Tree Physiology 39, 615–627
doi:10.1093/treephys/tpy149

Research paper

Oak genotype and phenolic compounds differently affect the performance of two insect herbivores with contrasting diet breadth

Thomas Damestoy[1,3], Benjamin Brachi[1], Xoaquín Moreira [2], Hervé Jactel[1], Christophe Plomion [1] and Bastien Castagneyrol[1]

[1]BIOGECO, INRA, Univ. Bordeaux, 69 route d'Arcachon, 33612 Cestas Cedex, France; [2]Misión Biológica de Galicia (MBG-CSIC), Apartado de correos 28, 36080 Pontevedra, Galicia, Spain; [3]Corresponding author (damestoythomas@hotmail.fr)

Received October 8, 2018; accepted December 19, 2018; published online January 22, 2019; handling Editor Jörg-Peter Schnitzler

Research on plant–herbivore interactions has long recognized that plant genetic variation plays a central role in driving insect abundance and herbivory, as well as in determining plant defense. However, how plant genes influence herbivore feeding performances, and which plant defensive traits mediate these effects, remain poorly understood. Here we investigated the feeding performances of two insect leaf chewers with contrasting diet breadth (the generalist *Lymantria dispar* L. and the specialist *Thaumetopoea processionea* L.) on different genotypes of pedunculate oak (*Quercus robur* L.) and tested the role of leaf phenolics. We used leaves from four clones of 30 *Q. robur* full-sibs grown in a common garden to estimate the performance of both herbivores in laboratory feeding trials and to quantify the concentration of constitutive chemical defences (phenolic compounds). We found that tree genetics influenced leaf consumption by *T. processionea* but not by *L. dispar*. However genetic variation among trees did not explain growth rate variation in either herbivore nor in leaf phenolics. Interestingly, all phenolic compounds displayed a positive relationship with *L. dispar* growth rate, and leaf consumption by both herbivores displayed a positive relationship with the concentrations of condensed tannins, suggesting that highly defended leaves could induce a compensatory feeding response. While genetic variation in oaks did not explain herbivore growth rate, we found positive genetic correlations between the two herbivores for leaf consumption and digestion. Overall, we found that oak genotype and phenolic compounds partly and independently contribute to variability in herbivore performance. We challenged the current view of plant–insect interaction and provided little support to the idea that the effect of plant genotype on associated organisms is driven by plant defences. Together, our results point to the existence of genetically determined resistance traits in oaks whose effects differ between herbivores and motivate further research on mechanisms governing oak–herbivore interactions.

Keywords: chemical defences, genetic variability, herbivory, oak, plant–insect interactions.

Introduction

Over the past decades, numerous studies have reported that genetically determined phenotypic variation within host plant species influences plant–insect interactions, some leading to coevolutionary processes (Crutsinger et al. 2006, Johnson and Stinchcombe 2007, Hughes et al. 2008, Bidart-Bouzat and Kliebenstein 2011, McArt and Thaler 2013), and that within-population genetic diversity in plants influences insect community composition (Crutsinger et al. 2006, Johnson 2008, Tack and Roslin 2011, Crawford and Rudgers 2013). To understand both the evolution of plant–insect interactions and the effect of plant diversity on arthropod communities, it is crucial to simultaneously (i) investigate the effect of genetic variation on plant–insect interactions, (ii) identify the host traits that influence insect herbivore performance and (iii) understand the impact of this variation on the performance of different functional groups of insects from the community.

Our current understanding of plant genetic effects on herbivores was largely based on studies in crops or non-woody plants comparing plant genotypes producing different phytochemical

defenses (but see Rubert-Nason et al. 2017, Barker et al. 2018, Falk et al. 2018). In plants, and in trees in particular, defense-related phytochemicals are often considered to be key traits driving the effect of within species diversity on arthropod communities (Wimp et al. 2007, Richards et al. 2015) as well as on the performance of individual herbivore species (Slinn et al. 2018). In particular, the specialized metabolism of plants produces hundreds or thousands of different molecules that, considered together, constitute phytochemical profiles that are often highly variable in natural populations (Geber and Griffen 2003, Barbour et al. 2015). Thus, secondary metabolites are likely a major mechanistic link between genetic variation in trees and levels of herbivory. Among specialized metabolites, phenolic compounds are commonly considered effective plant defences against many herbivores in several tree species (Feeny 1976, Lill and Marquis 2001, Forkner et al. 2004). These compounds are often toxic (Salminen and Karonen 2011, Mithöfer and Boland 2012) and some have been shown to reduce digestibility in herbivores, hence potentially reducing herbivore damage (Feeny 1970, Roslin and Salminen 2008, Abdala-Roberts et al. 2016, Moreira et al. 2018). For instance, condensed and hydrolysable tannins as well as flavonoids reduce plant digestibility by binding digestive enzymes and altering herbivores' digestive tissues through the production of reactive oxygen species (Barbehenn et al. 2009, Barbehenn and Constabel 2011, Falcone Ferreyra et al. 2012). In addition, lignins act as toxic compounds and also contribute to increased leaf toughness (Bidlack et al. 1992, Bonawitz and Chapple 2010), a common physical defensive trait (Clissold et al. 2010, Pearse 2011, Caldwell et al. 2016).

Importantly, the response of herbivores to plant chemical defences has proven variable among herbivore functional groups (Slinn et al. 2018). In particular, leaf phenolic compounds often have contrasting effects on generalist and specialist herbivore species (Cornell and Hawkins 2003, Lankau 2007, Bidart-Bouzat and Kliebenstein 2011, Ali and Agrawal 2012). Specialist herbivores are thought to overcome (or even benefit from) low concentrations of specific compounds while being more sensitive than generalists to molecules produced at high concentrations (Karban and Agrawal 2002, Coley et al. 2006, Després et al. 2007, Carmona et al. 2011). Beyond the effect of phytochemical defenses, other genetically determined plant traits (e.g., physical defenses, phenology, growth rate) may further contribute to resistance to herbivore species differing in their diet breath (Barbour et al. 2015). Thus, important insights may be gained by directly quantifying the effect of plant genetic variability on the performance of herbivores and the mediating role of phytochemical defenses.

We investigated the effect of the genetic and phenotypic variation in pedonculate oak (*Quercus robur L.*) on the performance of a generalist and a specialist herbivore species. The pedunculate oak is a broadleaved species that is widely distributed and native to Europe. This tree species supports a large community of insect herbivores, mainly leaf chewers (Southwood 1961) including the gypsy moth *Lymantria dispar* (GM) and the oak processionary moth *Thaumetopoea processionea* (OPM). These two herbivores are univoltine and sympatric species native to Europe. Their phenology (egg hatching) is synchronized with oak phenology (bud bursting) (Wagenhoff et al. 2013) such that larvae of both species can cause major defoliation in spring and early summer. The OPM is considered an oligophagous (specialist) species feeding mainly on *Quercus* sp. (with preferences for *Q. robur*, *Q. petreae* and *Q. cerris*) and occasionally on closely related Fagaceae species. The GM is a highly polyphagous (generalist) species that has been documented to feed on more than 500 host species belonging to different families (including conifers and broadleaved species) (Liebhold et al. 1995). Although the principal host species varies across its geographic range, GM larvae have marked preferences for oaks in south Western Europe, where the present study was conducted.

In the present study, we investigated how genetic variation in pedunculate oak affects the feeding behaviour of GM and OPM, including through the production of leaf phenolic compounds (flavonoids, lignins and condensed and hydrolysable tannins), by performing laboratory feeding experiments and assessing the consumption, growth and metabolic efficiency of the two herbivore species feeding on the leaves of 30 oak full-sibs. By doing so, our study builds towards a better understanding of the mechanisms underlying the effect of tree genetic variation on phytophagous insect performances.

Materials and methods

Oak common garden and leaf samples

We used 120 oak trees corresponding to four clonal replicates of 30 full-sib genotypes of a single family obtained from a cross between two parental trees (Bodénès et al. 2016). Clones were obtained by grafting and established in a common garden in 1998 following a randomized block design (INRA experimental station of Bourran, latitude 44.332492 °N, longitude 0.413993 °E). For this study, we randomly selected 30 genotypes among 207 full-sibs that have been intensively used to study the genetic architecture of plants traits (Brendel et al. 2008, Song et al. 2017). In early May 2017, we collected one branch per tree with pole pruners. Branches were ~1.5 m long and were collected at ~3–4 m height. Upon collection, we stored branches vertically, dipping in water in plastic bins to avoid desiccation. We then transported the branches back to the laboratory where the bins were placed in a dark, cold room at 4 °C. We used leaves from these branches for the laboratory trials described below.

Laboratory feeding trials

Oak processionary moth (OPM) larvae were obtained from eggs collected on mature *Q. robur* in North Eastern France in November 2016. Gypsy moth (GM) larvae were obtained from a laboratory

rearing initiated with eggs collected in Southwestern France in late 2015 (Castagneyrol et al. 2018). Egg masses of both species were kept in a climatic chamber at 4 °C prior to the experiment. On 10 April 2017 egg hatching was initiated by transferring eggs into a climatic room at constant temperature (20 °C) and photo-period (12:12 light:dark) for 4 days. Neonates were reared on leaves taken on one single mature *Q. robur* growing close to the laboratory until they reached the third instar stage.

