No evidence for genetic differentiation in juvenile traits between Belgian and French populations of the invasive tree *Robinia pseudoacacia*

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Background – The role of evolution in biological invasion studies is often overlooked. In order to evaluate the evolutionary mechanisms behind invasiveness, both quantitative and population genetics studies are underway on *Robinia pseudoacacia* L., one of the worst invasive tree species in Europe.

Methods – A controlled experiment was set up using 2000 seeds from ten populations in Southern France and ten populations in Belgium. Seedlings were cultivated in two climatic chambers set at 18°C and 22°C. Early development life history traits (e.g. seedling phenology) and functional traits (e.g. growth rates) were monitored. Genotyping using SNP markers was used to evaluate the genetic differentiation among the populations and a $Q_{ST} - F_{ST}$ comparison was done in order to test for the role of selection.

Results – Populations exhibited a strong plasticity to temperature for all measured traits, the warmer environment being generally more suitable, irrespective of their origin. No significant departure from neutral evolution was evidenced by the $Q_{ST} - F_{ST}$ comparisons, although we found a slightly significant differentiation at the molecular level.

Conclusion – Plasticity for the functional and life history traits was evidenced but no genetic interaction suggesting no possible evolution of plasticity at those traits. Moreover, no support for genetic differentiation and local adaptation was found among studied populations within invasive range, raising two main questions: first, what is the role of selection on functional and life-history traits; and second, is the elapsed time since first introduction sufficient to allow evolution and local adaptation?

Key words – Biological invasion, $Q_{ST} - F_{ST}$ comparisons, local adaptation, selection, temperature, *Robinia pseudoacacia*.

INTRODUCTION

Biological invasions are recognized as a major threat on biodiversity (Heywood 1989, Vitousek et al. 1996, 1997, Pimentel et al. 2001). Most studies have been concentrating on ecological aspects, and the role of Darwinian evolution was often overlooked in the study of invasive species (Colautti & Lau 2015). However, one interesting aspect of biological invasions is that they represent a natural experiment allowing to study evolutionary processes (Lee 2002, Prentis et al. 2008, Colautti & Lau 2015). Invasive species are indeed an opportunity to study contemporary evolution, several decades or hundreds of years after their introduction in a new range (Keller & Taylor 2008, Colautti & Lau 2015).

Following introductions, species may have experienced modifications in their selection regime (Keller & Taylor 2008). Genetically-based changes in phenotypic traits have already been observed (Lee 2002, Blair & Wolfe 2004, Maron et al. 2004, Keller & Taylor 2008, Barrett et al. 2008, Colautti & Barrett 2013, Monty et al. 2013); for instance, a latitudinal cline in flowering period similar in both the native and invasive ranges was evidenced for populations of *Lythrum salicaria* L. (Barrett et al. 2008). Using reciprocal transplant experiments with three common gardens to evaluate phenotypic changes (Colautti & Barrett 2013) and genetic structure analysis (Chun et al. 2009), Colautti & Barrett (2013) demonstrated that local adaptation in the new range

was the most likely process explaining the observed differentiation between invasive populations of this species.

Phenotypic plasticity is another mechanism that could enhance introduced populations' establishment (Agrawal 2001, Richards et al. 2006). Indeed, thanks to plasticity, organisms could express favourable phenotypes under a wider range of environments and maintain a higher fitness (Richards et al. 2006). Consequently, plasticity could be an important factor promoting invasions success (Davidson et al. 2011, Richards et al. 2006). In this context, phenotypic plasticity is defined at the population level (Valladares et al. 2006), as the ability of genetically-related individuals to express differences in phenotypes under different environments; this level of genetic diversity is particularly relevant for understanding the ecological role of plasticity in natural populations (Monty et al. 2013, Richards et al. 2006).

Coutts et al. (2011) underlined, using a modelling approach, the importance of understanding seedling development and survival for woody invasive relatively to herbaceous ones. Germination, although the earliest stage in plant development, influences survival, fitness and adaptive capacity of plants (Donohue et al. 2010). It has been demonstrated that natural selection acted on germination timing in response to the season of seeds dispersal in the model plant *Arabidopsis thaliana* (Donohue et al. 2005). On the same species, a reciprocal transplant experiment using recombinant inbred lines demonstrated that selection occurring during germination and juvenile stages promoted local genotypes and favoured local adaptation (Postma & Ågren 2016). Therefore, understanding germination is critical for understanding local adaptation of populations (Postma & Ågren 2016), and seems particularly relevant to evaluate on newly introduced populations.

At large spatial scales, temperature is a major factor controlling species distributions (Woodward 1987, De Frenne et al. 2010) and alteration in temperature can dramatically modify tree species distributions and impact their fitness (Prentice et al. 1992, Iverson & Prasad 1998). Moreover, temperature interacts strongly with germination and seedlings recruitment by driving dormancy (i.e. initiation and break), germination phenology or seedlings vigour (Walck et al. 2011).

Among the 100 worst invasive species in Europe, seven trees and shrubs were identified (DAISIE 2009) among which *Robinia pseudoacacia* L. (Fabaceae). Black locust is a deciduous tree species, native to North America. The first European introduction in 1601 in Paris was often attributed Jean Robin, the botanist of the French King Henri IV. However, this date is discussed and it seems that the tree was probably introduced later, around 1634 (Cierjacks et al. 2013, Vítková et al. 2017), in England and France simultaneously. It is now widely distributed in natural lands throughout the continent (DAISIE 2009). In Western Europe, the species grows under a wide range of temperatures; adaptive divergences among European populations in response to this abiotic constraint can thus be hypothesized.

Our objective was to test for genetic differentiation and local adaptation to temperature in the European invasive range of *R. pseudoacacia* by comparing juvenile growth of 20 populations sampled in Wallonia (Belgium) and Aquitaine (southern France). Specifically, we will address the following questions:

(i)   Can we detect plasticity on the studied life history traits and functional traits at the population level?
(ii) Are there phenotypic differences among populations in relation to temperature at their location of origin?
(iii) Can we detect any departure from neutral evolution on the life history traits and functional traits?

In order to test for local adaptation within ranges, a multiple common gardens experiment and a quantitative genetic analysis of $Q_{ST} - F_{ST}$ comparisons were combined (Keller & Taylor 2008). In common garden experiments, local adaptation is revealed when populations grown in their original environment outperform other populations. Moreover, the comparison of phenotypic Q_{ST} and genotypic F_{ST} differentiation indexes allows to propose some evolutionary inferences: under neutrality, the expectation is that Q_{ST} would be similar to F_{ST}, a Q_{ST} value significantly higher than a F_{ST} value would indicate a divergent selection acting among populations; on the contrary, a Q_{ST} value inferior to a F_{ST} value would signal a stabilizing selection (Whitlock & Guillaume 2009, Leinonen et al. 2013, Colautti & Lau 2015). The two methodological approaches are complementary to understand the evolutionary forces acting on traits.

