Ecological and evolutionary drivers of phenotypic and genetic variation in the European crabapple (Malus sylvestris (L.) Mill.), a wild relative of the cultivated apple

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Abstract (250 words max with bullet points)

- Characterizing the phenotypic and genetic variation among populations of crop wild relatives help understanding the ecological and evolutionary processes involved in population divergence, and better harness their diversity to mitigate the impact of climate change on crops. We assessed genetic and phenotypic diversity of the European crabapple, *Malus sylvestris*, a main contributor to the cultivated apple genome (*Malus domestica*), and investigated for ecological divergence.
- We assessed variation in growth rate and traits related to carbon uptake between seedlings measured in a common garden, and related it to the genetic ancestry of the seedlings, assessed using 13 microsatellite loci and Bayesian clustering method. The occurrence of patterns of isolation-by-distance, -by-climate and -by-adaptation that might have caused genetic and phenotypic differentiation among *M. sylvestris* populations was also tested.
- Seedlings belonged to seven *M. sylvestris* populations in Europe, with 11.6% of seedlings introgressed by *M. domestica*. Significant trait variation among *M. sylvestris* populations was observed, which for some was of moderate to high heritability. Lack of association between trait and genetic divergence suggests that this significant phenotypic variation is not adaptive, but strong association between genetic variation and the climate during the last glacial maximum suggests local adaptation of *M. sylvestris* to past climates.
- This study provides an insight into the ecological and evolutionary drivers of phenotypic and genetic differentiation among populations of a wild apple species and relative of cultivated apples, which is a starting point for future breeding programs.

Keywords: population structure, isolation-by-distance, isolation-by-ecology, local adaptation, climate change, apple tree, crop wild relatives.

Societal impact Statement (113 words needs to be reduced to 100)

Apple is a major fruit crop worldwide and a model species for understanding the evolutionary processes underlying perennial crop domestication. Several wild species have contributed to the genetic make-up of the cultivated apple, yet phenotypic and genetic diversity data across their natural distribution is lacking. This study revealed phenotypic variation between populations of the European crabapple, and showed that both geography, and surprisingly, past but not current climate, shaped its genetic structure. We provide a starting point for harnessing wild apple diversity for apple breeding programs to mitigate the impact of climate change on this perennial crop.

Introduction

Knowledge of the spatial phenotypic and genetic variation among populations is essential for understanding the ecological (biotic and abiotic) and evolutionary (gene flow, selection, drift, mutation) processes involved in population divergence and adaptation (Savolainen *et al.*, 2013; Sork, 2018). The global biodiversity crisis, and its consequences on ecosystem health and services, makes investigating these questions, and identifying the taxa most vulnerable to anthropogenic change, all the more relevant (Hoffmann *et al.*, 2021).

Plant species distributed across climatic gradients typically experience spatial variation in selection, genetic drift and gene flow, processes that drive genetic and phenotypic divergence among populations (Svenning *et al.*, 2015). Climate influence demography such as population expansion and contraction, the extent of gene flow among populations, and ultimately the extent of genetic divergence among populations (Edwards *et al.*, 2022). For instance, changes in the climate since the last glacial maximum (LGM) 20,000 years ago have driven the genetic composition of the European crabapple and many other tree species (Comes & Kadereit, 1998; Kremer *et al.*, 2002; Pyhäjärvi *et al.*, 2008; Cornille *et al.*, 2013a; Gugger *et al.*, 2013; Riordan *et al.*, 2016; Lander *et al.*, 2021; Yamada *et al.*, 2021; Parisod, 2021). Climate can also shape phenotypic variation among populations. Populations occurring under the same climate may share physiological tolerances to climatic conditions, including plant carbon uptake via photosynthesis. Carbon uptake traits condition plant size and growth, reproduction and survival under different climatic conditions (Nicotra *et al.*, 2010; Hartmann *et al.*, 2020). In some cases, local climate can impose divergent selection on carbon uptake traits and lead to long-term reduction in gene flow among populations and local adaptation (Keller *et al.*, 2011; Franks *et al.*, 2014; Aitken & Bemmels, 2015; Ramírez-Valiente *et al.*, 2017; Alexandre *et al.*, 2020). Whether the phenotypic variation observed in species distributed across large climatic ranges results from their demographic or adaptive history remains an intense topic of investigation (Li *et al.*, 2012; Tiffin & Ross-Ibarra, 2014). Furthermore, investigating this question can help predict how plants may respond to climate change and how species adapt to their environment.

There are multiple ways to investigate whether the genetic and phenotypic variation among populations distributed across climatic gradients results from selection, genetic drift and/or gene flow. A first step could be to use a common garden experiment to investigate the genetic basis of phenotypic variation among populations. Indeed, different populations occurring across a climatic gradient may display clinal variation, *i.e.*, differences in a trait that may be the result of plasticity or local genetic adaptation (Savolainen *et al.*, 2013; de Villemereuil *et al.*, 2016). Measuring candidate traits for adaptation to climate, *e.g.*, phenology (Brachi *et al.*, 2013) or traits related to plant carbon uptake (Savolainen *et al.*, 2013; de Villemereuil *et al.*, 2016) in individuals from different populations under the same environmental conditions can help elucidate the genetic basis of phenotypic variation across populations without the confounding effects of the environment. Ideally, common garden should include the main genetic groups across the species' distribution (de Villemereuil *et al.*, 2020). The

association of neutral genotypic variation with ecological variation can also be used as evidence of adaptive divergence among populations (Shafer & Wolf, 2013; Wang & Bradburd, 2014). The correlation between neutral genetic differentiation and environmental or phenotypic divergence among populations, independent of geographic distance, referred to as isolation-by-ecology (IBE), is an extension of the isolation-by-distance (IBD hereafter) model (Wright, 1943), and has increasingly used as an indicator of adaptive divergence between populations. In the IBE model, natural selection, which results from several factors including climate, can indirectly increase neutral genetic and phenotypic differentiation between populations by promoting general barriers to gene flow (Nosil *et al.*, 2009; Orsini *et al.*, 2013, p. 201; Shafer & Wolf, 2013; Wang & Bradburd, 2014). The IBE pattern is agnostic with respect to the underlying processes that generated it (Wang & Bradburd, 2014); this pattern can be generated by different processes including natural selection against immigrants, sexual selection against immigrants, reduced hybrid fitness and biased dispersal. Although it can be difficult to map one or more processes to this pattern, testing for the IBE pattern is valuable for better understanding the ways in which natural selection shapes neutral genetic and phenotypic variation. Evidence from common garden experiments and IBE patterns can therefore contribute to understanding how genotypes, phenotypes and the environment interact to ultimately influence population divergence and potentially local adaptation.

Fruit trees are a major component of terrestrial ecosystems (Petit & Hampe, 2011) and are grown in managed plantations and orchards to provide a variety of economically important products (Boyd *et al.*, 2013). Recent breeding efforts have involved the repeated use of a limited number of cultivars sources of genetic material leading to a reduction in genetic diversity and the loss of valuable alleles at genes that are not directly targeted by human selection (Myles *et al.*, 2011; Warschefsky & von Wettberg, 2019; Migicovsky *et al.*, 2021). Wild relatives of crop fruit trees (or "CWR" for crop wild relative) harbor phenotypic and genetic diversity that are potentially highly valuable for future breeding programs in the context of climate change (Zhang *et al.*, 2017; Hoban *et al.*, 2018; Hübner & Kantar, 2021). However, rare of the studies which thoroughly investigate the phenotypic variation of CWR of fruit trees in relation to response to climate; most studies so far have focused on forest trees (Kremer & Hipp, 2019). Key traits to study in this context are related to plant carbon uptake. Indeed, climate impacts plant carbon uptake (Aubin *et al.*, 2016), which latter known to impact fruit quality characteristics and production (Demestihas *et al.*, 2017). These questions are urgent as native CWRs can be threatened by crop-to-wild gene flow from nearby domesticated trees (Delplancke *et al.*, 2011; Cornille *et al.*, 2015; Diez *et al.*, 2015; Feurtey *et al.*, 2017; Flowers *et al.*, 2019; Liu *et al.*, 2019). Therefore, the study of the genetic and phenotypic variation among populations of CWR fruit tree species is timely to guide future breeding programs; in addition, it may contribute to our understanding of the evolutionary and ecological drivers of population divergence, including climate.

The European crabapple, *Malus sylvestris* (L.) Mill, is a CWR and a major contributor to the cultivated apple genome (Cornille *et al.*, 2012, 2014, 2019; Peace *et al.*, 2019). Substantial crop-to-wild

gene flow has been observed across *M. sylvestris* populations in Europe (up to 23.1% of natural populations are introgressed by *M. domestica* (Cornille *et al.*, 2015)). Crop-wild hybrids sampled in a forest in France and grown in controlled conditions showed superior fitness compared to wild seedlings (Feurtey *et al.*, 2017). Population genetics analyses also identified five pure (*i.e.*, not introgressed by *M. domestica*) populations in Scandinavia, western France, eastern France, Eastern Europe and Italy (Cornille *et al.*, 2015). These five populations resulted from past contractions and expansions associated with the LGM (Cornille *et al.*, 2013a, 2015). It remains unclear, however, whether these five populations, distributed across a large area with different climatic conditions, present phenotypic variation that could be the result of local adaptation to past and/or present climates.

