RESEARCH ARTICLE

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Parallel increases in insect herbivory and defenses with increasing elevation for both saplings and adult trees of oak (*Quercus*) species

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PREMISE: Herbivory is predicted to increase toward warmer and more stable climates found at lower elevations, and this increase should select for higher plant defenses. Still, a number of recent studies have reported either no evidence of such gradients or reverse patterns. One source of inconsistency may be that plant ontogenetic variation is usually not accounted for and may influence levels of plant defenses and herbivory.

METHODS: We tested for elevational gradients in insect leaf herbivory and leaf traits putatively associated with herbivore resistance across eight oak (*Quercus*, Fagaceae) species and compared these patterns for saplings and adult trees. To this end, we surveyed insect leaf herbivory and leaf traits (phenolic compounds, toughness and nutrients) in naturally occurring populations of each oak species at low-, mid- or high-elevation sites throughout the Iberian Peninsula.

RESULTS: Leaf herbivory and chemical defenses (lignins) were unexpectedly higher at mid- and high-elevation sites than at low-elevation sites. In addition, leaf chemical defenses (lignins and condensed tannins) were higher for saplings than adult trees, whereas herbivory did not significantly differ between ontogenetic stages. Overall, elevational variation in herbivory and plant chemical defenses were consistent across ontogenetic stages (i.e., elevational gradients were not contingent upon tree ontogeny), and herbivory and leaf traits were not associated across elevations.

CONCLUSIONS: These findings suggest disassociated patterns of elevational variation in herbivory and leaf traits, which, in turn, are independent of plant ontogenetic stage.

KEY WORDS elevational gradients; Fagaceae; Iberian Peninsula; nutrients; phenolic compounds; plant–herbivore interactions; plant ontogeny; *Quercus*.

Understanding the drivers of spatial variation in species interactions represents a major challenge in ecology (Linhart and Grant, 1996). A useful approach to this end has been to study interactions along environmental (i.e., along latitude or elevation) gradients (Schemske et al., 2009; Rasmann et al., 2014a). In particular, gradient-based analyses have been important for studying interactions between plants and their herbivores. Theory holds that herbivory (defined as the consumption of all or a part of a plant) increases toward warmer and more stable climates characteristic of lower elevations, and this increase has selected for greater investment in plant defenses at low relative to high elevations (e.g., Scheidel and Bruelheide, 2001; Zehnder et al., 2009; Garibaldi et al., 2011; Pellissier et al., 2012, 2016). However, the generality of this prediction has been called into question by a number of studies reporting no evidence of elevational gradients in herbivory and plant defenses or higher plant defense and herbivory with increasing elevation (reviewed by Rasmann et al., 2014b; Moreira et al., 2018a).

Mixed support for the predicted elevational gradients in herbivory and plant defenses can be explained in several ways. Most studies (ca. 65%) have assessed elevational gradients for only one or a few plant defensive traits, mostly chemical defenses (reviewed by Moreira et al., 2018a), without necessarily establishing clear criteria for the selection of target traits or their role in herbivore resistance (Rasmann et al., 2014b). As a result, assessments of plant defensive phenotypes have usually been incomplete or have failed to target ecologically important traits for the studied system. In this sense, other plant traits associated with plant quality for herbivores, such as physical features (e.g., toughness) or macronutrients (Mattson, 1980; Agrawal, 2007) could also influence herbivores, but so far they have been overlooked (Moreira et al., 2018a). A more comprehensive assessment of multivariate defensive phenotypes that includes secondary metabolites, physical defenses, and traits related to the nutritional value for herbivores will likely improve our understanding of the ecological mechanisms and evolutionary consequences of elevational clines in plant–herbivore interactions.

Another important factor to consider when studying plant–herbivore interactions along ecological gradients is plant developmental stage (Galmán et al., 2018). For long-lived plants, defense levels are expected to build up from the sapling to the adult stage, following an increase in available resources to be diverted to defensive functions (Boege and Marquis, 2005; Barton and Koricheva, 2010; Barton and Boege, 2017). If adult plants are better defended than saplings, we would expect less difference in herbivory between life stages at high elevations because herbivore pressure is already low and being more defended has less impact than at low elevation (Galmán et al., 2018). The resulting elevational change in herbivory should thus be weaker for adults than saplings (Galmán et al., 2018). Alternatively, adults may be more detectable and therefore more attacked than saplings (Castagneyrol et al., 2013; Moreira et al., 2017). Here we would also expect differences in herbivory (and, concomitantly, defenses) between adults and saplings to be weaker at high elevation where detectability has less impact because herbivore pressure is already low. However, the resulting elevational change in herbivory and defenses would be expected to be stronger for adults than saplings because increased detectability leads to a more pronounced increase in herbivory (and thus defenses) with increasing herbivore pressure at low elevations. Studies that explore how gradients in defense and herbivory depend on plant ontogeny are needed to test these predictions and in so doing increase our understanding of clinal variation in plant–herbivore interactions.

