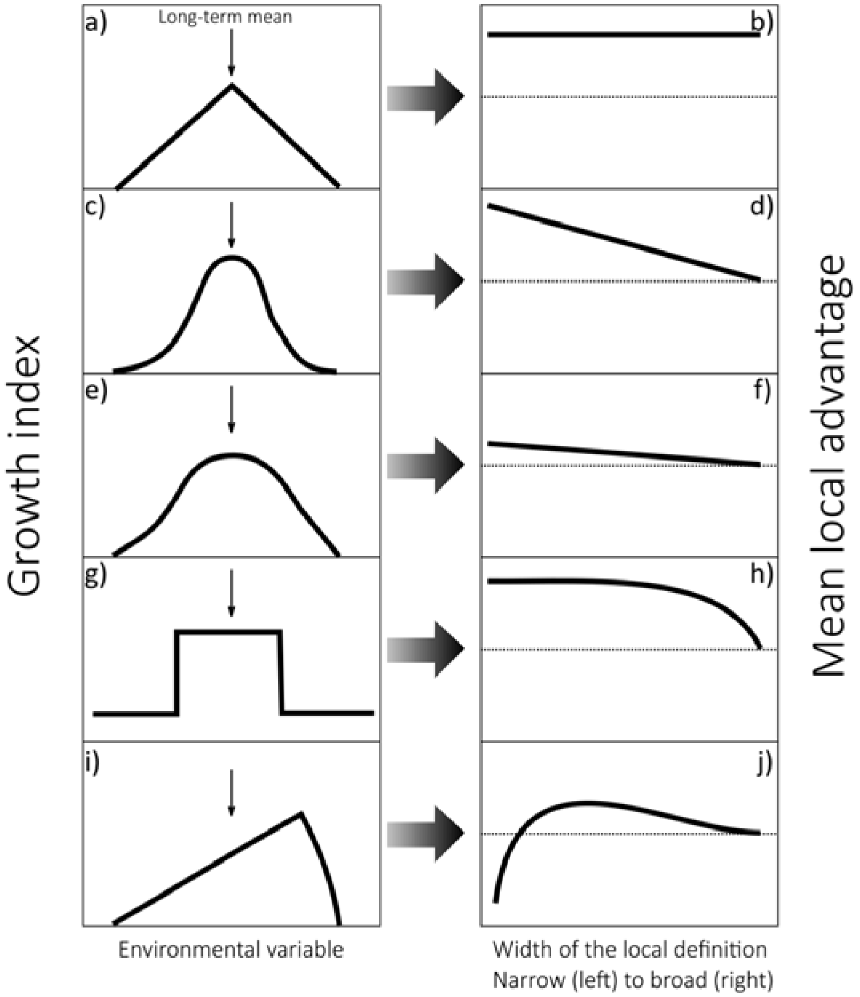


Figure 2 | Mean local advantage curves and the climate response curve that may create them. Hypothesized climate response curves caused by distinct patterns of local adaptation (a, c, e, g, i) and the associated mean local advantage curves (b, d, f, h, j): a) a linear decrease in fitness with increasing distance from the optimum climate gives b) a flat mean local advantage curve; c and e) normal distributions of performance around a climate optimum give d and f) a decline in the extent of local advantage with increasing width of the local climate definition; g) an extreme decline in performance outside a range of optimum conditions gives h) a nonlinear decline in local advantage with wider climate definitions and i) a skewed response to optimum climate gives j) a more complex response of local advantage to the width of climate definition. See Figure S2 for the pattern representation using simulated data for this response curve shapes.

