





## Research paper

# Inducibility of chemical defences in young oak trees is stronger in species with high elevational ranges

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Elevational gradients have been highly useful for understanding the underlying forces driving variation in plant traits and plant–insect herbivore interactions. A widely held view from these studies has been that greater herbivory under warmer and less variable climatic conditions found at low elevations has resulted in stronger herbivore selection on plant defences. However, this prediction has been called into question by conflicting empirical evidence, which could be explained by a number of causes such as an incomplete assessment of defensive strategies (ignoring other axes of defence such as defence inducibility) or unaccounted variation in abiotic factors along elevational clines. We conducted a greenhouse experiment testing for inter-specific variation in constitutive leaf chemical defences (phenolic compounds) and their inducibility in response to feeding by gypsy moth larvae (*Lymantria dispar* L., Lepidoptera) using saplings of 18 oak (*Quercus*, Fagaceae) species. These species vary in their elevational distribution and together span >2400 m in elevation, therefore allowing us to test for among-species elevational clines in defences based on the elevational range of each species. In addition, we further tested for elevational gradients in the correlated expression of constitutive defences and their inducibility and for associations between defences and climatic factors potentially underlying elevational gradients in defences. Our results showed that oak species with high elevational ranges exhibited a greater inducibility of phenolic compounds (hydrolysable tannins), but this gradient was not accounted for by climatic predictors. In contrast, constitutive defences and the correlated expression of constitutive phenolics and their inducibility did not exhibit elevational clines. Overall, this study builds towards a more robust and integrative understanding of how multivariate plant defensive phenotypes vary along ecological gradients and their underlying abiotic drivers.

**Keywords:** climate, defences, elevation, herbivory, phenolic compounds, *Quercus*.

## Introduction

Understanding the factors and mechanisms governing species interaction strength represents a major challenge in ecology (Linhart and Grant 1996). A powerful approach in this regard has been to study species interactions along environmental or geographical (i.e., latitudinal or elevational) gradients (Schemske et al. 2009). This gradient-based approach has been particularly

useful for studying interactions between plants and their insect herbivores, providing important insights and new theory in recent years (Anstett et al. 2016, Moreira et al. 2018a, Pellissier et al. 2018). With respect to elevation, it has been proposed that greater insect herbivory under warmer and less variable climates at low elevations is accompanied by stronger herbivore selection on plant defences (Galmán et al. 2018, Moreira et al. 2018a).

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This, in turn, has presumably resulted in presumably greater plant investment in defences compared with high elevations (Rasman et al. 2014a). Yet, recent work has questioned these predictions, opening a new debate on the underlying drivers of observed patterns. A review by Moreira et al. (2018a) reported that plant defences and herbivore damage decrease with elevation in 48% and 60% of cases (respectively), whereas insect herbivore abundance and species richness decrease with elevation in 48% of cases. Overall patterns thus indicate that the predicted association between elevation and plant defences is not universal and exhibits inherent variability due to unaccounted factors.

Variation among studies in the strength and direction of elevational gradients in herbivory and plant defences could be explained by differences in how plant defences are quantified and the type of traits measured. First, studies often assume that levels of constitutive plant secondary metabolites or physical defences are strongly associated with elevational variation in herbivore preference or performance (Rasman et al. 2014b). However, other defensive strategies such as induced defences (i.e., those that are only expressed after herbivore damage) are rarely considered but could nonetheless also co-vary with elevation (e.g., Pellissier et al. 2016, Defosse et al. 2018). Second, there may be negative (e.g., trade-offs) or positive correlations between defensive strategies depending on the costs and benefits of each under different ecological contexts (Agrawal 2000), such that two or more defensive strategies may co-vary along an elevational gradient. Ignoring correlations between defensive strategies may lead to erroneous conclusions about the nature and presence of elevational clines (Pellissier et al. 2016, Defosse et al. 2018). In particular, trade-offs between constitutive and induced defences have often been observed (Kempel et al. 2011, Rasman et al. 2011, Moreira et al. 2014), and shifts in the relative allocation to each strategy are likely to occur along environmental gradients (Moreira et al. 2014). Environments with low herbivore pressure (presumably at high elevations under the prevailing view) might select for low constitutive defences and high induction because the costs of continuously producing constitutive defences are high when damage is low and not persistent (i.e., costs of constitutive defences outweigh their benefits; Zangerl and Rutledge 1996). In contrast, environments with high herbivore pressure (at low elevations) would select for high constitutive but low induced defences because the fitness benefits of reduced losses to herbivory outweigh the costs of continuous production of constitutive defences (Zangerl and Rutledge 1996, Moreira et al. 2014). Overall, not accounting for multiple axes of defensive strategies or addressing plant defence from a multivariate perspective has likely contributed to inconsistent patterns or erroneous conclusions about the nature of elevational gradients in plant defensive investment.