In this study, we conducted a field test of elevational gradients in insect leaf herbivory and leaf defensive and nutritional traits for saplings and adult trees of eight oak (*Quercus*, Fagaceae) species. Specifically, we asked: (1) Are there elevational gradients in insect leaf herbivory and in plant defenses and nutritional traits? (2) Are any such elevational gradients contingent on plant ontogenetic stage? (3) Is elevational and ontogenetic variation in herbivory associated with variation in leaf traits? To this end, we surveyed herbivory and leaf traits in naturally occurring populations of each oak species found at low-, mid- and high-elevation sites in the Iberian Peninsula. For each population, we quantified leaf damage by chewing insects, specific leaf area (a physical trait correlated with toughness), and the concentration of phenolic compounds (putative chemical defenses in oaks), and phosphorus and nitrogen (proxies of nutritional content) in saplings and adult oak trees. Overall, this study builds toward a better understanding of the ecological mechanisms behind elevational gradients in plant–herbivore interactions.

MATERIALS AND METHODS

Natural history

To address whether elevation and plant ontogenetic stage affected insect leaf herbivory and leaf traits, we sampled eight oak species throughout the Iberian Peninsula that collectively encompass an elevational gradient of 1330 m: *Quercus robur*, *Q. petraea*, *Q. suber*, *Q. ilex*, *Q. pubescens*, *Q. lusitanica*, *Q. canariensis*, and *Q. coccifera* (Table 1). At the studied sites, leaf burst for these *Quercus* species usually occurs in April and leaves turn brown and drop off in October. The studied oaks are attacked by several insect herbivores, mainly leaf chewers such as *Tortrix viridana* (Lepidoptera: Tortricidae), *Lymantria dispar* (Lepidoptera: Lymantridae), and *Malacosoma neustria* (Lepidoptera: Lasiocampidae) (Abdala-Roberts et al., 2016). Leaf miners and gall formers were less common at the study sites (<5% of the leaves; A. Galmán, personal observation).

Field sampling and leaf herbivory measurements

At the end of the growing season, from late September to mid October 2017, we surveyed three populations of each species. Populations spanned virtually the entire elevational range of the studied oaks and corresponded to low-, mid- and high-elevation sites based on the elevational range reported for each species (Table 1). Each site included at least 15 adult oak trees for which we randomly selected five adult (reproductive) trees and five saplings (<1 m tall). In total, we sampled 240 trees (8 oak species × 3 elevations × 2 ontogenetic stages × 5 individuals). Most leaf damage was caused by insect leaf chewers, and we did not find signs of vertebrate leaf herbivory for either saplings or adult trees (A. Galmán, personal observation).

For each adult tree, we randomly selected two low-hanging branches (2–3 m from the ground) and collected 25 leaves per branch. For each leaf, we visually estimated the percentage of leaf area removed by insect leaf chewers using 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%

TABLE 1. Latitude, longitude (decimal degrees), and elevation (m a.s.l.) of populations sampled for each oak (*Quercus*) species. These populations were sampled following the optimal elevational ranges (low, mid and high elevations) of each oak species. Optimal elevational range of each oak species was estimated from literature.

	Latitude			Longitude			Elevation (m)			Elevation range (m)
Plant species	Low	Mid	High	Low	Mid	High	Low	Mid	High	
Q. canariensis	36.35928	37.31829	36.53187	−5.6445	−8.5427	−5.5872	120	400	600	0–600
Q. coccifera	42.68305	42.01907	42.01002	−1.7951	1.0294	1.0184	400	800	990	0–1000
Q. ilex	42.3882	42.41805	42.36947	−7.1851	−6.6602	−6.6385	300	680	1046	0–1400
Q. lusitanica	42.87298	37.35346	37.31002	−9.1065	−8.47320	−8.5287	124	350	790	0–600
Q. petraea	42.84838	42.86471	42.81339	−6.9194	−6.8604	−6.9016	829	1130	1450	600–1800
Q. pubescens	42.05505	42.70484	42.72323	0.9514	−0.7890	−0.3079	600	830	1140	500–1500
Q. robur	42.45307	42.46818	42.47016	−8.5828	−8.4995	−8.3487	120	347	743	0–1000
Q. suber	42.39777	42.34394	37.31362	−8.1384	−8.6772	−8.5363	224	430	650	0–800

damaged) ("leaf herbivory" hereafter; Moreira et al., 2019). Values were averaged across all leaves to obtain a mean value per branch, then averaged across branches to obtain a single mean per tree for statistical analyses. For saplings, we collected 15 leaves throughout the plant canopy and visually estimated the percentage of leaf area removed by insect leaf chewers (as above) for all leaves per plant. We then averaged values across leaves to obtain a single mean value per sapling for statistical analyses. All sampled leaves were transported to the laboratory where they were scored by the same person (A. Galmán) to avoid biases in estimating leaf damage. Our method could have underestimated leaf herbivory by mammals (e.g., deer), although direct and indirect evidence of mammal presence suggest they were rare at most of the studied sites.