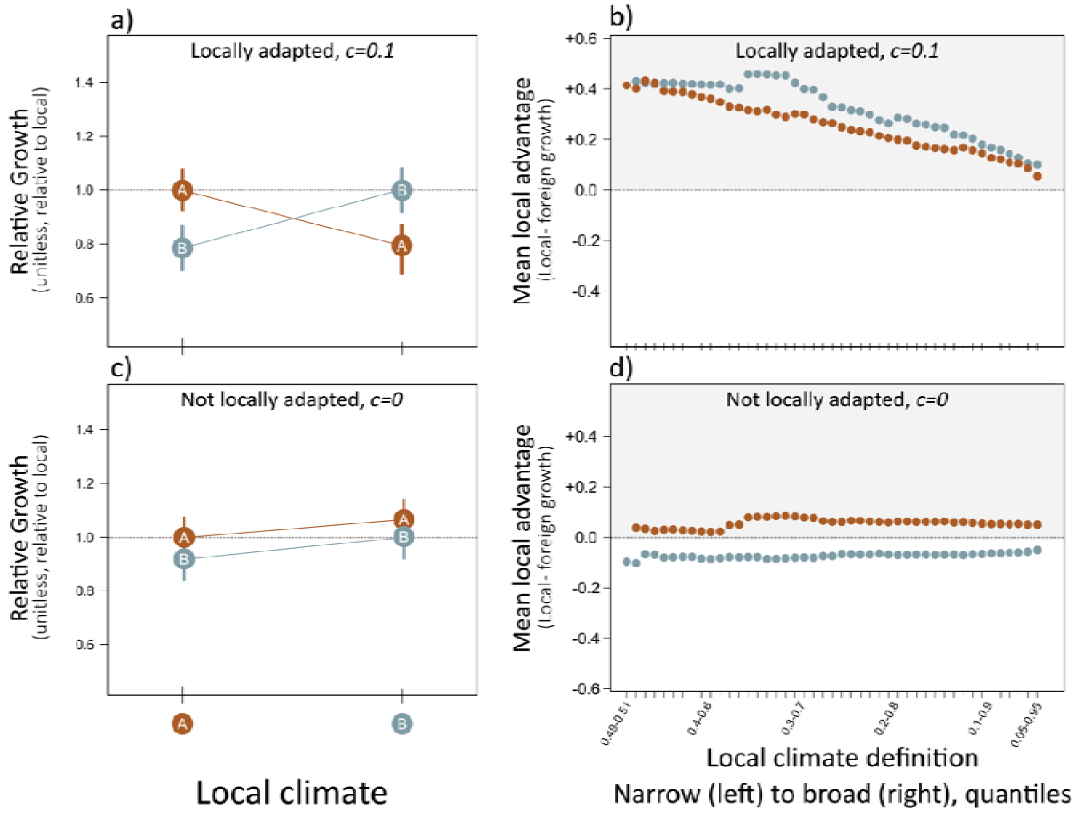


Figure 3 | Virtual transplant experiments using simulated data for two populations (A and B) with known levels of local adaptation. These show the historical performance of each population under climatic conditions representative of its own (local) climate and under conditions representative of the typical climate in the other location. a and b) The virtual transplants correctly identified locally adapted growth series (local adaptation parameter, c, was set to 0.1) in which each population is the best performer under its local climate, i.e. each has a local advantage. This is shown in a) as crossed lines (reaction norms) when the local climate is defined based on variation within the 25-75% quantile range and performance is expressed relative to the local population and in b) as a consistent local advantage for both populations across a range of local climate definition widths from narrow to broad. The VTEs also showed no evidence for local adaptation when this was absent from the simulated data (parameter c was set to 0). This is shown in c) as a lack of crossed reaction norms (parallel lines) for the 25-75% quantile local climate definition because population A outperforms B under both climates. d) This is consistent across a range of local climate definition widths. The standard error around the mean is displayed for each group in a and c.

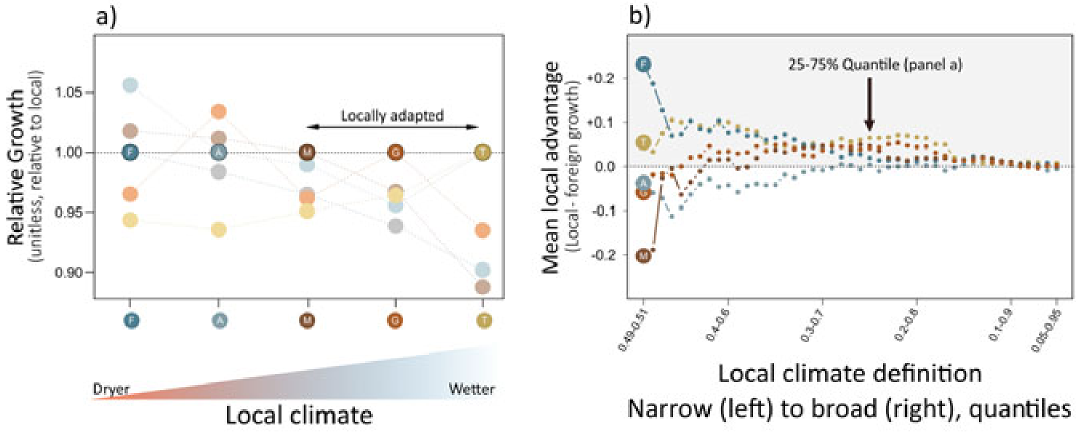


Figure 4 | Case study #1: Virtual transplant experiments (VTEs) on Pinus nigra populations from the southwestern edge of its distribution reveal local adaptation to mean annual aridity. F: Sierra de los Filabres, A: Sierra de Almijara, M: Sierra de María, G: Sierra de Mágina, in Spain, T: Talassemptane NP, in Morocco. Left panel (a) shows the results when the local climate is defined using a 25-75% quantile range, while the right panel (b) shows the local advantage patterns across a range of local climate definitions from narrow to broad. Local populations are highlighted in a). We used the De Martonne Aridity index, calculated from mean annual temperature and total annual precipitation.

