

Research

Elevational gradients in constitutive and induced oak defences based on individual traits and their correlated expression patterns

Andrea Galmán, Luis Abdala-Roberts, Pola Wartalska, Felisa Covelo, Gregory Röder, Mark A. Szenteczki, Xoaquín Moreira and Sergio Rasmann

A. Galmán (<https://orcid.org/0000-0001-6344-7721>) ✉ (andrea.cortegoso.g@gmail.com) and X. Moreira (<https://orcid.org/0000-0003-0166-838X>), Misión Biológica de Galicia (MBG-CSIC), Pontevedra, Galicia, Spain. – L. Abdala-Roberts, Depto de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias, Univ. Autónoma de Yucatán, Itzimmá, Mérida, Yucatán, México. – P. Wartalska, Malopolska Centre of Biotechnology, Jagiellonian Univ., Kraków, Poland. – F. Covelo, Depto de Sistemas Físicos, Químicos y Naturales, Univ. Pablo de Olavide, Sevilla, Spain. – G. Röder, M. A. Szenteczki and S. Rasmann, Inst. of Biology, Univ. of Neuchâtel, Neuchâtel, Switzerland.

Oikos

130: 396–407, 2021

doi: 10.1111/oik.07588

Subject Editor: Nina Fatouros

Editor-in-Chief: Dries Bonte

Accepted 23 November 2020

Elevational gradients are useful ecological settings for revealing the biotic and abiotic drivers of plant trait variation and plant–insect interactions. However, most work focusing on plant defences has looked at individual traits and few studies have assessed multiple traits simultaneously, their correlated expression patterns, and abiotic factors associated with such patterns across elevations. To address this knowledge gap, we studied elevational variation in direct (phenolic compounds) and indirect (volatile organic compounds) constitutive defences and their inducibility after feeding by a specialist beetle *Altica quercetorum* in saplings of 18 wild populations of *Quercus pyrenaica*. We tested for: 1) clines in each defensive trait individually, 2) their patterns of correlated expression and 3) associations between any such clines and climatic factors. We found that constitutive direct defences (lignins and hydrolysable tannins) decreased with increasing elevation. We observed no elevational gradient for constitutive indirect defences (volatile organic compounds) or the inducibility of direct or indirect defensive traits when looking at groups of compounds. However, at individual tree-level, increased induction of two monoterpenes (α -fenchene and camphene) at higher elevation was shown. Furthermore, we show a significant pattern of co-expression of constitutive and induced phenolics across populations, which weakened with increasing elevation. Finally, we found no evidence that climatic factors were associated with either individual or correlated trait expression patterns across elevations. Overall, these findings call for moving beyond elevational clines in individual plant defences, and argue that assessing elevational shifts in trait correlated expression patterns and their underlying mechanisms can increase our understanding of plant defence evolution and plant–herbivore interactions along environmental gradients.

Keywords: climatic gradients, flea beetles, northwestern Spain, plant defence syndromes, phytochemicals, volatiles



Introduction

Ecological gradients are potent tools for understanding how the environment shapes species traits, communities and ecosystem function (Dobzhansky 1950, Schemske et al. 2009). Changing environmental conditions along such gradients are characterized by variation in biotic (e.g. species abundances or composition) and abiotic (e.g. temperature, irradiance) factors, which shape clinal variation in species interactions and traits (Schemske et al. 2009, Rasmann et al. 2014a). Notably, higher abundance and diversity of insect herbivores (leading to greater herbivore pressure) toward warmer and more stable climatic conditions, such as those found at lower elevations, is expected to select for greater investment in plant defences (Scheidel and Bruelheide 2001, Rasmann et al. 2014b, Pellissier et al. 2016, Galmán et al. 2018, Moreira et al. 2018a). Despite multiple layers of empirical support for this prediction in early studies (Zehnder et al. 2009, Garibaldi et al. 2011, Pellissier et al. 2012), recent work has defied this paradigm by reporting either no evidence for decreasing interaction intensity with elevation, greater herbivory and defences at intermediate elevations, or even inverse gradients where plant defences and herbivory increase with elevation (reviewed by Rasmann et al. 2014b, Moreira et al. 2018a). Consequently, there is a need for further research to resolve inconsistencies along elevation gradients of plant–herbivore interactions and identify common generative mechanisms.

One way in which elevational research on plant defences can take a next step toward resolving these inconsistencies is by investigating clines in multiple defence traits simultaneously, rather than looking at individual traits (Pellissier et al. 2016, Kergunteuil et al. 2018, Moreira et al. 2018a). The main reason for this is that plant defensive phenotypes are usually integrated by multiple traits, such that assessing these patterns of concurrent variation can provide a more robust understanding of plant defence variation along ecological gradients (Defosse et al. 2018, Moreira et al. 2018a). While most elevational studies up to a decade ago had looked at variation within single plant defensive traits (reviewed by Moreira et al. 2018a), recent work has started to address this gap by conducting simultaneous measurements of direct (traits that deter or reduce herbivore feeding) and indirect (traits that recruit natural enemies of herbivores) defences, as well as a separation of constitutive (basal levels in the absence of attack) and induced (increased levels in response to attack) trait expression levels along elevational gradients (Rasmann et al. 2014c, Pellissier et al. 2016, Defosse et al. 2018). This research has shown that addressing multiple traits and their correlated expression patterns is key for achieving an improved assessment of plant defensive responses and can importantly expose underlying trait correlations which can shape plant defensive phenotypes along elevational gradients. These trait associations may be positive, leading to co-expression patterns (Pellissier et al. 2016), or negative, which often result from underlying allocation constraints (i.e. tradeoffs

between traits; Agrawal and Hastings 2019), and these correlations can be modulated by biotic or abiotic factors varying across elevations and ultimately shape plant defensive phenotypes.

Recent work indicates that elevational gradients in trait co-expression can arise and could be potentially common (Defosse et al. 2018, Kergunteuil et al. 2018). For example, changes in herbivore abundance or diversity with elevation may result in distinct patterns of plant defence co-expression, whereby more diverse and functionally complex insect communities found at lower elevations could select for a broader repertoire of simultaneously expressed direct or indirect defensive traits compared to higher elevations (Pellissier et al. 2016). Alternatively, elevational shifts in trophic structure (e.g. ratio of predator to herbivore abundance; Godschalx et al. 2019) or complexity (three- versus two-trophic level chains; Abdala-Roberts et al. 2019) might modulate differential patterns of allocation to direct and indirect defences. Greater herbivore and predator pressure at low elevations would favour increased plant allocation to both direct and indirect defences, whereas reduced predator to herbivore abundances or predator-free environments at high elevations would favour only direct plant defences (Rasmann et al. 2014a, Moreira et al. 2018a, Godschalx et al. 2019). Overall, the consideration of co-expression patterns in multiple traits is therefore an essential aspect for understanding how plant defences and plant–herbivore interactions evolve along environmental clines.

Abiotic variables can also be important predictors of elevational clines in plant defences and their correlated expression patterns, but until recently had not been explicitly evaluated. For example, changes in climate with elevation can shape plant defence levels through effects on abiotic stress or resource availability which modulate the costs of producing defensive traits (Johnson and Rasmann 2011, Abdala-Roberts et al. 2016, Moreira et al. 2018b). These abiotic effects on resource availability can also affect the concurrent expression of multiple defences if these traits are correlated due to genetic linkages, metabolic constraints or allocation tradeoffs (Rasmann et al. 2014a, Moreira et al. 2018a). For instance, two or more defensive traits are co-expressed under high resource availability (i.e. at low elevations) but when resources are low (at high elevations) they are no longer co-expressed, suggesting that resource allocation constraints are behind these shifts in trait correlations (Moreira et al. 2018a). In this sense, plant defence theory can be used to make specific predictions with respect to elevational changes in the expression of constitutive versus induced defence levels (Coley et al. 1985, Endara and Coley 2011), whereby harsher conditions at high elevations would favour increased constitutive defences but reduced inducibility, presumably because the cost of replacing consumed plant tissues is higher under limiting conditions. While this pattern has been supported by a few recent studies (Moreira et al. 2014, Pellissier et al. 2016), more work is needed to investigate how clinal variation in abiotic factors shapes patterns of constitutive and induced expression of both direct and indirect defences.