Another important consideration is that most studies have considered 'elevation' as a proxy for multiple co-varying abiotic (e.g., climatic) factors without a detailed analysis of these drivers (Körner 2007). Recent studies have demonstrated that an explicit consideration of climatic variables can shed light onto how the abiotic environment shapes elevational clines of herbivory and plant defences (Abdala-Roberts et al. 2016, Pellissier et al. 2014, 2016). For example, Abdala-Roberts et al. (2016) found that climatic conditions (temperature) influenced elevational variation in insect herbivory independently of chemical defences in English oak *Quercus robur*, a pattern that may arise when defences are influenced by resource availability or when these compounds play a role in abiotic tolerance. Similarly, Pellissier et al. (2016) found that temperature and precipitation strongly affected both herbivory and chemical defences across *Cardamine* species growing along an elevational gradient. Empirical evidence and theory thus argue for the dual consideration of climatic factors and herbivory, as their concurrent influences have likely shaped the evolution of plant defence traits along elevational gradients.

In this study we tested for elevational gradients in defensive traits in oak (*Quercus*, Fagaceae) species. We approached this by studying how individual defensive strategies vary with species elevational ranges, as well as whether the simultaneous or correlated expression of different defensive strategies also exhibits such clinal variation. Using a phylogenetic comparative approach we addressed the following questions: (i) Are there elevational gradients in constitutive chemical defences and their inducibility (i.e., difference between induced and constitutive levels of defences) across oak species? (ii) Are there elevational gradients in the correlated expression (negative or positive) of constitutive chemical defences and their inducibility? And (iii) do climatic factors account for elevational clines in individual defensive traits and strategies or their correlated expression? To address these questions, we quantified the concentration of constitutive levels of phenolic compounds in leaves as well as their inducibility after feeding by the generalist gypsy moth larvae (*Lymantria dispar*, Lepidoptera) by means of a greenhouse experiment with 18 oak species collectively spanning >2400 m in elevation. We further investigated the effects of climatic factors (temperature and precipitation) associated with the climatic niche of each species on plant defences. Overall, this study builds towards a more robust and integrative understanding of variation in plant multivariate defensive phenotypes along ecological gradients and their underlying abiotic drivers.

## Materials and methods

### Natural history

We used 18 species of the genus *Quercus*, of which 11 have a Palearctic distribution range, namely: *Quercus robur*, *Q. faginea*,

*Q. petraea*, *Q. suber*, *Q. ilex*, *Q. pubescens*, *Q. frainetto*, *Q. phillyraeoides*, *Q. cerris*, *Q. serrata* and *Q. pyrenaica*, and seven have a Nearctic distribution, namely: *Q. agrifolia*, *Q. macrocarpa*, *Q. coccolinea*, *Q. rubra*, *Q. shumardii*, *Q. palustris* and *Q. phellos*. These oak species are widespread along the northern hemisphere and encompass a 2423 m elevational gradient (on average, from  $-3$  for *Q. cerris* to up 2420 m above sea level for *Q. serrata*) (Manos et al. 1999, Hipp et al. 2018).

*Quercus* species have developed a broad spectrum of defence traits against phytophagous insects, including chemical defences such as phenolic compounds (Feeny 1970, Roslin and Salminen 2008, Pearse and Hipp 2012, Abdala-Roberts et al. 2016, 2018, Moreira et al. 2018b). These chemical defences are the most important chemical barrier against herbivory in oak trees as they function as digestibility reducers in insects (Pearse and Hipp 2012). They are present at high constitutive levels in all tissues, but can also be induced upon damage (Pearse and Hipp 2009, Mizumachi et al. 2012).