We investigated the spatial phenotypic and genetic variation among populations of a major wild contributor to the cultivated apple, *M. sylvestris*, to test for adaptive divergence. Variation in plant growth and traits related to carbon uptake was measured in 584 *M. sylvestris* seedlings grown under controlled conditions and genotyped for 13 microsatellite markers. We first assessed the genetic status of each seedling (pure *vs.* crop-wild hybrid). Then, we compared growth traits and traits related to plant carbon uptake among seedlings belonging to different European genetic groups. We also formally tested the impact of geography (IBD) and ecology (IBE tested with phenotypic traits and climate) on genetic variation observed from 13 microsatellite markers. We investigated the following questions: 1) Does growth rate and carbon uptake trait vary between populations of the European crabapple? Are those traits heritable, and thus can population history predict seedling phenotype?; 2) Is there any association between phenotypic variation and genetic variation, taking into account geographic distance?; and 3) Is climate associated with neutral genetic diversity of the European crabapple, which could suggest local adaptation (i.e., ecological/adaptive divergence)?

Materials and Methods

Plant material, experimental design and trait measurements

A total of 584 seeds were collected from 90 *M. sylvestris* mother trees (three to 15 seeds per mother tree, Table S1) from six different geographical regions in Europe: Austria (N = 89, two sites), Denmark (N = 91, three sites), Spain (N = 39, one site), France (N = 220, eight sites), Italy (N = 32, one site), Romania (N = 117, seven sites) (Table S1).

In mid-April 2019, the 584 seeds were washed, sterilized (in 0.5% chlorine for 20 min), and vernalized for three months at 4°C in the dark in a mix of damp sand and vermiculite. Then, seeds were sowed in jiffy pellets and each pellet was randomly placed in a 20-hole array. Seeds were grown in controlled conditions for two months (from mid-July to mid-September 2019: 22 ±1◦C, 60 ±5 % relative humidity, a 16:8 (L:D) photoperiod and a light level of 40–60 µmol $m^{-2}.s^{-1}$). Each 20-hole array was rotated daily in the growth chamber to avoid any micro-environmental variation in plant response, and plants were watered weekly.

During the course of the two-month experiment, the number of leaves and the height of each seedling were recorded. Some accessions, due to the low germination rate, could not be recorded (*i.e.*, height and number of leaves could not be recorded for 19 seedlings out of 584, $N = 565$, Table 1). Seedlings were measured every two or three days, starting from day 7-11 after the start of the experiment.

The last week of the experiment, the superficial flavonol and chlorophyll content and the nitrogen balance index (NBI) were measured in three leaves per seedling. Superficial chlorophyll content is the concentration of chlorophyll in the leaf epidermis (µg/cm²), and superficial flavonol content is an index of the flavonoid concentration (µg/cm²) in this upper layer and is related to phenol accumulation and UV protection. Leaf chlorophyll and flavonol content and NBI are parameters correlated with plant carbon uptake via photosynthesis. Flavonol is a phenolic compound that is also known to contribute to plant resistance, acclimation and adaptation to environmental constraints through various mechanisms, including its antioxidant activity. These traits were measured using a portable Dualex device (Force-A, Orsay, France), which uses a combination of fluorescence signals at various excitation bands to quantify pigments and chemical compounds. As carbon uptake related traits must be measured in the same day, a subsample of 257 seedlings out of the 565 seedlings (Table 1, numbers in brackets) was measured because of time limitation in a day. Seedlings measured for carbon uptake related traits were selected based on two criteria: having at least one seedling per mother tree and three seedlings per geographic site.

DNA extraction, microsatellite genotyping and genetic ancestry of the seedlings

At the end of the experiment, leaves of each seedling were sampled for microsatellite genotyping. Genomic DNA was extracted with the NucleoSpin plant DNA extraction kit II (Macherey & Nagel, Düren, Germany) according to the manufacturer's instructions. Microsatellites were amplified by multiplex PCR with the Multiplex PCR Kit (QIAGEN, Inc.). We used 13 microsatellite markers, Ch01f02, Ch01f03, Ch01h01, Ch01h10, Ch02c06, Ch02c09, Ch02c11, Ch02d08, Ch03d07, Ch04c07, Ch05f06, GD12 and Hi02c07 in four multiplexes (MP01 to MP04; (Cornille *et al.*, 2012)).

PCR was performed in a final reaction volume of 15 ml (7.5 ml of QIAGEN Multiplex Master Mix, 10–20 mM of each primer with the forward primer labelled with a fluorescent dye, and 10 ng of template DNA). We used a touch-down PCR program (initial annealing temperature of 60°C, decreasing by 1°C per cycle down to 55°C). Genotyping was performed at the GENTYANE platform (INRAE Clermont-Ferrand) on an ABI PRISM X3730XL, with 2 ml of GS500LIZ size standard (Applied Biosystems). Alleles were scored with the GENEMAPPER 4.0 software (Applied Biosystems). We retained only multilocus genotypes presenting less than 10% missing data.

Clones or closely related individuals can bias inferences of population structure. We estimated the kinship coefficient between pairs of individuals (*Fij*) with SPAGeDI 1.5d (Loiselle *et al.*, 1995; Hardy & Vekemans, 2002), and removed highly genetically related individuals with $Fij > 0.5$.

The individual-based Bayesian clustering method implemented in STRUCTURE 2.3.3 (Pritchard *et al.*, 2000) was used to estimate the admixture between *M. domestica* and *M. sylvestris*, and the population genetic structure of *M. sylvestris*. STRUCTURE uses Markov Chain Monte Carlo (MCMC) simulations to infer the proportion of ancestry of genotypes from *K* distinct clusters. The underlying algorithm attempts to minimize deviations from Hardy–Weinberg and linkage disequilibria. *K* ranged from 1 to 10. Ten independent runs were carried out for each *K* and 500,000 MCMC iterations after a burn-in of 50,000 steps were used. CLUMPAK (Greedy algorithm) (Kopelman *et al.*, 2015) was used to identify distinct modes in the 10 replicated runs for each *K*. STRUCTURE analyses were run for the full dataset ($N = 584$), plus 40 *M. domestica* genotypes included as a reference for the cultivated apple gene pool (Cornille *et al.*, 2013b). The *R* package pophelper v2.3.0 was used (Francis, 2016) to visualize bar plots. The amount of additional information explained by increasing *K* was determined using the ΔK statistic (Evanno *et al.*, 2005), as implemented in Structure Harvester (Earl & vonHoldt, 2012). However, ΔK provides statistical support for the strongest but not the finest population structure (Puechmaille, 2016). Natural populations can display a hierarchical genetic structure with fine-scale population structure. Visual inspection of the barplots was used to identify the *K* value for which all clusters have well assigned individuals, and where additional clusters at higher *K* values do not have well assigned individuals (indicating that we have reached the highest *K* value for which no new genuine clusters could be delimited). The *K* value we therefore considered corresponded to the finest one, which can be higher than the *K* value of the strongest population structure identified by ΔK.

Using the best *K* value inferred with STRUCTURE, we defined P_{dom}, the membership proportion of a seedling to the *M. domestica* gene pool; membership coefficients were used to define the genetic ancestry of each seedling: 1) seedlings with $P_{dom} > 0.9$, whose mother tree was likely misidentified in the field (referred to as "*dom*" hereafter); 2) seedlings with $P_{dom} > 0.1$, *i.e.*, crop-wild hybrids (referred to as "*cw*" hereafter); then 3) seedlings with a membership coefficient > 0.9 to a given wild apple cluster were considered to be « pure » wild seedlings (referred to as "*pure*" hereafter); and 4) seedlings with a membership coefficient < 0.9 to a given wild apple gene pool were considered to be wild-wild hybrids ("*ww*", hereafter). In addition, "*pure*" seedlings were assigned to different populations (*i.e.*, groups of seedlings with membership coefficient > 0.9 to a given wild gene pool). Two effects were then tested using statistical models below: the genetic status effect (*i.e.*, *dom*, *cw*, *ww*, *pure*), and the wild apple population effect (*i.e.*, corresponding to the "*pure*" populations detected with STRUCTURE).

Fitness proxy estimates

The fitness of each seedling was therefore estimated from growth and carbon related trait proxies.

The absolute growth rate (*AGR* (Radford, 1967)), relative growth rate (*RGR* (Briggs *et al.*, 1920)), and whole *AGR* were estimated as follows (the traits considered were the height and the number of leaves of the seedling):

$$AGR(cm/day) = \frac{(trait_{t+1} - trait_t)}{(date_{t+1} - date_t)} \quad (1)$$

$$RGR(cm/day/day) = \frac{AGR}{date_t} \quad (2)$$

$$WholeAGR(cm/day) = \frac{trait_{end} - trait_{beginning}}{date_{end} - date_{beginning}} \quad (3)$$

Note that for the whole AGR, the beginning of the experiment corresponded to days 7 and 11 for leaf and height measurements, respectively, while the last measurement was done at day 60. The *internode* ratio, which represents the ratio between the number of leaves and the height of the seedling at day 60, was also considered a fitness trait, as this value plays an important role in apple tree architecture (Ripetti *et al.*, 2008).