We also collected four fully expanded leaves per branch for adult individuals and six fully expanded leaves near the apical meristem for saplings to quantify plant traits (see ahead). We selected leaves with little or no evidence of herbivory to reduce variation in defenses or nutrients caused by site-specific induction (Moreira et al., 2019). We sampled completely expanded leaves, of roughly the same age (position on branch, color and consistency) and location in the canopy. Leaves were oven-dried for 48 h at 40°C, ground with liquid nitrogen, and stored at room temperature.

Quantification of specific leaf area

We estimated specific leaf area (SLA) for each leaf by dividing the surface area of a 9.5-mm disk by its dry mass. We selected mature leaves in a similar position along the branch, and only measured one leaf per plant because previous trials demonstrated relatively low leaf-to-leaf variation within individuals. SLA is correlated with leaf toughness (Mattson, 1980; Hanley et al., 2007) and was therefore taken as a proxy of leaf physical or structural resistance against herbivory.

Quantification of phenolic compounds

Phenolic compounds have been reported to confer resistance against insect herbivores in oaks (Feeny, 1970; Roslin and Salminen, 2008; Moreira et al., 2018b, c); therefore, they provide a suitable proxy of chemical defenses. Phenolic compounds were extracted from 20 mg of dry leaf tissue with 1 mL of 70% methanol in an ultrasonic bath for 15 min, followed by centrifugation (Moreira et al., 2014). We then transferred the extracts to chromatographic vials. For the chromatographic analyses, we used an ultra-high-performance liquid chromatograph (UHPLC Nexera LC-30AD; Shimadzu Corp., Kyoto, Japan) equipped with a Nexera SIL-30AC injector and one SPD-M20A UV/VIS photodiode array detector. Compounds were separated on a Kinetex 2.6 µm C18 82-102 Å, LC Column 100 × 4.6 mm (Phenomenex, Torrance, CA, USA), 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 10 µL. For phenolic compound identification, we used an ultra-performance liquid chromatograph coupled with electrospray ionization quadrupole (Thermo Dionex Ultimate 3000 LC; Thermo Fisher Scientific, Waltham, MA, USA) time-of-flight mass spectrometer (UPLC-Q-TOF-MS/MS; Bruker Compact, Bruker Corp., Billerica, MA, USA). We identified four groups of phenolic compounds: flavonoids, ellagitannins and gallic acid derivatives ("hydrolyzable 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, hydrolyzable tannins as gallic acid equivalents, and lignins as ferulic acid equivalents (Moreira et al., 2018c; Galmán et al., 2019). We quantified these phenolic compounds by external calibration using calibration curves at 0.25, 0.5, 1, 2 and 5 µg mL^{-1}. We expressed phenolic compound concentrations as milligrams per gram dried tissue.

Quantification of nutrients

Phosphorus and nitrogen in leaves represent good proxies of leaf nutrient status; both are strongly associated with levels of leaf herbivory across many plant taxa (Mattson, 1980; Huberty and Denno, 2006), and previous work has shown positive correlations between leaf herbivory and these nutrients in oaks (e.g., Forkner and Hunter, 2000; Eatough Jones et al., 2008; Moreira et al., 2018c). To quantify these nutrients, we digested approximately 0.1 g of ground dried leaf material in a mixture of selenous sulphuric acid and hydrogen peroxide (Moreira et al., 2012). Diluted aliquots of the digestion were analyzed by colorimetry to quantify nitrogen (indophenol blue method) and phosphorus (molybdenum blue method) concentration using a microplate reader (model 650, Bio-Rad Laboratories, Philadelphia, PA, USA) at 650 nm and 700 nm, respectively (Walinga et al., 1995). We expressed nitrogen and phosphorus concentrations as milligrams per gram dried tissue.