We used a feeding trial protocol adapted from Fernandez-Conradi et al. (2017) and Castagneyrol et al. (2018). On the same day we collected branches of the 120 oak trees in the field, we isolated 120 third instar larvae of each defoliator species into individual 354 ml plastic boxes. We kept the larvae without food for 24 h and then weighed them. We also weighed the totality of frass produced during the experiment. We randomly assigned each larva to one replicate of one of the 30 oak genotypes. The experiment therefore included 120 GM third instar larvae and 120 OPM third instar larvae, each reared on the leaves of one clone of each oak genotype (i.e., each larva received leaves from a single oak branch).

Every morning, we randomly collected six leaves per branch, choosing leaves with minimum signs of herbivory, disease or senescence. Two leaves were used to feed GM and OPM larvae (i.e., one leaf per larva) while the four remaining leaves were dried for chemical analyses (see below). Each larva received one fresh leaf per day, and partially consumed leaves were removed from rearing boxes and stored for further analysis. The experiment was carried out at room temperature (~20 °C) and lasted for 4 days. Leaves introduced into each rearing box were scanned before (day n) and after (day $n + 1$) to estimate consumption by GM and OPM larvae. Small lost fragments of leaves were carefully isolated from frass and scanned together with the consumed leaves. We scanned leaves using a standard desktop scanner and estimated leaf area before and after consumption with ImageJ software (Schneider et al. 2012). Consumed leaves were dried for 72 h at 45 °C and then weighed in order to establish the relationship between leaf dry weight and leaf area.

At the end of the fourth day, we kept larvae without food for 24 h and weighed them individually. Frass accumulated over the time of the experiment was collected, dried for 72 h at 45 °C and then weighed. Larval initial and final weight, frass weight, pre- and post-consumption leaf surface and post-consumption leaf dry weight were used to estimate leaf consumption (mg day^{-1}), larval growth (mg day^{-1}) and the amount of digested leaves (the difference between consumption rate and the daily frass production mg day^{-1}) for each larva fed on leaves from a single oak tree.

Quantification of leaf phenolic compounds

Chemical analyses were conducted at the level of individual branches by pooling all intact leaves collected from the same branch over the course of the 4-day experiment. We therefore analysed phenolic content of 120 samples of 16 leaves per branch encompassing the duration of the experiment (4 leaves per branch and per day for 4 days) to capture potential changes in the composition and amount of leaf phenolics over the time of the experiment. We only collected leaves with little or no herbivore damage; hence we considered the levels and composition of phenolics measured as constitutive defences (Abdala-Roberts et al. 2016). After collection, we oven-dried leaves for 48 h at 45 °C, ground them to a thin powder using a Labman high speed grinding station, and stored powder samples at room temperature in individual plastic vials. We extracted phenolic compounds using 20 mg of dry plant tissue with 1 ml of 70% methanol in an ultrasonic bath for 20 min, followed by centrifugation (Moreira et al. 2014). We diluted methanolic extracts (1:4 vol:vol) with an extraction solvent and transferred them to chromatographic vials to perform chromatographic analyses. We carried out chromatographic analyses with an Ultra-High-Performance Liquid-Chromatograph (UHPLC Nexera LC-30AD; Shimadzu Corporation, Kyoto, Japan) equipped with a Nexera SIL-30AC injector and one SPD-M20A UV/VIS photodiode array detector. The UHPLC column was a Kinetex™ 2.6 µm C18 82–102 Å, LC Column 100 × 4.6 mm, protected with a C18 guard cartridge. The flow rate was 0.4 ml min^{-1} and the oven temperature was set to 25 °C. The mobile phase consisted of two solvents: water-formic acid (0.05%) (A) and acetonitrile-formic acid (0.05%) (B), starting with 5% B and using a gradient to obtain 30% B at 4 min, 60% B at 10 min, 80% B at 13 min and 100% B at 15 min. The injection volume was 30 µl. We recorded chromatograms at 330 nm and processed data with the LabSolutions software (Shimadzu). We quantified flavonoids as rutin equivalents, condensed tannins as catechin equivalents, hydrolysable tannins as gallic acid equivalents and lignins as ferulic acid equivalents. We achieved the quantification of these phenolic compounds by external calibration using calibration curves based on chemical equivalent at 0.25, 0.5, 1 and 2 µg ml^{-1}. We expressed phenolic compound concentrations in mg g^{-1} tissue on a dry weight basis.

Herbivore performance and nutritional indices

We first estimated leaf biomass consumed by insect larvae using the linear regression of leaf dry weight against leaf area of consumed leaves. We estimated slope and intercept parameters for each genotype separately. We then used parameter estimates of genotype-specific regressions to estimate the amount of consumed biomass from the area of consumed leaves (Fernandez-Conradi et al. 2017, Castagneyrol et al. 2018).

Then, for statistical analysis (see below) we compared larval performance between oak genotypes by using ANCOVA equivalents to Waldbauer's nutritional indexes (Waldbauer 1968, Raubenheimer and Simpson 1992, Hägele and Rowell-rahier 1999) (Figure 1).

Larval relative growth rate (RGR) is the ratio between growth rate ($G = (w_f - w_{t=0})/d$, mg day^{-1}, where $w_{t=0}$ and w_f are initial

and final larval weights, respectively, and d the number of days of the experiment) and initial weight ($w_{t=0}$): RGR = $G/w_{t=0}$. It was analysed as ANCOVA equivalent by using growth rate (G) as response variable and initial weight ($w_{t=0}$) as covariate.

Larval relative consumption rate (RCR) is the ratio between leaf biomass consumption (C, mg day^{-1}) and the initial weight: RCR = $C/w_{t=0}$. It was analysed by using leaf consumption rate (C) as response variable and initial weight ($w_{t=0}$) as covariate.

Efficiency of conversion of ingested food (ECI) is the daily amount of ingested food that is converted into body biomass. The ECI is the ratio between growth rate (G) and consumption rate (C) and represents pre-regulatory mechanisms (e.g., consumption). It was analysed by using larval growth rate as response variable (G) and leaf consumption rate (C) as covariate.

Efficiency of conversion of digested food (ECD) is the daily amount of digested food ($D = C - F$, with F the daily frass production, mg day^{-1}) that is converted into body biomass. The ECD is the ratio between growth rate (G) and the amount of digested food (D) and represents post-ingestive regulatory mechanisms (e.g., enzymatic activity in midgut). It was therefore analysed by using growth rate as response variable (G) and the amount of digested food (D) as covariate.

Approximate digestibility (AD) represents digestion efficiency. It is the ratio between the amount of digested food (D) and the consumption rate (C): AD = D/C, such that a value close to 1 means a high digestion capacity (small amount of excreted food for a given amount of ingested food). It was analysed by using amount of digested food as response variable (D) and consumption rate (C) as covariate.

We estimated the variation of each nutritional index among oak genotypes by calculating the coefficient of variation (CV), which is the ratio between the overall standard deviation and the overall mean value across all genotypes.

Statistical analyses

Third instar GM larvae were larger and consumed much more leaf biomass than third instar OPM larvae. We therefore decided not to compare GM and OPM directly but to analyse their response to oak genotypes and leaf phenolic compounds in separate models.

For each herbivore species, we analysed three response variables: consumption rate (C), growth rate (G) and amount of digested food (D). The results were interpreted in terms of nutritional indices by using the appropriate covariate (see above).