Seven fitness proxies were therefore calculated for the full dataset (565 seedlings, Table 1): *height_AGR*, *height_RGR*, *whole_height_AGR*, *leaf_AGR*, *leaf_RGR*, *whole_leaf_AGR* and *internode*. In addition, chlorophyll (*Chl*) and flavonol (*Flav*) content, and *NBI*, were measured in the subsample (257 seedlings, Table 1). A preliminary exploration of correlation and variation among phenotypic traits was carried out using a principal component analysis (PCA) with the FactoMineR R package (Lê *et al.*, 2008).

Statistical analyses of fitness variation

A previous study demonstrated that crop-to-wild gene flow has an impact on early-stage growth rate (Feurtey *et al.*, 2017). The effect of the genetic status of seedlings (*i.e., dom*, *cw*, *ww*, *pure*, Table 1) on fitness variation among seedlings was therefore tested. A linear mixed model was fitted to the data as follows:

$$Fitness_{ijkl} \sim \mu + \text{wild population of origin}_i + \text{genetic ancestry status}_j + \text{wild population of origin}_{i*}\text{genetic ancestry status}_j + \text{mother}_k + e_{ijkl} \quad (4),$$

where μ is the overall mean, "wild population of origin" is the fixed effect of the population of origin of the seedling inferred with STRUCTURE, "status" is the fixed effect of the genetic status of the seedling (*i.e.*, "*pure*", "*dom*", "*ww*", "*cw*"), the interaction between the two fixed effects, and *"mother"* is a normally distributed random effect with its own mean and variance parameters, and *e* is the residual. The mother tree of each seedling was used as a random factor to avoid pseudo-replication due to the presence of multiple half-siblings (*i.e.*, from the same mother tree). We ran the model (4), but replaced the "status" effect by the "*Pdom*" fixed effect. We gradually removed interactions and effects depending on their significance. In addition, we evaluated the differences in the effect on trait variation using a contrast analysis. We fitted the data to the model using the *lm4e* R package (Bates *et al.*, 2015).

For fitness proxies defined from the number of leaves (*leaf_AGR*, *leaf_RGR* and *whole_leaf_AGR)*, a log link function was used and the residual distribution was fitted to a negative binomial distribution (function *glm.nb* in R package lme4). For fitness proxies defined from the height of the seedling (*height_AGR*, *height_RGR*, *whole_height_AGR)*, and for chlorophyll and flavonol content, and *NBI*, a similar linear mixed model was run, but with a residual term that was assumed to be normally distributed.

Heritability estimates

Heritability estimates were computed using only pure and wild-wild *M. sylvestris* seedlings detected as above. We fitted each fitness proxy with a linear mixed model as follows: $Y_{ijk} = \mu + F_i + C_j + e_{ijk}$ (5), where Y_{ijk} is the fitness proxy (growth rate or carbon uptake related trait) of the kth seedling belonging to family *i*, member of the j^{th} genetic cluster, μ the overall fixed mean of the population, F_i the random effect of the i^{th} family, C_j the fixed effect of the j^{th} genetic cluster and e_{ijk} the random error term. The model was fitted using REML (restricted maximum likelihood). Calculations were performed by the *lme*-function of the *R*-library *nlme* (Pinheiro *et al.*, 2022). The output of *lme* provides estimates for the variance components, the corresponding standard deviations (sd), and the best unbiased linear predictors (BLUP) for random effects. Genetic parameters were then calculated as follows:

- the additive genetic variance: $VA = 4\sigma_F^2$ *w*ith σ_F^2representing the between-family variance
- the corresponding coefficient of variation: $CVA = \frac{\sqrt{VA}}{\mu}$
- the phenotypic variation: $VP = \sigma_F^2 + \sigma_E^2$, with σ_E^2 representing the residual variance
- the corresponding coefficient of variation: $CVP = \frac{\sqrt{VP}}{\mu}$
- Narrow-sense heritability: $h^2 = \frac{VA}{VP}$
- Dickerson's approximation for its standard deviation: $sd(h^2) \approx \frac{4sd(\sigma_F^2)}{VP}$

Test for isolation-by-ecology

Only pure and wild-wild hybrid *M. sylvestris* seedlings were selected for IBE analysis (*N* = 449, 21 sites, Table 1). The IBE pattern, *i.e.*, the contribution of climate and phenotypic distances to the genetic structure taking into account geographical distance, was evaluated using a distance-based redundancy analysis (db-RDA). db-RDA can be used when the response variable is a distance matrix, here a genetic distance matrix (F_{ST}) across 21 sampled sites, and the explanatory variables are in vector form. Explanatory variables were as follows : (i) the geographical distance between sampled sites which underlies an IBD process, represented by vectors with positive eigenvalues of a principal coordinate of neighbor matrix (*PCNM*) (Borcard & Legendre, 2002), which was applied to the geographical pairwise distance matrix between sampled sites computed with SPAGeDI 1.5d (Hardy & Vekemans, 2002); 19 bioclimatic variables downloaded from the Worldclim2 database (30s resolution,

https://www.worldclim.org/data/worldclim21.html) representing annual and seasonal trends and extremes averaged (ii) over the years 1970-2000 and averaged (iii) for the Pleistocene period (20K years ago) (Gamisch, 2019), which were used to test for an isolation-by-climate pattern (IBC, hereafter); and (iv) growth rate (N = 551, Table 1) and carbon uptake related traits (N = 239, Table 1) averaged per site, as well as chlorophyll and flavonoid content, which were used to test for an isolation-by-adaptation pattern, referred as to IBA hereafter.

To identify the variables that explained the genetic structure of *M. sylvestris*, a db-RDA using the "capscale" function (Oksanen *et al.*, 2014) was run on a full model which included all investigated variables (*i.e.*, PCNM components, growth rates, carbon uptake related traits, 19 bioclimatic variables). The best variables were selected for an optimum model with the function "step" based on the Akaike Information Criterion (AIC). Because db-RDA does not provide information on the relative contribution of each variable of the model, a variance partitioning analysis was run using the "varpart" function from the R-package "vegan" (Peres-Neto *et al.*, 2006).

Results

Genetic ancestry of seedlings

No clones or closely related individuals were detected (Figure S1) and therefore all 584 seedlings were included in the STRUCTURE analyses.

STRUCTURE revealed a clear spatial population genetic structure of *M. sylvestris* in Europe as well as crop-wild admixture (Figures 1 and S2). For K = 2, the analysis recovered a group that included *M. domestica* and the western *M. sylvestris* samples (green) and another group that consisted of the Eastern European samples (red). For K = 3, the western group was split into two groups, one comprising *M. domestica* and Spanish and Italian *M. sylvestris* seedlings (black), and another comprising the remaining western samples (green); the Central European group was also recovered. For K = 4, the Eastern European samples were split into two groups: an Austrian group and a Romanian group. For K = 5, there was a clear east/west substructure in France. For K = 6, a sixth cluster comprising the Danish individuals was found. For K = 7, the Italian population split into two groups. For K = 8, the population from one site in eastern France (*Lor*) was identified as a new cluster. When K > 9, STRUCTURE did not reveal any further substructures, with only additional cluster with highly admixed individuals (Figure S2). Therefore, although the ΔK indicated that the most likely K value was five (Figure S3), K = 8 was the finest population structure and was therefore retained in subsequent analyses.

For K = 8, we found that the *M. domestica* reference varieties were admixed with the Italian and Western European *M. sylvestris*. Conversely, we detected 68 *M. sylvestris* seedlings with P_{dom} > 0.1 (considered as crop-wild hybrids), corresponding to 11.6% of the seedlings (N = 584, Figures 1, S4 and S5, Table 1). We also found 21 seedlings with a membership coefficient to the *M. domestica* gene pool > 0.9, corresponding to 4% of the seedlings. Nearly all Spanish seedlings were assigned to the *M. domestica* gene pool with membership coefficients > 0.1 (*i.e.*, 26 crop-wild hybrids and 13 individuals

assigned to the *M. domestica* gene pool) and showed admixture only with the wild Italian purple gene pool (Figure S4). 33 individuals could not be assigned to any cluster (*i.e.*, individuals with a membership coefficient < 0.5 to any cluster).

We therefore identified 68 "*cw*", 21 "*dom*", 167 "*ww*" and 282 "*pure*" seedlings (Table 1, $N = 551$). After removing *cw* hybrids ($N = 68$), seedlings sampled from misidentified mother trees ($N = 21$), the *M. domestica* reference samples ($N = 40$) and individuals with a membership coefficient < 0.5 to any cluster, seven wild apple populations (*i.e.*, groups of seedlings with a membership coefficient > 0.5 to a wild apple cluster) were defined: French Western ("*FR-W*", $N = 77$), French Eastern ("*FR-E*", $N = 50$), French Lorraine ("*FR-Lor*", $N = 28$), Danish (*DA*, $N = 78$), Italian ("*IT*", $N = 27$), Austrian ("*AUT*", $N = 81$) and Romanian ("*RO*", $N = 108$) (Figure S6). Each *M. sylvestris* population showed a high level of genetic variation (Table S2). The Romanian population was the most genetically differentiated population and was close to the Austrian population; the Danish and French Western populations were genetically similar (Figure S7).