MATERIALS AND METHODS

Sampling

Between January and March 2015, ten populations of *R. pseudoacacia* were sampled per range, in the Belgian region of Wallonia and in the French region of Aquitaine (fig. 1, electronic appendix 1). We sampled unplanted populations derived from natural regeneration. Mean, Minimal and Maximal temperatures of May were extracted from WorldClim version 2 (http://worldclim.org/version2) using the 30 seconds resolution raster. WorldClim version 2 (Fick & Hijman 2017) provides average monthly climate data for minimum, mean, and maximum temperature over the 1970–2000 period. The different populations were spread over geographic areas of the same dimensions (i.e. Gironde and Lot et Garonne, French departments in Aquitaine: 15 000 km² and Belgian Wallonia: 16 000 km²). Overall, French and Belgian populations were distant almost 800 km from one another. In each population, 10 to 100 pods were collected on ten trees with a tree pruner. Given that the species is known to spread by root-suckering (Cierjacks et al. 2013), a minimal distance of twenty meters was kept between two sampled trees in order to minimize the risk of collecting the same genotype.

Seeds were manually extracted from pods and placed into a tea paper filter to be stored in a cold room at 0–5°C (Forest Research 2015, Royal Botanic Gardens Kew 2015). Only well conserved seeds (i.e. seeds without mould stains or without damaged tegument) were kept and seeds with moist or empty appearance were systemically eliminated. Seeds were counted for each maternal tree and weighted with a 0.1mg accuracy (Practum 224-1S, Sartorius, Goettingen, Germany).

Experimental design for phenotypic measurements

A controlled experiment was set up in two climatic chambers (Fitoclima D1200, Aralab, S Domingos de Rana, Portugal) with a total of 2000 individuals: five seeds corresponding to half-sibs × 10 maternal trees × 10 populations × 2 geographic range × 2 climatic chambers. Prior to seeding, seeds were mechanically scarified using an automated sand blasting technique (Bouteiller et al. 2017) to ensure a controlled dormancy breaking. Seeds were sowed into plastic trays (QuickPot 35RW, HerkuPlast Kubern GmbH, Ering, Germany); each pot was filled with 25 g of substrate (Substrate 307, Peltracom, Gent, Belgium) plus 6 g after sowing to cover the seeds (Bonner & Karrfalt 2008).
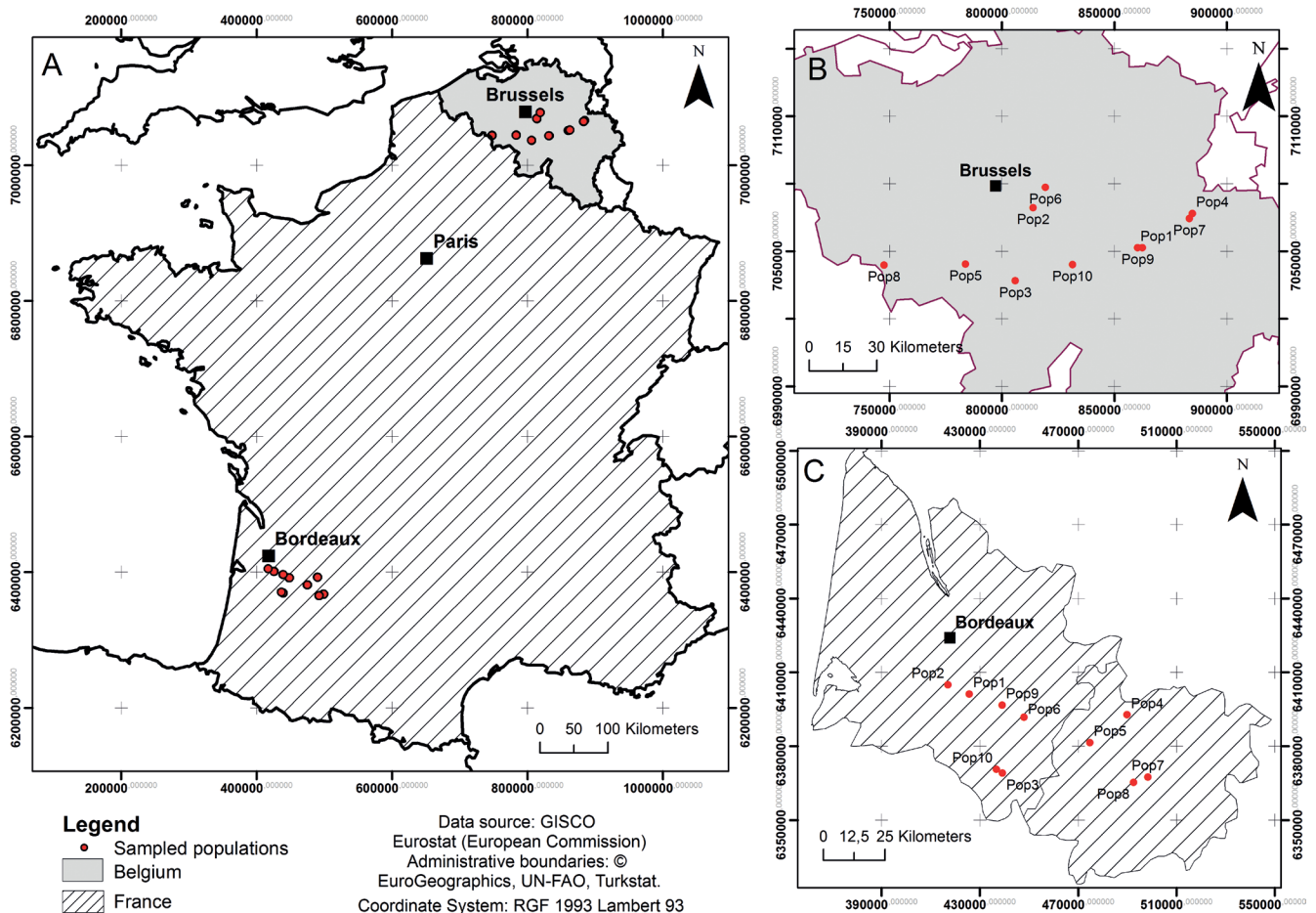
Environmental conditions within both chambers were set equal except for air temperature: a day/night 12/10 h photoperiod with progressive day/night transitions of one hour, 60 ± 5% of air relative humidity, 185 ± 45 µmol.m^2.s^{-1} photosynthetic photon flux density (LiCOR Li190, Lincoln, USA). CO_2 concentration was equal to ambient atmospheric concentration. Temperature conditions were corresponding to the average maximal temperature of May as measured at the weather station of INRA in Villenave d'Ornon (Aquitaine, France, 1987–2007) and at the weather station of Uccle (Brussels-Capital, Belgium, 1981–2010): 22°C/20°C day/night temperature in the first climatic chamber (French temperature conditions) and 18°C/16°C day/night temperature in the second climatic chamber (Belgian temperature conditions).

