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ECOSPHERE

The effect of tree genetic diversity on insect herbivory varies with insect abundance

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Abstract. Variance in edibility among plant genotypes is expected to be a key driver of plant genetic diversity (PGD) effects on abundance of insect herbivores and resulting herbivory. Yet, herbivore foraging behavior and leaf consumption may be also context-dependent and, in particular, influenced by herbivore density, which remains unexplored. We used a combination of field and laboratory experiments with saplings from four half-sib families (henceforth, families) of pedunculate oak (*Quercus robur*) to test how PGD and herbivore density interactively affect herbivory. Insect herbivory was assessed in a common garden experiment with plots containing all possible combinations of individuals from one to four oak families. Herbivore density was manipulated by spraying insecticide in a factorial design. Complementary feeding trials with gypsy moth larvae (*Lymantria dispar*) were used to further explore the mechanisms underlying observed patterns in the field. Herbivory decreased with increasing PGD under normal herbivore density, but not under reduced herbivore abundance. The most damaged oak family in the field was also the most consumed in non-choice tests and was consistently preferred over other families in choice tests. Trials showed that the presence of less edible families in the diet reduced overall consumption by gypsy moth larvae. Under field conditions, the most edible family consistently benefited most from being associated with less edible, neighboring genotypes. Our results demonstrate that small-scale PGD can provide associational resistance to insect herbivory, probably through change in herbivore foraging activity. Importantly, they also reveal that the magnitude of genetic diversity effect depends on herbivore density.

Key words: associational resistance; mixed-diet; oak; regeneration; variance in edibility.

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Introduction

Plant diversity is a key driver of terrestrial ecosystem functioning (Cardinale et al. 2011). Intraspecific genetic diversity is an essential component of biodiversity, and its significance for ecosystem functioning seems to be of similar magnitude than species diversity in many aspects (Cook-Patton et al. 2011). For instance, greater plant genetic diversity (PGD) has been shown to enhance biomass production (Stachowicz et al. 2013), community stability (Booth and Grime 2003), and resistance to stress or perturbation (Jung et al. 2014). Assemblages of different plant genotypes also shelter richer insect communities (Crutsinger et al. 2008). So far, herbivore richness and abundance were mainly considered as response variables and were shown to increase with PGD (Crutsinger et al. 2008, Cook-Patton et al. 2011, Crawford and Rudgers 2013, Pohjanmies et al.

2015). However, it remains uncertain to what extent PGD also influences the activity of insect herbivores and resulting plant consumption (Castagneyrol et al. 2012, McArt and Thaler 2013, Barton et al. 2014).

Plant associational effects against herbivores occur when damage on a given plant is a function of the identity and abundance of its heterospecific neighbors (Underwood et al. 2014). It is now clear that intraspecific variability in plant traits is large enough to explain why PGD could result in such associational effects (Hughes et al. 2008, Hughes 2014, Barbour et al. 2015). However, evidence about their direction and magnitude is still conflicting since genetically based associational effects range from associational resistance, when plants from a given genotype suffer less damage when surrounded by conspecifics of different genotypes (McArt and Thaler 2013, Barton et al. 2015), to neutral (Moreira et al. 2014) or even the opposite, that is, associational susceptibility, when a plant experiences more damage when surrounded by distinct genotypes of the same species (Castagneyrol et al. 2012, Moreira and Mooney 2013).

Discrepancies among studies may result from insect guild-specific responses to PGD (Abdala-Roberts et al. 2015, Barton et al. 2015), spatial factors such as host patch connectivity (Pohjanmies et al. 2015), or uncontrolled environmental factors (Tack et al. 2010, 2012) among which local herbivore density has so far been overlooked. Yet, it can critically change the direction and strength of associational effects. For instance, insect foraging behavior and relative preference for a given host plant may be influenced by the intensity of inter- and intraspecific competition between herbivores (Underwood 2010, Utsumi et al. 2011, Karban et al. 2013, Parent et al. 2014, Carrasco et al. 2015). The amount of competitors on a given plant, together with induced plant defenses, is likely to change rank order preference in foraging insects (Utsumi et al. 2013, Carrasco et al. 2015) and thus PGD associational effects on herbivory.

In addition to herbivore density, PGD-based associational effects may also depend on the genetic identity and relative abundance of conspecific neighbors. Assuming differences in palatability among plant genotypes, the "variance in edibility hypothesis" (Liebold 1989) posits that a genotypically more diverse plant population is more likely to experience reduced herbivory on some genotypes and exacerbated herbivory on others as some genotypes could be preferred by herbivores over the others (Hambäck et al. 2014). Less defended plants could benefit from the vicinity of more resistant neighbors that deter or repel herbivores, that is, associational resistance (McArt and Thaler 2013). In contrast, less preferred genotypes could be more attacked when growing near more edible and hence more attractive neighboring genotypes, as a result of herbivore spillover (White and Whitham 2000, Castagneyrol et al. 2012). Moreover, the strength of associational effects likely depends on the relative abundance of genotypes varying in palatability: Associational resistance is expected to be stronger for more palatable plants increasingly diluted among less palatable neighbors (Hahn and Orrock 2016).

