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Leaf chemical defences and insect herbivory in oak: accounting for canopy position unravels marked genetic relatedness effects

Elena Valdés-Correcher[1,*], Audrey Bourdin[1], Santiago C. González-Martínez[1], Xoaquín Moreira[2], Andrea Galmán[2], Bastien Castagneyrol[1,†] and Arndt Hampe[1,†]

[1]*INRAE, Univ. Bordeaux, BIOGECO, F-33610 Cestas, France and* [2]*Misión Biológica de Galicia (MBG-CSIC), Apartado de correos 28, 36080 Pontevedra, Galicia, Spain*
**For correspondence. E-mail elena.valdes.correcher@gmail.com*
[†]*Contributed equally to this paper*

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• **Background and Aims** Highly controlled experiments document that plant genetic diversity and relatedness can shape herbivore communities and patterns of herbivory. Evidence from the field is, however, scarce and inconsistent. We assessed whether a genetic signal underlying herbivory can be detected in oak woodlands when accounting for variation at smaller (within-tree) and larger (among-stand) scales.
• **Methods** We tested relationships between tree genetic relatedness, leaf chemical defences and insect herbivory for different canopy layers in 240 trees from 15 pedunculate oak (*Quercus robur*) forest stands. We partitioned sources of variability in herbivory and defences among stands, individuals and branches.
• **Key Results** Leaf defences, insect herbivory and their relationship differed systematically between the upper and the lower tree canopy. When accounting for this canopy effect, the variation explained by tree genetic relatedness rose from 2.8 to 34.1 % for herbivory and from 7.1 to 13.8 % for leaf defences. The effect was driven by markedly stronger relationships in the upper canopy.
• **Conclusions** Our findings illustrate that considerable effects of the host plant genotype on levels of leaf chemical defences and associated insect herbivory can be detected in natural tree populations when within-individual variation is properly accounted for.

Key words: Genetic relatedness, chemical defences, herbivory, *Quercus robur*, plant–insect interactions.

INTRODUCTION

A rapidly growing number of studies have shown that plant genetic diversity and genetic relatedness can influence herbivore communities and associated patterns of invertebrate herbivory (Crutsinger *et al.*, 2006; McArt and Thaler, 2013; Kagiya *et al.*, 2018). It has been proposed that the composition and activity of herbivore communities are heritable traits of the host plant that are partly driven by the heritability of its anti-herbivore chemical defences (Wimp *et al.*, 2005; Bangert *et al.*, 2006; Bustos-Segura *et al.*, 2017; Barker *et al.*, 2019). Indeed, plant families vary considerably in their edibility and resulting herbivore damage (Donaldson and Lindroth, 2007; Fernandez-Conradi *et al.*, 2017; Barker *et al.*, 2018; Damestoy *et al.*, 2019). However, most previous research has been performed using highly controlled experiments (e.g. common gardens), often with juvenile plants and minimized spatial and environmental effects, settings that could lead to overemphasis on the putative role of genetics in nature (Tack *et al.*, 2012; Lämke and Unsicker, 2018). Accordingly, more research in natural plant populations is needed for understanding the extent to which genetically based variation in plant chemical defences determines insect herbivory in real-world contexts (Wimp *et al.*, 2005; Carmona *et al.*, 2011).

Diverse biological mechanisms can contribute to the blurring of links between plant genotype, plant chemical defences and herbivory patterns in mature trees in the wild. Many secondary metabolites exhibit low heritability because their production is controlled by multiple genes and their interactions (Külheim *et al.*, 2011; Büchel *et al.*, 2016). Different plant parts experience different microclimates (e.g. irradiation, temperature, humidity) that can trigger extensive within-individual variation in leaf morphology and chemistry, especially along vertical gradients. For instance, upper canopy leaves are typically thicker, tougher, smaller and drier and contain higher levels of chemical leaf defences than lower canopy leaves (Murakami and Wada, 1997; Le Corff and Marquis, 1999; Murakami *et al.*, 2005; Ruhnke *et al.*, 2009; De Casas *et al.*, 2011; Castagneyrol *et al.*, 2019). More specifically, differences in microclimate should directly affect the expression of genes that encode the production of leaf chemical defences (Lämke and Unsicker, 2018). In turn, vertical gradients in insect herbivory can result from differences in herbivore dispersal (e.g. flying insects concentrated in the upper canopy; Ulyshen, 2011) or herbivore exposure to predators (e.g. lower predation rates in the upper canopy; Aikens *et al.*, 2013), which are not driven by leaf chemistry. The plethora of potential confounding factors underpins the need for careful study designs (from the tree level to the

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within-individual level) to thoroughly assess the effects of genetically based variation in leaf chemical defences on herbivory in natural plant populations.