The studied oak species are attacked by a large community of insect herbivores including generalist and specialist species, mainly leaf chewers and miners (Roslin and Salminen 2008, Pearse and Hipp 2009, Tack et al. 2010, Moreira et al. 2017, 2018b). Among these herbivores, the generalist gypsy moth (*L. dispar*, Lepidoptera) is one of the most destructive pests of broad-leaf and conifer trees in Europe and, following introduction, in North America (Milanovic et al. 2014). Larvae of this species reach densities that can cause extensive damage on a large proportion of an individual tree's leaves (Miller and Hanson 1989). Although co-evolution of oaks and the gypsy moth is thought to have mainly occurred in the Palearctic region, this phytophagous insect also feeds on many species of Nearctic *Quercus* (Elkinton and Liebhold 1990). In addition, our work has also showed that this species causes a similar induction of chemical defences in oaks of both regions, suggesting that this species represents a suitable model for studying inducibility of defences across both groups of oaks (Moreira et al. 2018c).

### Plant growth, greenhouse conditions and treatments

In March 2017, in a glasshouse at the Biological Mission of Galicia (Pontevedra, northwestern Spain), we individually planted 1-year-old plants (purchased from Planfor nursery, Uchacq-et-Parentis, France) in 4-l pots containing peat. All oak stocks belong to native provenances, but saplings of each species used in our experiment came from multiple source populations of unknown location (and thus elevation). Despite this, we expected that among-species variation due to adaptation to specific elevational ranges would be greater than within-species variation and followed a similar approach to previous studies for which variation for each species was based on single-population estimates or seed source locations were unknown (e.g., Rasmann and Agrawal 2011, Pearse and Hipp 2012, Moreira et al. 2014). Plants were grown under controlled conditions:

minimum of 12 h of light per day, temperature between 10 °C at night and 25 °C during the day and were watered daily. After 2 months of acclimatization, we randomly assigned half of the plants of each species to one of two herbivory treatments: (i) damaged plants by gypsy moth larvae and (ii) undamaged control plants. The experimental design followed a randomized split-plot design replicated across six blocks, with herbivory treatment applied at the whole factor and oak species as the split factor. This design comprised six blocks  $\times$  18 oak species  $\times$  two herbivory treatments (control and infestation by gypsy moth larvae), for a total of 216 plants. We placed a fourth-instar larva on each fully expanded leaf per plant assigned to the infestation treatment. Larvae fed on the plants for 4 days and were then removed and we estimated the percent of total leaf area damaged at the whole-plant level by visually assigning each plant to one of the following categories: 0 = undamaged; 1 = 1–10% damaged; 2 = 11–20% damaged; 3 = 21–30% damaged; 4 = 31–40% damaged; 5 = 41–50% damaged; 6 = 51–60% damaged; 7 = 61–70% damaged; 8 = 71–80% damaged; 9 = 81–90% damaged; 10 = 91–100% damaged (Moreira et al. 2018c). To avoid biases in our herbivory estimates, the same person (X.M.) scored all the leaves. After this we collected three random leaves per plant for quantification of phenolic compounds. Leaves were oven-dried for 48 h at 40 °C, ground with liquid nitrogen and stored at room temperature.

### Chemical analyses

Phenolic compounds were extracted from 20 mg of dry plant tissue with 1 ml of 70% methanol in an ultrasonic bath for 15 min, followed by centrifugation and subsequent dilution of the methanolic extract (Moreira et al. 2014). We diluted these methanolic extracts (1:5 vol:vol) with the extraction solvent and transferred them to chromatographic vials. We did not use an internal standard because previous trials with a subset of our samples using resorcinol (#398047; Sigma-Aldrich, St. Louis, MO, USA) as internal standard indicated that recovery rates were  $>90\%$  in all cases. To perform the chromatographic analyses we used Ultra-High-Performance Liquid-Chromatograph (UHPLC Nexera LC-30AD; Shimadzu Corporation, Kyoto, Japan) equipped with a Nexera SIL-30AC injector and one SPD-M20A UV/VIS photodiode array detector. The compound separation was carried out on a Kinetex™ 2.6  $\mu\text{m}$  C18 82–102 Å, LC Column 100  $\times$  4.6 mm, protected with a C18 guard cartridge. The flow rate was 0.4 ml min $^{-1}$  and the oven temperature was set at 25 °C. The mobile phase consisted of two solvents: water-formic acid (0.05%) (A) and acetonitrile-formic acid (0.05%) (B), starting with 5% B and using a gradient to obtain 30% B at 4 min, 60% B at 10 min, 80% B at 13 min and 100% B at 15 min. The injection volume was 30  $\mu\text{l}$ . We previously ran some of our samples with an ultrahigh-pressure liquid chromatography-quadrupole-time-of-flight mass spectrometry to identify the phenolic compounds. We quantified flavonoids as rutin equivalents,

condensed tannins as catechin equivalents, hydrolysable tannins as gallic acid equivalents, and lignins as ferulic acid equivalents (Moreira et al. 2018b). We achieved the quantification of these phenolic compounds by external calibration using calibration curves at 0.25, 0.5, 1, 2 and 5  $\mu\text{g ml}^{-1}$ . We quantified all compounds using a wavelength of 330 nm. We expressed phenolic compound concentrations in  $\text{mg g}^{-1}$  tissue on a dry weight basis.