Watering (50 mL) was provided every two days in order to supply a non-limiting resource. After 31 days, seedlings were fertilized with a liquid fertilizer (NPK 7.5.6, Florendi Jardin SAS, Dinard, France), renewed twice every 10 days.

Phenotypic life history traits and functional traits

Life history traits – Germination and seedling phenology were monitored daily for each individual over a period of 440 growing degree days (GDD). GDD were calculated by multiplying the climatic chamber day temperature condition (in °C) and the number of days spent in the climatic chamber, base temperature was considered equal to 0 since no reference value was found for black locust. Five phenological stages have been defined as follows: 1, emergence; 2, straight stem; 3, open cotyledons; 4, first leaf; 5, second leaf (see Bouteiller et al. 2017 for details and photographs of the phenological stages). According to air temperature, the phenological survey was stopped after 20 days in the warmer climatic chamber and after 25 days in the colder cli-

Figure 1 – A, map of France and Belgium presenting the sampled populations with a highlight of: B, Wallonia's Belgian populations; and C, of Aquitaine's French populations. © EuroGeographics for the administrative boundaries.

matic chamber. For each phenological stage and each individual, the minimum number of days required to reach the stage was noted. When a stage was missing, the number of days was calculated as the average between the numbers of days required to reach the previous and the stage. When an individual died after germinating, it was further recorded as missing value.

Functional traits – At the end of the phenological survey (440 GDD), total height was measured from collar to the apical bud using a ruler (cm ~1mm). Seedlings were thinned from 5 to 3 plants per pot in order to avoid competition, preferentially removing damaged individuals. After 1342 GDD corresponding to 61 and 75 days in the 22°C and 18°C climatic chamber, respectively, total eight (cm ~1mm), stem collar diameter (mm ~ 0.01mm) and the effective quantum yield of photosystem II (PSII yield) were measured using a ruler, an electronic calliper and a portable chlorophyll fluorometer (PAM 2100, Heinz Walz GmbH, Effeltrich, Germany) respectively.

The PSII yield was measured on the terminal leaflet of the youngest fully-developed. This trait provides information about the proportion of light absorbed by the PSII that is used in photochemistry (Genty et al. 1989, Santiso et al. 2015).

Then, seedlings were separated into leaves and stem, and oven dried at 65°C (Universal oven, Memmert, Swabach, Germany). After minimum one drying week to reach constant weight, leaves and stem were weighted with a 0.1 mg accuracy (Practum 224-1S, Sartorius, Goettingen, Germany). Growth rates in height, diameter and leaf and stem biomass were calculated by dividing the trait value by the number of days spent in each climatic chamber. Data are accessible on Open Science Framework repository (https://osf.io/q3ebp/).

DNA extraction and SNP genotyping

In order to evaluate molecular genetic diversity, a 1 cm^2 leaf sample was collected on one individual per family sampled randomly among the thinned individuals. DNA was extracted and isolated using DNAeasy 96 Plant Kit (Qiagen, Venlo, Netherlands) following the manufacturer's protocol. One negative control was set on each plate. DNA concentration was measured using an UV spectrophotometer NanoDrop 8000 (Thermo Fisher Scientific Inc., Wilmington, Delaware, USA) and confirmed using QuantiFluor dsDNA System (Promega, Fitchburg, Wisconsin USA). Besides DNA concentration, 260/280 and 260/230 absorbance ratio provide information about DNA purity. Since the concentration was about 10ng/µL, DNA was further used for SNP amplification without dilution. SNPs were recently developed on black locust using the RADseq approach (Verdu et al. 2016). Four multiplexes of 36 SNPs each were further designed with the MassArray assay design 4.1 software and genotyped with the Sequenom MassARRAY iPLEX platform at the Bordeaux Genome Transcriptome facility (https://pgtb.cgfb.u-bordeaux.fr), using the iPLEX Gold genotyping kit according to the manufacturer's instructions. Products were then analysed using the MassArray mass spectrophotometer (Sequenom) and data were acquired in real time using the MassArray RT software.

SNP data were then visualized and validated using ViClust, a R program that we implemented for Galaxy (https://usegalaxy.org/) and that is also available as a standalone R script for Linux or Windows at https://github.com/garniergere/ViClust/. Briefly, ViClust imports the unmodified xml file to export from the Typer-4_0_20 in-house Sequenom software. It then allows to visualize the clusters proposed by the Sequenom method based on Gaussian mixtures (Johansen et al. 2013), proposes additional and more or less stringent filters on the different allele signal magnitudes, and performs an alternative hierarchical clustering method using the Ward algorithm (Ward 1963). This method relaxes the constraining assumption of normal distribution for allele signals. The program gives SNPs plots across the different alternatives in batch, allows comparing and validating each SNPs more easily, and exports automatically the proposed genotype assignments, making the whole process much less time consuming that using Typer-4_0_20. Out of the initial 144 SNPs genotyped, 132 SNPs were finally, kept for analyses. Data are accessible on Open Science Framework repository (https://osf.io/q3ebp/).

Data analyses

Molecular genetic differentiation was explored using two approaches. First, the typology of populations was assessed using a Correspondence Analysis (CA) on SNP data; it was implemented using the adegenet library (Jombart 2008) in R version 3.2.2 (R Development Core Team 2015). As recommended by Jombart et al. (2009), rare alleles, defined as those recorded less than 5% of the total allele number for each marker, were removed from the analysis.

Second, population structure was analysed using STRUCTURE v2.3.4 (Pritchard et al. 2000). Using the admixture model with no a priori hypothesis, 20 independent Markov chains, each with K (number of assumed clusters) values allowed to range from 1 to 20, were run with a burn-in period of 500,000 iterations followed by 1 500 000 iterations. STRUCTURE runs were computed on the GenoToul bioinformatics facility (http://bioinfo.genotoul.fr/). The script StrAuto (Chhatre and Emerson 2017) was used to produce STRUCTURE mainparams and extraparams and to automatize and parallelise STRUCTURE analysis. STRUCTURE HARVESTER (Earl & vonHoldt 2012) was employed to calculate ΔK as described in Evanno et al. (2005) in order to determine the most likely K. Finally, CLUMPAK (Kopelman et al. 2015) was utilised to synthetize STRUCTURE outputs and to compute graphical STRUCTURE outputs.