No effect of seedling status on phenotypic variation

Variation and correlations among traits are presented in Figures S8 to S11. Heritability estimates were moderate to high for all traits except growth rate based on leaf number (Table S3). However, these estimates need to be taken with caution given the limited sample size, as reflected by the large standard deviations.

We did not find any significant effect of seedling status (*i.e.*, *pure, ww, cw, dom*) (Figure 5, Table S4, Figure S12) or P_{dom} (Table S5, Figure S13) on phenotypic traits (*i.e.*, leaf and height AGR, leaf and height RGR, whole leaf and height AGR). We therefore removed the seedling status and *Pdom* effects from the model 4, as well as *dom* and *cw* individuals. We therefore only considered wild apple seedlings (*i.e.*, pure and ww, $N = 449$), and focused on the 'wild population of origin 'effect (Table S6).

Significant variation in growth rates and chlorophyll content among populations

Mean height variation along the course of the experiment among seedlings from different populations is shown in Figure 2. There was significant variation among seedlings from different populations in certain growth-related traits (Table 2). On average, seedlings belonging to the Austrian population were taller (+11 cm, $P = 0.047$) whereas Romanian (-14.9 cm, $P = 0.008$) and Italian (-18.7 cm, $P = 0.044$) seedlings were shorter (Figure 2) than seedlings from other populations. Seedlings from other populations did not show a significant difference in height. In addition, the number of leaves and height traits were negatively correlated, r = -0.3, $P < 0.001$). The Austrian population presented the lowest number of leaves (average = 5, sd = 4) whereas seedlings belonging to the Romanian population had the highest number of leaves (average = 8, sd = 7, Figure S15). The Romanian population also had the largest *internode* (+ 0.02 leaf/cm, $P = 0.024$).

Chlorophyll content differed among populations with seedlings from the Italian population producing on average more chlorophyll (+4.14 µg/cm², P = 0.039, Figure S16) than seedlings from other populations. Flavonol content and NBI did not differ significantly among populations.

Significant IBD and IBC

Correlation plots between bioclimatic variables are provided in Figures S17 and S18; however, all variables were included in the analysis as db-RDA can cope with correlated variables. The optimal model was chosen according to its best AIC value. The optimal model explained up to 25.9% of the genetic structure ($Adj\text{-}R^2$ = 69.9%, P = 0.001) and contained seven variables (four geographic and three bioclimatic variables) (Table 3): the geographical distance is represented by the *1st, 2nd, 3rd* and *6th* axis of the PCNM analysis and three past climatic variables (Bio3: isothermality; Bio6: minimum temperature of coldest month; Bio 9: mean temperature of driest quarter). In total, IBD explained 47% of the variance of the wild apple tree population genetic structure, whereas IBC explained 22% (Figure 3). Taking geographical distance into account, we did not find a pattern of IBA i.e., covariation between phenotype and genetic divergences.

Discussion

This study is the first to take into account the population genetic structure as well as the phenotypic variation of a contributor to the cultivated apple genome (Cornille *et al.*, 2012), to investigate ecological divergence. Bayesian clustering revealed seven *M. sylvestris* populations across Europe with a substantial number of seedlings (11.6%, mainly from Western Europe) introgressed by *M. domestica*, although this figure is less substantial than previously reported (Cornille *et al.*, 2013b, 2015; Feurtey *et al.*, 2017). Although the crop-wild hybrid status of seedlings did not impact phenotypic variation, we observed phenotypic variation among crabapple populations when grown in controlled conditions. Phenotypic variation was found for growth and chlorophyll content among populations of the European crabapple from different climates in Europe. Based on the IBA pattern, this phenotypic variation was not adaptive. However, the IBC pattern revealed that climate was a driver of genetic differentiation between populations. Given that the IBC pattern was still found after accounting for IBD, this implies that there are sufficient levels of local adaptation to LGM climate to reduce gene flow among populations. The European crabapple may therefore be locally adapted to the past climate conditions of the LGM. The lack of signal of adaptive phenotypic divergence suggests that traits other than the ones we investigated in this study may be under divergent selection. The results of this study pinpoints adaptive divergence related to climate in a wild contributor to a fruit tree crop genome, which is a starting point for future breeding programs and mitigating the impact of climate change of CWR of an emblematic temperate fruit tree.

Ongoing crop-to-wild gene flow in the European crabapple

We revealed substantial gene flow from *M. domestica* to the European crabapple, with 11.8% of seedlings, mostly from Western Europe, introgressed by *M. domestica*. Introgression rates were lower compared to previous studies (*i.e*, 37% in Cornille *et al.* (2013b) and 23.1% in Cornille *et al.* (2015)). However, these studies genotyped more mother trees (*i.e.*, N = 756 and N = 1,889, respectively), which could explain the difference in estimates of crop-to-wild gene flow. The results described here highlight the fact that crop-to-wild gene flow is still ongoing in the European crabapple. The Spanish seedlings sampled here were the progeny of trees growing in a location that is known to have high levels of *M. domestica* introgression (pers. comment. G. Alins). It is even possible that the mother trees of these seedlings were *M. domestica* and not *M. sylvestris*. The inclusion of reference cultivated apple samples mainly from Western Europe may decrease the probability of detecting crop-to-wild introgression events in wild populations from Eastern and Northern Europe. The lower crop-to-wild introgression rates in wild seedlings from Eastern Europe can also be explained by their physical distance from cultivated apple orchards. Indeed, distance can be a natural barrier to hybridization between *M. domestica* and its wild relative (Larsen *et al.*, 2016), which we confirmed in this study. The position of a *M. sylvestris* individual in a forest may also impact its level of introgression. Indeed, *M. sylvestris* trees are often found in forest gaps and at the forest edge, corresponding to their ecophysiological preferences (*i.e.*, preference for light and low competition). The effect of the location of the trees in the forest on the level of crop-to-wild introgression needs to be studied further.

The consequences of crop-wild introgression on phenotypic variation between crop and wild individuals have been studied more in annual crops (*e.g.*, maize, wheat, lettuce, rice) (Ellstrand *et al.*, 2013) than in perennial fruit trees. One study has shown that crop-wild hybrid seedlings of the European crabapple have higher growth rates and showed earlier germination than wild apple seedlings (Feurtey *et al.*, 2017). We did not detect any effect of the status of a seedling (pure, wild, crop-wild, dom) or the level of introgression (*Pdom*) on growth and carbon uptake related fitness proxies. This could be due to the low number of samples from the *cw* and *dom* categories. Note that we did not test the variation in germination rate among seedlings as germination can be strongly impacted by stratification conditions.

No adaptive phenotypic variation among populations, but signs of local adaptation to past climate in the European crabapple

Under controlled conditions, seedlings from the different populations were found to have significantly different growth and morphology, but IBA analyses indicated that this variation was likely not adaptive. Seedlings belonging to the Austrian population were the tallest, had the highest absolute growth rate and the lowest number of leaves; by contrast, Romanian seedlings were the shortest, had the lowest absolute growth rate and the highest number of leaves. Italian seedlings had the highest chlorophyll content. The seedlings belonging to the Austrian population may be fitter in the climate conditions simulated in this experiment. We tested whether this phenotypic variation was adaptive. However, taking geographic distance into account, we did not find any significant covariation between genetic

and phenotypic variation. This suggests a lack of divergent selection on traits related to carbon uptake or growth that are often associated with plant responses to climate (Bussotti *et al.*, 2015). Therefore, the phenotypic variation we observed among populations under controlled conditions may be the result of genetic drift alone; alternatively, the traits we selected (thought to be related to responses to climate) are not good candidates for investigating divergent selection. Leaf mass per area (LMA) and foliar nitrogen content demonstrated can be future targeted parameter to assess the responses of apple seedling to environmental stress (Bussotti *et al.*, 2015). Another explanation could be that we did not phenotype enough seedlings from each genetic group. Indeed, we observed a high variation in each phenotypic trait and in their heritability estimates, suggesting that the traits we studied may be relevant but that a larger number of seedlings should be phenotyped and analyzed (*e.g.*, (Klein *et al.*, 1973)). However, some studies have found that even with large sample sizes, the standard error of heritability estimates can still be large and vary greatly between experimental designs (Visscher & Goddard, 2015). The fairly high heritability estimates for most of the traits considered here could be seen as consistent with rather weak within population selection, enabling the maintenance of ample additive genetic variation (Wheelwright *et al.*, 2014). Furthermore, high variation in seedling traits combined with high heritability estimates could suggest that there is large room of genetic material for adaptation to work on. In addition, the population of origin of the seedlings did not explain all phenotypic variation. Even though we found an effect of the population of origin on phenotypic traits, its contribution was relatively low (*e.g.*, model 4, R^2 for height = 0.119, and R^2 for height AGR= 0.047). Environment (*e.g.*, climate) and interaction between genotype and environment could also impact fitness.