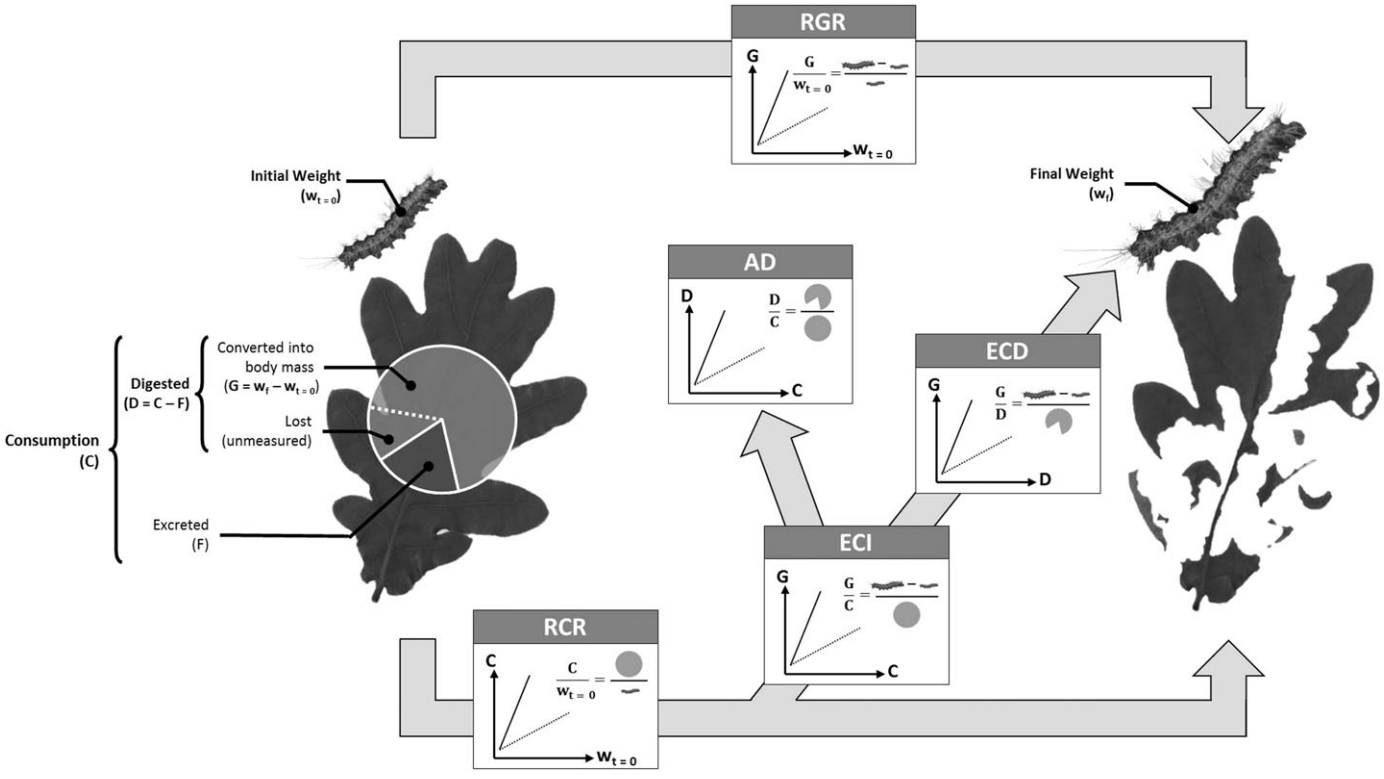

Figure 1. Schematic representation of nutritional indices and their ANCOVA equivalents. For each nutritional index (AD: approximate digestibility, ECI: efficiency of conversion of ingested food, ECD: efficiency of conversion of digested food, RGR: relative growth rate, RCR: relative consumption rate), formula and ANCOVA equivalents are shown. For instance, the ANCOVA equivalent of RGR takes growth rate (G) as a response variable and Initial Weight ($w_{t=0}$) as a covariate such that RGR is given by ($G/w_{t=0}$). G: larval growth ($w_f - w_{t=0}$), C: leaf biomass consumption, F: frass production, D: leaf biomass digestion ($C - F$).

We used linear mixed effects models (LMM) where genotype was declared as random factor. We first estimated proportion of variance in herbivore performance explained by oak genotype using a random effect models:

$$y_{i,j} = \alpha + g_j + \varepsilon_{i,j} \quad (1)$$

where $y_{i,j}$ was the response variable (*G*, *C* or *D*) for clone *i* and genotype *j*, α the model intercept, g_j the random intercept for genotype *j* and $\varepsilon_{i,j}$ residual errors. g_j and $\varepsilon_{i,j}$ were assumed to be normally distributed with mean 0 and variance σ_g^2 and σ_e^2, respectively. We used 1000 bootstraps to compute the 95% confidence interval (CI) around σ_g^2. We used the same modelling approach to analyse the genotype effect on the concentration of each group of phenolic compounds.

We tested the effect of leaf phenolic compounds on nutritional indices (RGR, RCR, ECI, ECD, AD) with random intercept models. For both GM and OPM and for each index separately, we first built a full LMM where the numerator of the index (*G*, *C* or *D*) was the response variable, and the denominator (*C*, *D* or $w_{t=0}$) was the appropriate covariate (fixed effects). We also included larval initial weight and the concentration of one of the four groups of phenolic compounds (condensed tannins, hydrolysable tannins, flavonoids or lignins, each at a time) as additional fixed effects. The term for oak genotype was a random factor. For instance, the model corresponding to the effect of hydrolysable tannins (*HT*) conditional to oak genotype on larval growth rate was:

$$G_{i,j} = \alpha + \beta_1 w_{t=0,\,i,j} + \beta_2 HT_{i,j} + g_j + \varepsilon_{i,j} \quad (2)$$

$G_{i,j}$ was the growth rate of larva fed on clone *i* of genotype *j*, α the model intercept, β_1 the effect of larval initial weight and $w_{t=0,\,i,j}$ is the initial weight measured for the larva fed on clone *i* of genotype *j*, β_2 the effect of hydrolysable tannins ($HT_{i,j}$ is the concentration of hydrolysable tannins estimated for clone *i* of genotype *j*), g_j is a random intercept for each tree genotype ($N = 30$) and $\varepsilon_{i,j}$ is the residual term ($N = 120$) following $N(0, \sigma_g^2)$ and $N(0, \sigma_e^2)$, respectively.

We then simplified the initial full model by sequentially removing terms with non-significant fixed effects, starting with the least significant, using *step* function of *lmerTest* package (Kuznetsova et al. 2017). When needed, we used logarithm (consumption of OPM) or square root (consumption of GM except when *HT* was a covariate) transformations of response variable to satisfy model assumptions. We estimated model fit by calculating marginal (R_m^2) and conditional (R_c^2) *R*-squares (Nakagawa and Schielzeth 2013). R^2 was interpreted as the amount of variance in the response variable explained by the fixed effects only (R_m^2) and by fixed effects conditional to random effects (R_c^2).

Finally, we tested whether the effect of oak genotype on herbivore performance was consistent between GM and OPM larvae.

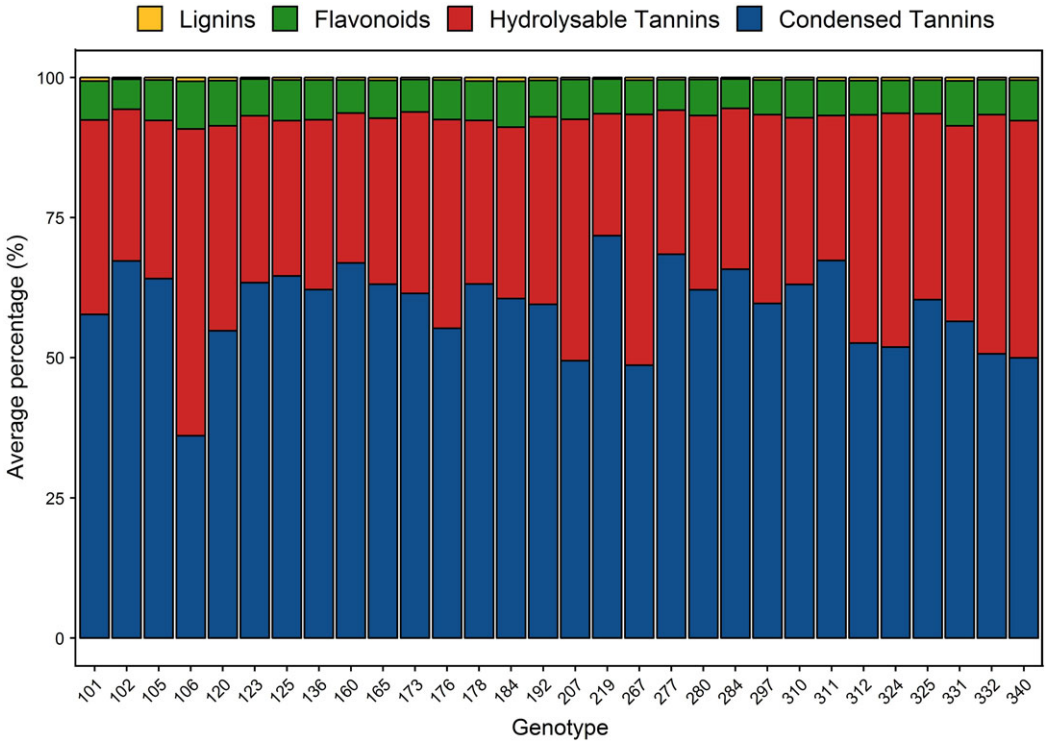

Figure 2. Oak phenolic profiles. Average percentage of the four groups of phenolic compounds in the leaf samples of each of the 30 pedunculate oak genotypes. Numbers on the *x*-axis correspond to oak genotype label.

We computed average performance traits (*G*, *C* and *D*) per oak genotype for each of the two herbivore species and tested genetic correlations across herbivore species for each performance trait using a Spearman rank sum test. Slopes and regression lines were estimated by performing linear regressions between trait values for GM and OPM.

All analyses were performed in R v3.5.1 (R Development Core Team 2018) with the following packages: *doBy*, *sciplot*, *plyr*, *ggplot2*, *cowplot*, *lme4*, *lmerTest* and *MuMIn* (Bates et al. 2015, Wickham 2011, 2016, Kuznetsova et al. 2017, Morales et al. 2017, Wilke 2017, Barton 2018, Højsgaard and Halekoh 2018).

Results

Effect of phenolics on performance of GM and OPM larvae

We identified four groups of phenolic compounds: flavonoids (13 compounds), condensed tannin (one compound), hydrolysable tannins (two compounds) and lignins (two compounds). Tannins, and in particular condensed tannins, were the most abundant phenolic compounds in leaf samples, regardless of oak genotype (Figure 2). Lignins and flavonoids only represented on average 0.65% and 8.68% of total phenolics (Figure 2). For the four groups of phenolics we observed coefficients of variation greater than 60% (see Table 2).