Some studies have shown that herbivores can adjust the amount of consumed leaf biomass according to plant quality (Mody et al. 2007, Kotowska et al. 2010, McArt and Thaler 2013). The "dietary mixing hypothesis" (Bernays et al. 1994) states that herbivores achieve better performance when feeding on a mix of plant resources due to complementary acquisition of deficient nutrients or reduced ingestion of toxins. The consequences of this process for plants are less well known. Having access to a mixed-diet could result in lower overall herbivory in more diverse plant assemblages as in monocultures herbivores would compensate suboptimal nutrition by consuming more plant tissues that they would need in a mix of plant resources (McArt and Thaler 2013). Alternatively, a mixed-diet may also result in higher plant consumption because of a reduction in toxins and better insect performances, although there is little evidence supporting this "toxins dilution hypothesis" (Marsh et al. 2006, Mason et al. 2014).

All these hypotheses suggest that the diversity of traits involved in plant defense could exert idiosyncratic effects on insect herbivore activity. However, the interpretation of herbivory pattern will also depend on whether the individual plant or the plant population is considered. Some individual plants may be more severely attacked in mixtures while overall herbivory at the population level is lower, if the rest of the plant population is less damaged (than in monocultures). Upscaling effects of PGD on herbivory from the

individual plant genotype to the population level thus remains an important challenge in community genetics (Utsumi et al. 2011, Barton et al. 2014).

In the present study, we performed a manipulative field experiment with different mixtures of one to four half-sib families of pedunculate oak (*Quercus robur*, Fagaceae) planted in a common garden in order to test the density-dependent effect of PGD on insect herbivory. Manipulation of herbivore abundance in the field was complemented by feeding bioassays and choice tests in the laboratory, in order to further explore mechanisms responsible for observed patterns in the field. In particular, we tested the following hypotheses: (1) Insect herbivory and herbivore preference and performance vary among oak families, (2) insect herbivory decreases with increasing PGD, and (3) the magnitude of PGD effects on herbivory changes with herbivore abundance. Our study is therefore one of the few that explicitly tested mechanisms responsible for associational effects resulting from PGD in the field.

Materials and Methods

Experimental site and field experiment

The study was carried out in 2013 in a previously established experimental common garden located 40 km southwest of Bordeaux (44°440 N, 00°460 W). The experimental design has been described in detail by Castagneyrol et al. (2012). Briefly, oak saplings were grown from acorns collected in 2007 on four pedunculate oak trees within a 10 km radius around the experimental site. Saplings had been kept in the greenhouse in 2008 and treated with insecticide to prevent herbivore damage until being planted into the field in March 2009. The field site was a clearing surrounded by pine (*Pinus pinaster*) and broadleaved (*Quercus robur*, *Q. rubra*, and *Betula pendula*) forest stands. It was fenced to prevent grazing by mammalian herbivores. The four source trees will hereafter be referred to as "mother trees" and saplings from the same mother tree (being either full-sibs or, more likely, half-sibs) as "family." Saplings from a same family were genetically and phenotypically more similar than those from different trees (see Castagneyrol et al. 2012). We therefore used the number of oak families per plot as a proxy of genetic diversity.

The common garden consisted of six different blocks established in a factorial design (see fig. S1 in Castagneyrol et al. 2012). Each block contained 15 plots with 12 saplings each (i.e., four rows of three saplings planted 0.2 m apart from each other), corresponding to one of the 15 possible family combinations of one to four families: four family monocultures, six mixtures of two families, four mixtures of three families, and one mixture of all four families. Saplings from different families were planted at equal distance in a regular alternate pattern so that saplings from the same family were never adjacent to each other in mixed plots. Plots were separated by a distance of 3 m and were randomly distributed within blocks. Blocks were located 4 m apart from each other.

In 2013, we manipulated herbivore density by applying three treatments to the experiment. Blocks 1 and 2 were kept as control. All plots in blocks 3 and 4 were sprayed with pyrethroid insecticides (alternating Decis Protech [Bayer AG, Leverkusen, Germany], 15 g of deltametrine per liter diluted at 3 mL/L, and Fastac [BASF SE, Ludwigshafen, Germany], 50 g of alphametrine per liter, diluted at 25 mL/L) every fortnight from March to September in order to reduce insect herbivore density. These insecticides have a large action spectrum ensuring an efficient reduction in abundance of herbivores belonging to different taxonomic groups. In blocks 5 and 6, each sapling received three fifth-instar larvae of gypsy moth (*Lymantria dispar*) in late May 2013 that fed in the plot for *ca.* 10 d until pupation. The gypsy moth is a generalist herbivore naturally present in our field area, which usually feeds on oaks. Larvae were obtained from eggs collected in the wild. From egg hatching to installation in the field, larvae were fed a wheat germ-based artificial diet in the laboratory (Bioserv product no. F9630B).