Statistical analyses

Effects of elevation and ontogeny on leaf herbivory and traits—We ran general linear mixed models (GLMMs) using data at the plant level testing for effects of elevation (low, mid, and high), plant ontogeny (saplings vs. adult trees), and their interaction (all fixed factors) on insect leaf herbivory, chemical defenses, nutrients, and SLA. In the case of chemical defenses and nutrients, we ran separate models for each group of phenolic compounds and type of nutrient. The elevation by ontogeny interaction tested whether the magnitude of change in herbivory or plant traits with elevation differed between adults and saplings. For each model, we also included the effect of oak species, the oak species × elevation interaction, and the oak species × elevation × ontogeny interaction as random factors in order to analyze the main effects of elevation and ontogeny with the appropriate error terms.

Leaf traits associated with effects of elevation and ontogeny on herbivory—Based on the previous analyses, if elevation and/or ontogeny showed significant effects on leaf herbivory, we ran the model again (with the same main fixed effects and their interaction), while additionally including leaf traits as covariates potentially associated with effects on leaf herbivory ("mechanistic model"; Abdala-Roberts et al., 2016; Moreira et al., 2017). We expected that if leaf traits account for a large fraction of the total variance when measuring the effect of elevation and plant ontogeny on herbivory, then a significant main effect in the initial model should turn nonsignificant after including leaf traits as covariates. We caution that our assessment of plant traits associated with herbivory is by no means exhaustive (i.e., other unmeasured traits could be equally or more important) and that these analyses do not test for causality. Rather, the analyses can identify candidate traits that should be addressed more robustly in subsequent work.

We performed all statistical analyses in R v 3.4.1 using the lme4 package (R Core Team, 2018). We log-transformed all data to achieve normality of the residuals. We report least-squares means and standard errors in the original scale as descriptive statistics.

RESULTS

Effects of elevation and ontogeny on leaf herbivory and traits

There was a significant effect of elevation but no effect of oak ontogeny on insect leaf herbivory (Table 2, Fig. 1). Contrary to expectations, mean herbivore damage was 23% higher for individuals at high elevations than for those at low elevations, whereas there was no significant difference in damage between high- and mid-elevation or between mid- and low-elevation (low elevation: 1.91 ± 0.21, mid-elevation: 2.12 ± 0.21, high elevation: 2.34 ± 0.21; Fig. 1). In addition, there was no elevation by ontogeny interaction on herbivory (Table 2, Fig. 1), indicating that the effect of elevation was similar for adults and saplings.

The mean concentration of lignins was significantly higher for individuals found at high and mid elevations relative to those at low elevations (40% and 30% respectively; Table 2, Fig. 2D). There was no significant difference between high and mid elevations (low elevation: 1.80 ± 0.36 mg g^{-1} d.w., mid-elevation: 2.54 ± 0.36 mg g^{-1} d.w., high elevation: 2.36 ± 0.36 mg g^{-1} d.w.; Fig. 2D). In contrast, there was no significant effect of elevation on leaf condensed or hydrolyzable tannins, flavonoids, nitrogen, phosphorus, or SLA (Table 2, Figs. 2A–C, 3A–C). Mean concentration of condensed tannins and lignins was 14% and 18% higher (respectively) for saplings than for adult trees (condensed tannins: saplings = 9.20 ± 1.19 mg g^{-1} d.w.; adult trees = 8.11 ± 1.19 mg g^{-1} d.w.; lignins: saplings = 2.42 ± 0.32 mg g^{-1} d.w.; adult trees = 2.05 ± 0.32 mg g^{-1} d.w.; Fig. 2A, D). In contrast, there was no significant effect of plant ontogeny on leaf hydrolyzable tannins, flavonoids, nitrogen, phosphorus, or SLA (Table 2; Figs. 2B, C, 3A–C). Finally, there was no significant elevation by ontogeny interaction for any of the leaf traits measured (Table 2; Figs. 2,3).

TABLE 2. Summary of results from linear mixed models testing for the effects of elevation (low, mid and high), ontogeny (saplings vs. adult trees) and their interaction on leaf herbivory by chewing insects (% damage), concentration of leaf chemical defenses (condensed and hydrolyzable tannins, flavonoids and lignins) and nutrients (nitrogen and phosphorus), and specific leaf area (SLA) in eight oak (*Quercus*) species. We also included the effect of oak species, the oak species × elevation interaction, and the oak species × elevation × ontogeny interaction as random factors. *F*-values with degrees of freedom (numerator, denominator) and associated significance levels (*P*-values) are shown. Significant *P*-values ($P < 0.05$) are in bold.