### Species distribution range and niche characterization

For each of the 18 oak species, we constructed a species distribution model and extracted climatic data from the estimated species range. We obtained georeferenced species presence data from the Global Biodiversity Information Facility database (<https://www.gbif.org/>). We used these occurrence data to construct a Maximum Entropy Species Distribution Model (SDM) for each oak species using the MaxEnt software (Phillips et al. 2006) as implemented via the DISMO package (Hijmans et al. 2010) for R v. 3.4.1 (R Development Core Team 2018). We randomly assigned each species presence record to one of five equal partitions for k-fold cross-validation of the model fit for each species. Four of these partitions were used to train the models with 30-arc-second resolution climate data from 19 BIOCLIM variables from the WorldClim database (Hijmans et al. 2005) as predictors (see Table S1 in the Appendix A available as Supplementary Data at *Tree Physiology* Online). The fifth data partition was used to assess the model fit using the area under the receiver-operator curve. For each oak species, this procedure was repeated such that each species presence data partition was used once as the validation dataset. We discretized the SDM by taking all areas with an occurrence probability  $\geq 75\%$  as the species range.

We extracted the elevation of each cell within the discretized species range using digital elevation data at 30 arc-second resolution from the NASA Shuttle Radar Topographic Mission database (<http://www.cgiar-csi.org>). We supplemented the elevational range for well-sampled species where we could get a better estimate from the literature (see Figure S1 in the Appendix B available as Supplementary Data at *Tree Physiology* Online). We also estimated the latitudinal range of each oak species. However, latitudinal range did not significantly affect any plant defence (analyses not shown) and was thus removed from the statistical analyses.

### Statistical analysis

**Summarizing constitutive and induced defences and climatic variables** In order to analyse all defensive traits without inflating type I error through multiple individual tests, species-level (i.e., species means) variation in the chemical defensive compounds studied (flavonoids, lignins, condensed tannins and hydrolysable tannins) was summarized by means of two independent principal component analyses (PCA) using Proc

FACTOR (rotation = varimax) in SAS 9.4 (SAS System, Cary, NC, USA), one for constitutive defences and another for inducibility of defences (Moreira et al. 2014, 2015). In each case, constitutive defences and their inducibility were summarized based on the first two principal components. The first two axes of the PCA explained 71% of the variance for constitutive defensive traits, with PC1 being positively associated with constitutive concentration of lignins and hydrolysable tannins, and PC2 being positively associated with constitutive concentration of flavonoids. Similarly, the first two axes of PCA explained 73% of the variance in inducibility of defences, with PC1 being positively associated with the inducibility of condensed tannins, and PC2 being positively associated with the inducibility of hydrolysable tannins. The standardized z-scores of the first two principal components from each analysis were subsequently used in univariate and multiple regression analyses testing for elevational gradients in plant defensive strategies (see below).

Similarly, we summarized the 19 climatic variables by conducting two independent PCAs using Proc FACTOR (rotation = varimax) in SAS 9.4, one for temperature variables and one for precipitation variables (Moreira et al. 2014, 2015). In each case, climatic variables were summarized with the first principal component. The first principal component explained 52% of the variance for the 11 temperature variables across oak species ('temperature' hereafter), and was positively related to mean temperature of coldest month, mean temperature of driest quarter and mean temperature of coldest quarter, and negatively related to temperature seasonality and temperature annual range. Similarly, the first principal component explained 55% of the variance for the eight precipitation variables across oak species ('precipitation' hereafter), and was positively related to annual precipitation, precipitation of the wettest month, precipitation of the wettest quarter and precipitation of the warmest quarter. The standardized z-scores of the first principal component of each analysis were then used to test for the effects of climate in the multiple regressions ahead.