In this study, we tested for elevational gradients in constitutive direct and indirect chemical defences and their inducibility (assessed as the difference between induced and constitutive levels of defences), as well as in their correlated expression patterns in the Pyrenean oak *Quercus pyrenaica* (Fagaceae). We also investigated whether climatic factors associated with elevation explained any such clinal patterns in plant defences. Specifically, we asked: 1) are there elevational gradients in constitutive direct and indirect defences? 2) Does herbivory induce direct and indirect defenses and are there elevational gradients in their inducibility? 3) Are there emergent patterns of correlated expression in traits and do these exhibit elevational clines? 4) Are climatic correlates of elevation (namely, precipitation and temperature) associated with concomitant clines in individual defensive traits and their correlated expression patterns? To address these questions, we sampled 18 populations of *Q. pyrenaica* distributed along a 1300 m-elevational gradient and quantified constitutive direct (phenolic compounds) and indirect (volatile organic compounds or VOCs) defences in leaves and their inducibility after in situ manipulation of feeding by the specialist oak flea beetle *Altica quercetorum* (Coleoptera: Chrysomelidae). This study builds toward a more complete understanding of elevational gradients by assessing realistically complex plant defensive phenotypes associated with multiple traits and by further inquiring on their underlying abiotic drivers.

Material and methods

Natural history

The Pyrenean oak *Quercus pyrenaica* is a deciduous tree native to southwestern Europe and northwestern Africa, and it grows from ca 400 m up to 1800 m a.s.l. A number of specialist and generalist insect herbivores attack *Q. pyrenaica* across its distribution range, many of which are leaf chewers and skeletonizers. Among these, the specialist oak flea beetle, *A. quercetorum*, is one of the most destructive pests of oak trees in southwestern Europe (Mijuskovic 1980, Fernandez de Ana et al. 1996), and is also a common and ecologically-important herbivore associated with *Q. pyrenaica*. This insect is a leaf skeletonizer which reaches peak abundances on *Q. pyrenaica* at the end of the summer season (mid to late August).

Quercus pyrenaica produces secondary metabolites in leaves, which are potentially important direct defensive barriers against herbivores. Among these, phenolic compounds have been shown to be toxic for a broad range of phytophagous insects (Feeny 1970, Pearse and Hipp 2012, Moreira et al. 2018b). Several oak species have also been shown to produce VOCs which attract natural enemies of herbivores (i.e. indirect defences; Ghirardo et al. 2012, Pearse et al. 2013). *Quercus pyrenaica* also produces and emits VOCs (data from this study, but also Ramilo et al. 2017), although their defensive role has not yet been demonstrated. Both direct and

indirect defences in oaks are inducible in response to herbivore damage (Moreira et al. 2018c, Galmán et al. 2019a).

Field sampling and induction of plant defences

At the end of the growing season, from late August to early September, and before leaf senescence, we sampled 18 *Q. pyrenaica* populations spanning a 1300 m elevational gradient (from 370 to 1614 m, Supporting information) in the mountains of Ancares (Galicia, north-western Spain). The sampled gradient covers most of the elevational gradient experienced by this oak species throughout its distribution range. Each population consisted of at least 10 adult oak trees, and for each population we selected six saplings (mean height = 70.5 ± 18.6 cm) separated by at least 2 m and with little to no signs of herbivory so that leaf defence levels largely represented constitutive defence levels of defence expression while also serving as baseline to experimentally assess inducible levels.

After selecting saplings at each site, we randomly assigned equal numbers to one of two treatments: 1) herbivore induction with *A. quercetorum* larvae or 2) no induction (control). We obtained *A. quercetorum* larvae from a population of *Q. robur* situated 200 km away from our study area. For both control and induced saplings, we selected one branch with three or four fully-expanded leaves. Although herbivory was absent or low in most cases for selected leaves, we estimated percent leaf area removed (mainly by miners and leaf chewers) prior to treatment application to account for pre-existing herbivory as this may influence defence levels due to site-specific induction. For this, we used the following scale: 0 = undamaged; 1 = 1–5% damaged; 2 = 6–10% damaged; 3 = 11–25% damaged; 4 = 26–50% damaged; 5 = 51–75% damaged; 6 = >75% damaged (Moreira et al. 2020). We used the average value across leaves per plant for statistical analysis.

For each beetle-induced sapling, we placed one third-instar larva on each leaf, and individually covered all leaves with a nylon bag to prevent herbivore dispersal or predation. For control saplings, we also covered the selected leaves with a nylon bag but without adding the larvae to control for the effect of bagging. Larvae were allowed to feed for two days, after which they were removed (with the nylon bags) and percent leaf area removed by the larvae was visually estimated, using the previously described scale. Prior baseline damage and the damage caused by the beetle larvae are clearly distinguishable, as the latter produces a characteristic leaf skeletonization pattern, whereas previous damage was caused mainly by leaf chewers and miners.

Shortly after removing the beetle larvae we collected leaf VOCs following Rasmann et al. (2011). Briefly, to collect VOCs we bagged the branches of the treatment leaves with a 1-l Nalophan bag, and we trapped VOCs on a charcoal filter for 3 h at a rate of 250 ml min^{-1} . After VOC collection, we stored all traps at -80°C until chemical analyses. Right after VOCs collection, we collected the same leaves to quantify leaf phenolic compounds. Leaves were oven-dried for 48 h at 40°C (Mahugo Santana et al. 2009).

Quantification of volatile organic compounds

To perform gas chromatography and spectrometric analyses of leaf VOCs, we first added 5 μl of naphthalene (20 ng ml^{-1}) as an internal standard (Pellissier et al. 2016) to the charcoal traps, and then eluted their contents with 400 μl of dichloromethane. We then injected 2 μl of the extract for each sample into a gas chromatograph (GC) coupled with a mass selective detector (MSD) fitted with a 30 m \times 0.25 mm \times 0.25 mm film thickness HP-5MS fused silica column. We operated the GC in splitless mode with helium as the carrier gas (flow rate 0.9 ml min^{-1}). The GC oven temperature program was: 1 min hold at 40°C, and then 10°C min^{-1} ramp to 240°C. We identified volatile terpenes using Kovats retention index from published work (Supporting information), the NIST Standard Reference Database 1A v17, and by comparison with commercial standards when available. We identified two groups of VOCs: monoterpenes and sesquiterpenes (Supporting information) for subsequent analyses. Finally, we quantified individual VOCs relative to the internal standard and used for statistical analyses those exhibiting a relative abundance higher than 1%.

Quantification of phenolic compounds

We extracted phenolic compounds from 20 mg of dry leaf tissue with 1 ml of 70% methanol in an ultrasonic bath for 15 min, followed by centrifugation (Galmán et al. 2019b). We then transferred the extracts to chromatographic vials. To perform the chromatographic analyses, we used ultra-high-performance liquid-chromatography 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 μm 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 15 μl .

For phenolic compound identification, we used an ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry. We identified four groups of phenolic compounds: flavonoids, ellagitannins and gallic acid derivatives ('hydrolysable tannins' hereafter), proanthocyanidins ('condensed tannins' hereafter) and hydroxycinnamic acid precursors to lignins ('lignins' hereafter). We quantified flavonoids as rutin equivalents, condensed tannins as catechin equivalents, hydrolysable tannins as gallic acid equivalents, and lignins as ferulic acid equivalents (Galmán et al. 2019a, Moreira et al. 2020). We achieved the quantification of these phenolic compounds by external calibration using the corresponding calibration curve at 0.25, 0.5, 1, 2 and 5 $\mu\text{g ml}^{-1}$ for each of the four standards used (rutin, catechin, gallic acid and ferulic acid).