To evaluate the germination rate of *R. pseudoacacia* seeds among ranges and temperatures, we constructed a germination curve on the fraction of germinated seeds, $G(t)$ (for more details see Bouteiller et al. 2017). A Gompertz model was fitted using a Bayesian procedure (equation 1). The code used in this study is provided on the open source GitHub platform (https://github.com/xbouteiller/GompertzFit).

$$G(t) = D * \exp(-\exp(-b(t-t_m))) \qquad (1)$$

where parameter D equals the maximum germination rate, b reflects the slope of the germination curve and t_m is the time at the inflexion point.

To estimate differentiation between populations in life history traits and functional traits between climatic chambers (i.e. the temperature effect), a Bayesian mixed model of ANCOVA was fitted for each trait, using the mothers' average seed weight as covariate. There is indeed a significant difference (electronic appendix 3A) among ranges for seed weight. French families' average seed weight (23.84 ± 3.33 mg ranging from 13.79 mg to 31.65 mg) is significantly higher than Belgium families' average seed weight (18.99 ± 3.34 mg ranging from 11.47 mg to 26.02 mg). Moreover, there are significant differences among populations (electronic appendix 3B) with some populations presenting heavier (e.g. Fr.Pop4, Fr.Pop1) and lighter (e.g. Bel.Pop8) seed weights.

The between-chambers model was defined as:

$$\underbrace{Y_{ijklm}}_{trait\ value} = b_o + \underbrace{b_{1,i}}_{temp} + \underbrace{b_{2,j}}_{range} + \underbrace{B_{3,jk}}_{population} + \underbrace{B_{4,jkl}}_{tree} + \underbrace{B_{5,ijk}}_{temp \times pop}$$

$$+ \underbrace{c\left(x_{ijkl} - \overline{x}\right)}_{maternal\ effect} + \varepsilon_{ijklm} \qquad (2)$$

With the residual as:

$$\varepsilon_{ijklm} \sim N\left(0, \sigma^2\right) \qquad (3)$$

Lowercases indicate the fixed effects and uppercases indicate random effects, with

$$i \in 1,2$$

for the 18 and 22°C temperature chamber conditions;

$$j \in 1,2$$

for the two ranges: Belgium, France;

$$k \in \llbracket 1, 10 \rrbracket$$

for the 10 populations in each range;

$$l \in \llbracket 1, 10 \rrbracket$$

for the 10 trees per population; and

$$m \in \llbracket 1, 5 \rrbracket$$

the 5 individuals (seeds) per tree.

x_{ijkl} represents the seed weight at the tree (i.e. family level) and $\overline{x}$ the global mean seed weight.

We used JAGS 3.4.0 (Plummer 2005), the R2jags package of R (Su & Yajima 2012) and R version 3.2.2 (R Development Core Team 2015) to compute the Bayesian model. For each life history traits and functional traits, 150,000 iterations were run with a burn-in of 125 000 iterations and a thinning interval of 10, using 4 different chains. Autocorrelation and convergence were assessed using the "autocorr. plot" and "gelman.plot" native JAGS functions and Rhat convergence criterion was inferior to 1.01 as recommended (Kruschke 2014).

Codes provided by O'Hara & Merilä (2005) and Kruschke (2014) were adapted to our design using uninformative inverse gamma conjugate priors for random effects and normal priors for fixed effects.

Genetic differentiation among populations can be defined from molecular markers analyses using F_{ST} indices calculated as follows (Wright 1949):

$$F_{ST} = \frac{\sigma_B^2}{(\sigma_B^2 + \sigma_W^2)} \qquad (4)$$

The F_{ST} value for the whole dataset was estimated using the HICKORY 1.1 software (Holsinger 1999). F_{ST} was estimated by running the software native's full model (i.e. assuming non-null inbreeding coefficient and significant genetic structure among populations) and compared to the non-inbreeding and no population structure models, as recommended by Holsinger (1999). The full model outperformed the other models, based on the DIC criterion values (see HICKORY documentation for more details on the models). Pairwise F_{ST} were calculated between all pairs of the 20 populations using Genepop v4.3.3 (Raymond & Rousset 1995, Rousset 2008).

For each quantitative life history traits and functional traits, the Q_{ST} index of quantitative genetic differentiation among populations was calculated as follows (Spitze 1993):

$$Q_{ST} = \frac{\sigma_B^2}{(\sigma_B^2 + 2\sigma_W^2)} \qquad (5)$$

where

$$\sigma_W^2$$

is the within population genetic variance and

$$\sigma_B^2$$

is the between population genetic variance estimated using

$$\widehat{\sigma}_{pop}^2$$

Assuming that individuals obtained from seeds of the same tree were half-sibs, within population variance can be estimated as

$$\widehat{\sigma}_W^2 = 4\widehat{\sigma}_{tree}^2$$

Q_{ST} values were computed per temperature chamber condition using the same model that presented above (equation 1) but without the temperature and temperature x population interaction effects.

The code used in this study is provided on the open source GitHub platform (https://github.com/xbouteiller/BayesMix). It was tested by comparison to the same model computed with SAS software using the frequentist PROC MIXED procedure (SAS, version 9.1, SAS Institute, Cary, NC, USA); Bayesian Q_{ST} estimations (mean + CI) were compared to estimates using first the delta method computed with SAS (O'Hara & Merilä 2005, Isik 2009) and second using the R package QstFstComp (Gilbert & Whitlock 2015). Comparisons are presented in electronic appendix 2. Results were similar in almost all traits. Frequentist methods can estimate weak negative variances, when those are close to zero at the population level, which we found in preliminary explorations. Given that our choice of priors in the Bayesian models (with positive support) made such negative estimates of variances impossible, and that the models showed overall a good performance, we adopted a Bayesian approach to estimate Q_{ST} (O'Hara & Merilä 2005).

<div style="text-align:center">RESULTS</div>

Molecular genetic differentiation: a weak structure and few outlying populations

CA on SNP data was first characterized by the grouping of most of the populations, both French and Belgian (fig. 2A & B). Nevertheless, respectively three populations (Fr.Pop2, 3, 10) and five populations (Fr.Pop3, 8 and Bel.Pop2, 7, 8) were separated from this melted group of populations when considering either the first two axes or axes 1 and 3. Fig. 2 shows the major mode identified by CLUMPAK on 20 STRUCTURE runs for K = 2 (fig. 2C). Higher ΔK was obtained for K = 2. Thus optimal clustering was most likely for K = 2 clusters (Earl & vonHoldt 2012, Evanno et al. 2005). Structure analysis exhibited a similar pattern than the one observed in the CA: a weak population structure has been revealed. Indeed the STRUCTURE analysis revealed a very

high level of admixture in most populations: less than 3.5% of the individuals had an inferred ancestry higher than 90%.

