



Effects of insularity on insect leaf herbivory and chemical defences in a Mediterranean oak species

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Abstract

Aim: Research on plant–herbivore interactions has shown that islands typically have low abundances and diversity of herbivores because of barriers to dispersal, isolation and reduced land area. Islands commonly have lower levels of herbivory relative to mainland regions, and, as a consequence, insular plants should exhibit lower levels of defences than their mainland counterparts. Despite these predictions, there are significant gaps in our understanding of insularity effects on plant–herbivore interactions. For instance, most work addressing the effects of insularity on plant–herbivore interactions have compared one or a few islands with a single mainland site. In addition, studies have measured herbivory or plant defences but not both, and the influence of abiotic factors has been neglected.

Location: Mediterranean Basin (from Spain to Greece).

Taxon: *Quercus ilex* L.

Methods: We conducted a large-scale study to investigate whether insect leaf herbivory and plant chemical defences in holm oak (*Quercus ilex* L.) differ between insular versus mainland populations. We further investigated mechanisms by which insularity effects on herbivory may take place by assessing the influence of defences and climatic variables on herbivory.

Results: We found that insular populations exhibited lower herbivory and higher defences (condensed tannins) than their mainland counterparts. Our analyses, however, suggest that these concomitant patterns of insect herbivory and plant defences were seemingly unrelated as island versus mainland differences in defences did not account for the observed pattern in herbivory. Furthermore, climatic factors did not explain insularity effects on either herbivory or plant defences.

Main conclusions: Overall, this study provides one of the most robust assessments to date on insularity effects on herbivory and builds towards a better understanding of the ecology and evolution of plant–insect interactions in insular ecosystems.

KEYWORDS

condensed tannins, islands, Mediterranean Basin, phenolic compounds, plant–herbivore interactions, *Quercus ilex*



1 | INTRODUCTION

Insular systems provide a valuable framework for elucidating the ecological mechanisms shaping biodiversity (Darwin, 1909; Gillespie, Claridge, & Goodacre, 2008; MacArthur & Wilson, 1967; Ricklefs & Bermingham, 2008; Wallace, 1880), species interactions (Spiller & Schoener, 1990; Traveset et al., 2013) and trait evolution (Grant & Grant, 1998). Research on plant–herbivore interactions has shown that islands typically have low abundances and diversity of vertebrate and invertebrate herbivores because of barriers to dispersal, isolation and reduced land area (Burns, 2014; Carlquist, 1974; MacArthur & Wilson, 1967). Islands are therefore expected to have lower levels of herbivory relative to mainland regions, and, as a consequence, insular plants should exhibit lower levels of physical (e.g., spines, thorns) and chemical (e.g., secondary metabolites) defences than their mainland counterparts (Adersen & Adersen, 1993; Bowen & Van Buren, 1997; Burns, 2014; Vourc'h, Martin, Duncan, Escarré, & Clausen, 2001). However, to date there have been very few robust tests of insularity effects on plant–herbivore interactions, and recent work has challenged expectations by reporting higher (rather than lower) levels of physical and chemical traits associated with herbivore resistance in insular plants (e.g., Hanse, Brimer, & Mølgaard, 2003; Monroy & García-Verdugo, 2019; Pardo, Cáceres, & Pulido, 2018).

There are significant gaps in our understanding of insularity effects on plant–herbivore interactions, some of which are common to other types of species interactions as well. First, most studies suffer from low sample sizes and have been conducted at small spatial scales comparing one or a few islands with an adjacent mainland site (e.g., Bowen & Van Buren, 1997; Vourc'h et al., 2001). These limitations have inevitably constrained our ability to reach broader conclusions based on the observed patterns. Second, abiotic factors play a key role in shaping spatial variation in plant–herbivore interactions (Rasman, Pellissier, Defosse, Jactel, & Kunstler, 2014), but have not been explicitly accounted for in island versus mainland comparisons despite the fact that insular ecosystems frequently differ in their climatic conditions relative to mainland counterparts (e.g., islands are usually cooler, wetter and less seasonal; Weigelt, Jetz, & Krefta, 2013). Explicitly accounting for these factors may help elucidate the mechanisms of abiotic control over insularity effects on herbivory, with the expectation that the degree of reduction in herbivore pressure on islands is contingent on the difference in abiotic (e.g., climatic) conditions between insular and mainland ecosystems. Third, studies have usually measured herbivory or plant traits associated with herbivore damage, but not both (e.g., Adersen & Adersen, 1993; Burns, 2014; Givnish, Sytsma, Smith, & Hahn, 1994; Kavanagh, 2015), leading to an incomplete understanding of insularity effects on plant–herbivore interactions. This aspect is also important in relation to the previous point, as abiotic factors may concurrently influence herbivores and plant traits (Moreira, Abdala-Roberts, et al., 2018). For example, climatic variables can directly influence herbivory by shaping herbivore abundance or activity, but at the same time may also exert indirect effects on herbivory by altering