We expressed phenolic compound concentrations in mg g^{-1} tissue on a dry weight basis.

Climatic variables

To estimate climatic conditions at sampling site, we extracted a subset of eight climatic variables from the WorldClim database (Hijmans et al. 2005) at a resolution of 30-arc-second (approximately 1 km distance) for the populations coordinates. Specifically, we selected BIO1 (annual mean temperature, °C), BIO4 (temperature seasonality, expressed as the standard deviation of temperature among months \times 100), BIO5 (maximum temperature of the warmest month, °C), BIO6 (minimum temperature of the coldest month, °C), BIO12 (annual precipitation, mm), BIO13 (precipitation of the wettest month, mm), BIO14 (precipitation of the driest month, mm), BIO15 (precipitation seasonality, expressed as standard deviation of precipitation across months) (Abdala-Roberts et al. 2016). We summarized these variables by conducting a principal component analyses (PCA) using the *prcomp* function in R ver. 3.4.1 (<www.r-project.org>). The first principal component ('PC climate' hereafter) explained 89% of the variance in the climatic variables across populations and was positively associated with annual mean temperature, maximum temperature of the warmest month, and minimum temperature of the coldest month but negatively associated with annual precipitation, precipitation of the wettest month, and precipitation of the driest month (Supporting information).

Statistical analyses

Elevational gradients in constitutive defences and their inducibility and climatic correlates

To test for elevational gradients in constitutive direct and indirect defences and their inducibility, we performed population-level multiple regressions (i.e. with population means) using the *lm* function in the *lme4* package in R ver. 3.4.1 (<www.r-project.org>). Specifically, we tested for an effect of elevation on constitutive phenolics and VOCs (i.e. using only control plants), as well as on their inducibility, i.e. mean level for induced plants minus the mean level for control plants at each site. For both constitutive and inducibility data, we ran separate models for each group of phenolic compounds (condensed tannins, hydrolysable tannins, flavonoids and lignins) and VOCs (monoterpenes and sesquiterpenes), as well as for total phenolics and total VOCs. In each of these models, we also included the amount of leaf herbivory on focal leaves to account for pre-treatment variation in natural herbivory (herbivory treatment above) as well as in the amount of experimentally-imposed damage by larvae. For control saplings, the herbivory estimate corresponded to damage that occurred before establishing herbivory treatments (as bagging prevented any further damage during the experiment). For herbivore-induced saplings, herbivory estimates represented damage prior to establishing the experiment plus the damage

caused by *A. quercetorum* larvae due to the induction experiment. In addition to the above population-level analyses, we also ran general linear mixed-effect models at the individual tree level testing for the effect of elevation, herbivory treatment and their interaction (all fixed factors) on direct and indirect defences. In this case, we ran individual models for each group of phenolic compounds (condensed tannins, hydrolysable tannins, flavonoids and lignins) and VOCs (monoterpenes and sesquiterpenes), as well as for individual phenolics and VOCs. Each of these tree-level models also included leaf herbivory as a covariate and population as a random factor. For models testing effects on individual phenolics and VOCs, we performed p-value adjustments using the false discovery rate for $p < 0.05$ to avoid inflating type I error due to multiple testing (Benjamini and Hochberg 1995).

We next investigated the association between climate and elevational variation in constitutive direct and indirect defences and their inducibility. To this end, in those cases where elevation had a significant effect on a trait (or totals for a given trait type) in the population-level analysis described above, we re-ran the model including (in addition to elevation and herbivory) the first principal component of the climate PCA (PC climate, above) as predictor. For these follow-up tests, it is important to keep in mind that temperature and precipitation variables were strongly correlated with elevation (temperature: $r = -0.96$, $p < 0.001$, $n = 18$; precipitation: $r = 0.95$, $p < 0.001$, $n = 18$). If a significant effect of elevation in the initial model for a trait (either totals or individual compounds groups) turned non-significant after accounting for climate, this would suggest that climate accounts for (i.e. underlies) the elevational gradient in such defence.

Elevational gradients in trait co-expression patterns

In addition to looking at trait types individually, we also tested for correlated trait expression patterns by means of co-inertia analyses using population means, using the *ade4* package in R (Dray et al. 2003, Dray and Dufour 2007). These analyses tested whether matrices of constitutive and induced direct and indirect defences using values for individual compound groups (above) were correlated (i.e. co-expressed) across oak populations (Pellissier et al. 2016, Defosse et al. 2018). First, we assessed whether there was a correlated expression between constitutive and induced trait levels separately for phenolics and VOCs. Namely, we tested for a correlated expression pattern between constitutive and induced phenolics (coinertia test 1) and between constitutive and induced VOCs (coinertia 2). Second, we assessed whether there was correlated expression between direct and indirect defences separately for constitutive and induced levels. Namely, we tested for a correlated expression between constitutive phenolics and constitutive VOCs (coinertia 3), and between induced phenolics and induced VOCs (coinertia 4). When one of the above coinertia analyses was significant we proceeded to run a multiple regression model using elevation and herbivory as predictors separately for each of the first two axes of the corresponding coinertia test. Then, if the effect of elevation was significant

for a given axis, we followed the same approach as above and ran a follow-up model including PC climate to test whether the observed pattern of trait correlated expression for a given axis of that coinertia test was underlain by climatic variation.

Results

Elevational gradients in constitutive defences and their inducibility and their climatic correlates

We found significant effects of elevation on constitutive levels of some groups of phenolic compounds, but not for others or total phenolics (Table 1, Fig. 1, Supporting information). Specifically, we found significant negative effects of elevation on constitutive lignins and hydrolysable tannins, whereas we observed no effect of elevation for flavonoids or condensed tannins (Table 1, Fig. 1). For indirect defences, we found no significant effect of elevation on the emissions of monoterpenes, sesquiterpenes or total VOCs (Table 1, Fig. 1, Supporting information).

Our assessment of climatic variation underlying the above gradients in plant defences showed that the effect of elevation on the concentration of constitutive hydrolysable tannins and lignins remained significant after accounting for climate in the models (Table 2), suggesting that climate did not underlie the elevational gradients observed for these compounds.

We found no significant effect of elevation on the inducibility of total phenolics, total VOCs or any of the groups of phenolic compounds or VOCs (Table 1, Fig. 2, Supporting information). Likewise, results from mixed-effect models at the tree level indicated no significant effects of herbivory treatment and the interaction between elevation and herbivory treatment for any of the groups of phenolics or VOCs (Supporting information). However, results concerning individual compounds showed elevation by herbivory treatment effects for a couple of individual VOCs, but not so for any of the individual phenolic compounds (Supporting information). Specifically, these significant interactions indicated that the magnitude of induction of α -fenchene and camphene was significantly greater at high elevations (Supporting information).

Elevational gradients in the correlated expression of defensive traits

Results from the coinertia analyses indicated a significant association between the correlated expression of constitutive and induced phenolics (coinertia 1, Table 3). We extracted the first two axes from this analysis, which were both associated with a mixture of constitutive and induced levels of different compounds (Supporting information). In contrast, the other coinertia tests indicated no significant association between the correlated expression of constitutive and induced VOCs (coinertia 2), constitutive phenolics and constitutive VOCs (coinertia 3) or induced phenolics and induced VOCs (coinertia 4) (Table 3).

Table 1. Multiple regressions showing the effects of elevation and leaf herbivory on the concentration of constitutive direct and indirect defences and their inducibility after *Altica quercetorum* feeding, as well as the correlated expression between constitutive and *A. quercetorum*-induced phenolics (coinertia 1), in leaves of saplings from 18 *Quercus pyrenaica* populations. β =slope estimator, r^2 =partial correlation coefficient. Significant p-values ($p < 0.05$) are in bold.