Additional treatments were applied at the block level for technical reasons. Given the short distance between plots, insecticide was spread on all plots of two adjacent blocks to reduce the risk of spray drift that might affect neighboring control and herbivore-enriched plots. We set up plastic barriers, 30 cm high and sprayed with glue, around blocks with gypsy moth larvae to prevent their spillover onto adjacent blocks. Herbivore-enriched (blocks 5 and 6) and control blocks (1 and 2) were separated by blocks sprayed with insecticide to further reduce the risk of spillover. This resulted in

a split-plot experiment (Altman and Krzywinski 2015), where additional factors (insecticide and herbivore addition) were applied at the whole-block level, while genetic diversity was manipulated at the sub-block (plot) level (see below, *Data analyses*).

Insect herbivory assessment

A total of 20 leaves were collected on each sapling in August 2013. Five leaves were picked up at the tip and five at the base of two randomly chosen branches from the top and two from the lower part of the sapling, respectively. Leaves were placed into a paper bag and dried for 48 h at 55°C for further examination in the laboratory. Preliminary tests confirmed that this treatment does not affect the assessment of herbivore damage. Herbivory was visually assessed as the percentage of leaf area removed by chewing and skeletonizing herbivores (% LAR), the most abundant insect herbivores. We used seven defoliation classes (0, 1–5, 6–15, 16–25, 26–50, 51–75, and >76%). The midpoint of each class was used to calculate the mean defoliation per tree. Given most of the damage at the leaf level is usually smaller than 25%, having more classes for small damage provides a better estimate of mean herbivory at the individual level (Johnson et al. 2016).

A total of 40 individual saplings of the initial experimental design were dead in previous years, and 43 of the 1040 remaining saplings had less than 20 leaves. The last ones were not collected to avoid complete defoliation, and herbivory was only assessed on 997 saplings. The missing data were randomly distributed among blocks and plots, and all families were represented by at least one individual in all plots.

Herbivore preference and performance

A feeding trial and choice test was carried out between 12 and 17 May 2014 with second-instar larvae of gypsy moth reared on a wheat germ-based artificial diet (Bioserv product no. F9630B). Tests were performed in a climatic chamber with L16:D8 photoperiod at 23°C. A complete description of the method is provided as supplementary material (Appendix S1). An overview is given here.

We designed five experimental feeding treatments, each one being replicated 10 times. Replicates consisted in three larvae feeding on four oak leaves in a transparent plastic box. The four leaves came either from the same family (four single-diet treatments) or from each of the four families (one mixed-diet treatment). The mixed-diet treatment was included to test the dietary mixing hypothesis and also to evaluate gypsy moth preferences among families (i.e., as choice test).

Every morning, 50 intact mature leaves were randomly collected from saplings in monoculture plots of each oak family. Plots were selected within a single block to avoid possible block effects on leaf quality. Leaves were scanned every day before and after consumption by larvae. Total remaining leaf area per family in single and mixed-diet treatments was measured using the software ImageJ. After consumption (24 h), leaves were dried at 55°C for 48 h and weighted. We estimated the leaf area:biomass ratio and used it to estimate biomass consumption from leaf area consumption (see Appendix S1 for details). Larvae were kept in starvation for 24 h before the experiment and weighted at the start and at the end of the feeding trial to calculate mean larval weight gain in each replicate. The relative growth rate of larvae was calculated as: RGR = (final weight − initial weight)/initial weight.

To test for preferences of gypsy moth larvae for a given oak family, we used the method developed by Larrinaga (2010) for simultaneous, multiple-choice food trials. This approach summarizes the relative consumption of a food item, given the total amount of available food (Eq. 1), and overcomes the lack of independence of data derived from repeatedly measuring the preference for several food types by the same individuals. We calculated a preference index (p_i) as:

$$p_i = (C_i/A_i)/T \tag{1}$$

where C_i and A_i are the total amount of consumed and available food for oak family i, respectively, and T is:

$$T = \frac{\sum_{i=1}^{N} C_i}{\sum_{i=1}^{N} A_i} \tag{2}$$

with N being the total number of families.

Values of $p_i > 1$ and $p_i < 1$ indicate relative preference and avoidance for the corresponding family, given the choice offered.

Data analyses

Herbivory in the field.—Preliminary analyses of defoliation data showed that the insecticide treatment did not kill all insect herbivores. However, it consistently reduced herbivory by 55% as compared to control (mean % leaf area consumed ± SE: 4.6% ± 0.2% and 10.2% ± 0.3%, respectively). By contrast, there was no difference in mean herbivory between the control and the herbivore addition treatment (10.2% ± 0.3% vs. 8.1% ± 0.2%). These were hence pooled and the following analyses only distinguished plots with no insecticide treatment (hereafter +H, as more herbivory) vs. plots with insecticide (hereafter −H as less herbivory).

There might be no effect of PGD on herbivory at the plot level if herbivores have opposite preferences for different families. In order to unravel likely hidden effects, we used two complementary analyses to assess insect herbivory at the level of individual plants and of experimental plots. Individual-level analysis allowed testing family-specific resistance to herbivores and interactions between family identity and PGD. Aggregating data at the plot level made it possible to test possible non-additive effects of PGD on herbivory (Barton et al. 2014).