This study investigated the relationships between tree genetic relatedness, leaf defences and insect herbivory in natural forest stands of pedunculate oak (*Quercus robur*). For this purpose, we genotyped 703 trees from 15 stands and quantified the concentration of leaf phenolic compounds and herbivory by leaf-chewing insects in the intermediate and upper canopy layers for a subset of 235 trees. Specifically, we addressed the following questions: (1) To what extent do leaf phenolics and insect leaf herbivory vary among trees within stands and between canopy layers within trees? (2) Do leaf phenolics and herbivory show a genetic signal when accounting for their scale-dependent variation? (3) To what extent does variation in leaf phenolics explain patterns of leaf herbivory? By addressing these questions, we aim to combine a thorough description of cross-scale patterns typical of natural systems with insights into the biological mechanisms that underlie plant–insect herbivore relationships in non-experimental contexts.

MATERIALS AND METHODS

Study system

We performed this study in the Landes de Gascogne region (south-western France) ~40 km south of Bordeaux (44°41′ N, 00°51′ W). The area is dominated by extensive maritime pine (*Pinus pinaster*) plantations with scattered small stands of broadleaf forest. These are usually dominated by pedunculate oak and contain other tree species, like birch (*Betula pendula*), Pyrenean oak (*Quercus pyrenaica*), holly (*Ilex aquifolium*) and willows (*Salix* spp.) in minor abundance. Such stands are not subjected to intensive forest management and many are actively expanding (Gerzabek *et al.*, 2017), favoured by a recent change in silvicultural management that tends to conserve oaks recruiting within adjacent pine plantations in order to increase biological pest management (Dulaurent *et al.*, 2012). Pedunculate oak supports a large community of specialist and generalist herbivore insects in these stands (Giffard *et al.*, 2012). Leaf chewers, skeletonizers, miners and gallers are the principal guilds responsible for background herbivory (i.e. the damage caused by a community of herbivores whose populations are at normal densities) that amounts to around 17.8 % (Giffard *et al.*, 2012).

Forest stands, sampling and herbivory measurements

We selected 15 forest stands of variable size and spatial isolation within the landscape (Fig. 1). All stands were second-growth forests that have established since the 1950s through natural tree regeneration (Valdés-Correcher *et al.*, 2019). They were strongly dominated by pedunculate oak and contained a variable but often rather sparse woody understorey vegetation. The number of established oak trees ranged from 16 to 124 individuals and their surface (as derived from the minimum polygon including all trees) from 0.04 to 0.5 ha. Further information can be found in Supplementary Data Table S1 (see also Valdés-Correcher *et al.*, 2019). Within each stand, we mapped and tagged every oak tree with a diameter at breast height >3 cm and collected leaf material that was stored in silica gel until DNA isolation

FIG. 1. Map of the study area in the Aquitaine region, south-western France, showing the 15 oak stands.

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for genotyping. This exhaustive sampling included a total of 703 individuals (Supplementary Data Table S1).

In September 2018 (i.e. late growing season), we randomly selected 16 individuals with a diameter at breast height >6 cm within each stand (total n = 240). On each tree, we randomly chose and cut two south-facing branches situated 4 and 8 m above ground level, respectively, which corresponds to the intermediate (shaded) and upper (sun-exposed) tree canopies in most of our trees (see also Castagneyrol *et al.*, 2019). In the smallest stands (B, L and P; Supplementary Data Table S1) five trees were excluded as they did not have enough leaves to perform the measurements of leaf phenolics and insect herbivory. Three of the 235 sampled trees did not reach 8 m, so we shifted the position of the intermediate and upper tree canopy layers 2 m downward (i.e. 2 and 6 m, respectively). Operators unaware of the study design systematically picked the 30 most apical leaves from each branch, resulting in a total of 60 leaves per tree. Samples were stored at −18 °C until insect herbivory measurement.