	Elevation			Leaf herbivory		
	β	r^2	p-value	β	r^2	p-value
Constitutive direct defences						
Condensed tannins	-0.29	0.08	0.262	-0.22	0.05	0.388
Hydrolysable tannins	-0.60	0.35	0.012	-0.59	0.34	0.012
Lignins	-0.57	0.32	0.018	-0.46	0.21	0.061
Flavonoids	-0.14	0.02	0.594	-0.63	0.40	0.007
Total phenolics	-0.38	0.14	0.134	-0.56	0.31	0.019
Constitutive indirect defences						
Monoterpenes	-0.17	0.03	0.517	-0.11	0.01	0.685
Sesquiterpenes	-0.09	0.01	0.725	-0.27	0.08	0.286
Total VOCs	-0.16	0.03	0.532	-0.09	0.01	0.717
Inducibility of direct defences						
Condensed tannins	0.12	0.01	0.654	-0.08	0.01	0.751
Hydrolysable tannins	0.39	0.15	0.119	0.01	< 0.01	0.978
Lignins	0.14	0.02	0.596	0.04	0.01	0.889
Flavonoids	0.08	0.01	0.771	0.01	< 0.01	0.963
Total phenolics	0.20	0.04	0.452	-0.02	< 0.01	0.948
Inducibility of indirect defences						
Monoterpenes	0.15	0.02	0.565	-0.40	0.16	0.114
Sesquiterpenes	0.34	0.12	0.181	0.04	0.01	0.891
Total VOCs	0.25	0.06	0.329	-0.35	0.12	0.166
Correlated expression of constitutive and induced defences						
Coinertia 1	-0.48	0.23	0.049	0.32	0.10	0.213

Based on the above results, we tested for and found a significant negative effect of elevation on the co-expression of constitutive and induced phenolics for one of the axes of the coinertia 1 test (Table 1, Fig. 3), indicating that this pattern of co-expression of direct defences weakened with increasing elevation. The effect of elevation on values for this coinertia 1 axis remained significant after accounting for climate in the model (Table 2), suggesting that climatic factors did not explain the elevational gradient in correlated expression of constitutive and induced phenolics depicted by this coinertia axis.

Discussion

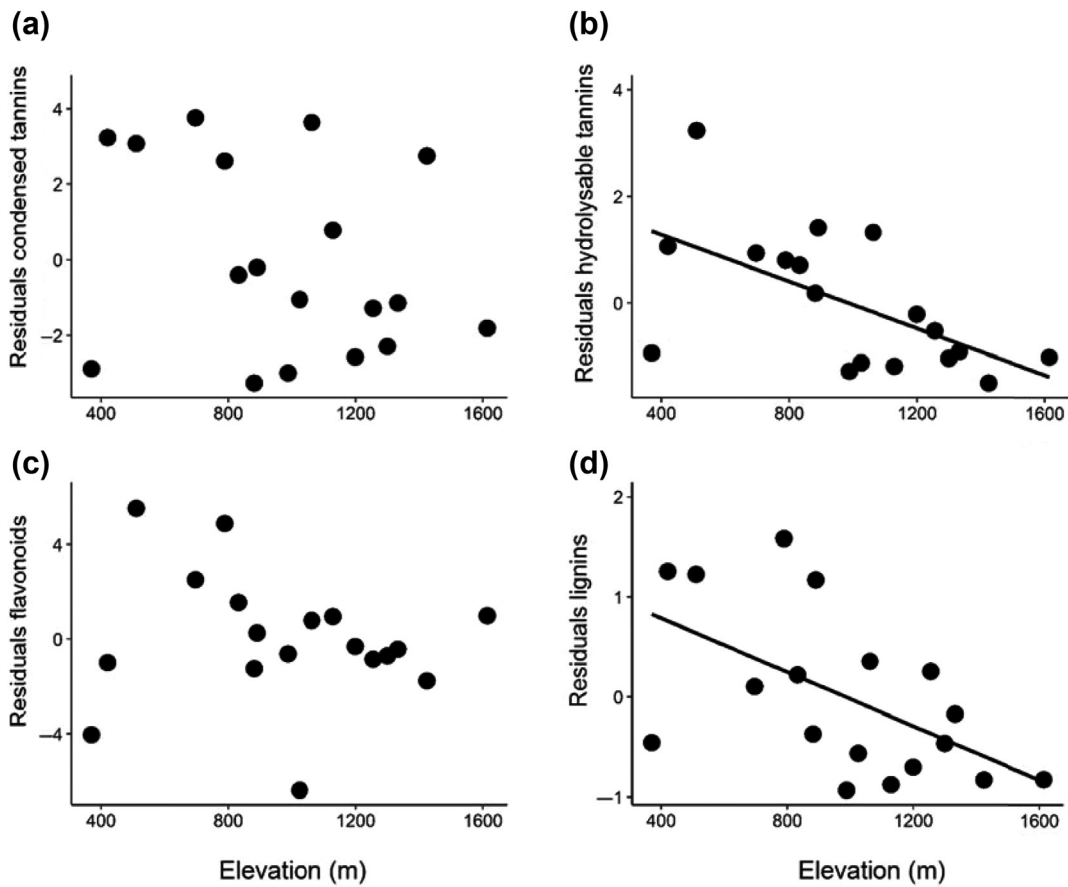
We found partial evidence for elevational gradients in direct defences in *Quercus pyrenaica*, whereby constitutive lignins and hydrolysable tannins (but not flavonoids or condensed tannins) significantly decreased with increasing elevation, and none of the analyzed groups of phenolic compounds exhibited a gradient in inducibility. Likewise, there was no elevational gradient in constitutive indirect defences or their inducibility (total monoterpenes and sesquiterpenes). The only exception came from results for individual compounds, in which case the induction of two monoterpenes (α -fenchene and camphene) increased toward higher elevations. On the other hand, the coinertia analysis indicated a significant pattern of co-expression of direct defences whereby the simultaneous expression of constitutive and induced phenolics weakened with increasing elevation, whereas no significant

patterns of co-expression involving VOCs were found. Lastly, and counter to expectations, climatic factors did not account for these elevational gradients in phenolic compounds or their correlated expression patterns.

Our analysis of constitutive direct defences indicated a decrease in lignins and hydrolysable tannins toward higher elevations. Condensed and hydrolysable tannins are well known to act as feeding deterrents reducing plant digestibility in both vertebrate and invertebrate herbivores (Misra and Fridovich 1972, Rosenthal and Berenbaum 1992, Thiboldeaux et al. 1998). Whilst condensed tannins have the ability to bind and precipitate digestive enzymes in the acid gut of vertebrate herbivores (Hagerman 1989), hydrolysable tannins might be more reactive against insect herbivores since their prooxidant activity is favored at basic pH, characteristics of insects gut (Barbehenn et al. 2006, Moilanen et al. 2013). Lignins are also important antiherbivore barriers, by acting as toxic compounds, and contribute to increased leaf toughness (Bonawitz and Chapple 2010, Pearse 2011). Interestingly, our previous work with the English oak *Q. robur* conducted in the same region indicated that flavonoids were the only group of phenolic compounds that varied with elevation and did so positively (Abdala-Roberts et al. 2016). This suggests oak species-specific differences in the types of constitutive phenolic compounds exhibiting elevational clines as well as the direction of such gradients.