With insecticide treatment being applied at the block level (i.e., whole block), our design corresponds to a split-plot experiment which requires adapting the calculation of degrees of freedom and mean sum of squares of residuals (Altman and Krzywinski 2015). This was achieved using linear mixed effect models (LMM), with Block and Block × Insecticide as random factors (1| Block: Insecticide in *R* syntax). At the individual sapling level, plot identity was included as an additional random factor, nested within block, to account for the fact that individual trees from the same family were pseudo-replicates within plots (Schielzeth and Nakagawa 2013). Mother tree identity (MT), insecticide treatment (+H vs. −H), PGD of the plot, and their interactions were declared as fixed effects. The full model was simplified by sequentially removing non-significant interactions terms, starting with the highest order interaction, to finally retain the least parameterized models including only simple terms and significant interaction terms. Significance of parameters was assessed using χ^2 tests by comparing models with and without the term to be tested. Parameters corresponding to fixed effects were estimated by maximum likelihood. A log+1 transformation was applied to herbivory data to meet assumptions of homogeneity in variance and normality in residuals.

Analyses at the plot level were carried out using the method developed by Loreau and Hector (2001) and adapted by Unsicker et al. (2008) to partition the net effect of PGD on herbivory into a complementarity effect (CE) and a selection effect (SE). Net, complementarity, and selection effects were used to upscale observations from the individual plant to the plot level while accounting for family-specific differences. The net effect compares observed vs. expected damage in a given mixture, where expected damage is the mean of damage observed in component monocultures weighted by the proportion of families in the mixture. The full description of these indices is provided as supplementary material (Appendix S2). We used analyses of variance (ANOVAs) to test for differences in herbivory for each effect (net, complementarity, and selection effects) and applied two-sided *t*-tests to determine whether these effects were significantly different from zero.

Herbivore performance and preferences in feeding trials.—Performance of gypsy moth larvae in single vs. mixed-diet treatments was compared using ANCOVA (Raubenheimer 1995) with diet type as factor, initial larval weight as continuous covariate, and final weight as dependent variable. Biomass consumption per diet type was assessed using LMM with the replicate (i.e., rearing box identity) as random factor to account for the repeated measurements of the same set of three larvae. Feeding preferences were tested using LMM with oak family as fixed effect factor, replicate as random factor, and preference index (p_i) as dependent variable.

All analyses were conducted in 3.0.2 version of R (R Core Team 2013), using the *lmer* function from the *lme4* package (Bates et al. 2013). Contrast analyses were used to compare factor levels. To estimate model adjustments, R^2 were calculated following Schielzeth and Nakagawa (2013). For each model, we calculated the marginal R^2 (R^2_m) corresponding to the proportion of variance explained by fixed effects and the conditional R^2 (R^2_c) corresponding to variance explained by fixed plus random effects.

Results

Herbivory in the field

Herbivory at individual sapling level.—In no insecticide plots (+H), herbivory was on average 9.1% ± 0.2% (mean ± SE) of leaf area removed and ranged between 1.3% and 37.8%. Application of insecticide resulted in 50% reduction in herbivory (4.6% ± 0.2%).

Herbivory differed among oak families with the family MT2 being on average 1.27 times more damaged than the other three families (among which no difference was observed; Fig. 1A). Family-specific differences in herbivory were independent of the number of families in the plot (PGD), as indicated by the non-significant interaction with the mother tree variable (MT × PGD; Table 1), and of insecticide treatment (no significant MT × IT interaction).

The effect of PGD on herbivory was dependent on insecticide treatment (significant IT × PGD; Table 1). In plots with no insecticide treatment (+H), herbivore damage decreased with the number of families (Fig. 1B). On the contrary, there was no effect of PGD on herbivory in blocks where the insecticide treatment had reduced herbivore density (−H).

Herbivory at plot level.—Insecticide treatment affected the net and complementarity effects of PGD on insect herbivory but not the selection effect (Table 2).

In blocks with high herbivore density (+H), mean defoliation was significantly lower in mixed plots than expected from the corresponding monocultures, that is, associational resistance. Three out of the four families showed a reduction in herbivory in mixtures compared to their respective monocultures (Fig. 2A). The negative net effect arose from both a negative complementarity and a negative selection effect (Table 2). The negative selection effect was mainly driven by MT2. It was the most susceptible family in monocultures (Fig. 1A) and the family for which the deviation from the 1:1 line (equal mean herbivory in monocultures and in mixtures) was the greatest, showing a large associational resistance effect (Fig. 2A).