For each leaf, we visually estimated the percentage of leaf area removed by chewing insects using the following scale: 0, 0 %; A, 1–5 %; B, 6–15 %; C, 16–25 %; D, 26–50 %; E, 51–75 %; F, >75 %. We used pre-established templates mimicking known levels of insect herbivory on oak leaves to increase the reliability and repeatability of herbivory measurements. Herbivory levels were always estimated by the same observer (A.B.) blind to leaf origin in order to maximize consistency of the estimates and reduce unconscious bias. We averaged values across all leaves to obtain a mean value per branch, and then used the median of each percentage class for statistical analyses (Castagneyrol *et al.*, 2019).

We also collected ten fully expanded leaves with no signs of herbivory or pathogen infection from each branch for quantification of phenolic compounds. We immediately oven-dried these leaves for 48–72 h at 45 °C and ground them to a thin powder before further chemical analyses (see below).

Molecular analyses

We isolated genomic DNA from the leaves using the Invisorb® DNA Plant HTS 96 Kit/C and the standard protocol and genotyped all trees using 141 single-nucleotide polymorphism (SNP) markers from the sets described in Guichoux *et al.* (2013) and Gerzabek *et al.* (2017). The list of loci is available via an open-access repository (https://doi.org/10.5281/zenodo.3786180). Loci were multiplexed using an iPLEX Gold kit on a MassARRAY Typer 4.0.26.75 (Agena Biosciences) at the Genomic and Sequencing Facility of Bordeaux (France), as described in detail in Gerzabek *et al.* (2017). We obtained high-quality data with a low proportion of missing calls for all markers and individuals, which we assembled into multilocus genotypes for the exhaustive sample (n = 703).

Chemical analyses

We extracted phenolic compounds from 20 mg of dry leaf tissue with 1 mL of 70 % methanol in an ultrasonic bath for 20 min, followed by centrifugation (Moreira *et al.*, 2014). Samples were centrifuged and transferred to chromatographic vials. We performed the chromatographic analyses in an ultra-high-performance liquid chromatograph (UHPLC Nexera LC-30AD; Shimadzu Corporation, Kyoto, Japan) equipped with a Nexera SIL-30AC injector and an SPD-M20A UV/VIS photodiode array detector.

For compound separation, we used 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 established at 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. We recorded chromatograms at 330 nm and processed data with LabSolutions software (Shimadzu, Kyoto, Japan). For phenolic compound identification, we used ultra-performance liquid chromatography coupled with electrospray ionization quadrupole (Thermo Dionex Ultimate 3000 LC; Thermo Fisher Scientific, Waltham, MA, USA) time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS; Bruker Compact™, Bruker, Billerica, MA, USA). 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 (Moreira *et al.*, 2018). We quantified these phenolic compounds with external calibration using calibration curves at 0.25, 0.5, 1, 2 and 5 µg mL^{-1}. We calculated total phenolics for each branch as the sum of flavonoids, lignins, condensed tannins and hydrolysable tannins, and expressed concentrations of each phenolic group in mg g^{-1} tissue on a dry weight basis.

Statistical analyses

Prior to the analysis of genetic relatedness, we first examined and visualized the landscape-scale genetic structure of our oak stands by calculating pairwise F_{ST} between stands (Weir and Cockerham, 1984) and applying principal components analysis (PCoA) to the obtained values with the dudi.pca function in the R package adegenet (Jombart and Ahmed, 2011). Overall low values (mean F_{ST} = 0.041, range 0.006–0.111) (Supplementary Data Table S2) and the absence of population structure (Supplementary Data Fig. S1) confirmed that the 15 stands can be considered a single gene pool and eventual confounding effects due to population genetic structure are negligible. Then, we quantified the level of genetic relatedness between each pair of trees relative to the full sample (n = 703). For this, we computed a kinship matrix using Nason's kinship coefficient (Loiselle *et al.*, 1995) with the software SPAGeDi version 1.2 (Hardy and Vekemans, 2002). We extracted the values for our 16 target trees per stand from the global matrix and used this information as a quantitative estimate of their genetic relatedness in the subsequent analyses (Van Horn *et al.*, 2008). Note that kinship-based estimates of relatedness, while commonly used in

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population genetics (Pemberton, 2008), are not directly comparable to those obtained through pedigree analyses.