The observed elevational patterns for constitutive hydrolysable tannins and lignins agree with previous studies showing that constitutive defences decrease with increasing elevation (Pellissier et al. 2012, 2014, Rasmann et al. 2014c),

I) Contitutive direct defences:



II) Contitutive indirect defences:

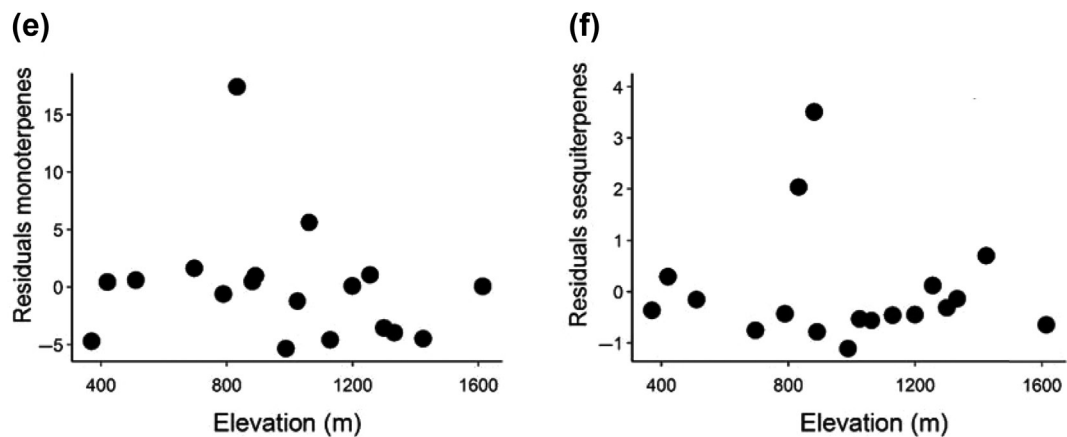


Figure 1. Partial residual plots from the multiple regressions controlling for the effect of leaf herbivory. The plots show the relationships between (I) elevation and constitutive direct defences, namely: (a) condensed tannins, (b) hydrolysable tannins, (c) flavonoids, (d) lignins; and (II) elevation and constitutive indirect defences, namely: (e) monoterpenes and (f) sesquiterpenes in leaves of saplings from *Quercus pyrenaica*. Dots represent *Q. pyrenaica* populations ($n = 18$). Predicted relationships come from simple linear regressions and the slope of each relationship is the partial correlation coefficient (r^2) for the effect of the predictor (x-axis) on a given plant trait (y-axis) (r^2 values are reported in Table 1). Black solid lines represents a significant correlation ($p < 0.05$).

Table 2. Multiple regressions showing the effects of elevation, leaf herbivory and climate (based on a principal component analysis summarizing a subset of bioclimatic variables) on constitutive hydrolysable tannins and lignins and the correlated expression between constitutive and *Altica quercetorum*-induced phenolics (coinertia 1) in leaves of saplings from 18 *Quercus pyrenaica* populations. β =slope estimator, r^2 =partial correlation coefficient. Significant p-values ($p < 0.05$) are in bold.

	Elevation			Leaf herbivory			PC climate		
	β	r^2	p-value	β	r^2	p-value	β	r^2	p-value
Constitutive hydrolysable tannins	-0.003	0.38	0.010	-1.668	0.37	0.012	-0.274	0.06	0.374
Constitutive lignins	-0.002	0.40	0.008	-0.808	0.26	0.045	-0.266	0.12	0.183
Coinertia 1	-0.546	0.30	0.030	0.307	0.09	0.247	-0.292	0.09	0.271

possibly because constitutive defences are favored at low elevations where herbivore pressure is greater and more predictable. In contrast, our previous studies on *Pinus* spp. and *Cardamine* spp. found that constitutive chemical defences increased with elevation possibly due to a higher cost of tissue loss and regrowth in more limiting environments found at high elevation (Moreira et al. 2014, Pellissier et al. 2016). These contrasting patterns suggest that different cost-saving strategies are at play shaping direct defences along elevational gradients: one driven by low resource availability selecting for increased constitutive levels at high elevation due to higher costs of tissue loss to herbivory, and the other where high and consistent herbivore pressure favors greater levels of constitutive defences at low elevations due to costs associated with lagged induced defences. Further work on elevational gradients that explicitly manipulates the effects of resource availability, herbivore pressure and measures the costs of defence expression is necessary to tease apart these mechanisms for other plant taxa.

In contrast to constitutive direct defences, we found no evidence of an elevational cline in constitutive VOCs for *Q. pyrenaica*. These findings run counter to previous studies showing that constitutive VOCs in *Vicia* (Rasmann et al. 2014c) and extra-floral nectar in *Inga* (Koptur 1985) decrease at high elevations, possibly due to lower herbivory and reliance on predator- or parasitoid-mediated defence. Our findings suggest that predator pressure does not vary substantially across elevations, or if it does, that this variation is inconsequential in shaping concomitant clines in constitutive volatile emissions.

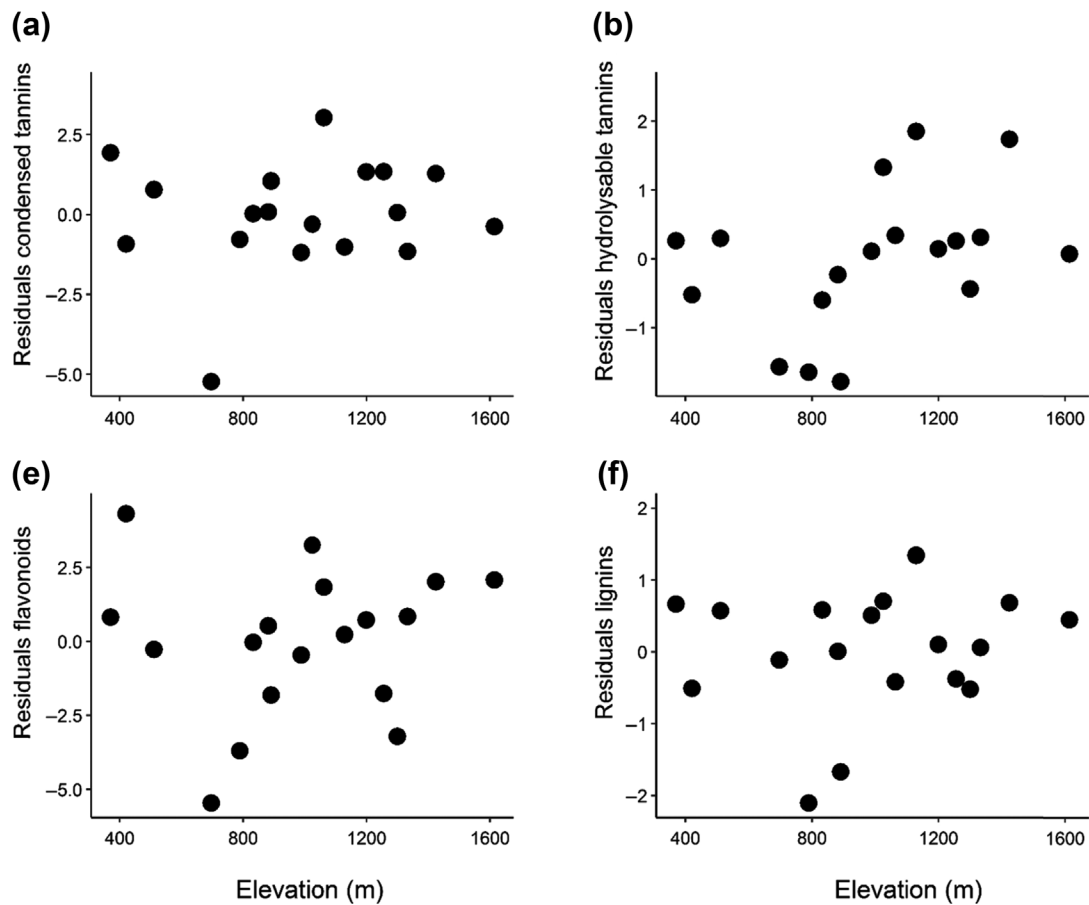
Contrary to expectations, our population-level analyses indicated no elevational gradients in the inducibility of either direct or indirect defences (totals or by group). As for constitutive levels, weak or unpredictable predator pressure would favor inducibility at high elevation (Rasmann et al. 2014c), whereas limiting conditions at high elevation would favor constitutive over induced indirect defences due to greater costs of tissue loss after herbivory (Moreira et al. 2014, Buckley et al. 2019). Our null findings suggest that neither of these mechanisms is at work (or at least not strongly enough) for either direct or indirect induced defences in the studied *Q. pyrenaica* populations. Moreover, these results contrast with our previous work showing greater inducibility for oak species with higher elevational ranges (analysis included *Q. pyrenaica*; Galmán et al. 2019a), underscoring differential

intra- versus inter-specific patterns of clinal variation in oak defences (Moreira et al. 2018a).