There was a significant positive effect of condensed tannins on the growth (*G*) and consumption rates (*C*) of GM larvae (Figure 3a and b, Table 1). However, this effect was not significant when consumption (i.e., ECI) or digestion (i.e., ECD) were included as covariates (Table 1). This result suggests that condensed tannins only influenced GM growth rate through pre-ingestive regulatory mechanisms (i.e., increased consumption). Other phenolic compounds (i.e., hydrolysable tannins, flavonoids and lignins) had a significant positive effect on GM growth rate (Figure 3a, Table 1), even when the amount of digested food (*D*) was included as a covariate in models (i.e., ECD, Table 1), suggesting that their effect involved post-digestive (i.e., metabolic) regulatory mechanisms.

There was a significant, positive effect of condensed tannins on OPM consumption rate (Figure 3c, Table 1), and a significant positive effect of consumption on OPM growth rate (Table 1). However, there was no significant effect of phenolic compounds, including condensed tannins, on OPM growth rate, even when consumption or digestion were included as covariates (Table 1).

In both GM and OPM, consumption was significantly and positively affected by initial weight (i.e., RCR, Table 1). Likewise, regardless the phenolic compounds, growth rate of OPM increased with initial weight (i.e., RGR, Table 1), and growth rate of both herbivores increased with consumption (i.e., ECI, Table 1) and digestion (i.e., ECD, Table 1). The amount of food digested by the two herbivore species increased with the amount of ingested food (i.e., AD, Table 1).

Effect of genetic variation in oaks on herbivore performance and oak phenolics

The three herbivore physiological responses (i.e., growth, consumption, digestion) varied greatly in our dataset (CV >60%), especially growth and digestion (Table 2). The oaks included in our study were full-sibs and shared on average 50% genetic identity. Despite this high degree of genetic similarity, tree genetics greatly influenced herbivore leaf consumption and digestion. In particular, the tree genotype explained large proportions of variance for consumption (*C*) and digestion (*D*) in OPM, as well as digestion in GM (Figure 4a). Oak genotype did not influence larval growth in both insect species, and the 30 tree genotypes did not display significant differences for the four groups leaf phenolics (95% CI overlapping with 0, Figure 4b)

Genetic correlations between GM and OPM performance

We found significant positive genetic correlations between performance of GM and OPM for the consumption rate, and the amount of digested food. This suggests that consumption rate or the amount of digested food of the two insect species were impacted by the same or physically linked genetic factors. In other words, oak genotypes that were more consumed or better digested by GM larvae also tended to be more consumed and better digested by OPM larvae (Figure 5a and b).

Discussion

In this study, we showed that two herbivore species with contrasting diet breadth (the generalist GM and the specialist OPM) had different ways to cope with oak phenolic compounds, suggesting different behavioural and physiological responses to plant defences. We observed that consumption and digestion of herbivores, but not growth, were largely influenced by host genotype, but that leaf phenolic compounds did not contribute to the effect of host genotype on herbivore behavioural and physiological responses. We also detected a significant positive correlation between oak genotype susceptibility to both insect species, suggesting that host resistance to both insects is driven at least in part by a set of common or physically linked loci (genes or genomic regions). Our results shed light on the role of host genotype and associated defences on food processing by insect herbivores and show that genetic variation in trees, even among full-sibs, can influence herbivore responses related to feeding.

Oak genotype influences herbivore consumption and digestion, but not growth

Our results demonstrated that oak genotypes differed in the way they drive behavioural and physiological responses of leaf feeding insects. Some studies have reported a strong influence of plant genotype on damage by insect herbivores (McArt and Thaler 2013). However, how these host genetic effects influence the

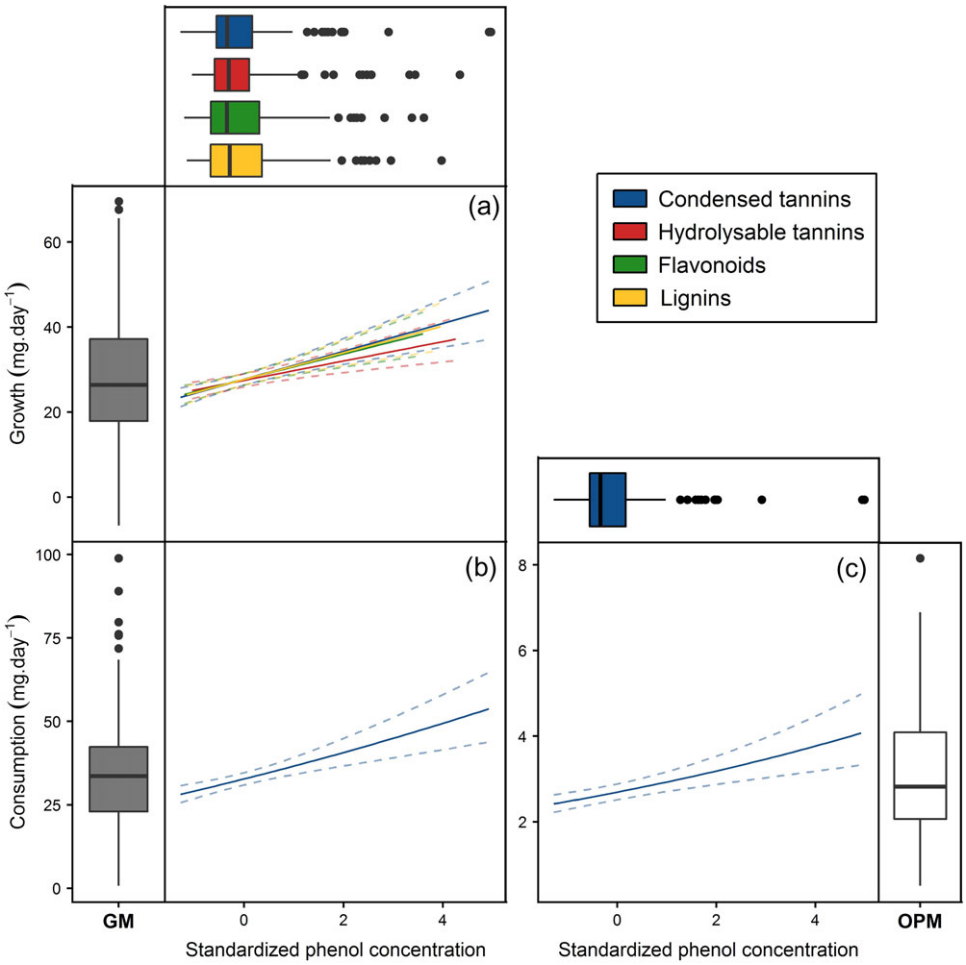

Figure 3. Effect of oak leaf phenolic compounds (*x*-axes) on gypsy moth (GM, left panels) growth (a) and consumption (b) and on oak processionary moth (OPM, right panels) consumption (c). Concentrations of phenolic compounds (mg g^{-1}) were standardized to make their effects comparable. For the sake of visibility, the figure only represents model predictions (solid lines) and corresponding standard errors (dashed lines) for those polyphenolics that had a significant effect of herbivore growth or consumption. Box and whiskers plots represent the distribution of response (*y*-axes) and explanatory (*x*-axes) variables.

performance of herbivores has been rarely tested (but see McArt and Thaler 2013, Utsumi et al. 2013). Here, we showed that the way in which herbivore response to plant genotype is evaluated does matter (Whitlock 2014). In particular, we found that the amount of food consumed and digested by the two herbivore species differed between tree genotypes, but that larval growth was, on the contrary, largely independent of oak genotype. This finding partially conflicts with previous studies reporting consistent or stronger effects of host genotype on GM growth relative to consumption (Osier and Lindroth 2001, Fernandez-Conradi et al. 2017). This discrepancy could be attributed to the duration of the feeding trials. Although we cannot exclude that oak genotype could have had a stronger effect on herbivore growth should we have prolonged feeding trials, a likely reason for these contrasting results could be that different oak genotypes differed in traits that drove pre- and post-ingestive regulatory processes. Thus, our findings suggest a compensation of lower nutritive quality of some oak genotype by increased consumption and/or efficiency of assimilation, so as to maintain a constant growth regardless of the oak genotype on which they feed (Milanović et al. 2014). Furthermore, the positive genetic correlations between the performances of both herbivores indicated that identical or linked loci impact consumption or digestibility in both the specialist and generalist herbivore. This is consistent with previous work by Reymond et al. (2004), who showed an almost identical transcript profile in plant responses to damage by generalist or specialist insect herbivores.

Leaf phenolics have contrasting effects on generalist and specialist herbivore species

Overall, we found that leaf phenolics had increased herbivore growth and consumption, but that these effects differed among the classes of phenolic compounds and, for some of them, between herbivore species. Leaf consumption by both the generalist and the specialist herbivore increased with increasing concentration of condensed tannins in oak leaves. This result is

Table 1. Summary of linear mixed effect models testing the effects of oak phenolic compounds on nutritional indices for gypsy moth (GM) and oak processionary moth (OPM) larvae. R^2 are given for the simplified model, R_m^2 is the marginal R^2 associated with the fixed effects and R_c^2 is the conditional R^2 associated with the fixed effects plus the random effects. Model coefficient parameter estimates are reported for significant effects only (Estimate, SE). Significant coefficients ($P < 0.05$) are in bold. RCR: relative consumption rate, RGR: relative growth rate, ECI: efficiency of conversion of ingested food, ECD: efficiency of conversion of digested food, AD: approximate digestibility.