We modelled patterns of insect leaf herbivory and leaf phenolics at the whole-tree level (i.e. pooling the two canopy layers) and at the canopy layer level (i.e. for each layer separately) by means of linear mixed-effect models (LMMs). At the whole-tree level, we built two independent LMMs with stand ID and the kinship values of the target trees as random factors in order to estimate the variance and the percentage of the overall variance explained by the local environment (stand ID) and by the genetic relatedness among trees (the kinship matrix). The first model was an intercept-only model with (tree-level mean) concentration of leaf phenolics as response variable (eqn 1). The second model included (tree-level mean) insect herbivory as response variable and leaf phenolics as an additional explanatory variable (eqn 2).

$$\text{Model 1: Phenolics}_{i,j} = \beta_0 + S_i + T_j + \varepsilon_{i,j} \quad (1)$$

$$\text{Model 2: Herbivory}_{i,j} = \beta_0 + \beta_1 \times \text{Phenolics}_{i,j} + S_i + T_j + \varepsilon_{i,j} \quad (2)$$

where β_0 is the model intercept, β_1 the fixed effect of leaf phenolics, S_i the random effect of stand i, T_j the random effect of tree genetic similarity j (entered as the kinship matrix) and $\varepsilon_{i,j}$ the error, with S_i Є $N(0, \sigma_S^2)$, T_j Є $N(0, \sigma_T^2)$ and $\varepsilon_{i,j}$ Є $N(0, \sigma_E^2)$. For each model, we computed the variance of the fixed effects (if any, σ_F^2) and calculated the percentage of variance explained by each random factor (e.g. $100 \times \sigma_T^2/(\sigma_S^2 + \sigma_T^2 + \sigma_E^2)$ for the random effect of tree genetic similarity in model 2).

The second group of models, performed at canopy layer level, adopted the same approach with two independent LMMs modelling the response of total leaf phenolics and herbivory, respectively (eqns 3 and 4):

$$\text{Model 3: Phenolics}_{i,j,k} = \beta_0 + \beta_1 \times \text{Canopy layer}_{i,j,k} + S_i + T_j + \varepsilon_{i,j,k} \quad (3)$$

$$\text{Model 4: Herbivory}_{i,j,k} = \beta_0 + \beta_1 \times \text{Phenolics}_{i,j,k} + \beta_2 + \text{Canopy layer}_{i,j,k} + S_i + T_j + \varepsilon_{i,j,k} \quad (4)$$

where k indicates the branch, β_0 the intercept, β_1 and β_2 the coefficient parameters of the fixed effects and S_i, T_j and $\varepsilon_{i,j,k}$ as above. Again, we computed the variance of the fixed effects (if any, σ_F^2) and calculated the percentage of variance explained by each random factor. Prior analyses revealed that including axes 1, 2 and 3 from our PCoA on the landscape-scale genetic structure (which explained 1.05 % of the total variation) did not change the outcomes, so we decided not to include the axes in the final models.

All analyses were done in R version 3.5.2 (R Core Team, 2018). LMMs including a kinship matrix as random factor were fitted with the function lmekin in package coxme (Therneau, 2018).

RESULTS

Leaf phenolics and insect herbivory at whole-tree level

Leaf phenolic concentration was on average (± s.e.) 14.69 ± 0.39 mg g^{-1} (Fig. 2). The random factors collectively explained 26.9 % of the overall variation, with stand ID accounting for 19.7 % and tree genetic relatedness for 7.1 %. Insect leaf herbivory was on average 12.27 ± 0.29 % and decreased significantly with increasing leaf phenolic concentration (model coefficient parameter estimate −0.12 ± 0.05, $z = -2.48$, $P = 0.013$). However, the effect size was small (2.0 %). Stand ID explained 38.1 % of the overall variation in herbivory and genetic similarity among trees only accounted for another 2.9 %.

Leaf phenolics and insect herbivory at canopy layer level

Leaf phenolic concentration was significantly lower in the intermediate than in the upper canopy layer (mean ± s.e.