### Testing for elevational gradients in oak defensive strategies and their underlying climatic drivers

First, to assess the presence of elevational gradients in constitutive chemical defences and in their inducibility, we individually regressed the oak species' PC scores for (i) constitutive defences and (ii) defence inducibility onto the mean of the species elevational range. In the case of inducibility, we included leaf damage score as a covariate [so that the inducibility of each species was compared on the same scale (per-unit leaf loss) in light of significant leaf consumption differences among oak species ( $F_{1,6,88} = 5.52, P < 0.001$ )]. Second, to investigate the influence of climatic correlates of elevational variation in plant defensive traits, we performed multiple regressions including elevation and climatic variables (temperature PC1 z-scores, precipitation PC1 z-scores) as predictors of constitutive chemical defences and their inducibility. If any such



significant effect of species elevational range in the former regression models not accounting for climatic variables turned non-significant and the slope estimator for the elevation effect exhibited a substantial change (i.e., reduction) after accounting for climatic factors, this would suggest that climate underlies the elevational gradient in defences.

For all the previous analyses, we accounted for phylogenetic non-independence among oak species by performing phylogenetically corrected generalized least square analyses (pGLS) with the `pGLS` function in the `CAPER` package for R (Orme 2013). These pGLS were based on a phylogenetic tree of *Quercus* species using Single Nucleotide Polymorphism matrices by ddRAD sequencing (see Moreira et al. 2018c).

**Testing for elevational gradients in the correlated expression of oak defensive strategies and their underlying climatic drivers** We tested for a shared structure between constitutive chemical defences and their inducibility, which would represent a correlated expression of both defensive strategies, using a coinertia analysis (Pellissier et al. 2016, Defosse et al. 2018). In other words, here we tested whether the matrices of constitutive chemical defences and their inducibility were correlated

Table 1. Descriptive statistics (least square means  $\pm$  standard error and species range) showing variation among oak (*Quercus*) species in constitutive leaf phenolic compounds (condensed tannins, hydrolysable tannins, lignins and flavonoids, in  $\text{mg g}^{-1}$  dry weight) and their inducibility after feeding by the generalist gypsy moth larvae (*Lymantria dispar*, Lepidoptera).

	Mean $\pm$ SE	Range
Constitutive phenolics		
Condensed tannins	2.07 $\pm$ 0.21	0.30–4.22
Hydrolysable tannins	55.80 $\pm$ 15.47	8.57–283.31
Lignins	4.63 $\pm$ 0.85	0.00–11.38
Flavonoids	11.04 $\pm$ 0.36	8.93–14.73
Inducibility of phenolics		
Condensed tannins	−0.37 $\pm$ 0.18	−1.92–1.46
Hydrolysable tannins	−15.34 $\pm$ 9.39	−162.63–24.34
Lignins	−0.81 $\pm$ 0.49	−4.52–4.36
Flavonoids	−0.83 $\pm$ 0.23	−2.66–0.92

Table 2. Effects of species elevational range on constitutive leaf chemical defences, their inducibility (based upon a principal components analyses summarizing a suite of phenolic compounds) and the correlated expression of constitutive defences and their inducibility (coinertia) in 1-year-old trees from 18 *Quercus* species. Leaf damage score by gypsy moth (*Lymantria dispar*) was used as a covariate in the inducibility models. Degrees of freedom, Student's *t*-tests (*t*) and *P*-values are shown. We accounted for phylogenetic non-independence among *Quercus* species by performing phylogenetic correct generalized least square analyses (pGLS). Significant coefficients ( $P < 0.05$ ) are in bold.

Response variable	DF	Elevational range		Leaf damage	
		<i>t</i>	<i>P</i> -value	<i>t</i>	<i>P</i> -value
Constitutive lignins and hydrolysable tannins	1,16	−1.02	0.321		
Constitutive flavonoids	1,16	0.32	0.757		
Inducibility of condensed tannins	1,15	1.33	0.205	2.17	<b>0.048</b>
Inducibility of hydrolysable tannins	1,15	2.21	<b>0.044</b>	−2.08	0.057
Coinertia	1,16	−0.38	0.710		

across species (Pellissier et al. 2016). These analyses were performed using the `ADE4` package for R (Dray et al. 2003, Dray and Dufour 2007), with the significance of the shared variance assessed using a Monte-Carlo test (10,000 simulations).

If the coinertia analysis was significant, we performed species-level univariate regressions to test for effects of species elevational range on the coinertia axis. Next, and following the same approach as in the previous section testing for effects climate on trait PCs, we performed multiple regression analyses to test for effects of both species elevational range and climate on the coinertia axis. If this significant effect of species elevational range in the univariate regressions turned non-significant after accounting for climatic variables, we interpreted this as evidence that climate underlies the elevational gradient in the correlated expression of constitutive defences and their inducibility.