In blocks with lower herbivore density (−H), complementarity and selection effects had opposite signs, resulting in a non-significant net effect of family mixtures (Table 2). The significant, positive complementarity effect indicates that on average, all families suffered higher herbivory than expected from component monocultures (indicating the existence of associational susceptibility). The negative selection effect indicates that the most resistant oak family in monoculture

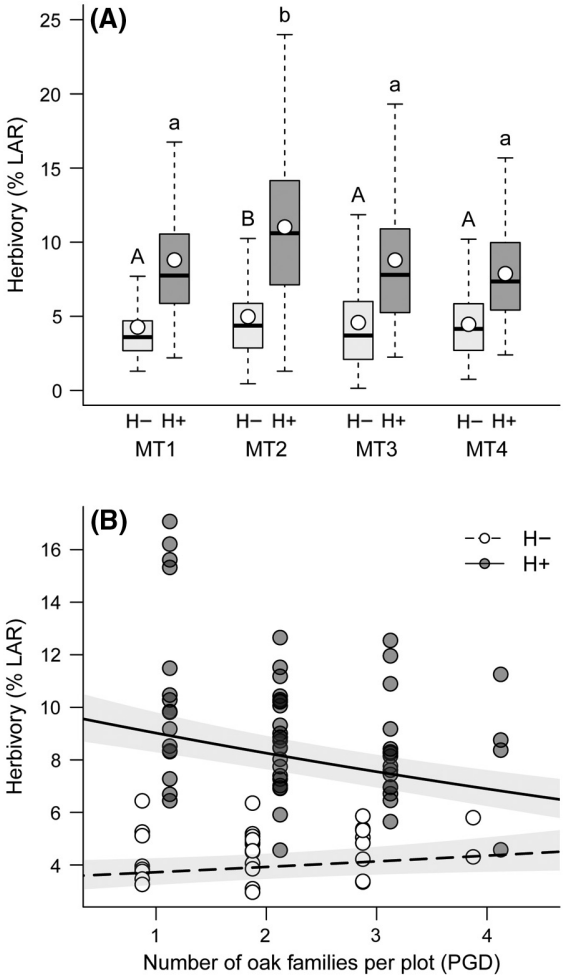


Fig. 1. Effects of mother tree identity (A), number of oak families per plot (B), and insecticide treatment on insect herbivory at individual oak sapling level. (A) Boxes represent first and third quartiles. The horizontal line represents the median, while dots correspond to the mean, across all plots. Different letters above boxes indicate significant differences in herbivory among families. (B) Dots represent mean herbivory in plots with different herbivore abundance. Regression lines and corresponding SE (indicated as shaded area) are predictions from mixed effect models averaged across the four families. PGD, plant genetic diversity; LAR, leaf area removal.

Table 1. Summary of linear mixed effect models evaluating the effects of insecticide treatment (IT, i.e., +H vs. −H), mother tree identity (*MT*, i.e., MT1, MT2, MT3, and MT4), plant genetic diversity (PGD), and their interactions on insect herbivory on oak saplings.

Explanatory variables	χ^2	Parameter	Estimate	SE	df
		Intercept (MT4, −H)	1.541	0.145	69,8
IT	41,16***	IT: H+	0.753	0.178	69,7
MT	35,01***	MT: MT3	−0.056	0.175	991
		MT: MT2	−0.057	0.178	991,1
		MT: MT1	0.003	0.177	991
PGD	6,67*	PGD	0.022	0.053	991
IT × MT	5,64 (ns)	IT: H+ × MT: MT3	−0.039	0.214	991
		IT: H+ × MT: MT2	0.504	0.217	991,1
		IT: H+ × MT: MT1	0.012	0.217	991,1
IT × PGD	12,93***	IT: H+ × PGD	−0.108	0.066	991
MT × PGD	4,67 (ns)	MT: MT3 × PGD	0.019	0.075	991
		MT: MT2 × PGD	0.063	0.076	991
		MT: MT1 × PGD	−0.008	0.076	991
IT × MT × PGD	5,72 (ns)	IT: H+ × MT: MT3 × PGD	0.061	0.092	991
		IT: H+ × MT: MT2 × PGD	−0.143	0.093	991,1
		IT: H+ × MT: MT1 × PGD	0.032	0.094	991,1

Notes: Explanatory variables in bold character correspond to those retained in the final model after model simplification. Significance thresholds for χ^2 values: (ns) $P < 0.1$, $^{*}P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$. Model parameter estimates and standard error for the intercept correspond to the reference level for IT (−H) and *MT* (MT4). Marginal R^2_m represents the variance explained by fixed factors, while conditional R^2_c is interpreted as variance explained by both fixed and random factors. For the final model retained after model selection, they equaled 0.32 and 0.41, respectively.

experienced disproportionally less damage in mixtures. Yet, family-specific differences in herbivory between monocultures and mixtures were less pronounced than in plots with no insecticide treatment (Fig. 2B). Only MT2, the most susceptible family overall, showed a reduction in herbivory in mixed plots compared to monocultures.

Herbivore preferences and performance in feeding trials

Gypsy moth larvae clearly distinguished among the four oak families when offered in mixed-diet ($F_{3,214} = 29.8$, $P < 0.0001$). The relative consumption of MT2 and MT3 leaves was higher than their proportion in the offered diet, indicating preferential feeding on these families (Fig. 3A). Conversely, larvae avoided feeding on leaves of MT1, while they were indifferent to leaves of MT4 (Fig. 3A). This preference pattern mirrored the difference in herbivory observed on oak families in the field experiment.