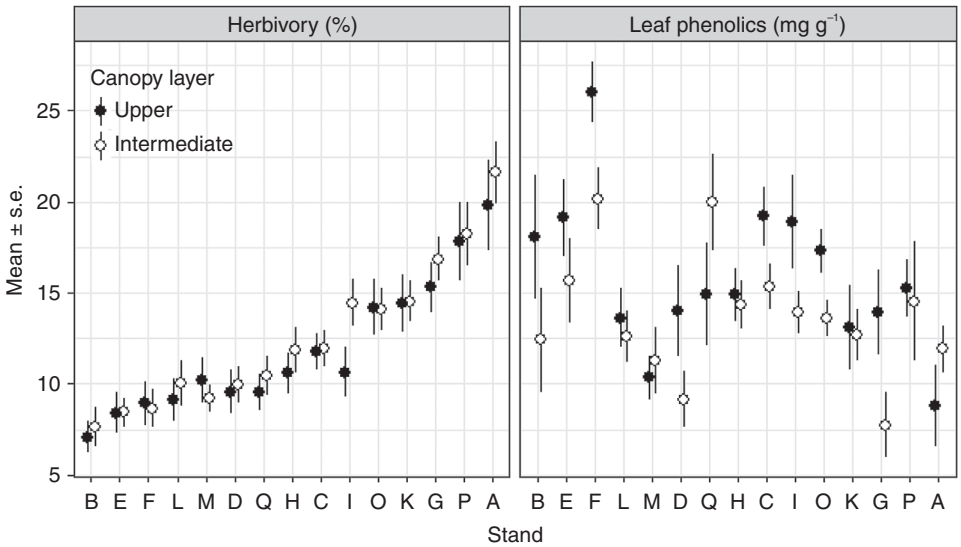

Fig. 2. Percentage of insect herbivory and concentration of total leaf phenolics in the intermediate and upper tree canopy. Symbols and error bars represent means ± s.e. aggregated at the level of oak stands (A–Q). Note that stands are ordered according to mean insect herbivory and the same order is used to display stand-level variability in leaf phenolics.

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13.63 ± 0.50 versus 15.79 ± 0.59 mg g^{-1}) (Fig. 2, Table 1). Stand ID accounted for 13.7 % and tree genetic relatedness for 13.9 % of the overall variation (Table 1). Insect leaf herbivory did not differ significantly between tree canopies (12.53 ± 0.39 versus 11.99 ± 0.43 %) and was independent of leaf phenolic concentration (Table 1). Stand ID and tree genetic relatedness accounted for 32.0 and 34.7 % of the overall variability in insect herbivory, respectively (Table 1).

In the intermediate canopy layer, stand ID and genetic relatedness accounted for 13.5 and 0.03 % of the overall variation in leaf phenolics, respectively. Leaf phenolics had no significant effect on herbivory (Fig. 3). Stand ID explained 40.5 % of the overall variation in herbivory, while tree genetic relatedness accounted for less than 0.02 %.

In the upper canopy layer, stand ID and genetic relatedness explained 17.4 and 24.8 % of the overall variation in leaf phenolics, respectively. There was a significant, albeit weak, negative effect of leaf phenolic concentration on herbivory (coefficient parameter estimate ± s.e. −0.12 ± 0.05, $z = -2.63$, $P = 0.009$) (Fig. 3). Leaf phenolics accounted 2.8 % of the overall variation in herbivory while stand ID and tree genetic relatedness accounted for 25.3 and 14.5 %, respectively.

DISCUSSION

Tree genetic relatedness explained a noteworthy part of the overall variation in leaf phenolics and associated insect leaf herbivory. However, this effect was only evident in the upper tree canopy, where concentrations of leaf phenolics were consistently higher. To our knowledge, our work represents one of the first confirmations of genotype–phenotype–herbivory links in natural tree populations and highlights that increased consideration of canopy effects is needed to improve our understanding of ecological and evolutionary factors driving plant–herbivore interactions in long-lived plants.