plant physical or chemical traits that affect herbivores (Huberty & Denno, 2004; Jactel et al., 2012). A combined approach measuring herbivory, plant defences, and abiotic factors is thus likely to explain the most variation in insularity effects on plant–herbivore interactions. Finally, to date most studies have investigated the effect of insularity on vertebrate (e.g., mammalian) herbivores (e.g., Bowen & Van Buren, 1997; Monroy & García-Verdugo, 2019; Skaïen & Arcese, 2018; Vourc'h et al., 2001), whereas research on insect herbivory is less common but may be key for understanding insularity effects on plant defences and herbivory (Cyr & Pace, 1993).

In this study, we investigated whether levels of insect leaf herbivory and leaf chemical defences (phenolic compounds) in holm oak (*Quercus ilex* L.) populations found on islands differ from those observed among their mainland counterparts. We additionally investigated whether plant chemical defences and climatic variables (temperature and precipitation) underlie any such effect of insularity on herbivory. To this end, we sampled 28 populations found on Mediterranean islands and 30 populations found on nearby mainland sites (Figure 1). Sampling sites were distributed from north-western Spain to Greece, covering most of the latitudinal and elevational distribution of this species (Figure 1). We predicted that oak trees found on islands would have lower levels of insect leaf herbivory and phenolic compounds than their mainland counterparts, and that such patterns would be underlain by differences in climatic factors. Phenolic compounds have been found to confer resistance against insect herbivores in *Quercus* species (Feeny, 1970; Moreira, Abdala-Roberts, et al., 2018; Moreira, Abdala-Roberts, et al., 2018; Roslin & Salminen, 2008), and were thus considered a suitable proxy for chemical defences in holm oak. Overall, this study contributes to better understand the ecological and evolutionary effects of insularity on plant–herbivore interactions and its underlying drivers.

2 | MATERIALS AND METHODS

2.1 | Natural history

Holm oak *Q. ilex* is a long-lived, evergreen tree native to the Mediterranean region, extending from Portugal to Turkey, also found on most islands of the Mediterranean basin (Figure 1). Phylogeographical analyses have delimited three clearly differentiated genetic groups: the western Mediterranean, the central Mediterranean and the Aegean region (see Figure 1), which are the result of an east-to-west colonization pattern (Vitelli et al., 2017). The species grows in pure stands or mixed forests at low (close to sea level) to moderate (up to 1,200 m asl) elevations, and in its native range is attacked by several specialist and generalist insect herbivores. Leaf chewers are particularly important herbivores on this species, among which the most common are caterpillars belonging to Noctuidae and Tortricidae (Giffard, Jactel, Corcket, & Barbaro, 2012; Ruiz-Carbayo, Bonal, Espelta, Hernández, & Pino, 2017; Southwood, Wint, Kennedy, & Greenwood, 2005).

2.2 | Field sampling and leaf herbivory measurements

Approaching the end of the growing season (from late August to early September), we surveyed 58 holm oak populations distributed from north-western Spain to Greece, of which 30 were found on mainland locations and 28 on insular locations (Figure 1). The sampled sites spanned most of the latitudinal (37–45°) and elevational (9–1,133 m asl) ranges of this species (Figure 1), and varied substantially in climatic conditions including a 2.6-fold gradient in mean annual precipitation (442–1,155 mm) and 7.5°C variation in mean annual temperature (10.4–17.9°C). We sampled all populations at the end of the growing season at each site; this was late August in northernmost populations and early September in southernmost populations. This approach was aimed at reducing phenological differences in herbivory and plant defensive traits across sites (Moreira, Abdala-Roberts, et al., 2018). In addition, we restricted our sampling to fully expanded mature leaves located on branches of similar height (see ahead) to minimize microhabitat effects on leaf chemistry and herbivory. We also took care in sampling leaves on branches of a similar size and at a similar position within the branch to control for within-plant allocation patterns influencing leaf chemistry. We opted to sample trees at the end of the growing season because this provided an assessment of cumulative leaf herbivory occurring over the entire growing season (Giffard et al., 2012).