As *M. sylvestris* is distributed across gradient, we further investigated the role of climate in shaping the genetic variation among populations of the European crabapple, without considering phenotypic trait variation. We tested for an IBE pattern, where the pattern of neutral genetic variation covaries with ecological variables (here climate). There was no combined effect of geographic and climatic distance (IBD ∩ IBC), which allowed us to assess the contribution of these processes separately (Wang & Bradburd, 2014). We showed that IBD and IBC played a significant role (R^2_{adj} = 47% and R^2_{adj} = 22%, respectively) on the genetic differentiation of European crabapple populations. Weak but significant IBD has been previously identified in wild apple relatives of the cultivated apple (*i.e.*, *M. sylvestris*, *M. orientalis* and *M. sieversii*) suggesting they have high dispersal capacities (Cornille *et al.*, 2013b,a, 2015). Weak IBD is explained by self-incompatibility systems that prevent self-fertilization (Brown, 1992), pollen dispersal by bees and flies (*Syrphidae*) and endozoochorous seed dispersal by large mammals such as ungulates, wild pigs, brown bears or humans (Larsen *et al.*, 2006). We show that in addition to IBD, IBC persisted after taking geographic distance into account. Climate can impose divergent selection pressures on different locations and thus reduce gene flow between populations, so that IBC contributes to genetic differentiation. The main variables explaining genetic differentiation in the European crabapple were related to temperature during the LGM. This suggests that the European crabapple may be locally adapted to its past temperature but not to its current climate. Local adaptation

to current climate is well studied in wind-dispersed trees (Savolainen *et al.*, 2013; Kremer & Hipp, 2019; Pyhäjärvi *et al.* 2020). However, to our knowledge no study has shown local adaptation to past climate conditions in a tree species.

Additional factors other than climate can also shape adaptive divergence between populations. *Malus sylvestris* is a pioneer species that needs high levels of light and is not very competitive. Local adaptation to biotic factors such as the presence of other species is possible. Competition for light with other species such as the European beech (*Fagus sylvatica*) could be a source of divergence between populations. Local adaptation of fruit trees to biotic factors, including parasites (Olvera-Vazquez *et al.*, 2021), deserves further investigations. Besides selection, the potential role of phenotypic plasticity in enabling growth and optimal fitness in changing environments also needs to be carefully evaluated (Benito Garzón *et al.*, 2011).

Further investigations needed on local adaptation and plasticity in response to climate in the European crabapple

Our study raises concerns regarding the future of wild apple populations and their current vulnerability to current climate change. However, the adaptation of tree species to climate remains complex (Bussotti *et al.*, 2015). For instance, in *Eucalyptus camaldulensis*, variation in leaf traits and performance proxies was unrelated to the climate of genotype provenance (Asao *et al.*, 2020), whereas variation in several photosynthetic traits was clearly related to the climate of genotype provenance across Australia (Dillon *et al.*, 2018). In contrast, collective differences in leaf morphology and photosynthetic physiology, in several *Populus* species may be adaptive for differences in growth season length, temperature and insolation (Keller *et al.*, 2011; Kaluthota *et al.*, 2015). Further investigations on local adaptation and plasticity to climate or biotic factors in the European crabapple are therefore needed. Genomic data will be particularly useful for determining the relative influence of adaptive and neutral processes on climate- or biotic- driven divergence by screening genomes from different populations in Europe. Comparing the fitness of seedlings from different populations in reciprocal transplants will also be important to further test for local adaptation. Our study therefore raises questions regarding the processes of local adaptation of fruit trees, and is a starting point for apple breeding programs.

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Authors contributions

AC, GV, AR, SB, TU conceived and designed the experiments; AC, GV, AT, TU, TK obtained the funding; AC, GV, AT, TU, KAO, SV, RR, XC, TK, CR sampled the material; XC, CR, AV, AR, GL, KAO, RR, MLG, HB, VC, HC, SV, MF performed the molecular biology analyses; AC, AF, KomAvi and XC analyzed the data. The manuscript was written by AC, KomAvi and AF, with essential input from other co-authors.

Figures and Tables

Figure 1. Bayesian clustering of the *Malus sylvestris* seedlings sampled in this study (*N* = 584) and the reference samples of *Malus domestica* (*N* = 40) inferred with STRUCTURE for *K* = 8, and its associated map of mean membership per sampled site. Each individual is represented by a vertical bar partitioned into clusters. Visualization was improved by sorting genotypes by country; countries are separated by a white line. The reference *M. domestica* reference samples are shown on the far left of the map in the Atlantic. Circle size is proportional to the number of individuals within the cluster (scale shown on the top right-hand corner).

Figure 2. Mean height of apple seedlings measured over the time of the experiment in controlled conditions (including pure and wild-wild hybrid seedlings, *N* = 449, and seedlings assigned to the *M. domestica* gene pool, *N* = 21, as detected with STRUCTURE for *K*=8)**.** The 40 reference *M. domestica* individuals were not measured under controlled conditions thus are not shown here. Vertical lines represent the standard deviation. Populations: AUT (*N* = 81), DA (N = 78), DOM (*N* = 21, includes 13 Spanish genotypes and seedlings from other countries), FR-E (*N* = 50), FR-Lor (*N* = 28), FR-W (*N* = 77), IT (*N* = 27), RO (*N* = 108).

Figure 3. Variance partitioning analysis of the db-RDA results obtained for *Malus sylvestris* (N_{sites} = 21, 13 microsatellite markers). Variation of the site pairwise genetic differentiation (F_{ST}) is explained by the variables generating isolation-by-distance (geographical distance) and isolation-by-climate (with three bioclimatic variables during the last glacial maximum: Bio3, isothermality; Bio6, minimum temperature of coldest month; Bio 9: mean temperature of driest quarter).

Table 1. Number of *M. sylvestris* seedlings used in this study for population genetic analyses inferred with STRUCTURE for *K* = 8 with 13 microsatellite markers and phenotyping (growth and carbon-uptake related traits).

Clusters	N_{pure}	N_{ww}	N_{cw}	N_{dom}	$N_{no\ cluster}$	Total measured for phenotypic traits	Wild population name
Q1 (light green)	32	46	7	0	10	92	FR-W
Q2 (yellow)	0	52	5	0	4	57	FR-E
Q3 (lor)	28	1	0	0		28	FR-Lor
Q4 (blue)	61	21	1	0	6	85	DA
Q5 (purple)	23	4	3	0	4	34	IT
Q6 (dark green)	66	17	1	0	1	83	AUT
Q7 (red)	77	34	5	0	0	113	RO
Q8 (black – M. domestica)	40	0	46	21	8	73	-
Total	287	175	68	21	33	551 (584)	
Total measured for height and number of leaves	282	167	63	21	32	533 (565)	

Total measured for leaf chlorophyll, flavonol and NBI contents	129	82	22	6	18	239 (257)

N_{pure}: number of seedlings assigned to a wild gene pool with a membership coefficient > 0.9 ; N_{ww}, number of wild-wild hybrids (*i.e.*, seedlings with a membership coefficient > 0.1 to a wild gene pool other than its own wild gene pool and a membership coefficient < 0.1 to the *M. domestica* gene pool); N_{cw}: number of crop-wild hybrids (*i.e.*, seedling assigned to the *M. domestica* gene pool with a membership coefficient > 0.1). $N_{no\ cluster}$: seedlings that could not be assigned to any defined gene pool; Total measured for phenotypic traits : number of individuals measured for each phenotypic trait and included in the statistical analyses, the number in brackets represents the initial sample size before data were filtered for statistical analyses. Wild population name: populations defined with STRUCTURE at *K*=8 excluding crop-wild hybrids and seedlings from misidentified mother trees (*i.e.*, including only wild pure and wild-wild hybrids).

Table 2. Final model depicting effects of the *Malus sylvestris* population to which each seedling belonged (*i.e.*, cluster inferred with STRUCTURE at *K*=8) on phenotypic traits (*i.e.*, height, number of leaves, internode, chlorophyll and flavonol content, NBI) measured in 533 individuals. Variables in green are significant ($P < 0.05$).