Trees lost between 7 and 22 % of their leaf area to insect herbivores, a range of defoliation similar to previous estimates (Giffard *et al.*, 2012; Castagneyrol *et al.*, 2019; Valdés-Correcher *et al.*, 2019). Our analysis at the whole-tree level attributed most of the overall variation in leaf herbivory to differences among forest stands, whereas the contributions of tree genetic relatedness and leaf phenolics were very weak. This result might suggest that insect leaf herbivory in our system would be basically driven by the nature of the forest stand, which encapsulates diverse environmental drivers acting at the local (e.g. stand size, tree density and species composition, vegetation structure; Maguire *et al.*, 2016; Fuller *et al.*, 2018; van Schrojenstein Lantman *et al.*, 2018) to landscape (e.g. stand isolation, nature of matrix habitats; Morante-Filho *et al.*, 2016) scale. Valdés-Correcher *et al.* (2019) actually reported for the same study stands that their size and isolation affected patterns of herbivory by different insect guilds. Limiting our analyses to the whole-tree level would hence have led to the conclusion that insect herbivory is primarily determined by extrinsic drivers and shaped by the ecological neighbourhood of the focal tree.

While tree genetic relatedness had little effect on herbivory (2.9 %), it was somewhat more influential in the case of leaf phenolics (7.1 %) (Fig. 4). Together with the likewise weak but statistically significant negative association between leaf phenolics and herbivory, one might argue that our results mirror – albeit extremely faintly – experimental studies that have consistently identified plant chemistry as the phenotypic link between the host plant genotype and the structure of associated arthropod communities (Bangert *et al.*, 2006; Barbour *et al.*, 2009, 2015) or patterns of herbivory (Bailey *et al.*, 2006; Andrew *et al.*, 2007; Donaldson and Lindroth, 2007). But consistent empirical support for this linkage from natural tree populations remains very scarce. In one of the few available studies, Kagiya *et al.* (2018) found that genetic relatedness of alder (*Alnus hirsuta*) trees largely influenced associated arthropod communities, although the effect was stronger for herbivore predators than for the herbivores themselves. Maldonado-López *et al.* (2015) observed that tree genetic relatedness of *Quercus castanea* trees was significantly associated with chemical defences but not with insect herbivory. In turn, Tack *et al.* (2012) and Gossner *et al.* (2015) failed to detect any relationships between tree genetic relatedness and herbivory in *Q. robur* populations and concluded that genetic effects tend to be overwhelmed by environmental and spatial factors.

In line with the predominant trend reported in the literature (e.g. Yamasaki and Kikuzawa, 2003; Poorter *et al.*, 2006; Lämke and Unsicker, 2018; but see Roslin *et al.*, 2006), we observed that upper canopy leaves systematically contained higher concentrations of leaf phenolics than those from the intermediate canopy. Extensive within-individual variation in leaf morphological and chemical traits is an inherent feature of plants (Niklas *et al.*, 2009; Herrera, 2017). For leaf phenolics, the phenomenon has been primarily explained as an ecophysiological, enzymatic and transcriptomic consequence of the higher irradiance that upper canopy leaves receive, given that diverse phenolic compounds are involved in protection from UV-B damage (reviewed in Jenkins and Brown, 2018; see also Lämke and Unsicker, 2018). This vertical gradient in leaf phenolics could have important consequences for plant–insect herbivore

TABLE 1. *Summary of LMM testing the effect of canopy layer (upper versus intermediate) on leaf phenolics or insect herbivory. For insect herbivory, the effect of leaf phenolics was also included in the model. Significant variables are indicated in bold σ² (%), variance and percentage of variance explained by the random factors of stand ID, genetic similarity introduced as a kinship matrix, and the residuals.R^2m and R^2c, variance explained by fixed and fixed plus random factors, respectively.*

Response	Predictors	Coefficient ± s.e.	*z*-value	*P*-value	σ² (%)			R^2m (R^2c)
					Stand ID σ_S^2	Genetic relatedness σ_T^2	Residuals σ_E^2	
Phenolics	Canopy layer	2.15 ± 0.66	3.22	**0.001**	9.27 (19.7)	9.64 (13.8)	50.22 (70.9)	1.63 (29.1)
Herbivory	Phenolics	−0.458 ± 0.24	−1.9	0.057	13.44 (32.0)	14.57 (34.7)	13.95 (33.3)	0.79 (66.6)
	Canopy layer	−0.585 ± 0.36	−1.62	0.1				