			Gypsy moth (*Lymantria dispar*)				Oak processionary moth (*Thaumetopoea processionea*)			
Phenolic compounds	Response (nutritional ratio equivalent)	Predictors	*F* value (df)	*P*-value	Estimate (SE)	R_m^2 (R_c^2)	*F* value (df)	*P*-value	Estimate (SE)	R_m^2 (R_c^2)
Condensed tannins	Consumption (RCR)	Initial weight	7.09 (1, 112.6)	**0.009**	0.012 (0.004)	0.096 (0.175)	34.55 (1, 92.6)	**<0.001**	0.216 (0.037)	0.191 (0.540)
		Phenols	5.35 (1, 113.5)	**0.023**	0.010 (0.004)		4.69 (1, 99.1)	**0.033**	0.084 (0.039)	
	Growth (RGR)	Initial weight	3.89 (1, 113)	0.051	–	0.048 (0.048)	19.74 (1, 115)	**<0.001**	3e-04 (6e-05)	0.145 (0.145)
		Phenols	5.84 (1, 114)	**0.017**	0.003 (0.001)		2.23 (1, 114)	0.138	–	
	Growth (ECI)	Initial weight	0.03 (1, 100.6)	0.853	–	0.702 (0.802)	5.41 (1, 114)	**0.022**	1e-04 (5e-05)	0.401 (0.401)
		Consumption	333.71 (1, 103.9)	**<0.001**	0.013 (7e-04)		49.71 (1, 114)	**<0.001**	4e-04 (5e-05)	
		Phenols	1.88 (1, 103.1)	0.173	–		0.31 (1, 113)	0.578	–	
	Growth (ECD)	Initial weight	1.88 (1, 103.8)	0.173	–	0.345 (0.493)	13.74 (1, 114)	**<0.001**	2e-04 (5e-05)	0.260 (0.260)
		Digested food	60.00 (1, 113.8)	**<0.001**	0.009 (0.001)		18.06 (1, 114)	**<0.001**	2e-04 (5e-05)	
		Phenols	2.38 (1, 107.8)	0.126	–		0.61 (1, 113)	0.435	–	
	Digestion (AD)	Initial weight	3.44 (1, 100)	0.067	–	0.752 (0.840)	35.27 (1, 114)	**<0.001**	−2e-04 (3e-05)	0.933 (0.933)
		Consumption	439.89 (1, 104.7)	**<0.001**	0.009 (4e-04)		1541.33 (1, 114)	**<0.001**	0.001 (3e-05)	
		Phenols	0.20 (1, 101.3)	0.657	–		1.20 (1, 113)	0.178	–	
Hydrolysable tannins	Consumption (RCR)	Initial weight	7.08 (1, 115)	**0.009**	0.004 (0.002)	0.057 (0.073)	34.99 (1, 93.8)	**<0.001**	0.220 (0.037)	0.162 (0.530)
		Phenols	1.22 (1, 114)	0.272	–		1.43 (1, 98.4)	0.234	–	
	Growth (RGR)	Initial weight	3.76 (1, 114)	0.055	–	–	19.74 (1, 115)	**<0.001**	3e-04 (6e-05)	0.145 (0.145)
		Phenols	3.20 (1, 113)	0.076	–		0.17 (1, 114)	0.685	–	
	Growth (ECI)	Initial weight	0.04 (1, 100.4)	0.842	–	0.702 (0.802)	5.41 (1, 114)	0.022	1e-04 (1e-04)	0.401 (0.401)
		Consumption	333.71 (1, 103.9)	**<0.001**	0.013 (7e-04)		49.71 (1, 114)	**<0.001**	4e-04 (1e-04)	
		Phenols	3.17 (1, 102.8)	0.078	–		0.06 (1, 113)	0.807	–	
	Growth (ECD)	Initial weight	1.85 (1, 102.6)	0.177	–	0.363 (0.514)	13.74 (1, 114)	**<0.001**	2e-04 (5e-05)	0.260 (0.260)
		Digested food	60.16 (1, 112.9)	**<0.001**	0.009 (0.001)		18.06 (1, 114)	**<0.001**	2e-04 (5e-05)	
		Phenols	4.06 (1, 106)	**0.047**	0.002 (0.001)		0.02 (1, 113)	0.898	–	
	Digestion (AD)	Initial weight	3.44 (1, 100)	0.067	–	0.752 (0.840)	35.27 (1, 114)	**<0.001**	−2e-04 (3e-05)	0.933 (0.933)
		Consumption	439.89 (1, 104.7)	**<0.001**	0.009 (4e-04)		1541.33 (1, 114)	**<0.001**	0.001 (3e-05)	
		Phenols	1.45 (1, 101.2)	0.232	–		1.83 (1, 113)	0.275	–	
Flavonoids	Consumption (RCR)	Initial weight	6.79 (1, 113.9)	**0.010**	0.012 (0.004)	0.054 (0.138)	34.99 (1, 93.8)	**<0.001**	0.220 (0.037)	0.162 (0.530)
		Phenols	2.58 (1, 111.6)	0.111	–		2.18 (1, 97)	0.143	–	
	Growth (RGR)	Initial weight	3.40 (1, 113)	0.068	–	0.039 (0.039)	19.74 (1, 115)	**<0.001**	3e-04 (1e-04)	0.145 (0.145)
		Phenols	4.71 (1, 114)	**0.032**	0.003 (0.001)		0.12 (1, 114)	0.734	–	
	Growth (ECI)	Initial weight	0.07 (1, 100.2)	0.788	–	0.702 (0.802)	5.41 (1, 114)	**0.022**	1e-04 (5e-05)	0.401 (0.401)
		Consumption	333.71 (1, 103.9)	**<0.001**	0.013 (7e-04)		49.71 (1, 114)	**<0.001**	4e-04 (5e-05)	
		Phenols	3.18 (1, 100.6)	0.078	–		0.15 (1, 113)	0.700	–	

Growth (ECD)	Initial weight	1.63 (1, 102.7)	0.204	–	0.364 (0.510)	13.74 (1, 114)	**<0.001**	2e-04 (5e-05)	0.260 (0.260)
	Digestion	58.61 (1, 112.9)	**<0.001**	0.009 (0.001)		18.06 (1, 114)	**<0.001**	2e-04 (5e-05)	
	food								
	Phenols	4.24 (1, 103.8)	**0.042**	0.002 (0.001)		0.08 (1, 113)	0.772	–	
Digestion (AD)	Initial weight	3.44 (1, 100)	0.067	–	0.752 (0.840)	35.27 (1, 114)	**<0.001**	−2e-04 (3e-05)	0.933 (0.933)
	Consumption	439.89 (1, 104.7)	**<0.001**	0.009 (4e-04)		1541.33 (1, 114)	**<0.001**	0.001 (3e-05)	
	Phenols	0.86 (1, 98.7)	0.357	–		3.55 (1, 113)	0.062	–	
Lignins	Initial weight	6.79 (1, 113.9)	**0.010**	0.012 (0.004)	0.054 (0.138)	34.99 (1, 93.8)	**<0.001**	0.220 (0.037)	0.162 (0.530)
	Phenols	2.37 (1, 113.1)	0.127	–		0.98 (1, 98.8)	0.325	–	
Growth (RGR)	Initial weight	3.10 (1, 113)	0.081	–	0.044 (0.044)	19.74 (1, 115)	**<0.001**	3e-04 (1e-05)	0.145 (0.145)
	Phenols	5.27 (1, 114)	**0.024**	0.003 (0.001)		0 (1, 114)	0.994	–	
Growth (ECI)	Initial weight	0.12 (1, 100.4)	0.732	-	0.702 (0.802)	5.41 (1, 114)	**0.022**	1e-04 (5e-05)	0.401 (0.401)
	Consumption	333.71 (1, 103.9)	**<0.001**	0.013 (7e-04)		49.71 (1, 114)	**<0.001**	4e-04 (5e-05)	
	Phenols	2.79 (1, 103.5)	0.098	–		0.06 (1, 113)	0.814	–	
Growth (ECD)	Initial weight	1.41 (1, 103.5)	0.238	–	0.360 (0.493)	13.74 (1, 114)	**<0.001**	2e-04 (1e-04)	0.26 (0.26)
	Digested	56.81 (1, 112.3)	**<0.001**	0.009 (0.001)		18.06 (1, 114)	**<0.001**	2e-04 (1e-04)	
	food								
	Phenols	4.07 (1, 107.9)	**0.046**	0.002 (0.001)		0.09 (1, 113)	0.760	–	
Digestion (AD)	Initial weight	3.44 (1, 100)	0.067	–	0.752 (0.840)	35.27 (1, 114)	**<0.001**	−2e-04 (3e-05)	0.93 (0.93)
	Consumption	439.89 (1, 104.7)	**<0.001**	0.009 (4e-04)		1541.33 (1, 114)	**<0.001**	0.001 (3e-05)	
	Phenols	0.29 (1, 101.1)	0.593	–		2.76 (1, 113)	0.100	–	

in line with previous studies (e.g., Foss and Rieske 2003) and can be interpreted as a compensatory feeding response of herbivores to plant defences (Lazarević et al. 2002, Barbehenn et al. 2009) whereby herbivores increase their consumption to compensate the ingestion of leaves of poorer quality (highly defended). The growth of the generalist herbivore (GM) was enhanced under high concentrations of condensed tannins, but this effect became non-significant when consumption or digestion was accounted for. This result indicates that leaf condensed tannins primarily influenced pre-ingestive regulatory processes in the generalist herbivore (Barbehenn et al. 2009). In contrast, condensed tannins had no effect on the growth of the specialist herbivore (OPM), suggesting that post-ingestive processes (e.g., detoxification) have evolved to avoid the need for overconsumption to maintain growth.