	Elevation (E)		Ontogeny (O)		E × O	
Variable	$F_{2, 14}$	P	$F_{1, 21}$	P	$F_{2, 21}$	P
Insect leaf herbivory	3.95	**0.044**	0.29	0.595	0.82	0.453
Condensed tannins	0.37	0.698	4.88	**0.038**	0.61	0.552
Hydrolyzable tannins	1.55	0.246	0.08	0.776	0.20	0.823
Flavonoids	1.42	0.274	0.15	0.700	0.47	0.629
Lignins	4.50	**0.031**	5.69	**0.027**	1.82	0.189
Nitrogen	1.99	0.173	0.09	0.768	2.76	0.086
Phosphorus	0.19	0.829	0.53	0.476	0.37	0.697
SLA	0.67	0.528	2.60	0.122	0.41	0.667

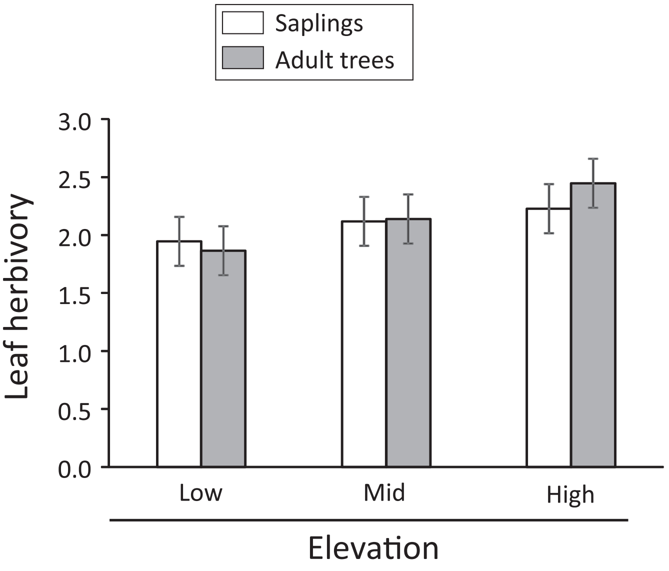

FIGURE 1. Leaf herbivory by insect chewers in adult reproductive trees (gray bars) and saplings (white bars) of eight oak (*Quercus*) species growing at low, mid and high elevations. Leaf chewer damage was visually estimated using an ordinal scale based on percentage of leaf area removed. Bars are least square means ± standard error. Results of the linear mixed models are presented in Table 2.

A scheme summarizing the effects of elevation and plant ontogeny on leaf herbivory and traits is shown in the Appendix S1.

Leaf traits underlying elevation and ontogenetic effects on herbivory

The significant effect of elevation on leaf herbivory observed in the initial model (Table 2) remained significant after including leaf traits as covariates (Table 3). This result suggests that the included leaf chemical and structural traits did not account for the observed increase in leaf herbivory from low to high elevations. None of the covariates had a significant effect on herbivory (Table 3).

DISCUSSION

Here we documented elevational gradients for insect leaf herbivory and lignin concentration across the eight studied oak species in the Iberian Peninsula. Unexpectedly, both leaf herbivore damage and chemical defenses (lignins) increased toward higher elevations. In addition, elevational variation in herbivory was not associated with variation in any of the leaf traits measured. Also contrary to expectations, we found that the concentrations of lignins and condensed tannins were higher in saplings than in adults, with no ontogenetic difference in herbivory. Together, these results suggest independent elevational patterns in herbivory and oak defenses and that these elevational patterns are independent of plant ontogenetic stage.

Counter to classic predictions on elevational gradients in plant defense and herbivory, our results showed that insect leaf herbivory and leaf chemical defenses (lignins) increased toward higher elevations. In our recent review of clinal studies of herbivory (Moreira