Explanatory variable	Cluster			Mother		Model			Mother
Fitness	X^2	*P-value*	df	REML	Standard Deviation	AIC	R^2	Corrected R^2	R^2
Height_AGR	17.863	0.007***	6	1,229	0.204	1248	0.047	0.091	0.044
Height_RGR	12.846	0.045*	6	-2,264	0.006	-2245	0,041	0.147	0.106
Leaf_AGR	-	-	-	-	-	-	-	-	-
Leaf_RGR	-	-	-	-	-	-	-	-	-
Height whole AGR	22.243	1.00e-03***	6	630	0.175	650	0.074	0.192	0.118
Leaf whole AGR	36.326	2.38e-06***	6	-1,277	0.009	-1,258	0.09	0.118	0.028

Height	31.623	1.93e-05***	6	4,113	11.69	4,131	0.119	0.301	0.182
Number of leaves	22.285	0.001***	6	-	0.084	2,659	0.052	0.064	0.012
Chlorophyll	14.418	0.025*	6	1,181	1.352	1,199	0.074	0.171	0.097
Flavonol	-	-	6	-	-	-	-	-	-
NBI	-	-	6	-	-	-	-	-	-
Internode (nbleaf/height)	17.768	0.007***	6	-1,328	0.009	-1,309	0.044	0.073	0.029

***: P-value <0.001; **: 0.01< P-value <0.001; *: 0.05< P-value <0.01; AIC: Akaike Indice Criterion.

Table 3. Contribution of geography and climate to the genetic variation observed among *M. sylvestris* seedlings. Distance-based redundancy analyses tested the effects of geography, climate and phenotype on genetic differentiation among 21 sites from 13 microsatellites in the European crabapple. Only significant variables are presented.

	db-RDA			
	% of variance explained	**d.f.**	**p-value**	**Adj-R^2**
Global analysis	25.9	7	0.001	69.9
Residuals	11.3	13	-	
Marginal test				
Geography (IBD)	14.9	4	<0.015	
PCNM 1-2-3-6				
Environment (IBC_LGM)	11.04	3	<0.015	
BIO3_LGM				
BIO6_LGM				
BIO9_LGM				
Interaction			-	

Residuals	11.3%	-	-	

BIO3_LGM: isothermality (BIO2/BIO7) (×100)); BIO6_LGM: minimum temperature of coldest month; BIO9_LGM: mean temperature of driest quarter, IBD: isolation-by-distance; IBC_LGM: isolation-by-climate during the last glacial maximum.

References

Aitken SN, Bemmels JB. 2015. Time to get moving: assisted gene flow of forest trees. *Evolutionary applications* **9**: 271–290.

Alexandre H, Truffaut L, Klein E, Ducousso A, Chancerel E, Lesur I, Dencausse B, Louvet J-M, Nepveu G, Torres-Ruiz JM, *et al.* 2020. How does contemporary selection shape oak phenotypes? *Evolutionary Applications* **13**: 2772–2790.

Asao S, Hayes L, Aspinwall MJ, Rymer PD, Blackman C, Bryant CJ, Cullerne D, Egerton JJG, Fan Y, Innes P, *et al.* 2020. Leaf trait variation is similar among genotypes of Eucalyptus camaldulensis from differing climates and arises in plastic responses to the seasons rather than water availability. *New Phytologist* **n/a**.

Aubin I, Munson AD, Cardou F, Burton PJ, Isabel N, Pedlar JH, Paquette A, Taylor AR, Delagrange S, Kebli H, *et al.* 2016. Traits to stay, traits to move: a review of functional traits to assess sensitivity and adaptive capacity of temperate and boreal trees to climate change. *Environmental Reviews* **24**: 164–186.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, Articles* **67**: 1–48.

Borcard D, Legendre P. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling* **153**: 51–68.

Boyd IL, Freer-Smith PH, Gilligan CA, Godfray HCJ. 2013. The Consequence of Tree Pests and Diseases for Ecosystem Services. *Science* **342**.

Brachi B, Villoutreix R, Faure N, Hautekèete N, Piquot Y, Pauwels M, Roby D, Cuguen J, Bergelson J, Roux F. 2013. Investigation of the geographical scale of adaptive phenological variation and its underlying genetics in Arabidopsis thaliana. *Molecular Ecology* **22**: 4222–4240.

Briggs AW, Kidd F, West C. 1920. A quantitative analyses of plant growth: part II. *Annals of Applied Biology* **7**: 202–223.

Brown SK. 1992. Genetics of apple. In: Plant breeding reviews vol 9. John Wiley & Sons New York, 333–366.

Bussotti F, Pollastrini M, Holland V, Brüggemann W. 2015. Functional traits and adaptive capacity of European forests to climate change. *Environmental and Experimental Botany* **111**: 91–113.

Comes HP, Kadereit JW. 1998. The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science* **3**: 432–438.

Cornille A, Antolín F, Garcia E, Vernesi C, Fietta A, Brinkkemper O, Kirleis W, Schlumbaum A, Roldán-Ruiz I. 2019. A Multifaceted Overview of Apple Tree Domestication. *Trends in Plant Science* **24**: 770–782.

Cornille A, Feurtey A, Gélin U, Ropars J, Misvanderbrugge K, Gladieux P, Giraud T. 2015. Anthropogenic and natural drivers of gene flow in a temperate wild fruit tree: A basis for conservation and breeding programs in apples. *Evolutionary Applications* **8**: 373–384.

Cornille A, Giraud T, Bellard C, Tellier A, Le Cam B, Smulders MJM, Kleinschmit J, Roldan-Ruiz I, Gladieux P. 2013a. Post-glacial recolonization history of the European crabapple (Malus sylvestris Mill.), a wild contributor to the domesticated apple. *Molecular Ecology* **22**: 2249–63.

Cornille A, Giraud T, Smulders MJM, Roldán-Ruiz I, Gladieux P. **2014**. The domestication and evolutionary ecology of apples. *Trends in Genetics* **30**: 57–65.

Cornille A, Gladieux P, Giraud T. **2013b**. Crop-to-wild gene flow and spatial genetic structure in the wild closest relatives of the cultivated apple. *Evolutionary Applications* **6**: 737–748.

Cornille A, Gladieux P, Smulders MJM, Roldán-Ruiz I, Laurens F, Le Cam B, Nersesyan A, Clavel J, Olonova M, Feugey L, *et al.* 2012. New insight into the history of domesticated apple: secondary contribution of the European wild apple to the genome of cultivated varieties. *PLoS Genet* **8**: e1002703.

Delplancke M, Alvarez N, Espíndola A, Joly H, Benoit L, Brouck E, Arrigo N. **2011**. Gene flow among wild and domesticated almond species: insights from chloroplast and nuclear markers. *Evolutionary Applications* **5**: 317-329.

Demestihas C, Plénet D, Génard M, Raynal C, Lescourret F. **2017**. Ecosystem services in orchards. A review. *Agronomy for Sustainable Development* **37**: 12.

Diez CM, Trujillo I, Martinez-Urdiroz N, Barranco D, Rallo L, Marfil P, Gaut BS. **2015**. Olive domestication and diversification in the Mediterranean Basin. *New Phytologist* **206**: 436–447.

Dillon S, Quentin A, Ivković M, Furbank RT, Pinkard E. **2018**. Photosynthetic variation and responsiveness to CO2 in a widespread riparian tree. *PLOS ONE* **13**: e0189635.

Earl DA, vonHoldt BM. **2012**. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**: 359–361.

Edwards SV, Robin VV, Ferrand N, Moritz C. **2022**. The Evolution of Comparative Phylogeography: Putting the Geography (and More) into Comparative Population Genomics. *Genome Biology and Evolution* **14**: evab176.

Ellstrand NC, Meirmans P, Rong J, Bartsch D, Ghosh A, de Jong TJ, Haccou P, Lu B-R, Snow AA, Neal Stewart C, *et al.* 2013. Introgression of Crop Alleles into Wild or Weedy Populations. *Annual Review of Ecology, Evolution, and Systematics* **44**: 325–345.

Evanno G, Regnaut S, Goudet J. **2005**. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611–2620.

Feurtey A, Cornille A, Shykoff JA, Snirc A, Giraud T. **2017**. Crop-to-wild gene flow and its fitness consequences for a wild fruit tree: Towards a comprehensive conservation strategy of the wild apple in Europe. *Evolutionary Applications* **10**: 180–188.

Flowers JM, Hazzouri KM, Gros-Balthazard M, Mo Z, Koutroumpa K, Perrakis A, Ferrand S, Khierallah HSM, Fuller DQ, Aberlenc F, *et al.* 2019. Cross-species hybridization and the origin of North African date palms. *Proceedings of the National Academy of Sciences* **116**: 1651–1658.

Francis R. **2016**. POPHELPER: An R package and web app to analyse and visualise population structure. *Molecular Ecology Resources* **17**: n/a-n/a.

Franks SJ, Weber JJ, Aitken SN. **2014**. Evolutionary and plastic responses to climate change in terrestrial plant populations. *Evolutionary Applications* **7**: 123–139.

Gamisch A. **2019**. Oscillayers: A dataset for the study of climatic oscillations over Plio-Pleistocene time-scales at high spatial-temporal resolution. *Global Ecology and Biogeography* **28**: 1552–1560.

Gugger PF, Ikegami M, Sork VL. **2013**. Influence of late Quaternary climate change on present patterns of genetic variation in valley oak, Quercus lobata Née. *Molecular Ecology* **22**: 3598–3612.

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

Hartmann H, Bahn M, Carbone M, Richardson AD. **2020**. Plant carbon allocation in a changing world – challenges and progress: introduction to a Virtual Issue on carbon allocation. *New Phytologist* **227**: 981–988.

Hoban S, Volk G, Routson KJ, Walters C, Richards C. **2018**. Sampling Wild Species to Conserve Genetic Diversity. In: Greene SL, Williams KA, Khoury CK, Kantar MB, Marek LF, eds. North American Crop Wild Relatives, Volume 1: Conservation Strategies. Cham: Springer International Publishing, 209–228.