## Results

### Species variation in constitutive chemical defences and their inducibility

Constitutive defensive traits exhibited 1.7-fold (flavonoids) to 33.0-fold (hydrolysable tannins) variation among species, whereas the inducibility of these defensive traits exhibited 4.4-fold (condensed tannins) to 188-fold (hydrolysable tannins) variation (Table 1).

### Elevational gradients in oak defensive strategies and their underlying climatic drivers

We found no significant associations between either PC of constitutive defences and species elevational range (Table 2, Figure 1a and b), and the subsequent model including climatic variables indicated that neither temperature nor precipitation significantly predicted constitutive defences (Table 3). In contrast, there was a significant positive association between PC2 of inducibility and species elevational range (Table 2), i.e., oak species with higher elevational ranges exhibited a greater inducibility of hydrolysable tannins (Figure 1d). This effect of species elevational range turned marginally significant ( $P = 0.062$ ) after accounting for temperature and precipitation (Table 3), though this represented

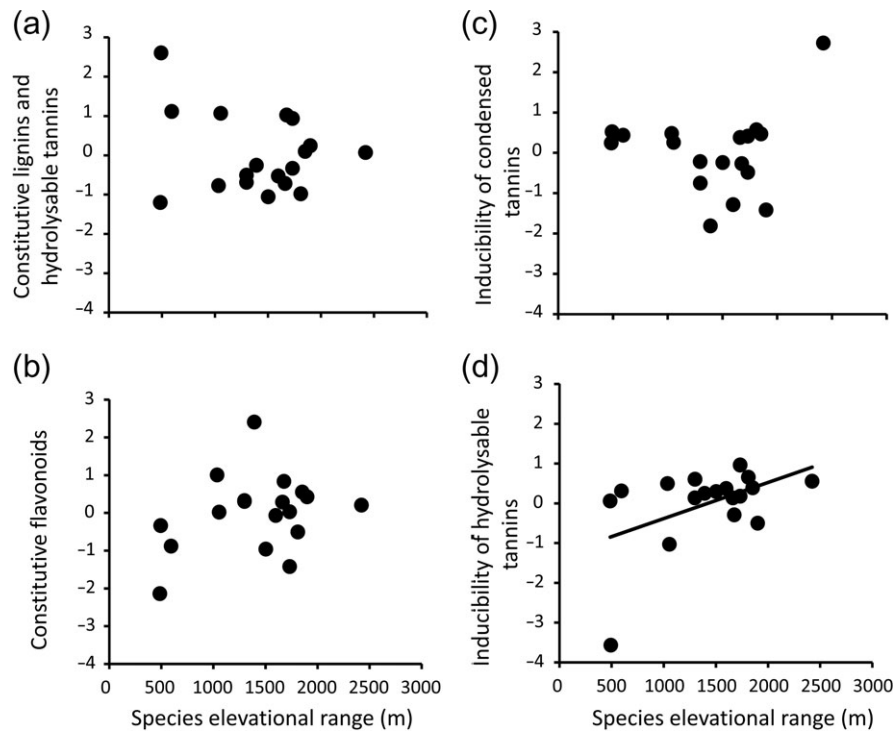


Figure 1. Correlations between species elevational range and (a) constitutive lignins and hydrolysable tannins, (b) constitutive flavonoids, (c) inducibility of condensed tannins and (d) inducibility of hydrolysable tannins in leaves of 1-year-old trees from 18 *Quercus* species. Constitutive phenolics and their inducibility were grouped based upon a principal components analysis so the y axis shows the standardized z-scores of the first two principal components from each analysis. Black solid line represents a significant correlation (phylogenetic correct generalized least square analyses, pGLS;  $P < 0.05$ ; see Table 2).

Table 3. Multiple regressions showing the effects of species elevational range and climate (based upon a principal components analysis summarizing a suite of variables associated to precipitation or temperature) on constitutive leaf chemical defences, their inducibility (based upon a principal components analysis summarizing a suite of phenolic compounds) and correlated expression of constitutive defences and their inducibility (coinertia) in 1-year-old trees from 18 *Quercus* species. Leaf damage score by gypsy moth (*Lymantria dispar*) was used as a covariate in the inducibility models. We accounted for phylogenetic non-independence among *Quercus* species by performing phylogenetic correct generalized least square analyses (pGLS). Degrees of freedom, Student's *t*-tests (*t*) and *P*-values are shown. Significant coefficients ( $P < 0.05$ ) are in bold. Marginally significant coefficients ( $0.05 < P < 0.10$ ) are in italics.