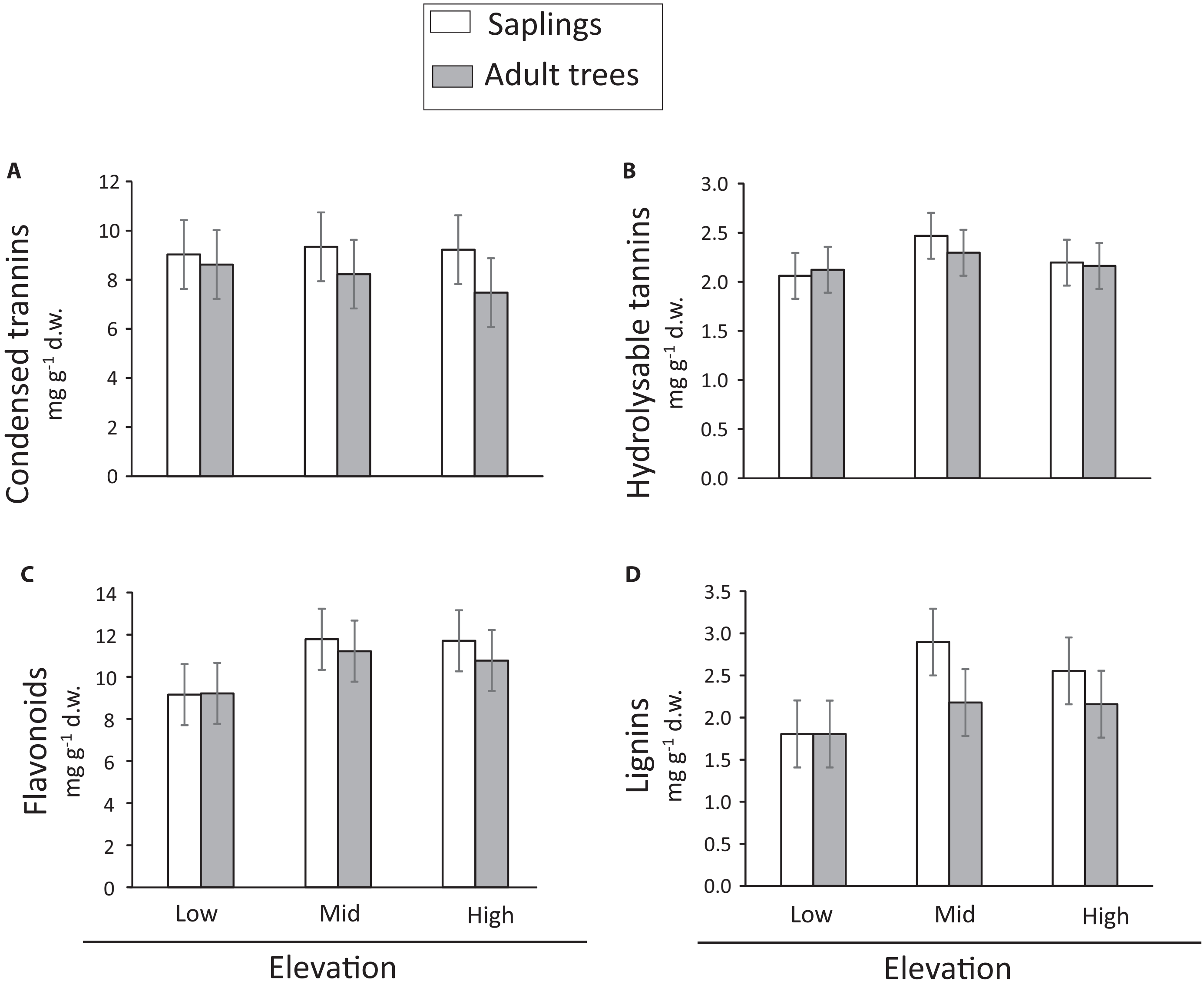

FIGURE 2. Concentration of leaf (A) condensed tannins, (B) hydrolyzable tannins, (C) flavonoids, and (D) lignins in adult reproductive trees (gray bars) and saplings (white bars) of eight oak (*Quercus*) species growing at low, mid and high elevations. Bars are least square means ± standard error. Results of the linear mixed models are presented in Table 2.

et al., 2018a), we reported that 30% of the studies found higher herbivory with increasing elevation, and 40% found higher plant defenses with increasing elevation, highlighting that reverse gradients in plant–herbivore interactions are common. In the case of herbivores, lower predator pressure at higher elevations could release herbivores from top-down control and result in greater herbivory at higher elevations (Moreira et al., 2018a). Alternatively, an increase in herbivore diet breadth with increasing elevation (Rasmann et al., 2014a) could also lead to greater herbivory by generalist species or guilds of insect herbivores at high elevations. In the case of defenses, plant defensive traits respond not only to herbivore pressure, but could also be adaptive for tolerance to climatic conditions and resource availability (Abdala-Roberts et al., 2016; Moreira et al., 2018b). Accordingly, more stressful environments (e.g., at higher elevations) are commonly associated with slow-growing species with high tissue value (i.e., resource availability hypothesis; Coley et al., 1985; Endara and Coley, 2011). Consequently, plant species adapted to more stressful environments would be expected to increase allocation to plant defenses because the cost of replacing tissues consumed by herbivores is higher when resources are low.