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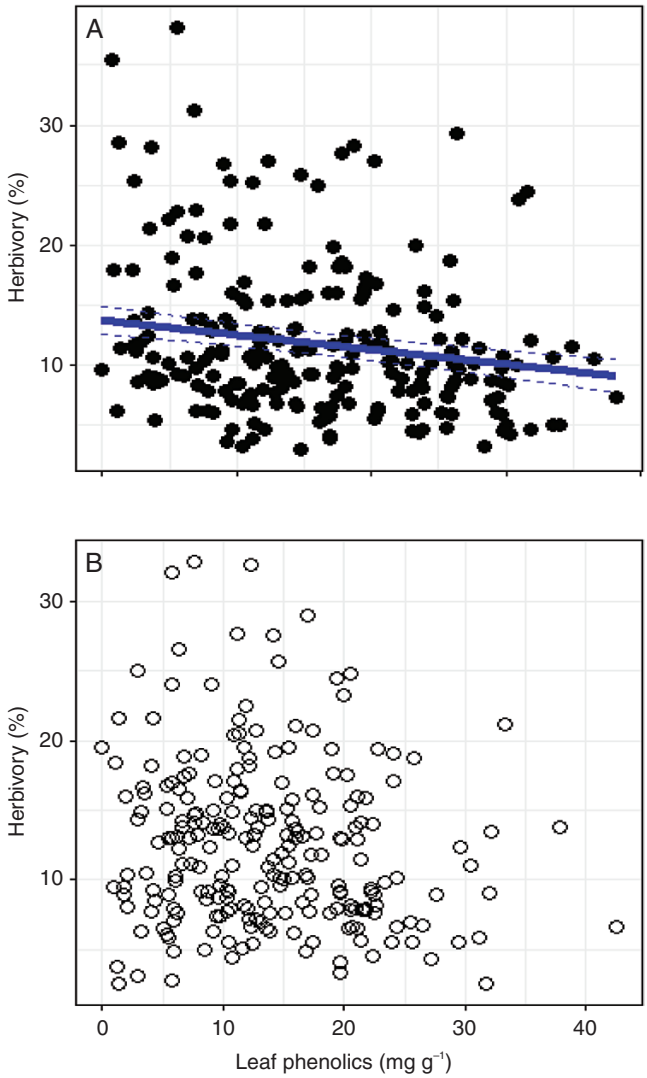

FIG. 3. Effects of total leaf phenolics on insect herbivory in the upper (A) and intermediate (B) canopy layer. Data points represent individual trees. The thick solid line and the thin dashed lines in (A) represent model predictions and corresponding standard errors, respectively.

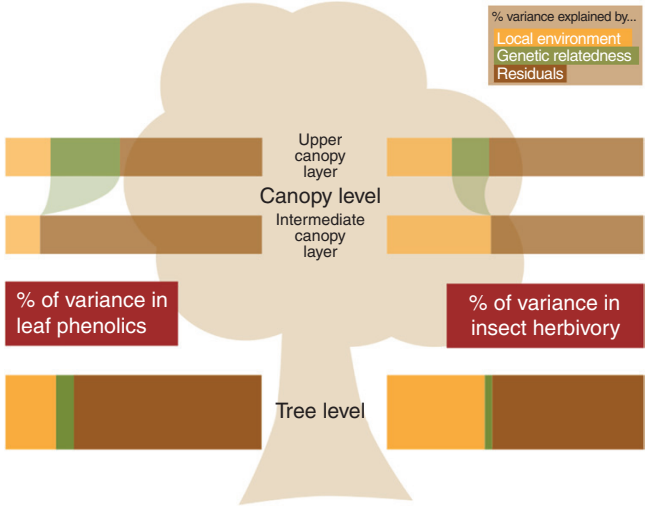

FIG. 4. Summary of variance partitioning among random effects and model residuals for leaf phenolics (left) and insect herbivory (right). Box length is proportional to the percentage of variance explained by each component.