Response variable	DF	Elevational range		Leaf damage		Temperature		Precipitation	
		<i>t</i>	<i>P</i> -value	<i>t</i>	<i>P</i> -value	<i>t</i>	<i>P</i> -value	<i>t</i>	<i>P</i> -value
Constitutive lignins and hydrolysable tannins	1,14	-1.26	0.227			0.99	0.337	-0.66	0.517
Constitutive flavonoids	1,14	0.65	0.528			-0.35	0.733	1.18	0.258
Inducibility of condensed tannins	1,13	0.37	0.721	1.71	0.113	3.83	<b>0.002</b>	1.93	0.078
Inducibility of hydrolysable tannins	1,13	2.05	0.062	-1.84	0.091	-0.39	0.707	-0.22	0.830
Coinertia	1,14	0.10	0.924			-1.79	0.096	0.69	0.501

a slight change in the significance value and the estimator of the elevation effect remained similar after including climatic factors (Table 2 vs 3), which suggests that climate did not play an important role in mediating the elevational gradient in defence inducibility. There was no significant association between species elevational range and PC1 of inducibility (i.e., inducibility of condensed tannins) (Table 2, Figure 1c), though the subsequent model with climatic variables indicated that both temperature and precipitation were (marginally) significantly and positively correlated with this PC1 of inducibility (Table 3).

### Elevational gradients in the correlated expression of oak defensive strategies and their underlying climatic drivers

The co-inertia analysis showed a marginally significant correlated expression of constitutive chemical defences and their inducibility ( $P = 0.058$ ). However, we found no significant association between the correlated expression of constitutive chemical defences and their inducibility and species elevational range (Table 2, Figure 2), and the subsequent model including climatic variables indicated that neither temperature nor precipitation significantly predicted this correlated expression (Table 3).

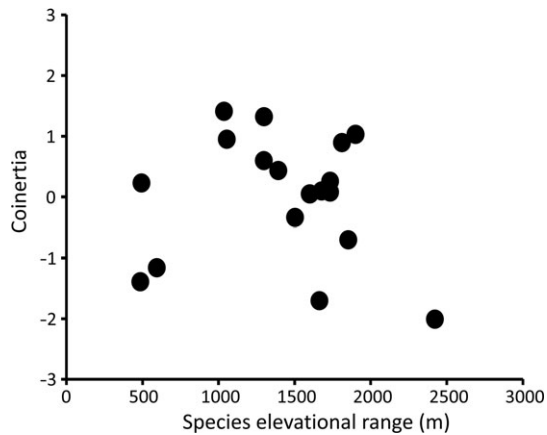


Figure 2. Correlation between species elevational range and the correlated expression of constitutive defences and their inducibility (coinertia axis) in 1-year-old trees from 18 *Quercus* species.

## Discussion

By studying multiple defensive traits and strategies and their underlying climatic drivers, this work provides several important insights into the nature of elevational gradients in oak defence, particularly for early life stages (i.e. seedlings and saplings). First, we found evidence for an elevational gradient in the inducibility of leaf chemical defences, but not in constitutive chemical defences nor in the correlated expression of constitutive chemical defences and their inducibility. In the former case, the inducibility of leaf hydrolysable tannins was stronger in species with high elevational ranges. Second, the observed elevational gradient in the inducibility of chemical defences remained mostly unchanged after accounting for temperature and precipitation variables, suggesting that climate does not underlie such gradient. Overall, these results bring us closer to understanding the underlying factors and processes behind elevational patterns in plant defences and, in doing so, contributes to a more robust theory of biogeographical patterns of plant–herbivore interactions.

It is often assumed that plant–herbivore interactions are stronger towards warmer and more stable climates found at lower elevations, and that this selects for higher plant constitutive defences at low relative to high elevations (Hegland et al. 2009, Rasmann et al. 2014a). In contrast to this commonly held view, we found no evidence for elevational gradients in constitutive leaf phenolics across the studied oak species. One plausible explanation for our findings might be that, in order to increase seedling establishment, young oak trees should maximize the production of constitutive defensive traits regardless of their elevation. Constitutive defences are the first barrier to herbivory and are considered more costly to produce than induced defences (Franceschi et al. 2005), so that they should be maximized during the earliest and most vulnerable stages of plant and leaf growth (Boege and Marquis 2005). Although leaf

phenolics are thought to be a primary defence in oaks, our results do not preclude elevational gradients in other types of traits including chemical (e.g., terpenes) and physical (e.g., toughness, trichomes) defences, as well as leaf nutrients (e.g., nitrogen, phosphorus).