Hoffmann AA, Weeks AR, Sgrò CM. **2021**. Opportunities and challenges in assessing climate change vulnerability through genomics. *Cell* **184**: 1420–1425.

Hübner S, Kantar MB. **2021**. Tapping Diversity From the Wild: From Sampling to Implementation. *Frontiers in Plant Science* **12**: 38.

Kaluthota S, Pearce DW, Evans LM, Letts MG, Whitham TG, Rood SB. **2015**. Higher photosynthetic capacity from higher latitude: foliar characteristics and gas exchange of southern, central and northern populations of Populus angustifolia. *Tree Physiology* **35**: 936–948.

Keller SR, Soolanayakanahally RY, Guy RD, Silim SN, Olson MS, Tiffin P. **2011**. Climate-driven local adaptation of ecophysiology and phenology in balsam poplar, Populus balsamifera L. (Salicaceae). *American Journal of Botany* **98**: 99–108.

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I. **2015**. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* **15**: 1179–1191.

Kremer A, Hipp AL. **2019**. Oaks: an evolutionary success story. *New Phytologist* **226**: 943–946.

Kremer A, Kleinschmit J, Cottrell J, Cundall EP, Deans JD, Ducousso A, König AO, Lowe AJ, Munro RC, Petit RJ, *et al.* 2002. Is there a correlation between chloroplastic and nuclear divergence, or what are the roles of history and selection on genetic diversity in European oaks? *Range wide distribution of chloroplast DNA diversity and pollen deposits in European white oaks: inferences about colonisation routes and management of oak genetic resources* . **156**: 75–87.

Lander TA, Klein EK, Roig A, Oddou-Muratorio S. **2021**. Weak founder effects but significant spatial genetic imprint of recent contraction and expansion of European beech populations. *Heredity* **126**: 491–504.

Larsen B, Ørgaard M, Toldam-Andersen TB, Pedersen C. **2016**. A high-throughput method for genotyping S-RNase alleles in apple. *Molecular breeding : new strategies in plant improvement* **36**: 24–24.

Lê S, Josse J, Husson F. **2008**. FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software* **25**: 1–18.

Li J, Li H, Jakobsson M, Li SEN, Sjödin PER, Lascoux M. **2012**. Joint analysis of demography and selection in population genetics: where do we stand and where could we go? *Molecular Ecology* **21**: 28–44.

Liu S, Cornille A, Decroocq S, Tricon D, Chague A, Eyquard J-P, Liu W-S, Giraud T, Decroocq V. 2019. The complex evolutionary history of apricots: species divergence, gene flow and multiple domestication events. *Molecular Ecology* **28**: 5299–5314.

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

Migicovsky Z, Gardner KM, Richards C, Chao CT, Schwaninger HR, Fazio G, Zhong G-Y, Myles S. 2021. Genomic consequences of apple improvement. *Horticulture Research* **8**: 1–13.

Myles S, Boyko AR, Owens CL, Brown PJ, Grassi F, Aradhya MK, Prins B, Reynolds A, Chia J-M, Ware D, *et al.* 2011. Genetic structure and domestication history of the grape. *PNAS* **108**: 3530–3535.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, *et al.* 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science* **15**: 684–692.

Nosil P, Funk DJ, Ortiz-barrientos D. 2009. Divergent selection and heterogeneous genomic divergence. *Molecular Ecology* **18**: 375–402.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2014. *Vegan: Community ecology package*.

Olvera-Vazquez SG, Alhmedi A, Miñarro M, Shykoff JA, Marchadier E, Rousselet A, Remoué C, Gardet R, Degrave A, Robert P, *et al.* 2021. Experimental test for local adaptation of the rosy apple aphid (Dysaphis plantaginea) to its host (Malus domestica) and to its climate in Europe. *PCI Ecology* **Pre-registration version**.

Orsini L, Vanoverbeke J, Swillen I, Mergeay J, De Meester L. 2013. Drivers of population genetic differentiation in the wild: isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology* **22**: 5983–5999.

Parisod C. 2021. Plant speciation in the face of recurrent climate changes in the Alps. *Alpine Botany*.

Peace CP, Bianco L, Troggio M, van de Weg E, Howard NP, Cornille A, Durel C-E, Myles S, Migicovsky Z, Schaffer RJ, *et al.* 2019. Apple whole genome sequences: recent advances and new prospects. *Horticulture Research* **6**: 59.

Peres-Neto PR, Legendre P, Dray S, Borcard D. 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology* **87**: 2614–2625.

Petit RJ, Hampe A. 2011. Some evolutionary consequences of being a tree. *Annu. Rev. Ecol. Evol. Syst.* **37**: 187–214.

Pinheiro J, Bates D, R Core Team. 2022. *nlme: Linear and Nonlinear Mixed Effects Models*.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Puechmaille SJ. 2016. The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* **16**: 608–627.

Pyhäjärvi T, Kujala ST, Savolainen O. 275 years of forestry meets genomics in Pinus sylvestris. *Evolutionary Applications* **n/a**.

Pyhäjärvi T, Salmela MJ, Savolainen O. **2008**. Colonization routes of Pinus sylvestris inferred from distribution of mitochondrial DNA variation. *Tree Genetics & Genomes* **4**: 247–254.

Radford PJ. **1967**. Growth Analysis Formulae - Their Use and Abuse1. *Crop Science* **7**: cropsci1967.0011183X000700030001x.

Ramírez-Valiente JA, Center A, Sparks JP, Sparks KL, Etterson JR, Longwell T, Pilz G, Cavender-Bares J. **2017**. Population-Level Differentiation in Growth Rates and Leaf Traits in Seedlings of the Neotropical Live Oak Quercus oleoides Grown under Natural and Manipulated Precipitation Regimes. *Frontiers in Plant Science* **8**: 585.

Riordan EC, Gugger PF, Ortego J, Smith C, Gaddis K, Thompson P, Sork VL. **2016**. Association of genetic and phenotypic variability with geography and climate in three southern California oaks. *American Journal of Botany* **103**: 73–85.

Ripetti V, Escoute J, Verdeil JL, Costes E. **2008**. Shaping the shoot: the relative contribution of cell number and cell shape to variations in internode length between parent and hybrid apple trees. *Journal of Experimental Botany* **59**: 1399–1407.

Savolainen O, Lascoux M, Merila J. **2013**. Ecological genomics of local adaptation. *Nat Rev Genet* **14**: 807–820.

Shafer ABA, Wolf JBW. **2013**. Widespread evidence for incipient ecological speciation: a meta-analysis of isolation-by-ecology. *Ecology letters* **16 7**: 940–50.

Sork VL. **2018**. Genomic Studies of Local Adaptation in Natural Plant Populations. *Journal of Heredity* **109**: 3–15.

Svenning J-C, Eiserhardt WL, Normand S, Ordonez A, Sandel B. **2015**. The Influence of Paleoclimate on Present-Day Patterns in Biodiversity and Ecosystems. *Annual Review of Ecology, Evolution, and Systematics* **46**: 551–572.

Tiffin P, Ross-Ibarra J. **2014**. Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology & Evolution* **29**: 673–680.

de Villemereuil P, Gaggiotti OE, Goudet J. **2020**. Common garden experiments to study local adaptation need to account for population structure. *Journal of Ecology* **n/a**.

de Villemereuil P, Gaggiotti OE, Mouterde M, Till-Bottraud I. **2016**. Common garden experiments in the genomic era: new perspectives and opportunities. *Heredity* **116**: 249–254.

Visscher PM, Goddard ME. **2015**. A General Unified Framework to Assess the Sampling Variance of Heritability Estimates Using Pedigree or Marker-Based Relationships. *Genetics* **199**: 223–232.

Wang IJ, Bradburd GS. **2014**. Isolation by environment. *Molecular Ecology* **23**: 5649–5662.