interactions. Under experimental settings, herbivores tend to forage preferentially on upper canopy leaves owing to their higher nutritive value (Fortin and Mauffette, 2002; Oishi *et al.*, 2006). Yet field surveys typically report higher levels of leaf removal in lower canopy layers (e.g. Yamasaki and Kikuzawa, 2003; Stiegel *et al.*, 2017; Castagneyrol *et al.*, 2019), which is in line with a higher abundance and diversity of herbivores in these layers (reviewed in Ulyshen, 2011). The present study did not, however, detect differences in herbivory between the upper and the intermediate canopy layer. This could be due to the combined effects of different vertical gradients in bottom-up (e.g. leaf nutritional quality and defences) and top-down (e.g. predation and parasitism) forces that act simultaneously upon insect herbivores. For instance, in upper canopy layers herbivores are favoured by high leaf nutrient quality (Fortin and Mauffette, 2002) but constrained by high levels of leaf defences (Moreira *et al.*, 2017), while in low canopy layers herbivores are also negatively influenced by higher abundances of predators (Aikens *et al.*, 2013). On the other hand, differences in herbivore community composition might also contribute to the blurring of vertical trends in herbivory (Murakami *et al.*, 2005). Extensive research on within-individual variation in leaf traits and associated herbivory has given rise to the hypothesis that variance in nutritional quality itself could act as a defence mechanism that reduces insect herbivore performance by forcing herbivores to actively forage for suitable food (e.g. Wetzel *et al.*, 2016; Wetzel and Meek, 2019). Yet few if any studies have addressed the implications of this within-individual variability for genotype–phenotype–herbivory relationships.

The effect of tree genetic relatedness on leaf phenolics and insect herbivory was contingent on the canopy layer. Effects were considerable in the upper canopy but negligible in the lower canopy. The higher average levels of phenolic compounds in the upper canopy layer indicate that the genes encoding the production of leaf defences are generally more strongly expressed in this part, probably owing to their stronger exposure to solar radiation (Lämke and Unsicker, 2018). This effect would exacerbate phenotypic differences between more and less effective gene variants. In contrast, light transmittance tends to be lower and more variable in the intermediate compared with the upper canopy layer (Parker *et al.*, 2002), which would weaken genotype–phenotype relationships in this part. Sun leaves are far more productive in terms of carbon fixation than shade leaves (Poorter *et al.*, 2006) and their defence against herbivores is therefore disproportionately important for overall tree performance. Our finding that tree genotypes with high phenolic compound contents in the upper canopy systematically experience lower herbivory hence suggests that such genotypes could have a non-negligible fitness advantage. In turn, leaf defence levels in the intermediate canopy layer would be overall too low and too much driven by small-scale variation in light transmittance to generate notable among-genotype differences in insect herbivory. On the other hand, the extent of intra-individual variability in phenolic compounds can be heritable (Herrera, 2017) and might act as an indirect defensive trait (Wetzel *et al.*, 2016; Wetzel and Whitehead, 2020). If this were

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the case in our study system, we would expect that trees with large differences in defence allocation between upper and lower canopy leaves would tend to experience reduced herbivory. Our data did not, however, confirm such a trend (results not shown), suggesting that the strength of within-individual variation in leaf defences either lacks a genetic basis or has no effect on (tree-level) herbivore activity. Finally, the genetic signal in leaf herbivory that we detected suggests that leaf defences may differentially drive herbivory community heritability across different parts of the canopy. The phenomenon has been thoroughly documented at the whole-plant level in common garden experiments (e.g. Andrew *et al.*, 2007; Robinson *et al.*, 2012), whereas studies in natural populations have reported lower or non-significant levels of genetic variation and heritability. One important reason may be that most previous studies investigating the role of tree genetics in defences and associated herbivory have not explicitly addressed the role of the canopy layer (but instead pooled leaf samples from different heights; e.g. Gossner *et al.*, 2015; Maldonado-López *et al.*, 2015; Kagiya *et al.*, 2018).

Conclusions

Our study shows that failing to properly take within-individual variability in defences and herbivory into account can easily mask underlying genetic signals in natural populations. Our study therefore calls for a re-interpretation of the debated role of plant genetics in determining herbivore damage. Based on our findings, we recommend that future studies adopt hierarchical sampling designs and properly consider within-individual variability in both plant traits and insect herbivory when exploring their genetic basis in real-world contexts. Finally, we also recommend that further studies include other defence traits (e.g. physical defences such as trichomes and toughness or indirect defences such as volatile organic compounds) and strategies (e.g. induced defences or tolerance). Distinguishing between all these traits or strategies would make it possible to fully characterize multivariate defensive phenotypes (i.e. syndromes) and to better understand within and among-individual variation in genotype–phenotype–herbivory relationships.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Figure S1: results of principal components analysis depicting axes PC1 versus PC2 and PC2 versus PC3 for the 15 stands. Table S1: information about the location and size of the oak stands included in the study. Table S2: pairwise F_{ST} values of the 15 oak stands.

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