The type of diet (four single plus one mixed-diets) had a clear effect on leaf consumption ($F_{4,283} = 12.5$, $P < 0.0001$). MT1 was the least consumed family in single-diet treatments (Fig. 3B). Interestingly, the mixed-diet treatment was the one with lowest overall leaf consumption (Fig. 3B).

The relative growth rate of gypsy moth larvae differed among the five diet types ($F_{4,44} = 4.5$, $P = 0.004$; Fig. 3B). Larvae grew best on MT2 leaves and least on MT1 leaves, which was in accordance with preferences observed in the

Table 2. Effects of insecticide treatment on the net (NE), complementarity (CE), and selection effects (SE) of plant genetic diversity (PGD) on insect herbivory on oak saplings.

Test	Treatment	NE	CE	SE
ANOVAs	Insecticide treatment	$\mathbf{F_{1,60} = 34.81}^{***}$	$\mathbf{F_{1,60} = 29.58}^{***}$	$F_{1,60} = 0.26$ (ns)
	Block	$\mathbf{F_{4,60} = 7.36}^{***}$	$\mathbf{F_{4,60} = 7.68}^{***}$	$F_{4,60} = 0.9$ (ns)
t-tests	Insecticide (−H)	4.8 [−1.14, 10.73]	**8.45** [2.17, 14.74]	**−3.66** [−5.01, −2.3]
	No insecticide (+H)	**−26.86** [−36.63, −17.08]	**−20.66** [−30.78, −10.53]	**−6.2** [−8.62, −3.78]

Notes: *F* values from ANOVAs and estimated means and 95% CI from *t*-tests ($\mu = 0$) are reported. Bold characters indicate significant differences between factor levels. Significance thresholds: (.)$P < 0.1$, $^{*}P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$.

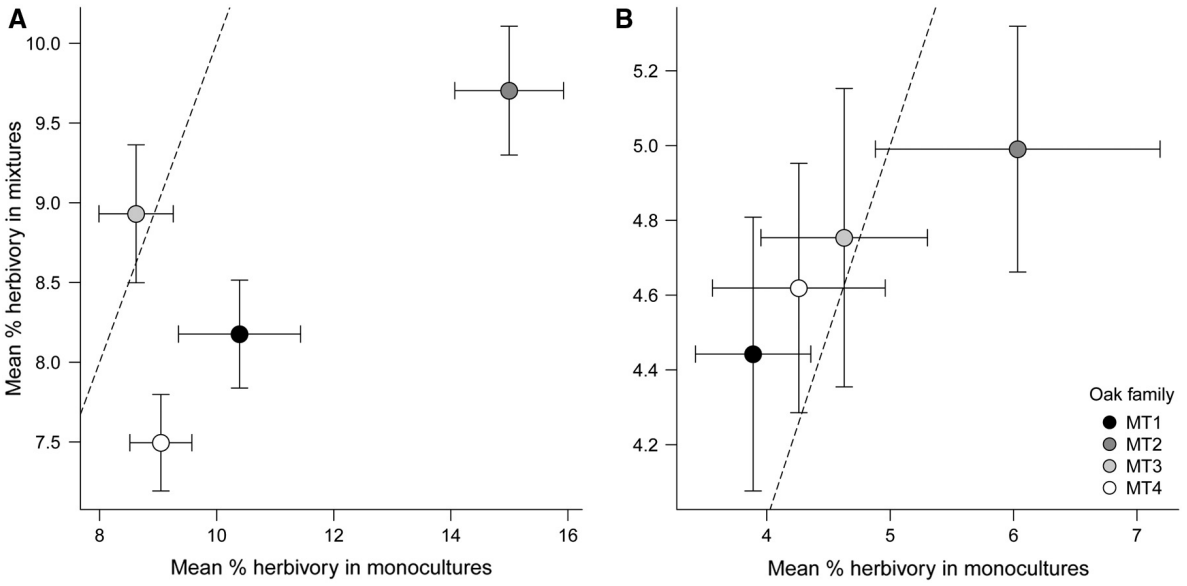


Fig. 2. Mean insect herbivory on oak saplings of each family growing in monoculture or in mixtures in plots with no insecticide treatment (A) and insecticide plots (B). Dotted lines correspond to the $y = x$ line (not shown in diagonal to improve the distinction of families). Error bars indicate standard errors.

feeding choice experiment. Larvae consuming a mixture of leaves from the four families showed an intermediate growth rate (Fig. 3B).

Discussion

We found experimental evidence that the amount of damage caused by insect herbivores on a given plant varies with the number of neighboring conspecific genotypes. However, the magnitude and direction of this relationship depend on both herbivore density (i.e., through insecticide treatment) and identity of plant genotypes.