Genetic vs environment effect on life history traits and functional traits: a strong response to temperature without genetic interaction

Germination curves per range and temperature as well as the fitted Gompertz model are presented on fig. 3. Germination was faster under the warmest temperature condition but maximum germination rates, D, were similar whatever the temperature and the range: total germination rate was established at 84.9 ± 7.4%.

Robinia pseudoacacia seedlings demonstrated a strong positive response to temperature for all considered traits, whatever the populations and range of origin in Europe. Fig. 4A–L show the distributions of trait values within each
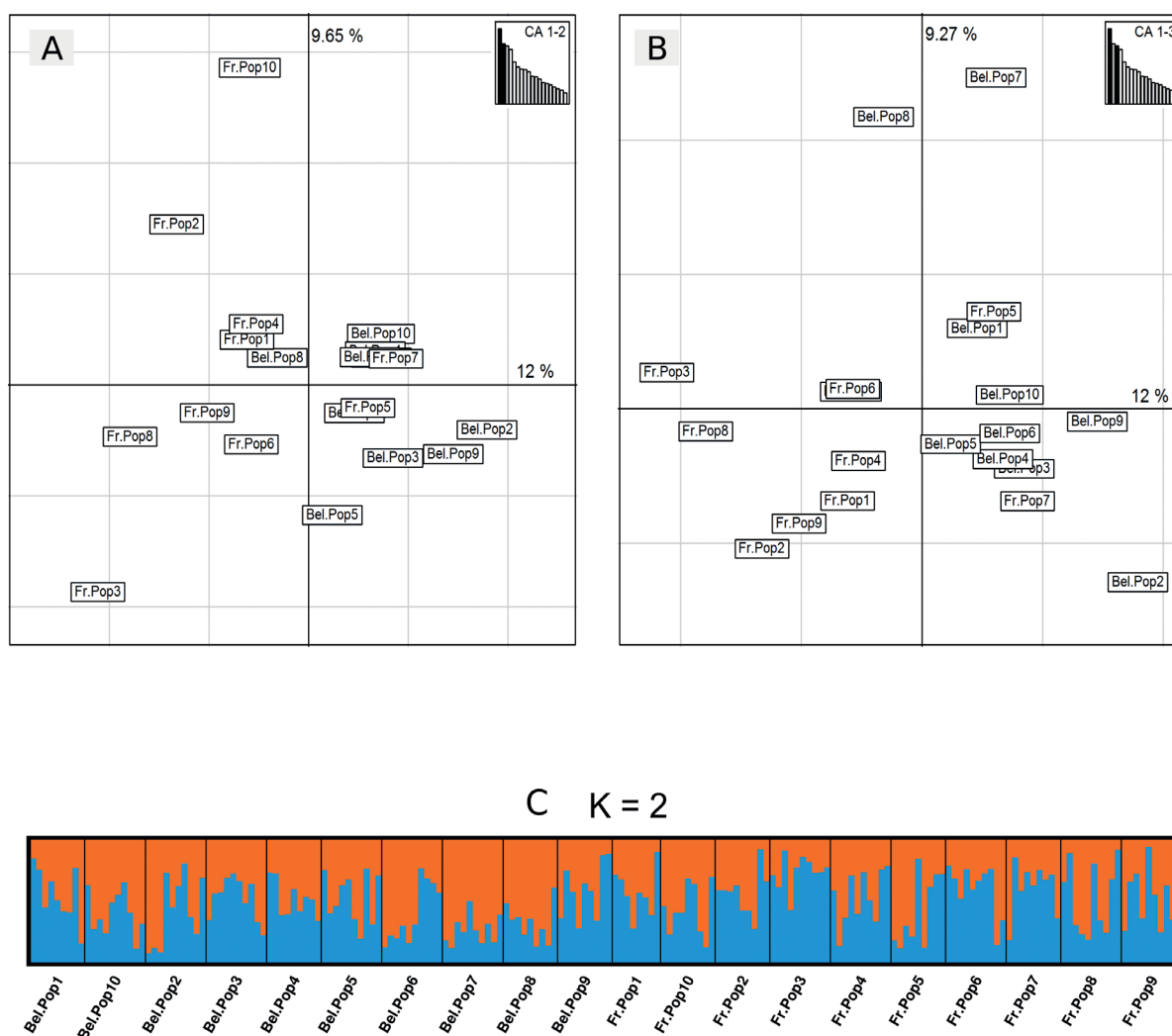

Figure 2 – Correspondence analysis (A & B) and STRUCTURE analysis (C) and on SNP genotyping data. For the Correspondence Analysis, first (A) axis 1 was plotted against axis 2, second (B) axis 1 was plotted against axis3. First axis contains 13% of variation, second axis contains 10.3% of variation and third axis contains 9.54% of variation. STRUCTURE plots represent major mode for K = 2 (C) identified by CLUMPAK on 20 STRUCTURE runs for 190 individuals from 20 populations using 132 SNP markers. K = 2 corresponds to the optimal K number based on ΔK calculated using STRUCTURE HARVESTER. Labels are indicating sampled populations. Fr: French, Bel: Belgian.

Table 1 – Mean values (± standard deviations) of all the surveyed traits within each climatic chamber (i.e. 18°C and 22°C).
Bold characters indicate significant differences between chambers in response to temperature. The temperature effect was always significant except for the biomass traits. GR: Growth Rate

Climatic chamber	18°C	22°C
Stage 1 (d)	8.2 ± 2.5	6.0 ± 1.7
Stage 2 (d)	10.0 ± 2.8	7.5 ± 1.7
Stage 3 (d)	13.0 ± 3.0	9.6 ± 2.1
Stage 4 (d)	20.0 ± 2.7	14.0 ± 1.9
Stage 5 (d)	25.0 ± 1.5	18.0 ± 1.5
Height GR 1 (cm.d^{-1})	0.16 ± 0.034	0.2 ± 0.045
Height GR 2 (cm.d^{-1})	0.15 ± 0.055	0.18 ± 0.059
PSII Yield	0.0014 ± 0.0011	0.003 ± 0.0012
Leaf weight GR (g.d^{-1})	0.0032 ± 0.002	0.0034 ± 0.0023
Stem weight GR (g.d^{-1})	0.0010 ± 0.00073	0.0011 ± 0.00099
Total weight GR (g.d^{-1})	0.0042 ± 0.0027	0.0045 ± 0.003
Diameter GR (mm.d^{-1})	0.026 ± 0.0066	0.030 ± 0.0088

climatic chamber and among populations: a strong response to temperature could be evidenced, but no clear population effect or range effect was easily identifiable. Seedling development was faster under warmer temperature conditions (fig. 5, electronic appendix 4A–La). For example, (table 1), number of days for seeds to germinate (to reach phenological stage 1) was 6.0 ± 1.7 days at 22°C versus 8.2 ± 2.5 days at 18°C; similarly, time to reach phenological stage 5 with a fully developed composed leaf was shorter at 22°C compared to 18°C (18 ± 1.5 days and 25 ± 1.5 days, respectively). For functional and physiological traits, increases in trait values with increases in temperature were observed for most of the traits: height and diameter growth rates were significantly higher at 22°C as well as PSII yield (table 1). However, biomass showed no significant increase with increased temperature.