Each population included at least 15 mature, reproductive individuals. We randomly selected five trees per population (mean diameter at breast height = 50.33 ± 2.33 [$\pm SE$] cm). For each tree, we randomly selected two low-hanging branches (2–3 m above the

ground) and collected 25 leaves from each of them. Leaves measured at our study sites were mostly damaged by insect chewers, whereas leaf miners and gall formers were rare (<2% of the leaves; results not shown). Because branches were sampled above the browsing height of ungulates, we did not find signs of leaf herbivory by mammals (X. Moreira, personal observation). For each leaf, we visually estimated per cent leaf area removed by insect leaf chewers using the following scale: 0 = undamaged; 1 = 1%–5% damaged; 2 = 6%–10% damaged; 3 = 11%–25% damaged; 4 = 26%–50% damaged; 5 = 51%–75% damaged; 6 = >75% damaged) ("leaf herbivory" hereafter) (Moreira et al., 2019), and averaged values across leaves to obtain a mean value per branch. We then averaged values across these two branches to obtain a mean value per individual for statistical analyses. To avoid biases in our herbivory estimates, the same person (XM) scored all the leaves.

At each site, we collected four fully expanded (mature) leaves per tree, transported samples to the lab in ice coolers, and immediately preserved them at -30°C for subsequent chemical analyses. We took special care to collect leaves with no (or very little) herbivore damage in order to minimize variation in defence levels caused by site-specific induction (Abdala-Roberts, Moreira, Rasmann, Parra-Tabla, & Mooney, 2016). However, systemic induced responses may still take place and are not accounted for using this procedure. Accordingly, measured levels of chemical defences presumably represented a combination of constitutive defences and an unknown level of systemic induction due to herbivory occurred over the growing season (Abdala-Roberts, Moreira, et al., 2016). After collection, we oven-dried leaves for 48 hr at 40°C , ground them with liquid nitrogen, and stored the samples for subsequent quantification of phenolic compounds. Leaves from each tree were pooled into a single sample for chemical analyses.