Despite the overall lack of elevational changes in the induction of direct and indirect defences, when individual compounds were analyzed we found two monoterpenes (namely α -fenchene and camphene) were more strongly induced at high elevations. These results counter patterns for *Cardamine* spp. for which VOCs showed greater induction at low elevation (Pellissier et al. 2016), but agree with Rasmann et al. (2014c) who found greater inducibility of VOCs at high elevations for *Vicia sepium*. Camphene was shown to be involved in egg parasitoid attraction to stink bug-induced soybean (Moraes et al. 2008) and entomopathogenic nematode attraction to root herbivores (Rasmann et al. 2011, Grunseich et al. 2020), whereas α -fenchene is also involved in attraction of bark beetle parasitoids to infested pines (Sullivan et al. 2000). Thus, at least for these monoterpenes, greater inducibility at high elevation could be explained by defence cost-saving strategy under weak or unpredictable predator and parasitoid effects (assuming these compounds mediate herbivore enemy attraction in *Quercus*).

Recent studies have attributed contrasting elevational clines in constitutive versus induced defences to allocation constraints leading to tradeoffs (reviewed by Moreira et al. 2018a). Likewise, other studies have also reported on shifts in the co-expression of direct and indirect defences along elevation gradients, arguing changes in selective forces favoring single versus simultaneously expressed traits which in turn shape the unfolding of defensive syndromes along elevational gradients (Pellissier et al. 2016, Defossez et al. 2018). Our coinertia analysis explicitly addressed these patterns of correlated trait expression and uniquely revealed an elevational change in the nature of the co-expression of induced and constitutive phenolics, whereby the simultaneous expression of constitutive and induced phenolics weakened toward higher elevations (i.e. greater co-expression of these direct defences at low elevations). This finding contrasts with our previous work showing no elevational gradient in the correlated expression of induced and constitutive direct defences across 18 oak species (Galmán et al. 2019a), which again points at differences when analyzing intra- versus inter-specific patterns. Nonetheless, our result is similar to that found in a previous work with *Cardamine* spp. showing increasing co-expression of induced direct and indirect defences across species toward low elevations (Pellissier et al. 2016).

I) Inducibility of direct defences:



II) Inducibility of indirect defences:

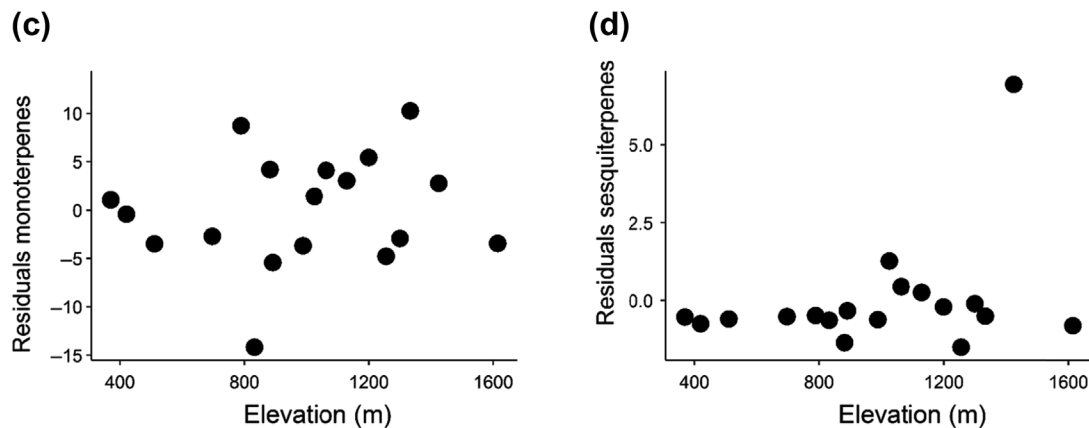


Figure 2. Partial residual plots from the multiple regressions controlling for the effect of leaf herbivory. The plots show the relationships between (I) elevation and inducibility of direct defences after *Altica quercetorum* feeding, namely: (a) condensed tannins, (b) hydrolysable tannins, (c) flavonoids, (d) lignins; and (II) elevation and inducibility of indirect defences after *Altica quercetorum* feeding, namely: (e) monoterpenes and (f) sesquiterpenes in leaves of saplings from *Quercus pyrenaica*. Dots represent *Q. pyrenaica* populations ($n=18$). Predicted relationships come from simple linear regressions and the slope of each relationship is the partial correlation coefficient (r^2) for the effect of the predictor (x-axis) on a given plant trait (y-axis) (r^2 values are reported in Table 1).

Table 3. Results of the coinertia analyses between constitutive and *Altica quercetorum*-induced phenolics (coinertia 1), constitutive and *A. quercetorum*-induced volatiles (VOCs) (coinertia 2), constitutive phenolics and constitutive VOCs (coinertia 3) and *A. quercetorum*-induced phenolics and *A. quercetorum*-induced VOCs (coinertia 4). Significant p-values ($p < 0.05$) are in bold.

Coinertia	Defence (coinertia) axis	$P_{\text{Coinertia}}$
1	Constitutive phenolics – Induced phenolics	0.003
2	Constitutive VOCs – Induced VOCs	0.112
3	Constitutive phenolics – Constitutive VOCs	0.666
4	Induced phenolics – Induced VOCs	0.663

Concerning the *Cardamine* system, the authors argued that herbivore abundance and richness are often higher at lower elevations (Pellissier et al. 2012, 2014), which would select for multiple defensive traits or strategies with synergistic or complementary effects in order to maximize resistance. While this could explain our result, it is also possible that higher resource availability at low elevations favors the simultaneous deployment of both strategies due to lower costs of defences, whereas their co-expression weakens under lower resource availability at high elevations. In addition, unlike Pellissier et al. (2016), we did not find any evidence that indirect defences (VOCs) were involved in the *Q. pyrenaica* defence co-expression patterns. The presence of clines in indirect defences and their involvement in correlated expression patterns would be expected for plant species or communities that rely more heavily on natural enemies for indirect defence (Rasmann et al. 2014c), which might not be the case for the

studied *Q. pyrenaica* populations. Nonetheless, as mentioned above, a more detailed assessment and functional evaluation of VOCs that includes other geographical areas is needed to test whether these compounds structure (and if so in what way) elevational clines in *Q. pyrenaica* multi-trait defensive phenotypes.