Neither a genetic nor a genetic x environment interaction could be found, irrespective of the trait (fig. 4, electronic appendix 4A–L b & c). The genetic variability was observed at the tree level for all traits (electronic appendix 4A–L d), variances between trees being 18 to 37 times higher than variances between populations. For instance, for phenological stage 3 and diameter growth rate, variance between trees equalled and , whereas variance between populations only represented and . Finally, as observed on fig. 5 and in electronic appendix 4d, no temperature × population interaction was observed. The temperature × population interaction variance was of the same magnitude than the between populations variance, and always lower than the between trees variance, for all traits.

To sum up, populations exhibited a strong plasticity to temperature for all measured traits, the warmer environment being generally more suitable. Thus, at the population and range levels, similar responses have been observed in response to an increase in temperature. Belgian populations did not outperform French populations when in their natural temperature range and reciprocally.

Q_{ST} – F_{ST} comparisons: no evidence for deviation from neutrality

A moderate but significant genetic differentiation among populations was evidenced at the molecular level, with a F_{ST} value of 0.026 (fig. 5, 95%CrI = 0.021 – 0.030). Using all measured life history traits and functional traits, no genetic differentiation was evidenced, with Q_{ST} values not significantly different from 0 (fig. 5), whatever the temperature conditions. Beware that Q_{ST} values for phenological stage 5
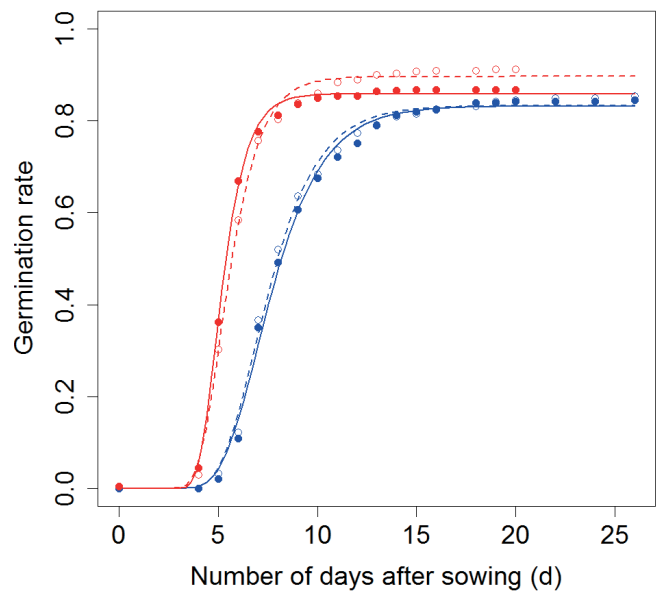

Figure 3 – Germination curves of *Robinia pseudoacacia* seeds per temperature condition (blue: 18°C, red: 22°C) and per range (solid lines, full circles: Belgian range, dashed lines, open circles: French range). Data were reported with circles and fitted curves using a Gompertz function with lines.
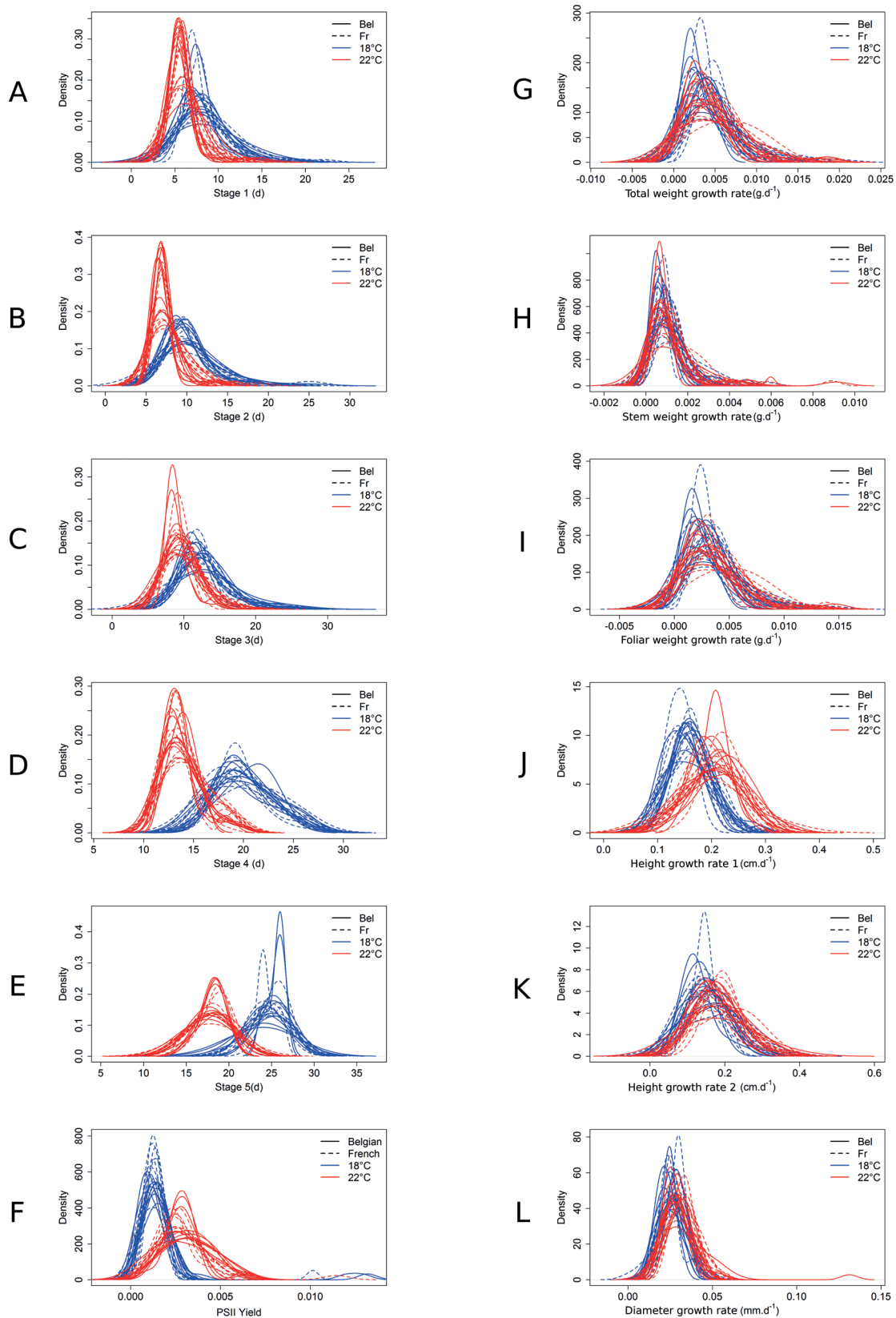
Figure 4 – Density distribution functions for each life history trait and functional trait according to the temperature conditions (red line 22°C, blue line 18°C) and the range (full line Belgium, dotted line France). A–E, phenological stages 1 to 5; F, PSII Yield, G, total weight GR, H, stem weight GR, I: Foliar weight GR, J–K, height GR 1 and 2, L, diameter GR. GR: Growth Rate.