Insect herbivory varies with oak genetic identity and diversity in interaction with herbivore density

In accordance with our first hypothesis, herbivory and herbivore preference and performance varied between oak families. In particular, oaks grown from mother tree MT2 experienced more damage than the three other families in the field. Its leaves were also more consumed in non-choice feeding trials and were preferred over leaves of other families in choice trials. These results confirm the genetically based variability in plant susceptibility to insect herbivores that has been observed in many other systems (Barbour et al. 2009, Barton et al. 2014) and suggest that despite the low number of families in the experiment, intraspecific variability in oak traits could be large enough to allow associational effects. Gypsy moth larvae fed on MT2 leaves also had greater growth rate as compared to larvae fed on other leaves, which confirms that differences in plant resistance are consistent with differences in herbivore performances. Although patterns in the field were consistent with results from feeding trials, not all herbivores respond in the same way to plant genotype identity and the response of a single species (here *Lymantria dispar*) may not be representative of the response of the whole herbivore community.

In addition to this identity effect, we detected a diversity effect. Increasing the number of oak families per plot caused an overall decrease in herbivory, at both the individual and plot levels. This was however only observed when herbivore density was medium (i.e., in no insecticide plots, +H), whereas the effect of PGD was null in case of low herbivore density (i.e., in insecticide plots, −H). The observed decrease in insect herbivory with increasing PGD is consistent with previous studies on willow (Peacock et al. 2001), evening primrose (Parker et al. 2010, McArt and Thaler 2013) and different crops (Tooker and Frank 2012). These results contrast with other studies

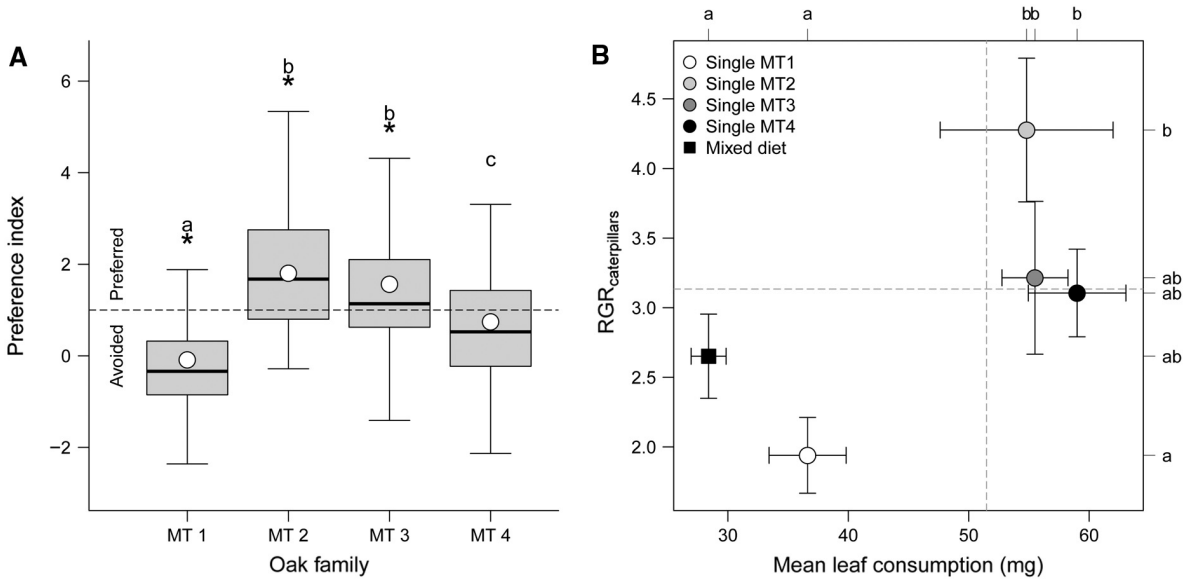


Fig. 3. Preference and performance of gypsy moth larvae in single vs. mixed-diet treatments. (A) The preference index indicates the relative consumption of a specific oak family by gypsy moth larvae in the mixed-diet treatment. Boxplots represent median, 25th, and 75th percentiles, respectively. The dashed horizontal line at $y = 1$ corresponds to the null hypothesis of neither preference nor avoidance. Asterisks indicate significant differences from $\mu = 1$ according to *t*-tests ($P < 0.05$). Different letters indicate differences between families in the mixed-diet treatment. (B) Effects of diet type on leaf consumption and larval growth. Circles represent consumption and RGR (Relative Growth Rate) in single-diet treatments, with corresponding SE. Mean consumption and RGR in single-diet treatments are shown by vertical and horizontal dashed lines, respectively. The effect of diet type was tested separately for consumption and growth using ANCOVAs. Letters on the top and right edges of the panel refer to contrast comparisons between treatments for leaf consumption (top) and RGR (right). Different letters indicate significant differences between treatments (at $P < 0.05$).

reporting opposite or neutral effects of PGD on insect herbivory (Tack and Roslin 2011, Castagneyrol et al. 2012, Barton et al. 2014, Maldonado-López et al. 2015). None of these studies (to the best of our knowledge) has however assessed PGD effects under contrasted herbivore densities. Yet, it is increasingly acknowledged that herbivore population dynamics may depend on both herbivore density and variance in plant quality (Underwood 2004, 2010, Parent et al. 2014).