Our results also showed that elevational variation in herbivory was not explained by leaf traits across the studied oak species, suggesting that other (unmeasured) plant traits (e.g., indirect defenses, induced defenses) or abiotic factors (e.g., temperature, precipitation, soil conditions) could play an important role in generating variation in herbivory pressure. We similarly found in a previous study that insect leaf herbivory and the concentration of phenolic compounds in pedunculate oak (*Q. robur*), one of the species included in the present study, increased toward higher elevations, but phenolics did not underlie elevational variation in herbivory (Abdala-Roberts et al., 2016). In addition, Pellissier et al. (2016) reported increasing herbivory but decreasing chemical defenses

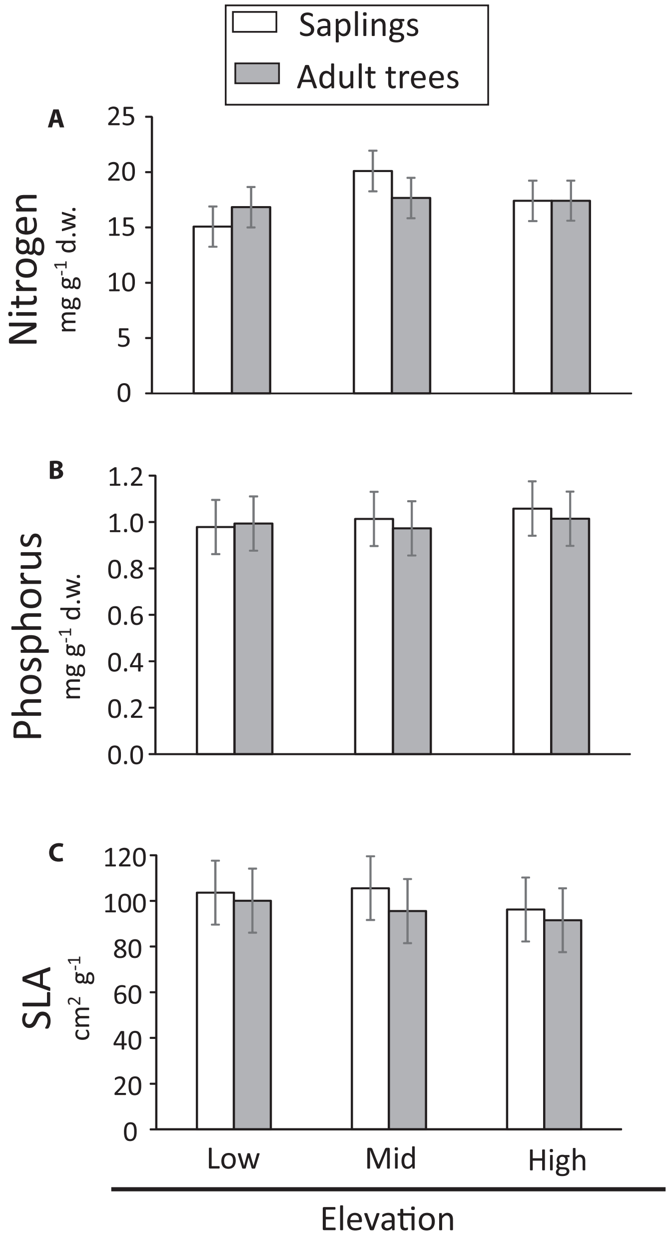

FIGURE 3. Concentration of leaf (A) nitrogen and (B) phosphorus, and (C) specific leaf area (SLA) in adult reproductive trees (gray bars) and saplings (white bars) of eight oak (*Quercus*) species growing at low, mid and high elevations. Bars are least square means ± standard error. Results of the linear mixed models are presented in Table 2.

TABLE 3. Summary of results from linear mixed models testing for the effects of elevation (low, mid and high elevations), ontogeny (saplings vs. adult trees) and their interaction on leaf herbivory by chewing insects (% damage) in eight oak (*Quercus*) species. We included chemical (condensed and hydrolyzable tannins, flavonoids, lignins) and structural (specific leaf area) defenses and nutritional (nitrogen and phosphorus) traits as covariates to test whether elevational effects on insect leaf herbivory were determined by these leaf traits. We also included the effect of oak species, the oak species × elevation interaction, and the oak species × elevation × ontogeny interaction as random factors. *F*-values, degrees of freedom (numerator, denominator) and associated significance levels (*P*-values) are shown. Significant *P*-values ($P < 0.05$) are in bold.

	Insect leaf herbivory		
Variable	***F***	**$\mathrm{df}_{\mathrm{num,\,den}}$**	***P***
Elevation	4.97	2, 14	**0.023**
Ontogeny	0.03	1, 21	0.855
Elevation × ontogeny	0.90	2, 21	0.422
Condensed tannins	0.57	1, 184	0.451
Hydrolyzable tannins	0.04	1, 184	0.843
Flavonoids	3.83	1, 184	0.052
Lignins	0.48	1, 184	0.489
Nitrogen	0.31	1, 184	0.582
Phosphorus	0.40	1, 184	0.530
Specific leaf area	0.06	1, 184	0.804

(glucosinolates) at low elevations across 16 *Cardamine* species, but again these chemical defenses did not explain the elevational gradient in herbivory. Together, our present work and these studies indicate decoupled elevational gradients in plant defenses and herbivory but at the same time (given contrasting gradients) suggest different mechanisms underlying elevational variation in herbivory and plant defenses across systems. Patterns might be reconciled by conducting measurements at multiple time points because most studies use single time-point measurements and thus provide a limited or inaccurate assessment of plant defense levels and herbivore pressure (Rosado-Sánchez et al., 2018). In addition, combining these observational results with reciprocal transplant experiments and bioassays of herbivore performance on leaf tissue from different populations is necessary to causally link targeted plant traits and herbivory, a necessary task to elucidate the ecological mechanisms and evolutionary implications of elevational changes in plant–herbivore interactions (Moreira et al., 2018a).