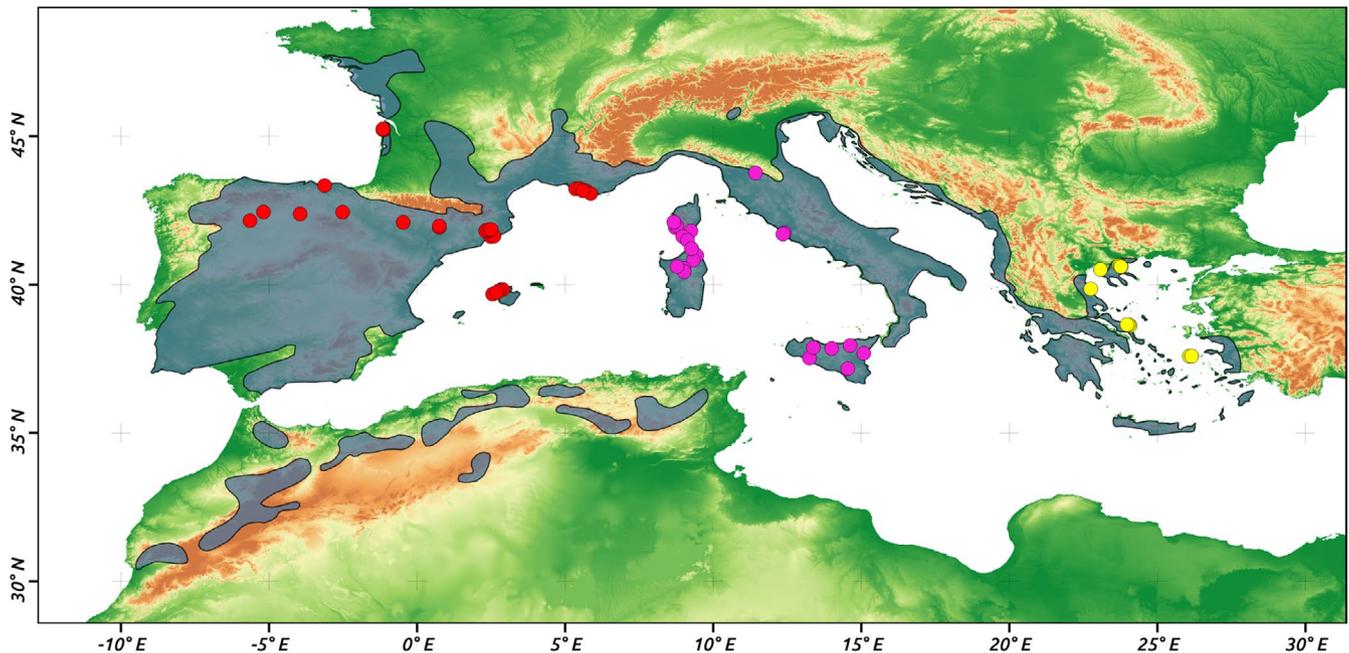


FIGURE 1 Map including a spatial layout of holm oak (*Quercus ilex*) populations sampled from Spain to Greece. Dots represent the location of each population ($N = 58$). Different colours for dots represent the three phylogeographic groups inferred for *Q. ilex* in previous studies (red for the western Mediterranean, pink for the central Mediterranean and yellow for the Aegean region) (Vitelli et al., 2017). The blue-shaded area represents the distribution range of this species [Colour figure can be viewed at wileyonlinelibrary.com]



2.3 | Quantification of phenolic compounds

Phenolic compounds were extracted from 20 mg of dry leaf tissue with 1 ml of 70% methanol in an ultrasonic bath for 15 min, followed by centrifugation (Moreira et al., 2014). We then transferred the extracts to chromatographic vials. To perform the chromatographic analyses we used Ultra-High-Performance Liquid-Chromatograph (UHPLC Nexera LC-30AD; Shimadzu) equipped with a Nexera SIL-30AC injector and one SPD-M20A UV/VIS photodiode array detector (Moreira, Abdala-Roberts, et al., 2018). Compound separation was carried out on a Kinetex™ 2.6 μm C18 82-102 Å, LC Column 100 × 4.6 mm, protected with a C18 guard cartridge. Flow rate was 0.4 ml/min and oven temperature was set at 25°C. The mobile phase consisted of two solvents: water-formic acid (0.05%) (A) and acetonitrile-formic acid (0.05%) (B), starting with 5% B and using a gradient to obtain 30% B at 4 min, 60% B at 10 min, 80% B at 13 min and 100% B at 15 min. Injection volume was 15 μl. We quantified flavonoids as rutin equivalents, condensed tannins as catechin equivalents and hydrolysable tannins as gallic acid equivalents (Moreira, Abdala-Roberts, et al., 2018). We achieved quantification of these phenolic compounds by external calibration using calibration curves at 0.25, 0.5, 1, 2 and 5 μg/ml. We quantified all compounds using a wavelength of 330 nm. We expressed phenolic compound concentrations in mg/g tissue on a dry weight basis.

2.4 | Geographic and climatic variables

We obtained geographic coordinates of each holm oak population using a Global Positioning System device (Garmin). To characterize the climatic conditions present at each site, we extracted the mean annual precipitation (mm) and mean annual temperature (°C) from the WorldClim database (<http://www.worldclim.org/>) at the 30-s resolution (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). Previous studies have demonstrated that both climatic variables are key drivers of herbivory and plant defensive traits for other oak species (Abdala-Roberts et al., 2018; Moreira, Abdala-Roberts, et al., 2018).

2.5 | Statistical analyses

2.5.1 | Effects of insularity on leaf herbivory and chemical defences

We ran general linear models (GLMs) testing for the effect of insularity (two levels: mainland vs. insular locations, fixed factor) and phylogeographic group (three levels: central and western Mediterranean, and the Aegean region; fixed factor) on insect leaf herbivory and chemical defences (with separate models for each group of phenolic compounds). For all models, we analysed data at the level of population by using mean values across trees at each site. Preliminary analyses including latitude and elevation as covariates indicated no significant effects of these predictors (results not show) and we therefore did not consider them in subsequent tests. Likewise, for insular populations we also ran a GLM testing for the effect of island size and distance to

mainland on herbivory and found no significant effects of these predictors ($F_{1,25} = 0.72$, $p = 0.403$ and $F_{1,25} = 0.14$, $p = 0.713$ respectively). We performed all analyses with PROC GLM in SAS ver. 9.4 (SAS Institute) (Littell, Milliken, Stroup, Wolfinger, & Schabenberger, 2006). All variables were log-transformed to achieve normality of residuals, and we report model least-squares means and standard errors as descriptive statistics. We additionally ran general linear mixed models testing the effect of insularity and phylogeographic group on herbivory and each group of phenolic compounds using data at the tree level, in each case accounting for population as random effect (PROC MIXED in SAS 9.4). Results from these models were highly consistent to those from models using population means (Table S1).