at 18°C and stem weight increment at 22°C are not robust estimates due to a large number of missing values.

Moreover, no deviation from neutrality was evidenced for all traits among the twenty populations. Indeed, all Q_{ST} values were not significantly different from the F_{ST} value except for the PSII yield at 22°C that was significantly lower (0.0026, 95%CrI = 0 – 0.013).

DISCUSSION

A strong response to temperature for all traits

A strong response to temperature for most traits was evidenced inducing a faster phenological development and superior trait values in the warmest environment.

The strong plasticity at the population level in response to temperature observed on juvenile *R. pseudoacacia* in this study could help understand its large invasion range in Europe. Indeed, broad environmental tolerance species are more susceptible to encounter favourable conditions when facing a new environment (Davidson et al. 2011). Invasive plants are commonly more phenotypically plastic than co-occurring native species for a wide variety of traits such as growth or fitness-related traits (Maron et al. 2007, Hyldgaard & Brix 2012, Molina-Montenegro & Naya 2012, Lamarque et al. 2015). For instance, a strong plasticity in response to temperature has been demonstrated for five invasive populations of *Taraxacum officinale* F.H.Wigg. in several ecophysiological and fitness related traits (Molina-Montenegro & Naya 2012); this plasticity could be an important promoting factor of in-

vasion success. Moreover, plasticity in germination traits could be an important factor for successful establishment and persistence of invasive species impacting their spreading and persisting capacities in new spaces (Gioria & Pyšek 2017). A study comparing germination plasticity among twelve native and twelve invasive species using three environmental regimes based on temperature, day length, and soil moisture demonstrated that invasive species had higher performances (germination speed and rate) and plasticity than native species (Wainwright & Cleland 2013).

Plasticity of phenotypic traits is adaptive when it improves survival and reproduction (Richards et al. 2006). In the context of biological invasions, phenotypic plasticity could evolve rapidly during the lag phase after introduction for ecologically important traits and thus contribute to the invasion success (Agrawal 2001, Richards et al. 2006). However, it seems that the majority of phenotypic plasticity is selectively neutral (Davidson et al. 2011), although it could be particularly beneficial during the early stages of the invasion. In order to assess the adaptive role of plasticity of juvenile traits of *R. pseudoacacia* during the invasion further comparisons need to be made using both native and invasive populations (Richards et al. 2006, Davidson et al. 2011).

No signal of local adaptation among European populations of *R. pseudoacacia*

In the present study, local adaptation was tested by growing populations in climatic chambers at two different temperatures; we also compared genetic structure using molecular markers.
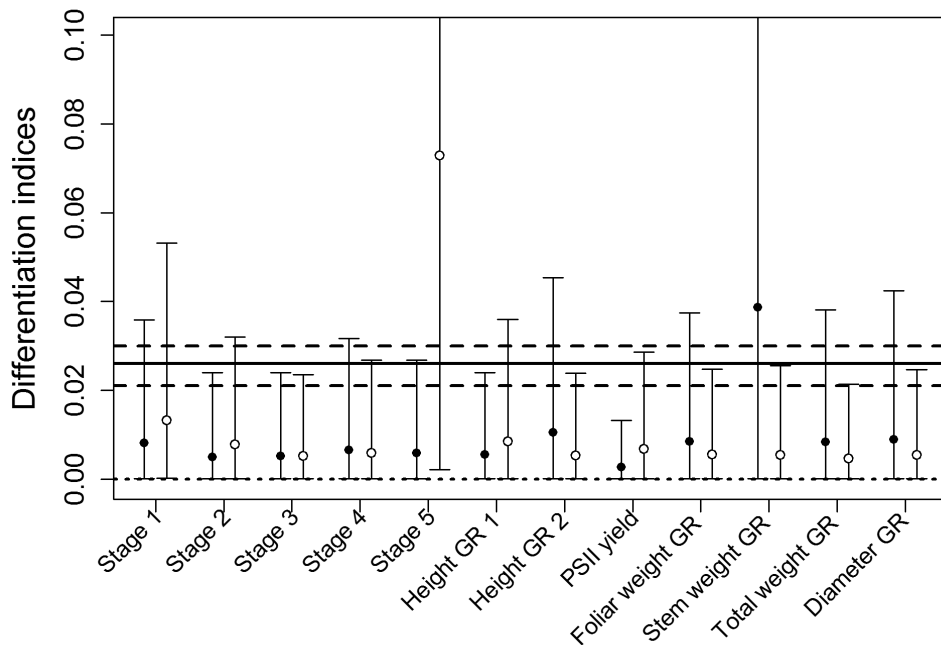

Figure 5 – Differentiation indices calculated using phenotypic life history traits and functional traits (Q_{ST}) and using SNP markers (F_{ST}). Q_{ST} values (mean and 95% confidence interval) were calculated on the 20 populations per trait and growing chamber (closed circle: 22°C, open circle: 18°C). The full line represents the mean F_{ST} and the dashed lines its 95% confidence interval based on allelic variation in SNP's loci. Estimations were calculated using Bayesian methods. GR: Growth Rate.