Mechanisms underlying associational resistance

Lower herbivory in mixed plots could arise from three non-exclusive mechanisms acting at different spatial scales: (1) a relocation of herbivores within plots, sparing the three most resistant families at the expense of the most susceptible one; (2) an overall reduced consumption due to more effective exploitation of mixed-diets by herbivores; and/or (3) an active avoidance of plots containing less edible individuals. All three mechanisms rely on the same two premises: Oaks from different families should differ in edibility and herbivores should be able to choose among them. Our field and laboratory experiments indicate that both premises are met in our study system, although the three ecological mechanisms received varying empirical support.

We cannot formally exclude the alternative hypotheses that variability in herbivory resulted from neighbor-mediated changes in plant traits such as anti-herbivore defenses (Moreira et al. 2014) or differential pressure of natural enemies upon herbivores (Moreira and Mooney 2013, Abdala-Roberts and Mooney 2014). However, we do not have data to test these assumptions.

Choice of individual plants within plots.—A relocation of herbivores within plots was not supported by our individual-level analysis, as we observed no family (MT) × PGD interaction. This

lack of interaction suggests that, on average, all oaks benefited from growing among neighbors from other families, regardless of their family identity. This interpretation is further supported by the observed negative complementarity effect, which indicates that, on average, all saplings experienced less damage in family mixtures than in component monocultures. Such an associational resistance has been observed in several other studies (Unsicker et al. 2008, Barbosa et al. 2009, McArt and Thaler 2013).

Avoidance of less suitable plots.—We found some support for an active avoidance of entire plots containing less edible individuals. Theory predicts that associational resistance would be stronger for most sensible plants, and weaker for more resistant ones (Hambäck et al. 2014, Hahn and Orrock 2016). Family MT2 was consistently preferred in feeding trials and the most damaged in monocultures in the field. At the same time, the negative selection effect observed at the plot scale suggests that more susceptible families (i.e., especially MT2) benefited most from growing together with more resistant neighbors. Our result is therefore in line with the "variance in edibility hypothesis" (Liebold 1989): More resistant plants can contribute to reduce herbivore recruitment in mixed plots more than expected from their sole abundance by "protecting" more edible neighboring plants (Jiang et al. 2008).

Dietary mixing and reduced herbivore consumption.—We found clearer evidence that the observed relationship between PGD and herbivory could have been driven by an overall reduced consumption of mixed-diets by herbivores, as predicted by the dietary mixing hypothesis (Bernays et al. 1994, McArt and Thaler 2013). Our feeding trials revealed that leaf consumption was on average lower in the mixed-diet treatment than in any single-diet treatments (although not significantly different from consumption of the less edible family, MT1). Despite this reduced consumption, the growth of gypsy moth larvae was not lower in the mixed-diet treatment than in the single-diet treatments.

Effects of host genetic diversity on herbivory are weak and herbivore density-dependent

The effect of PGD on insect herbivory was significant, but weak. It was only observed in "no insecticide" plots, where defoliation decreased from 10% in monocultures to 8% in four-family mixtures. Although low, such defoliation levels are quite common and consistent with background herbivory observed in trees at a global scale (Kozlov et al. 2015). Yet, even low levels of herbivory may have substantial negative effect on plant growth, especially in long-living trees (Zvereva et al. 2012).

So far, herbivore density has been studied as a response variable to local conditions, and it was shown to be better explained by local environmental drivers than by host genetic diversity (Tack et al. 2010, Pohjanmies et al. 2015). Yet, the response of herbivore abundance and damage to plant diversity are poorly related (Rhainds and English-Loeb 2003, Barbosa et al. 2009, Utsumi et al. 2011, Karban et al. 2013, Parent et al. 2014, Carrasco et al. 2015). The distinction between both aspects of herbivore response to PGD is not trivial because herbivore recruitment and actual plant consumption likely respond to different drivers (e.g., relative frequency of more or less palatable plants, plant nutritional quality, top-down control of natural enemies; Moreira et al. 2016). By addressing herbivory while controlling for herbivore density, our results provide new evidence that PGD effects on herbivory are density-dependent. We cannot completely exclude that observed pattern resulted from the specificity of the insecticide action on particular herbivore species, and further research will be needed to fully disentangle the effects of herbivore density from the composition of herbivore community. However, assuming that more herbivores exert a stronger pressure upon host plants, the density-dependent effect is consistent with other studies highlighting that the effects of PGD on ecosystem functioning vary along ecological gradients and are often stronger in harsher environments where plants have to face stronger biotic (e.g., herbivory) or abiotic (e.g., drought) pressures (Hughes and Stachowicz 2009, Kanaga et al. 2009, Parker et al. 2010, but see Drummond and Vellend 2012).

The overlooked density-dependency of plant–herbivore interactions may explain why previous studies addressing effects of PGD on insect herbivory provided conflicting results. It is a promising direction for unraveling causes of "context-dependency" in diversity–resistance relationships (Moreira et al. 2016). However, a deeper

understanding of mechanisms at play will require a better experimental control of herbivore density. In particular, larger gradient of herbivore abundance should be used in order to compare the effects of PGD under background herbivory vs. outbreak conditions.

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Supporting Information

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