Previous research has shown that abiotic factors are important drivers of plant defence allocation along elevation gradients (Moreira et al. 2014, Pellissier et al. 2014, Kergunteuil et al. 2019). Specifically, these studies found that climatic variables (e.g. temperature and precipitation) correlate with individual defensive traits (reviewed by Moreira et al. 2018a) as well as patterns of correlated trait expression (Pellissier et al. 2016, Galmán et al. 2019a). Our findings indicated that the observed elevational clines in constitutive levels of phenolic compounds (lignins and hydrolysable tannins), and the correlated expression of constitutive and induced phenolic compounds remained significant after accounting for climatic variables. This suggests that climatic forcing did not underlie the observed elevational patterns of direct chemical defences in *Q. pyrenaica*. One possible limitation to keep in mind is that the range of variation in temperature and precipitation across the studied gradient may have not been large enough to affect defensive traits. Thus, whereas annual mean temperature varied 5.6°C from the lowest to the highest elevation sites, annual precipitation varied only 357 mm. Despite this, the observed negligible effects of climate are not unique as previous research involving broader gradients has similarly found that climate only partially accounts for elevational gradients in plant defences (Pellissier et al. 2016, Galmán et al. 2018, 2019a). Instead, other abiotic factors such as soil conditions could underlie variation in plant defence across elevations (De Long et al. 2016, Pellissier et al. 2016). For instance, Pellissier et al. (2016) found that soil fertility positively correlates with constitutive glucosinolates in plants of *Cardamine* plant species growing at higher elevations. In addition, De Long et al. (2016) reported that soil nitrogen availability drove elevational gradients in plant community-level chemical defences in a subarctic tundra heath. Although observational approaches such our current study are fruitful for understanding the concurrent effects of abiotic factors (Moreira et al. 2014, Abdala-Roberts et al. 2016, Pellissier et al. 2016, Galmán et al. 2019a), they also carry several limitations (discussion in Abdala-Roberts et al. 2016). Experimental manipulations of candidate abiotic drivers are needed to disentangle their effects on plant–herbivore systems (Pellissier et al. 2014). In addition, phenological changes in leaf chemical composition may also take place and strongly

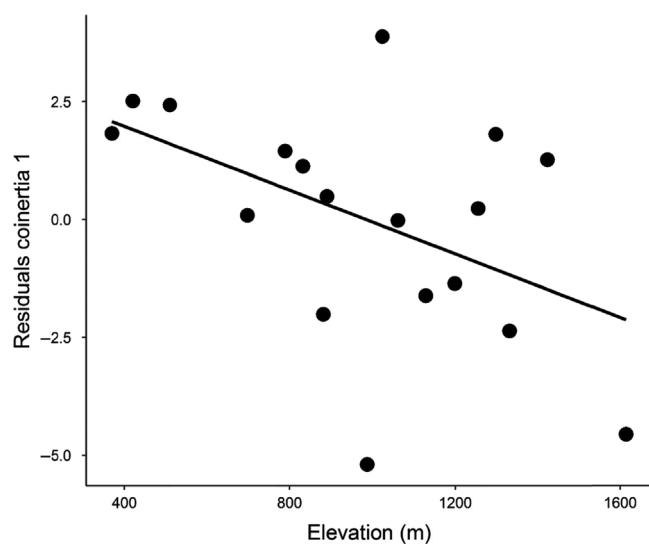


Figure 3. Correlation between elevation and the correlated expression of constitutive and *Altica quercetorum*-induced phenolics (second axis of the coinertia analysis 1; Methods) in leaves of saplings from *Quercus pyrenaica*. Dots represent *Q. pyrenaica* populations ($n = 18$). Predicted relationship comes from simple linear regression and the slope of each relationship is the partial correlation coefficient (r^2) for the effect of the predictor (x-axis) on a given plant trait (y-axis) (r^2 values are reported in Table 1). The observed relationship depicted a pattern whereby at populations at lower elevation invested more in the simultaneous expression of constitutive and induced phenolics. Black solid line represents a significant correlation ($p < 0.05$).

shape defence allocation and herbivory (Zehnder et al. 2009). Depending on the season, the consequences of leaf herbivore for the plant are not the same and the plant may shift between the different defensive strategies. Further studies should therefore investigate the differences in phenology of plant defences across elevation by sampling at several time points, especially during the start and middle of the season.

Our results emphasize the importance of looking at multiple plant defensive traits and their correlated expression patterns for achieving a more complete understanding of clinal variation in plant defensive phenotypes as these are inherently multivariate. By doing so, we reveal a unique elevational pattern of correlated expression of constitutive and induced chemical defences in *Q. pyrenaica*. It is possible that increased herbivore pressure and/or resources at low elevations favors the simultaneous expression of these strategies, and that increasing resource limitation at high elevations prevents co-expression and leads to tradeoffs. Further manipulations of herbivore pressure and resource availability are necessary to disentangle these mechanisms. Additionally, research on genetic factors (e.g. gene linkage) underlying patterns of trait-correlated expression is also needed to assess the evolutionary implications of our results. Controlled experiments under greenhouse conditions or in common gardens could help to reveal the genetic basis and mechanisms potentially linking plant defensive traits. Likewise, experimental manipulations under controlled conditions can also overcome potential biases or logistical limitations in field experiments (e.g. the selection of trees with low damage could have resulted in a bias for better defended oak genotypes), and serve as a basis for comparison to field patterns and to isolate and test specific ecological mechanisms.

Data availability statement

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.j3tx95xcj>> (Galmán et al. 2020).

Acknowledgements – We thank Silvana Pouceiro and María Lores for their help in the field.

Funding – This research was financially supported by a Spanish National Research Grant (AGL2015-70748-R), a Regional Government of Galicia Grant (IN607D 2016/001) and the Ramón y Cajal Research Programme (RYC-2013-13230) to XM. MAS was supported by the Swiss National Science Foundation Projects 31003A_163334 and 31003A_179481.

Author contributions

Xoaquín Moreira and Sergio Rasmann share last authorship. **Andrea Galmán**: Conceptualization (equal); Data curation (lead); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – original draft (lead). **Luis Abdala-Roberst**: Conceptualization (supporting); Investigation (supporting); Writing – review and editing (supporting). **Pola Wartalska**: Formal analysis (equal); Methodology (equal); Writing – review and

editing (supporting). **Felisa Covelo**: Formal analysis (equal); Methodology (equal). **Gregory Roder**: Data curation (supporting); Formal analysis (equal); Methodology (equal); Writing – review and editing (supporting). **Mark A. Szenteczki**: Formal analysis (equal); Methodology (equal); Writing – review and editing (supporting). **Xoaquín Moreira**: Conceptualization (equal); Formal analysis (supporting); Investigation (equal); Writing – review and editing (lead). **Sergio Rasmann**: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Writing – review and editing (lead).

References

- Abdala-Roberts, L. et al. 2016. Biotic and abiotic factors associated with altitudinal variation in plant traits and herbivory in a dominant oak species. – *Am. J. Bot.* 103: 2070–2078.
- Abdala-Roberts, L. et al. 2019. Tri-trophic interactions: bridging species, communities and ecosystems. – *Ecol. Lett.* 22: 2151–2167.
- Agrawal, A. A. and Hastings, A. P. 2019. Tradeoffs constrain the evolution of an inducible defense within but not between plant species. – *Ecology* 100: e02857.
- Barbehenn, R. V. et al. 2006. Ellagitannins have greater oxidative activities than condensed tannins and galloyl glucoses at high pH: potential impact on caterpillars. – *J. Chem. Ecol.* 32: 2253–2267.
- Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. – *J. R. Stat. Soc.* 57: 289–300.
- Bonowitz, N. D. and Chapple, C. 2010. The genetics of lignin biosynthesis: connecting genotype to phenotype. – *Annu. Rev. Genet.* 44: 337–363.
- Buckley, J. et al. 2019. Variation in growth and defence traits among plant populations at different elevations: implications for adaptation to climate change. – *J. Ecol.* 107: 2478–2492.
- Coley, P. D. et al. 1985. Resource availability and plant antiherbivore defense. – *Science* 230: 895–899.
- De Long, J. R. et al. 2016. Effects of elevation and nitrogen and phosphorus fertilization on plant defence compounds in sub-arctic tundra heath vegetation. – *Funct. Ecol.* 30: 314–325.
- Defossez, E. et al. 2018. The unfolding of plant growth form-defence syndromes along elevation gradients. – *Ecol. Lett.* 21: 609–618.
- Dobzhansky, T. 1950. Evolution in the tropics. – *Am. Sci.* 38: 209–221.
- Dray, S. and Dufour, A. B. 2007. The ade4 package: implementing the duality diagram for ecologists. – *J. Stat. Softw.* 22: 1–20.
- Dray, S. et al. 2003. Co-inertia analysis and the linking of ecological data tables. – *Ecology* 84: 3078–3089.
- Endara, M. J. and Coley, P. D. 2011. The resource availability hypothesis revisited: a meta-analysis. – *Funct. Ecol.* 25: 389–398.
- Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. – *Ecology* 51: 565–581.
- Fernandez de Ana, F. J. et al. 1996. *Alitica quercetorum* Foudras, 1860 (Col.:Chrysomelidae), oak's defoliator in Galicia (NW Iberian Peninsula). – *For. Syst.* 5: 275–303.
- Galmán, A. et al. 2018. A global analysis of elevational gradients in leaf herbivory and its underlying drivers: effects of plant