Our study is one of the first showing an elevational gradient in defence inducibility. We found that oak species with high elevational ranges exhibited a greater inducibility of hydrolysable tannins. This result agrees with Rasmann et al. (2014c), who found that *Vicia faba* plants growing at high elevations displayed increased inducibility of volatile organic compounds, an indirect defence that facilitates natural enemy recruitment. In contrast, Pellissier et al. (2016) found that inducibility of glucosinolates (a chemical anti-herbivore defence) in *Cardamine* species increased towards lower elevations, whereas Moreira et al. (2014) found no elevational gradients in the inducibility of chemical defences (resin and phenolics) in pine species. Multiple ecological factors may explain these discrepancies among studies, including variation in the strength of trade-offs between defensive strategies shaping the relative strength and direction in constitutive vs induced defence gradients. In oaks, our results indicate that these two defensive strategies did not trade-off but rather they are expressed simultaneously along the elevational gradients. These findings suggest that resource allocation constraints, or negative co-variance between constitutive and induced defences, are not influencing elevational gradients in phenolic compounds across the studied oak species.

The realization that plant defensive phenotypes are multivariate in nature has clarified how biotic and abiotic factors shape the concurrent expression of multiple defensive traits and mechanisms. However, few studies to date have tested for elevational gradients in the correlated expression of different defensive traits or strategies (Pellissier et al. 2016, Defosse et al. 2018). In one of these studies, Pellissier et al. (2016) found that direct and indirect defences in *Cardamine* species were positively correlated and that their co-expression was stronger at lower elevations. The authors argued that higher herbivore pressure at lower elevations has selected for multiple defences such that synergistic or complementary interactions between defensive strategies provides the most effective means of resistance against a diverse herbivore fauna (Pellissier et al. 2016). In our case, we found positively correlated expression of constitutive chemical defences and their inducibility (i.e., no trade-offs) in oak species, but such correlated expression was not significantly associated with species elevational range (Rasmann and Agrawal 2011). In light of findings by Pellissier et al. (2016), we would have expected an increase in the strength of correlated expression of constitutive and induced defences towards lower elevations, where herbivore loads and diversity would be presumably greater. This, however, was not the case. A robust characterization of oak-associated herbivore communities

and herbivory levels across elevations would be a valuable step forward for explaining the observed patterns.

Recent studies have demonstrated that climatic correlates of elevation underlie elevational gradients in herbivory and plant defences (e.g., [Moreira et al. 2014](#), [Pellissier et al. 2014](#), [Abdala-Roberts et al. 2016](#)). However, we found that temperature and precipitation did not explain the observed elevational gradient in the inducibility of chemical defences. In particular, our results showed that species elevational range still (marginally) predicted plant defence allocation after accounting for these climatic factors, suggesting that the gradient was not underlain by climatic conditions that often change most dramatically over elevation—temperature and precipitation. Other abiotic factors such as soil characteristics may be important drivers of elevational gradients in plant defences. For instance, [Pellissier et al. \(2016\)](#) found that soil fertility partially explained elevational gradients in plant chemical defences in *Cardamine* species, whereby plant species adapted to nutrient-poor soils were better defended. In another study, [De Long et al. \(2016\)](#) reported that soil nitrogen availability drove elevational gradients in plant community-level chemical defences in a subarctic tundra heath, whereby plant species growing in soils with low nitrogen concentration had higher defence levels. Overall, although correlative approaches have contributed to our understanding of the concurrent effects of abiotic factors, these studies—including our own work ([Moreira et al. 2014](#), [Abdala-Roberts et al. 2016](#))—are inherently limited in assigning causality. Experimental manipulations of candidate abiotic drivers are ultimately needed to disentangle their effects on plant–herbivore systems ([Pellissier et al. 2014](#)).

In conclusion, our findings emphasize the value of measuring multiple types of defensive traits and strategies along ecological gradients as a means for understanding the drivers of plant biotic resistance. Observed patterns of variation across the studied oak species suggest that the presence and magnitude of elevational gradients in plant defences are contingent upon the defensive strategy under study: we find that inducibility of defences varies with species elevational range, but not constitutive defences. Surprisingly, climatic factors appeared not to underlie the elevational gradient in induced defences, suggesting that other sources of abiotic (or biotic) variation are at work and should be investigated.

## Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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## Conflict of interest

None declared.

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