Hydrolysable tannins, lignins and flavonoids had a positive effect on the growth of the generalist herbivore, when digestive processes were accounted for (i.e., ECD), indicating that their effects occurred through post-digestive (i.e., metabolic) regulatory processes. The positive effect of leaf chemical defences on larval growth in the generalist herbivore may have been primarily driven by an increase in gut enzymatic activity (Lazarević et al. 2002, Lazarevic and Peric-Mataruga 2003, Milanović et al. 2015). In contrast, these phenolic compounds had no significant effects on the growth of the specialist herbivore. Insect herbivores, and in particular generalist herbivores (Karban and Agrawal 2002), can adjust the production of gut enzymes in response to the nutritional quality of their diet (Clissold et al. 2010, Milanović et al. 2014, Mrdaković et al. 2014). However, this adaptation may result in a metabolic cost (Lazarevic and Peric-Mataruga 2003). Such a cost may be more important for specialist herbivores, which cannot switch onto other host species. They may have then greater difficulty adapting to changes in the diet quality of the main host (Wetzel et al. 2016, Pearse et al. 2018), such as those imposed by our experimental approach. The lack of effect on the growth in the specialist herbivore could be explained by an increased consumption compensating for metabolic costs of enzymatic adjustment (Lazarevic and Peric-Mataruga 2003).

Herbivore response to oak genotype is not primarily mediated by leaf phenolics

Previous studies showed that the effect of plant genotype on herbivore damage can be driven by differences in plant secondary metabolites (Kersten et al. 2013, Brachi et al. 2015) whereby plants having more similar secondary metabolite profiles also tend to be more sensitive to the same herbivore. In contrast, we found that, although leaf phenolic content greatly varied among oak trees, oak genotype only explained ~6% of this variability. As such, it is unlikely that the observed effect of oak genotype on the consumption and digestion by the two herbivore species was driven by oak leaf phenolics. However,

Table 2. Summary of oak and herbivore trait means and variances ($n = 30$). Min and Max are the mean of the minimum and maximum values calculated across clones of the same genotype (i.e., $n = 4$ replicates). Overall mean represents the mean calculated across all clones. The coefficient of variation (CV) is the ratio between the standard deviation and mean. GM: gypsy moth, OPM: oak processionary moth.

Organisms	Traits	Min (mean ± SD)	Max (mean ± SD)	Overall mean (mean ± SD)	CV
Oak	Total phenolics (mg g^{-1})	2.44 ± 0.63	8.36 ± 6.90	4.68 ± 3.61	0.77
	Condensed tannins (mg g^{-1})	1.18 ± 0.81	5.88 ± 5.37	2.79 ± 2.39	0.86
	Hydrolysable tannins (mg g^{-1})	0.66 ± 0.14	3.10 ± 2.57	1.58 ± 1.35	0.86
	Flavonoids (mg g^{-1})	0.17 ± 0.03	0.48 ± 0.32	0.29 ± 0.17	0.60
	Lignins (mg g^{-1})	0.01 ± 0.002	0.04 ± 0.04	0.02 ± 0.02	0.83
GM	Growth (mg day^{-1})	13.50 ± 14.22	45.84 ± 19.03	27.56 ± 16.77	0.61
	Consumption (mg day^{-1})	15.34 ± 12.17	52.91 ± 21.28	35.10 ± 20.24	0.57
	Digested food (mg day^{-1})	−3.43 ± 7.91	25.50 ± 14.88	12.42 ± 11.14	0.90
OPM	Growth (mg day^{-1})	−0.04 ± 0.55	1.24 ± 0.38	0.53 ± 0.78	1.48
	Consumption (mg day^{-1})	1.15 ± 0.50	5.3 ± 1.20	3.08 ± 1.67	0.54
	Digested food (mg day^{-1})	0.42 ± 0.67	4.56 ± 1.38	2.23 ± 1.36	0.60

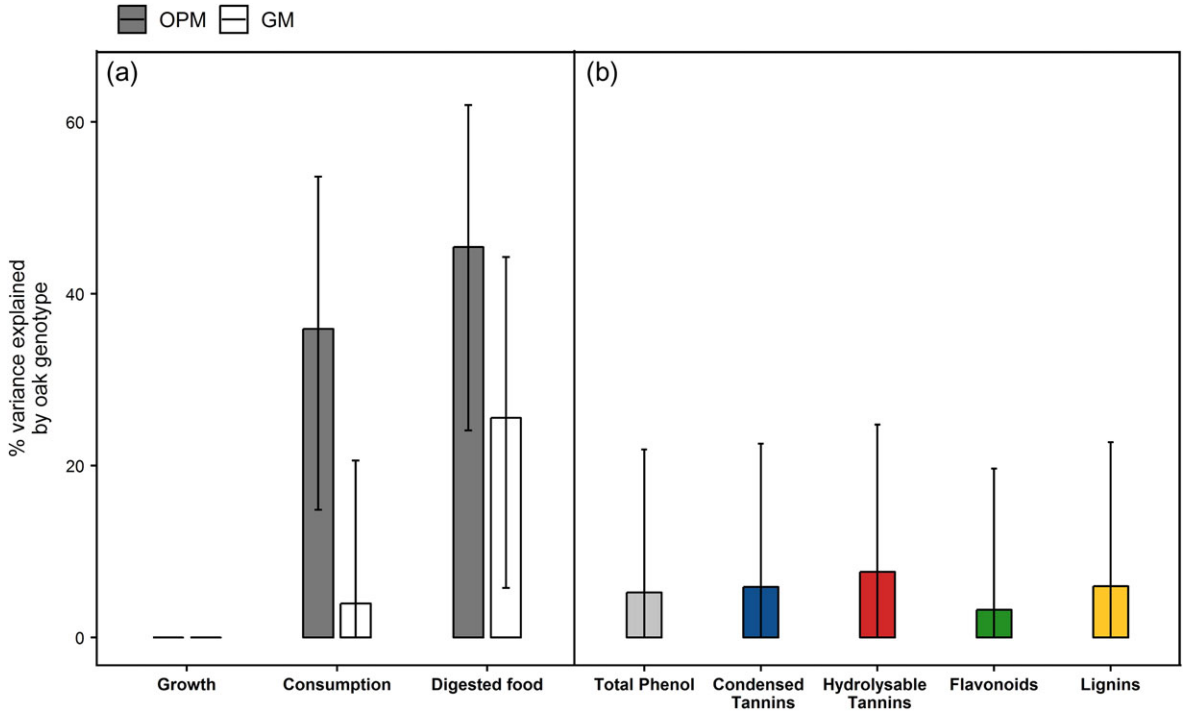

Figure 4. Percentage of variance explained by oak genotype on herbivore physiological traits (*G*, *C* and *D*) (a) and leaf phenolic compounds (b). Histograms represent the percentage of variance explained by oak genotype for each trait in intercept only linear mixed effects models. Error bars represent 95% confidence interval (estimated with bootstraps). GM: gypsy moth, OPM: oak processionary moth.

we cannot exclude the possibility that the amount of phenolic compounds that we measured was affected by induced systemic responses in distant leaves, or, to some extent, by the fact that we used excised leaves in our feeding trials. Thus, phenolic compounds that we measured presumably represented a combination of constitutive defences, plus an unknown level of systemic induction (Abdala-Roberts et al. 2016). Induced defences represent an important part of overall plant defences. We cannot exclude that oak's ability to induce specific defences in response to initial herbivores or pathogens attacks is genetically controlled (Arimura et al. 2000, Agrawal et al. 2002, Fürstenberg-Hägg et al. 2013, Moreira et al. 2012, 2015), or that the effect of oak genotype on chemical defences would have been stronger on induced defences than on constitutive defences. Therefore, it is possible that oak genotype did influence herbivores' consumption through induced systemic response that may have remained unnoticed in our experiments. Additionally, it is important to note that secondary metabolites might have lower effects on herbivore performance than other plant defensive traits (Carmona et al. 2011), maybe due to the ability of some herbivores to overcome chemical defences, through detoxification, secretion or degradation of toxins (Karban and Agrawal 2002, Després et al. 2007). We cannot exclude that the 30 oak genotypes of our experiment also differed in other chemical (e.g., terpenes,