- growth form, leaf habit and climatic correlates. – *J. Ecol.* 106: 413–421.
- Galmán, A. et al. 2019a. Inducibility of chemical defences in young oak trees is stronger in species with high elevational ranges. – *Tree Physiol.* 39: 606–614.
- Galmán, A. et al. 2019b. Parallel increases in insect herbivory and defenses with increasing elevation for both saplings and adult trees of oak *Quercus* species. – *Am. J. Bot.* 106: 1558–1565.
- Galmán, A. et al. 2020. Data from: Elevational gradients in constitutive and induced oak defences based on individual traits and their correlated expression patterns. – Dryad Digital Repository, <<http://dx.doi.org/10.5061/dryad.j3tx95xcj>>.
- Garibaldi, L. A. et al. 2011. Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. – *Oecologia* 167: 117–129.
- Ghirardo, A. et al. 2012. Function of defensive volatiles in pedunculate oak *Quercus robur* is tricked by the moth *Tortrix viridana*. – *Plant Cell Environ.* 35: 2192–2207.
- Godschalx, A. et al. 2019. Contribution of different predator guilds to tritrophic interactions along ecological clines. – *Curr. Opin. Insect Sci.* 32: 104–109.
- Grunseich, J. M. et al. 2020. Risky roots and careful herbivores: sustained herbivory by a root-feeding herbivore attenuates indirect plant defences. – *Funct. Ecol.* 34: 1779–1789.
- Hagerman, A. E. 1989. Chemistry of tannin–protein complexation. – In: Hemingway, R. W. et al. (eds), *Chemistry and significance of condensed tannins*. Springer, pp. 323–333.
- Hijmans, R. J. et al. 2005. Very high resolution interpolated climate surfaces for global land areas. – *Int. J. Climatol.* 25: 1965–1978.
- Johnson, M. T. J. and Rasmann, S. 2011. The latitudinal herbivory defence hypothesis takes a detour on the map. – *New Phytol.* 191: 589–592.
- Kergunteuil, A. et al. 2018. Plant physical and chemical defence variation along elevation gradients: a functional trait-based approach. – *Oecologia* 187: 561–571.
- Kergunteuil, A. et al. 2019. Plant adaptation to different climates shapes the strengths of chemically mediated tritrophic interactions. – *Funct. Ecol.* 33: 1893–1903.
- Koptur, S. 1985. Alternative defenses against herbivores in *Inga* (Fabaceae: Mimosoideae) over an elevational gradient. – *Ecol. Lett.* 66: 1639–1650.
- Mahugo Santana, C. et al. 2009. Methodologies for the extraction of phenolic compounds from environmental samples: new approaches. – *Molecules* 14: 298–320.
- Mijuskovic, M. 1980. *Haltica quercetorum* Foudr., a pest of Macedonian oak in Montenegro. – *Poljopr. Sumar.* 26: 3–12.
- Misra, H. P. and Fridovich, I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. – *J. Biol. Chem.* 247: 3170–3175.
- Moilanen, J. et al. 2013. Characterization of bioactive plant ellagitannins by chromatographic, spectroscopic and mass spectrometric methods. – *Chemoecology* 23: 165–179.
- Moraes, M. C. B. et al. 2008. Response of the parasitoid *Telenomus podisi* to induced volatiles from soybean damaged by stink bug herbivory and oviposition. – *J. Plant Interact.* 3: 111–118.
- Moreira, X. et al. 2014. Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. – *Ecol. Lett.* 17: 537–546.
- Moreira, X. et al. 2018a. Elevational gradients in plant defences and insect herbivory: recent advances in the field and prospects for future research. – *Ecography* 41: 1485–1496.
- Moreira, X. et al. 2018b. Latitudinal variation in plant chemical defences drives latitudinal patterns of leaf herbivory. – *Ecography* 41: 1124–1134.
- Moreira, X. et al. 2018c. Assessing the influence of biogeographical region and phylogenetic history on chemical defences and herbivory in *Quercus* species. – *Phytochemistry* 153: 64–73.
- Moreira, X. et al. 2020. Ontogenetic consistency in oak defence syndromes. – *J. Ecol.* 108: 1822–1834.
- Pearse, I. S. 2011. The role of leaf defensive traits in oaks on the preference and performance of a polyphagous herbivore, *Orgyia vetusta*. – *Ecol. Entomol.* 36:635–642.
- Pearse, I. S. and Hipp, A. L. 2012. Global patterns of leaf defenses in oak species. – *Evolution* 66: 2272–2286.
- Pearse, I. S. et al. 2013. Headspace volatiles from 52 oak species advertise induction, species identity and evolution, but not defense. – *J. Chem. Ecol.* 39: 90–100.
- Pellissier, L. et al. 2012. Shifts in species richness, herbivore specialisation and plant resistance along elevation gradients. – *Ecol. Evol.* 2: 1818–1825.
- Pellissier, L. et al. 2014. High elevation *Plantago lanceolata* plants are less resistant to herbivory than their low elevation conspecifics: is it just temperature? – *Ecography* 37: 950–959.
- Pellissier, L. et al. 2016. The simultaneous inducibility of phytochemicals related to plant direct and indirect defences against herbivores is stronger at low elevation. – *J. Ecol.* 104: 1116–1125.
- Ramilo, P. et al. 2017. Volatile organic compounds emitted by *Quercus pyrenaica* Willd. and its relationship with saproxylic beetle assemblages. – *Arthropod–Plant Interact.* 11: 221–234.
- Rasmann, S. et al. 2011. Direct and indirect root defences of milkweed *Asclepias syriaca*: trophic cascades, trade-offs and novel methods for studying subterranean herbivory. – *J. Ecol.* 99: 16–25.
- Rasmann, S. et al. 2014a. The altitudinal niche breadth hypothesis in plant–insect interaction. – *Annu. Plant Rev.* 47: 339–359.
- Rasmann, S. et al. 2014b. Climate-driven change in plant–insect interactions along elevation gradients. – *Funct. Ecol.* 28: 46–54.
- Rasmann, S. et al. 2014c. Differential allocation and deployment of direct and indirect defences of *Vicia sepium* along elevation gradients. – *J. Ecol.* 102: 930–938.
- Rosenthal, G. and Berenbaum, M. 1992. *Herbivores: their interactions with secondary plant metabolites*. Vol. 1. The chemical participants. – Academic Press.
- Scheidel, U. and Bruelheide, H. 2001. Altitudinal differences in herbivory on montane Compositae species. – *Oecologia* 129: 75–86.
- Schemske, D. W. et al. 2009. Is there a latitudinal gradient in the importance of biotic interactions? – *Annu. Rev. Ecol. Evol. Syst.* 40: 245–269.
- Sullivan, B. T. et al. 2000. Attraction of the bark beetle parasitoid *Roptrocercus xylophagorum* (Hymenoptera: Pteromalidae) to host-associated olfactory cues. – *Environ. Entomol.* 29: 1138–1151.
- Thiboldeaux, R. L. et al. 1998. Effects of juglone (5-hydroxy-1,4-naphthoquinone) on midgut morphology and glutathione status in Saturniid moth larvae. – *Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol.* 120: 481–487.
- Zehnder, C. B. et al. 2009. Elevational and seasonal variation in the foliar quality and arthropod community of *Acer pensylvanicum*. – *Environ. Entomol.* 38: 1161–1167.