A weak structure was detected between French and Belgian populations since only few individuals had an inferred ancestry higher than 90%. Moreover, Belgian populations have not outperformed French populations under the coolest temperature conditions resembling the Belgian conditions, nor reciprocally for the French populations under the warmer environment. Thus, based on the measures made on very early stage life history traits and functional traits, no signal of local adaptation was evidenced among the twenty European populations studied. On the contrary, signs of local adaptation have already been observed in invasive populations of the herbaceous species *Hypericum perforatum* L. (Maron et al. 2004), *Eschscholzia californica* Cham. (Leger & Rice 2007) and *Lythrum salicaria* (Colautti & Barrett 2013). However, the opposite result was also evidenced among invasive populations of *Polygonum cespitosum* Blume (Matesanz et al. 2012) or when testing introduced populations of invasive shrubs as *Buddleja davidii* Franch. whose populations responded similarly to environmental changes but exhibited high phenotypic plasticity (Ebeling et al. 2011).

In addition, our results showed no differences between Q_{ST} and F_{ST} values: no deviation from neutrality can be inferred for the measured traits. They would only be influenced by genetic drift, mutation and migration (Keller & Taylor 2008, Whitlock 2008). However, as presented in the introduction, early life stages are likely to be under selection so we can wonder if methodological biases could impede detection of local adaptation in $Q_{ST} - F_{ST}$ comparisons. This could happen if any maternal, environmental, dominance or epistasis effects are present (Whitlock 2008, Leinonen et al. 2013). To avoid these biases, a robust 20 populations of half sibs experiment was designed, which was suitable to estimate additive variance and Q_{ST} (O'Hara & Merilä 2005, Goudet & Büchi 2006, Whitlock 2008, Leinonen et al. 2013). Similarly to our study, Merilä & Crnokrak (2001) found that life history traits could exhibit no significant divergence between Q_{ST} and F_{ST} whereas functional traits showed significant differences between Q_{ST} and F_{ST}; they suggested that life history traits contain more non additive effects, and are consequently more subject to biases downward or upward the Q_{ST}. Still, in our study, both functional and life history traits were surveyed, and we used the mean seed weight per family as a covariate to control for maternal effects (Whitlock 2008, Chun et al. 2011). As a conclusion, we can consider that methodological biases are unlikely to explain our results that are consistent across all the traits considered.

A handful studies used $Q_{ST} - F_{ST}$ comparisons to infer the role of directional selection occurring during biological invasion, and their results could therefore be compared to ours, but their results are so far contrasted. For instance, Chun et al. (2011) found a Q_{ST} for reproductive allocation superior to the F_{ST} by comparing ten invasive populations of *Ambrosia artemisiifolia* L., introduced to Europe in the second part of the 19th century, suggesting that divergent selection is acting among populations (Chun et al. 2011). Similarly, populations of the invasive *Phalaris arundinacea* L. exhibited Q_{ST} superior to F_{ST} in the invasive range (Lavergne & Molofsky 2007). Lastly, a similar pattern was observed for *Centaurea solstitialis* L. (Eriksen et al. 2012). In contrast, a biological invasion study on *Lythrum salicaria* failed to demonstrate

Q_{ST} superior to F_{ST} even though recent local adaptation in the new range was demonstrated (Chun et al. 2009, Colautti & Barrett 2013). This highlights that in the case of invasive species, Q_{ST} F_{ST} comparisons can be difficult to interpret, especially if F_{ST} is small or if highly differentiated native populations are introduced into narrower environmental range or because of the history of introduction (Colautti & Lau 2015). Other methods exist to estimate Q_{ST} such as multivariate approaches that can strengthen precision when population number is low, or could allow to disentangle genetic drift and selection with a better accuracy when neutral differentiation is high (Ovaskainen et al. 2011, Leinonen et al. 2013). Considering that we used a robust experimental design and that we observed a very weak genetic differentiation both with neutral molecular markers and with quantitative traits, we kept a trait by trait approach (O'Hara & Merilä 2005, Leinonen et al. 2013).

In our study, the most probable hypothesis is thus that evolution in the local range did not play a role yet in the genetic differentiation and local adaptation of the studied European populations of *R. pseudoacacia*. Indeed, ability to detect a $Q_{ST} - F_{ST}$ difference evolves with time since the introduction (Hendry 2002) and in young systems in which selection would not have enough time to act and to produce divergent populations, similar results with Q_{ST} not exceeding F_{ST} would be expected (Whitlock 1999). In invasion studies, it has been shown that selection and local adaptation can act fast over a few generations (Koskinen et al. 2002, Dlugosch & Parker 2008). Since its first introduction in the early 17th century in Europe, *R. pseudoacacia* probably produced a maximum of 70 generations, considering a first flowering age of approximately six years (Burns & Honkala 1990). Although that could appear to be a sufficient number of generations when compared to results observed in *Hypericum canariense* L. (Dlugosch & Parker 2008), the number of generations required to evidence local adaptation through adaptive evolution may be increased in *R. pseudoacacia* due to the contribution of its clonal reproduction. To fully conclude on the role of evolution in shaping the diversity of *R. pseudoacacia* in Europe, a broader sampling including both the native and invasive range would be required (Keller & Taylor 2008, Colautti & Lau 2015).

CONCLUSION

In this study we studied the phenotypic differentiation of the invasive tree *Robinia pseudoacacia* among 20 populations from two parts of the invasive range (i.e. Aquitaine, France and Wallonia, Belgium). We followed germination and juvenile stages development. We demonstrated that most variability was within populations at the family (i.e. tree) level without significant differentiation among populations. Moreover, a $Q_{ST} - F_{ST}$ comparison highlighted no deviation from neutrality. Studies including a broader sampling both in the native and invasive ranges are needed to investigate the role of evolution during the invasion by *R. pseudoacacia*.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingenta-connect.com/content/botbel/plecevo/supp-data) and consist of: (1) summary of the sampled populations with their geographic coordinates; (2) results of the mixed model computed using frequentist delta method with SAS and comparisons of the estimated Q_{ST} computed with 3 different methods; (3) results of the Bayesian analysis for the seed weight trait; and (4) results of the Bayesian analyses for all phenotypic traits computed using the full between chambers mixed model.

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AUTHORS CONTRIBUTIONS

L. Lassois, S. Mariette, A. Monty, A.J. Porté and C. Verdu conceived the experiments; X.P. Bouteiller conducted the experiments with the contribution of C. Verdu, S. Mariette, A.J. Porté, A. Raimbault and R. Segura; P. Garnier-Géré, N. Harmand, Y. Laizet and S. Mariette developed the program ViClust for SNP validation; X.P. Bouteiller analysed the SNP and phenotypic data with the contribution of A. Raimbault, S. Mariette, F. Barraquand and A.J. Porté; X.P. Bouteiller, S. Mariette and A.J. Porté wrote the paper, S. Mariette and A.J. Porté are both senior authors of the study. All authors read and approved the final version of the manuscript. They declare no conflict of interest.

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