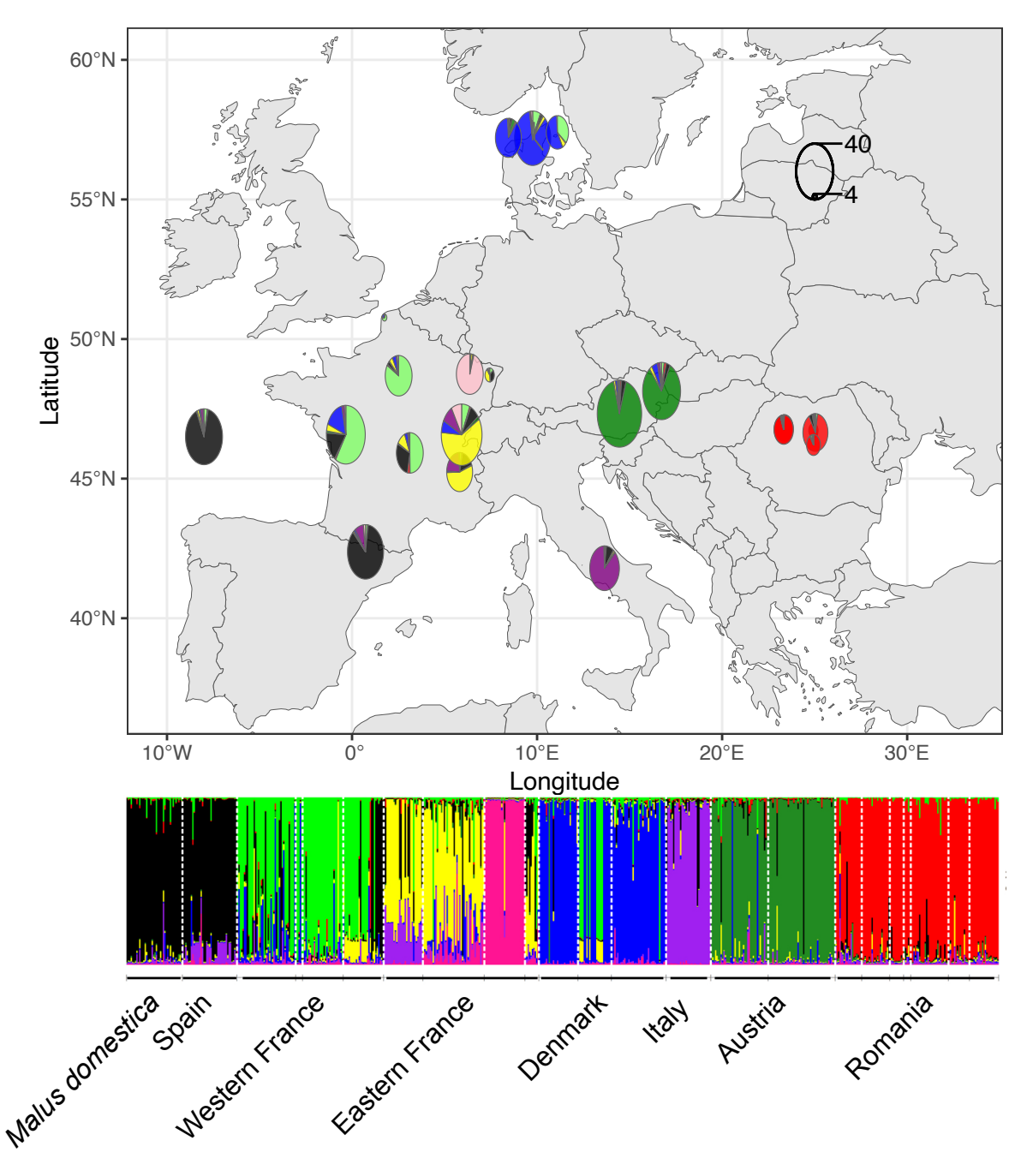
Warschefsky EJ, von Wettberg EJB. **2019**. Population genomic analysis of mango (Mangifera indica) suggests a complex history of domestication. *New Phytologist* **222**: 2023–2037.

Wheelwright NT, Keller LF, Postma E. **2014**. The effect of trait type and strength of selection on heritability and evolvability in an island bird population. *Evolution* **68**: 3325–3336.

Wright S. **1943**. Isolation by distance. *Genetics* **28**: 114–138.

Yamada T, Kokubugata G, Fujii S, Chen C-F, Asakawa A, Ito T, Maki M. **2021**. Refugia during the last glacial period and the origin of the disjunct distribution of an insular plant. *Journal of Biogeography* **48**: 1460–1474.

Zhang H, Mittal N, Leamy LJ, Barazani O, Song B-H. **2017**. Back into the wild—Apply untapped genetic diversity of wild relatives for crop improvement. *Evolutionary Applications* **10**: 5–24.


60°N
55°N
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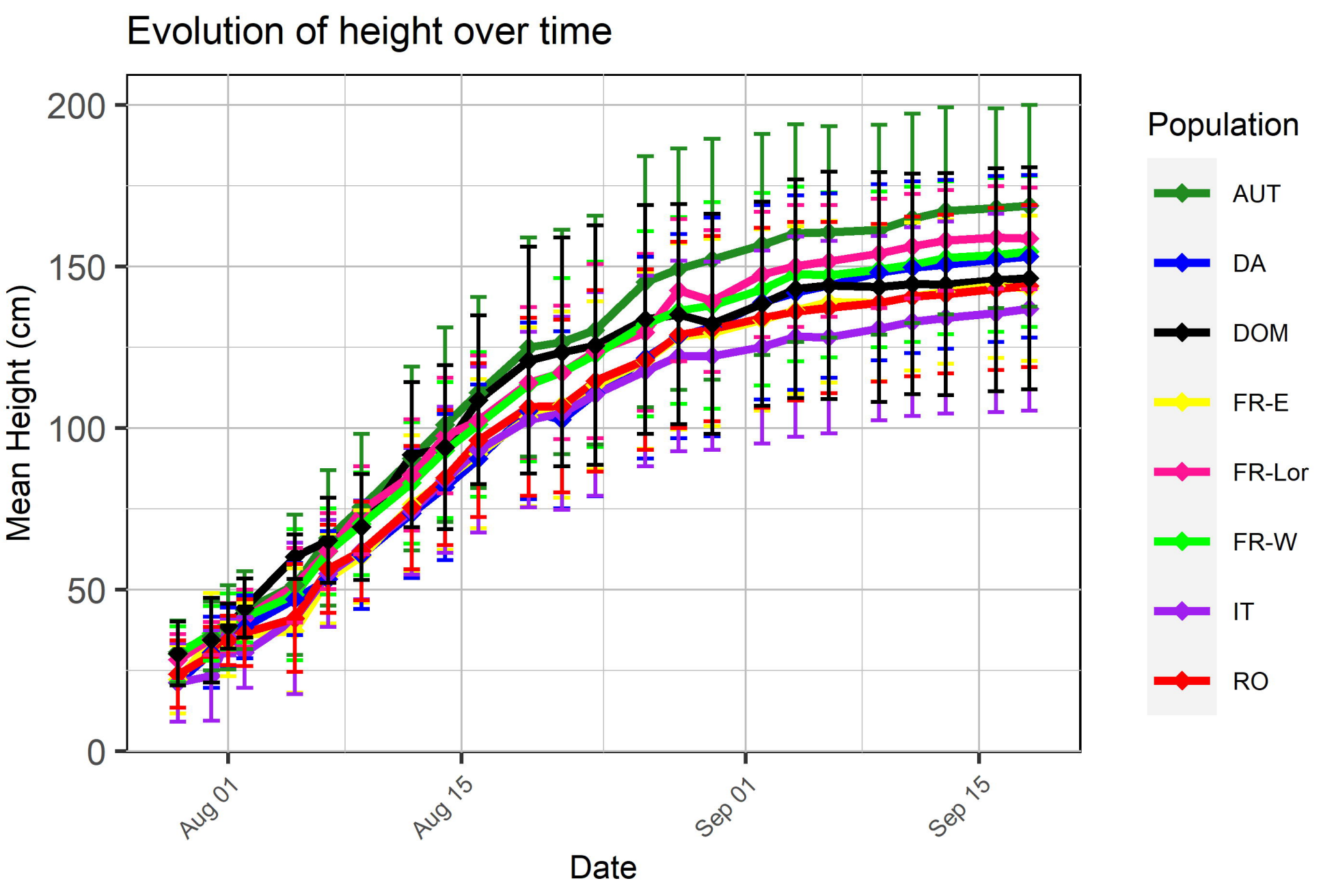
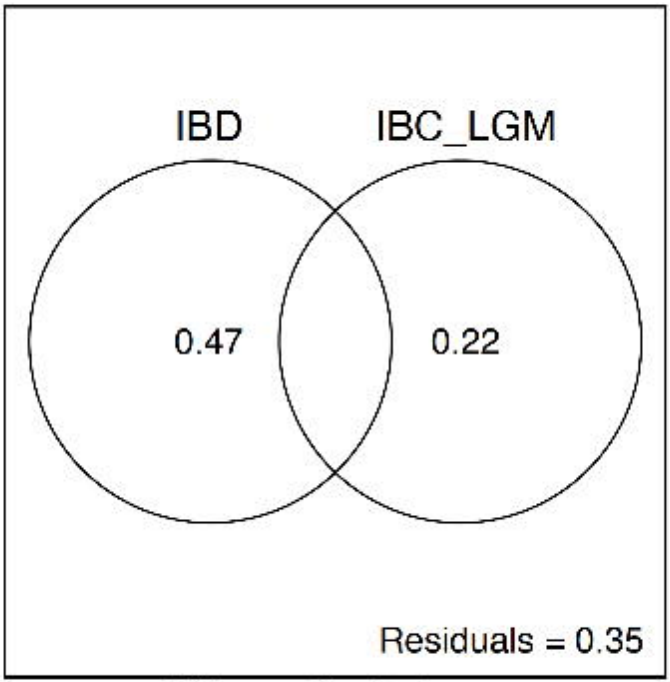
4
Malus domestica
Spain
Western France
Eastern France
Denmark
Italy
Austria
Romania


Evolution of height over time
Mean Height (cm)
200
150
100
50
0
Aug 01
Aug 15
Sep 01
Sep 15
Date
Population
AUT
DA
DOM
FR-E
FR-Lor
FR-W
IT
RO

Variance partitioning of the db–RDA results



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