Surprisingly, we found no effect of plant ontogeny on insect leaf herbivory, contradicting the prediction that plants that are easier to locate (i.e., adult trees in our case) exhibit higher herbivory (Feeny, 1976; Strauss et al., 2015). Although adult trees would presumably be more easily located by herbivores and offer large amounts of leaf tissue, most of the leaves are mature with relatively low nutritional value (thicker and tougher) that are not used by insect herbivores (Barton and Koricheva, 2010). Thus, the amount of herbivory on adults might be lower despite their increased detectability and ultimately diminish the difference in herbivory between life stages. On the other hand, we also found that the concentration of leaf defense chemicals (condensed tannins and lignins) was greater for saplings than for adult trees, similar to our finding that saplings of *Q. robur* also exhibited greater levels of defense chemicals (lignins and hydrolyzable tannins) than in adult trees (Moreira et al., 2017). This pattern counters the prediction that levels of defense chemicals build up from the sapling to the adult stage (Boege and Marquis, 2005; Barton and Koricheva, 2010). One explanation for our findings could be that saplings can rapidly increase their photosynthetic capacity (Huijser and Schmid, 2011) and in turn allocate more resources to defense (relative to their total energetic budget) than adult trees. Alternatively, the sapling stage is crucial for subsequent plant survival and establishment in trees, such that growing tissues should be better defended in saplings than in adult trees (i.e., optimal defense theory; McCall and Fordyce, 2010).

Contrary to expectations, we found that differences in insect leaf herbivory and plant defenses across elevations were consistent for adults and saplings. These results do not support that differences in allocation to defenses or in detectability lead to weaker differences in herbivory and plant defenses between adults and saplings at high elevations. Alternatively, it is possible that if adults are both better defended and more easily detected, the proposed mechanisms cancelled each other out. Further work explicitly testing and separating them is necessary to determine whether they are actually occurring and to what degree they interact. For example, the influence of tree defense levels on elevational clines in herbivory could be assessed in common gardens with plants of two early ontogenetic stages (e.g., seedlings and saplings) across an elevational gradient. At each elevation, defense induction elicitors (e.g., jasmonic acid) could be used to both reduce and increase ontogenetic differences in defense levels. This experimental design would allow to test whether differences in overall defense levels (combination of constitutive and induced) shape ontogenetic differences in herbivory and if this effect varies with elevation. Experiments involving adults are not feasible for long-lived trees, but this type of scheme including all ontogenetic stages (from seedlings to adults) could be applied to herbs and shrubs.

Limitations and future work

Previous work with oaks has demonstrated that leaf phenology is an important factor driving leaf herbivory (Pearse and Karban, 2013; Pearse et al., 2015a, b). Notably, early leaf phenology increased leaf miner and chewer damage in valley oak (*Q. lobata*) trees (Pearse et al., 2015a, b) and leaf miner damage among 55 oak species in a common garden (Pearse and Karban, 2013). In the present study, we sampled leaves at the end of the growing season across the entire elevational gradient to obtain an estimate of cumulative damage before leaf drop, thus reducing the influence of elevational differences in the phenology of plant defenses and herbivory, which could have come into play by sampling in the early or mid-season. If phenology had played a decisive role, we would have expected sites with earlier phenology found at lower elevations to exhibit higher levels of herbivory, but we instead found the opposite pattern. Unfortunately, it is not possible to separate the effects of total growing season length from those of season onset, and only by conducting further measurements at multiple time points during the season can the influence of different phenological variables be assessed. Finally, it is also important to note that, because allocation to plant defenses and growth can vary among years, having only two ontogenetic categories may not be enough to understand plant ontogenetic effects on herbivory and defenses on centennial trees. Accordingly, further work should consider a more careful subdivision involving multiple ontogenetic stages as well as multi-annual measurements to test for the consistency in allocation patterns to growth and defense.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Scheme summarizing the effects of (A) elevation and (B) plant ontogeny on leaf herbivory by chewing insects and plant traits associated with herbivore resistance in eight oak (*Quercus*) species.

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