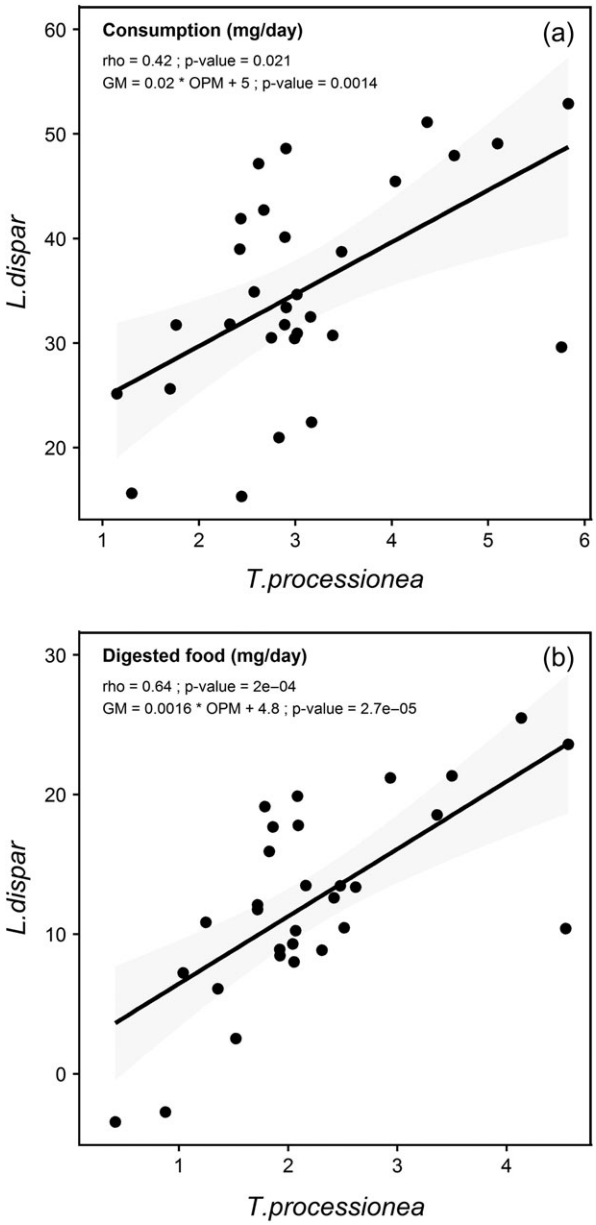

Figure 5. Genetic correlations between consumption (a), and digestion (b) of both herbivore species. Dots represent genotype-specific means. Regression line and grey areas represent predictions from linear models and corresponding SE. Spearman's correlation coefficients, rho, and the equation of regression line and their *P*-values are indicated in each panel. GM: gypsy moth; OPM: oak processionary moth.

nutrients, carbohydrates) or physical (e.g., leaf toughness) traits, which could drive herbivores' response to oak genotypes. For instance, a number of previous studies have reported that low nutrient (e.g., nitrogen, phosphorus) concentrations in plant tissues drastically reduce herbivore performances (Milanović et al. 2014, Wetzel et al. 2016). Similarly, leaf thickness and leaf toughness or hairiness are structural traits that have been reported to negatively affect the performance of leaf-chewing herbivores (Clissold et al. 2009, Caldwell et al. 2016). Considering both leaf chemical and structural traits will thus be needed to better characterize the physiological mechanisms linking plant genotype with the performance of feeding herbivores.

Conclusion

Overall, our study builds towards a better understanding of the relationships between host genotype and the performance of associated insect herbivores. In particular, our findings challenge two common views. First, we found that oak leaf phenolic compounds did not reduce the growth of the two herbivore species studied here. In particular, oak leaf phenolic compounds increased herbivore consumption and growth rates, probably as a result of overconsumption. Second, we provide little support to the idea that the effect of tree genotype on associated organisms is driven by tree chemical defences. Yet, we did observe large variability in herbivore performance and production of phenolic compounds among individuals and among genotypes, but these effects were partially independent. The fact that we observed genetic correlations between the performance of both herbivores suggests that other, unmeasured plant defensive traits under genetic control may provide general resistance to herbivores. In conclusion, by addressing the effect of host plant genotype on both the amount of damage (i.e., a plant perspective) and the physiology of two insect herbivores with contrasting diet breadth (i.e., herbivore perspective), we highlight the need for further research on mechanisms driving plant resistance to herbivores.

Acknowledgments

The authors thank Victor Rébillard, Christophe Poileux, Benjamin Dencausse and Yannick Mellerin for their superb technical assistance in branch sampling, Hubert Schmuck, Louis-Michel Nageleisen and colleagues of INRA Avignon for their help in egg masses sampling, and Inge Van-Halder and Fabrice Vetillard for their precious help in rearing of the caterpillars. T.D. and B.C. conceived the study and acquired the data. T.D. and X.M. performed the chemical analyses. T.D. and B.C. analysed the data. B.B. helped with data analysis. T.D. and B.C. drafted the first version of the manuscript. All authors wrote the final version of the manuscript.

Conflict of interest

None declared.

Funding

T.D. was funded by the French National Institute for Agronomy Research (INRA) and French National Forest Office (ONF) under Grant agreement 22001052. This work was supported by the French Department of Forest health (DSF) under Grant agreement E04/2017.

References

Abdala-Roberts L, Hernández-Cumplido J, Chel-Guerrero L, Betancur-Ancona D, Benrey B, Moreira X (2016) Effects of plant intraspecific diversity across three trophic levels: underlying mechanisms and plant traits. Am J Bot 103:1810–1818.

Agrawal AA, Conner JK, Johnson MTJ, Wallsgrove R (2002) Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. Evolution 56: 2206–2213.

Ali JG, Agrawal AA (2012) Specialist versus generalist insect herbivores and plant defense. Trends Plant Sci 17:293–302.

Arimura G, Tashiro K, Kuhara S, Nishioka T, Ozawa R, Takabayashi J (2000) Gene responses in bean leaves induced by herbivory and by herbivore-induced volatiles. Biochem Biophys Res Commun 277: 305–310.

Barbehenn RV, Constabel CP (2011) Tannins in plant-herbivore interactions. Phytochemistry 72:1551–1565.

Barbehenn RV, Jaros A, Lee G, Mozola C, Weir Q, Salminen JP (2009) Hydrolyzable tannins as 'quantitative defenses': limited impact against *Lymantria dispar* caterpillars on hybrid poplar. J Insect Physiol 55: 297–304.

Barbour MA, Rodriguez-Cabal MA, Wu ET, Julkunen-Tiitto R, Ritland CE, Miscampbell AE, Jules ES, Crutsinger GM (2015) Multiple plant traits shape the genetic basis of herbivore community assembly. Funct Ecol 29:995–1006.

Barker HL, Holeski LM, Lindroth RL (2018) Genotypic variation in plant traits shapes herbivorous insect and ant communities on a foundation tree species. PLoS One 13:1–21.

Barton K (2018) MuMIn: Multi-Model Inference. R package version 1.40.4. https://cran.r-project.org/package=MuMIn.

Bates D, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48.

Bidart-Bouzat MG, Kliebenstein D (2011) An ecological genomic approach challenging the paradigm of differential plant responses to specialist versus generalist insect herbivores. Oecologia 167: 677–689.

Bidlack J, Malone M, Benson R (1992) Molecular structure and component integration of secondary cell walls in plants. Proc Oklahoma Acad Sci 72:51–56.

Bodénès C, Chancerel E, Ehrenmann F, Kremer A, Plomion C (2016) High-density linkage mapping and distribution of segregation distortion regions in the oak genome. DNA Res 23:115–124.

Bonawitz ND, Chapple C (2010) The genetics of lignin biosynthesis: connecting genotype to phenotype. Annu Rev Genet 44:337–363.

Brachi B, Meyer CG, Villoutreix R, Platt A, Morton TC, Roux F, Bergelson J (2015) Coselected genes determine adaptive variation in herbivore resistance throughout the native range of *Arabidopsis thaliana*. Proc Natl Acad Sci USA 112:4032–4037.

Brendel O, Le Thiec D, Scotti-Saintagne C, Bodénès C, Kremer A, Guehl J-M (2008) Quantitative trait loci controlling water use efficiency and related traits in *Quercus robur* L. Tree Genet Genomes 4:263–278.

Caldwell E, Read J, Sanson GD (2016) Which leaf mechanical traits correlate with insect herbivory among feeding guilds? Ann Bot 117:349–361.

Carmona D, Lajeunesse MJ, Johnson MTJ (2011) Plant traits that predict resistance to herbivores. Funct Ecol 25:358–367.

Castagneyrol B, Moreira X, Jactel H (2018) Drought and plant neighbourhood interactively determine herbivore consumption and performance. Sci Rep 8:1–11.

Clissold FJ, Sanson GD, Read J, Simpson SJ (2009) Gross vs. net income: how plant toughness affects performance of an insect herbivore. Ecology 90:3393–3405.

Clissold FJ, Tedder BJ, Conigrave AD, Simpson SJ (2010) The gastrointestinal tract as a nutrient-balancing organ. Proc R Soc B 277:1751–1759.

Coley PD, Bateman ML, Kursar TA (2006) The effects of plant quality on caterpillar growth and defense against natural enemies. Oikos 115: 219–228.

Cornell HV, Hawkins BA (2003) Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. Am Nat 161: 507–522.

Crawford KM, Rudgers JA (2013) Genetic diversity within a dominant plant outweighs plant species diversity in structuring an arthropod community. Ecology 94:1025–1035.

Crutsinger GM, Collins MD, Fordyce JA, Gompert Z, Nice CC, Sanders NJ (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. Science 313:966–969.

Després L, David J, Gallet C (2007) The evolutionary ecology of insect resistance to plant chemicals. Ecol Evol 22:298–307.