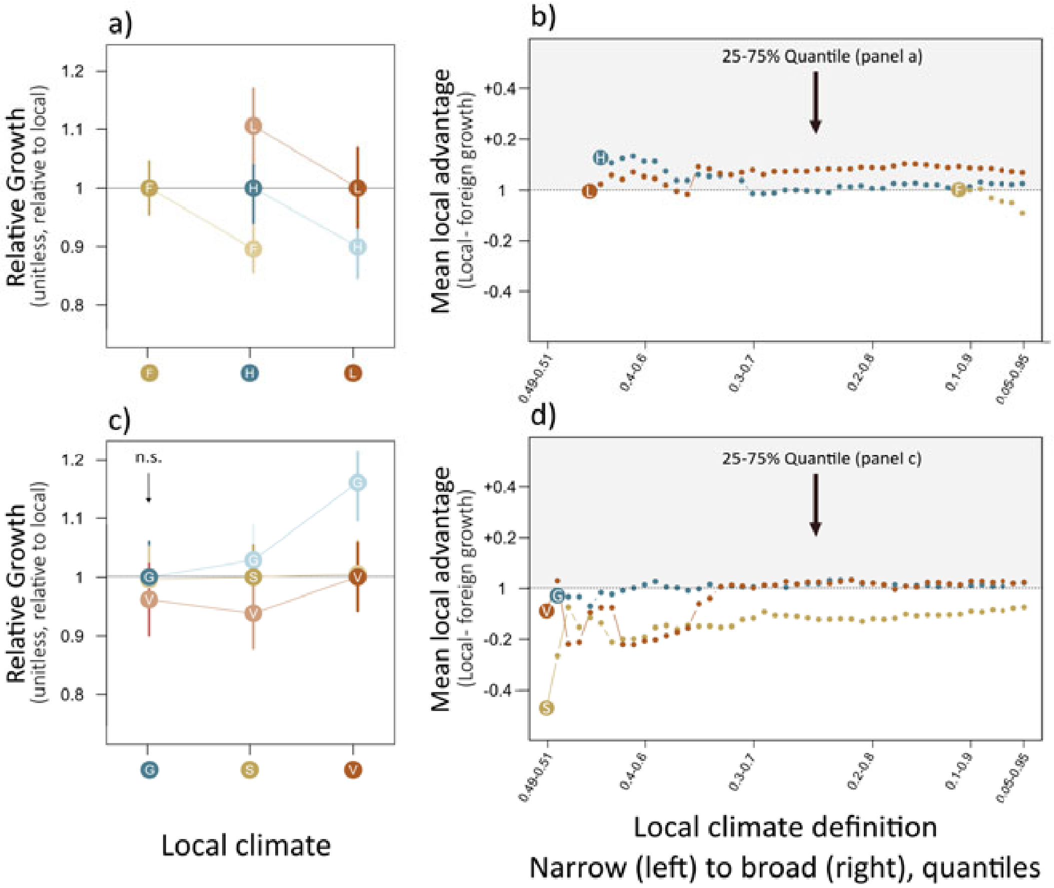


Figure 5 | Case study #2: Virtual transplant experiments (VTEs) applied to mesic Fagus sylvatica populations showed little evidence for local adaptation to climate (aridity). F: Freiburg Schwarzwald, H: Hainich NP, and L: Leipzig, in Germany (a,b). G: Gorovei, S: Suceava, and V: Valea Neagra, in Romania (c,d). Local populations are highlighted. The standard error around the mean is displayed for each group. Only combinations with at least 3 overlapping records are displayed. VTEs were not carried out across countries due to low overlap in climatic conditions. Panels a and c display the results with a 25-75% quantile local climate definition, while panels b and d show the mean local advantage curve across a range of local definitions, from narrow to broad. We used the De Martonne Aridity index, calculated from mean annual temperature and total annual precipitation, for the patterns of adaptation to precipitation and temperature see also Figure S6.