Falcone Ferreyra ML, Rius SP, Casati P (2012) Flavonoids: biosynthesis, biological functions, and biotechnological applications. Plant Sci 3: 1–15.

Falk MA, Lindroth RL, Keefover K, Kenneth R (2018) Genetic variation in aspen phytochemical patterns structures windows of opportunity for gypsy moth larvae. Oecologia 187:471–482.

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.

Feeny P (1976) Plant apparency and chemical defense. Recent Adv Phytochem 10:1–40.

Fernandez-Conradi P, Jactel H, Hampe A, Leiva MJ, Castagneyrol B (2017) The effect of tree genetic diversity on insect herbivory varies with insect abundance. Ecosphere 8:1–13.

Forkner RE, Marquis RJ, Lill JT (2004) Feeny revisited: condensed tannins as anti-herbivore defences in leaf chewing herbivore communities of *Quercus*. Ecol Entomol 29:174–187.

Foss LK, Rieske LK (2003) Species-specific differences in oak foliage affect preference and performance of gypsy moth caterpillars. Entomol Exp Appl 108:87–93.

Fürstenberg-Hägg J, Zagrobelny M, Bak S (2013) Plant defense against insect herbivores. Int J Mol Sci 14:10242–10297.

Geber MA, Griffen LR (2003) Inheritance and natural selection on functional traits. Int J Plant Sci 164:s21–s42.

Hägele BF, Rowell-rahier M (1999) Dietary mixing in three generalist herbivores: nutrient complementation or toxin dilution? Oecologia 119:521–533.

Højsgaard S, Halekoh U (2018) doBy: Groupwise Statistics, LSmeans, Linear Contrasts, Utilities. R package version 4.6-1. https://cran.r-project.org/package=doBy

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. Ecol Lett 11:609–623.

Johnson MTJ (2008) Bottom-up effects of plant genotype on aphids, ants, and predators. Ecology 89:145–154.

Johnson MTJ, Stinchcombe JR (2007) An emerging synthesis between community ecology and evolutionary biology. Trends Ecol Evol 22: 250–257.

Karban R, Agrawal AA (2002) Herbivore offense. Annu Rev Ecol Syst 33:641–664.

Kersten B, Ghirardo A, Schnitzler JP, Kanawati B, Schmitt-Kopplin P, Fladung M, Schroeder H (2013) Integrated transcriptomics and metabolomics decipher differences in the resistance of pedunculate oak to the herbivore *Tortrix viridana* L. BMC Genomics 14:737.

Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: tests in linear mixed effects models. J Stat Softw 82:1–26.

Lankau RA (2007) Specialist and generalist herbivores exert opposing selection on a chemical defense. New Phytol 175: 176–184.

Lazarevic J, Peric-Mataruga V (2003) Nutritive stress effects on growth and digestive physiology of *Lymantria dispar* larvae. Jugosl Med Biokem 22:53–59.

Lazarević J, Perić-Mataruga V, Stojković B, Tucić N (2002) Adaptation of the gypsy moth to an unsuitable host plant. Entomol Exp Appl 102:75–86.

Liebhold AM, Gottschalk KW, Muzika R-M, Montgomery ME, Young R, O'Day K, Kelley B (1995) Suitability of North American tree species to the gypsy moth: a summary of field and laboratory tests. United States Department of Agriculture Forest Service, Northeastern Forest Experimental Station, General Technical Report NE-211.

Lill JT, Marquis RJ (2001) The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia 126:418–428.

McArt SH, Thaler JS (2013) Plant genotypic diversity reduces the rate of consumer resource utilization. Proc Biol Sci 280: 20130639.

Milanović S, Lazarević J, Popović Z, Miletić Z, Kostić M, Radulović Z, Karadžić D, Vuleta A (2014) Preference and performance of the larvae of *Lymantria dispar* (Lepidoptera: Lymantriidae) on three species of european oaks. Eur J Entomol 111:371–378.

Milanović S, Janković-Tomanić M, Kostić I, Kostić M, Morina F, Živanović B, Lazarević J (2015) Behavioural and physiological plasticity of gypsy moth larvae to host plant switching. Entomol Exp Appl 158:152–162.

Mithöfer A, Boland W (2012) Plant defense against herbivores: chemical aspects. Annu Rev Plant Biol 63:431–450.

Moreira X, Zas R, Sampedro L (2012) Differential allocation of constitutive and induced chemical defenses in pine tree juveniles: a test of the optimal defense theory. PLoS One 7:e34006.

Moreira X, Mooney KA, Petry WK, Carrillo-Gavilán A, Zas R, Sampedro L (2014) Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. Ecol Lett 17:537–546.

Moreira X, Abdala-roberts L, Hernández-Cumplido J, Cuny MAC, Glauser G, Benrey B (2015) Specificity of induced defenses, growth, and reproduction in lima bean (*Phaseolus lunatus*) in response to multispecies herbivory. Am J Bot 102:1300–1308.

Morales M, with code developed by the R Development Core Team, with general advice from the R-help listserv community, especially Duncan Murdoch (2017) sciplot: Scientific Graphing Functions for Factorial Designs. R package version 1.1-1. https://cran.r-project.org/package=sciplot.

Moreira X, Castagneyrol B, Abdala-roberts L, Berny-Mier JC, Timmermans BGH, Bruun HH, Covelo F, Glauser G, Rasmann S (2018) Latitudinal variation in plant chemical defences drives latitudinal patterns of leaf herbivory. Ecography 41:1124–1134.

Mrdaković M, Stojković B, Ilijin L, Vlahović M, Perić-Mataruga V, Lazarević J (2014) Testing the adaptive plasticity of gypsy moth digestive enzymes in response to tannic acid using phenotypic selection analysis. Genetika 46:883–894.

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol Evol 4:133–142.

Osier TL, Lindroth RL (2001) Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. J Chem 27:1289–1313.

Pearse IS (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 IS, Paul R, Ode PJ (2018) Variation in plant defense suppresses herbivore performance. Curr Biol 28:1–6.

R Development Core Team (2018) R: a language and environment for statistical computing. https://www.r-project.org/

Raubenheimer D, Simpson SJ (1992) Analysis of covariance: an alternative to nutritional indices. Entomol Exp Appl 62:221–231.

Reymond P, Bodenhausen N, Van Poecke RMP, Krishnamurthy V, Dicke M, Farmer EE (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell 16:3132–3147.

Richards LA, Dyer LA, Forister ML, Smilanich AM, Dodson CD, Leonard MD, Jeffrey CS (2015) Phytochemical diversity drives plant–insect community diversity. Proc Natl Acad Sci USA 112:10973–10978.

Roslin T, Salminen J (2008) Specialization pays off: contrasting effects of two types of tannins on oak specialist and generalist moth species. Oikos 117:1560–1568.

Rubert-Nason KF, Couture JJ, Gryzmala EA, Townsend PA, Lindroth RL (2017) Vernal freeze damage and genetic variation alter tree growth, chemistry, and insect interactions. Plant Cell Environ 40:2743–2753.

Salminen JP, Karonen M (2011) Chemical ecology of tannins and other phenolics: we need a change in approach. Funct Ecol 25:325–338.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675.

Slinn HL, Richards LA, Dyer LA, Hurtado PJ, Smilanich AM (2018) Across multiple species, phytochemical diversity and herbivore diet breadth have cascading effects on herbivore immunity and parasitism in a tropical model system. Front Plant Sci 9:1–12.

Song J, Brendel O, Bodénès C, Plomion C, Kremer A, Colin F (2017) X-ray computed tomography to decipher the genetic architecture of tree branching traits: oak as a case study. Tree Genet Genomes 13:5.

Southwood TR (1961) The number of species of insect associated with various trees. J Anim Ecol 30:1–8.

Tack AJM, Roslin T (2011) The relative importance of host-plant genetic diversity in structuring the associated herbivore community. Ecology 92:1594–1604.

Utsumi S, Ando Y, Roininen H, Takahashi JI, Ohgushi T (2013) Herbivore community promotes trait evolution in a leaf beetle via induced plant response. Ecol Lett 16:362–370.

Wagenhoff E, Blum R, Engel K, Veit H, Delb H (2013) Temporal synchrony of *Thaumetopoea processionea* egg hatch and *Quercus robur* budburst. J Pest Sci (2004) 86:193–202.

Waldbauer GP (1968) The consumption and utilization of food by insects. Adv Insect Physiol 5:229–288.

Wetzel WC, Kharouba HM, Robinson M, Holyoak M, Karban R (2016) Variability in plant nutrients reduces insect herbivore performance. Nature 539:425–427.

Whitlock R (2014) Relationships between adaptive and neutral genetic diversity and ecological structure and functioning: a meta-analysis. J Ecol 102:857–872.

Wickham H (2011) The split-apply-combine strategy for data analysis. J Stat Softw 40:1–29.

Wickham H (2016) ggplot2: create elegant data visualisations using the grammar of graphics. Springer-Verlag, New York. https://cran.r-project.org/package=ggplot2.

Wilke CO (2017) cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'.R package version 0.9.2. https://cran.r-project.org/package=cowplot.

Wimp GM, Wooley S, Bangert RK, Young WP, Martinsen GD, Keim P, Rehill B, Lindroth RL, Whitham TG (2007) Plant genetics predicts intra-annual variation in phytochemistry and arthropod community structure. Mol Ecol 16:5057–5069.