2.5.2 | Test of underlying predictors associated with insularity effects on leaf herbivory and plant chemical defences

We tested whether the effect of insularity on insect herbivory was mediated by climate and/or changes in plant defences by running “mechanistic” models with insularity and phylogeographic group as fixed factors, as well as climatic factors or leaf chemical defences as covariates (PROC GLM in SAS 9.4). We tested for effects of defences on herbivory rather than the inverse because our analyses indicated that plant defences were negatively correlated with leaf damage (see section 3.1), suggesting that defences drove herbivory (see Abdala-Roberts, Moreira, et al., 2016). Specifically, we ran separate models including either climatic variables (temperature and precipitation) or plant defences, because running a single model with both types of predictors would not permit to tease apart the relative influences of climate versus leaf traits underlying the effect of insularity on herbivory. To assess whether these predictors mediate the effect of insularity on leaf herbivory, we compared the significance of the insularity effect in each of these models relative to the initial model without covariates to interpret whether such effect was associated (and potentially underlain) by leaf traits or climate. If climate or plant defences mediate the effect of insularity on herbivory, then a significant effect of insularity in the model without covariates should turn non-significant after including these predictors in the mechanistic model (Abdala-Roberts, Moreira, et al., 2016; Moreira, Abdala-Roberts, et al., 2018). Rather than including all groups of phenolic compounds in the GLM with leaf defences, we selected the most relevant traits to include in this model by running a separate multiple regression including all three groups of phenolics as predictors of herbivory (PROC REG in SAS 9.4). Results indicated that condensed tannins was the only significant predictor of herbivory (Table S2), with higher amounts of these compounds being associated with lower herbivory. We therefore only included this group of phenolic compounds in the above GLM assessing the influence of plant defences on herbivory.

Finally, we also tested whether the effect of insularity on leaf chemical defences was mediated by climate conditions by performing GLMs including the effect of insularity, phylogeographic group, and climatic variables as covariates for each group of phenolic compounds. We followed the same approach as above by comparing the

significance of the insularity effect in models without versus with covariates (climatic variables). We ran mechanistic models with covariates (and thus performed the model comparison) only for phenolic compounds (i.e., condensed tannins) for which the effect of insularity was significant in the initial model without covariates.

3 | RESULTS

3.1 | Effect of insularity on leaf herbivory and plant chemical defences

We found a significant effect of insularity on insect herbivory (Table 1a). In accordance with predictions, leaf damage was 1.9-fold lower on holm oaks found on islands (per cent leaf damage score: 0.50 ± 0.05) than on mainland sites (0.96 ± 0.05) (Figure 2a). We also found a significant effect of insularity on leaf condensed tannins (Table 1a), but in an inverse manner to herbivory as the concentration of these compounds was 1.9-fold greater for holm oak trees on islands (1.84 ± 0.21 mg/g

d.w.) than on mainland locations (0.98 ± 0.20 mg/g d.w.) (Figure 2b). There was no effect of insularity on flavonoids (Table 1a, Figure 2c) or hydrolysable tannins (Table 1a, Figure 2d). In all cases, we found no effect of phylogeographic group on herbivory or plant chemical defences (Table 1a).

3.2 | Underlying predictors associated with insularity effects on herbivory and leaf defences

Results from the mechanistic model for herbivory indicated that the effect of insularity on leaf herbivory remained significant after including condensed tannins on the one hand, or temperature and precipitation on the other (Table 1b), suggesting that neither chemical defences nor climate accounted for lower levels of insect herbivory on islands relative to mainland. Results from the herbivory model including condensed tannins indicated no significant effect of this predictor (Table 1b), whereas the model including climatic factors indicated a significant positive effect of precipitation on herbivory

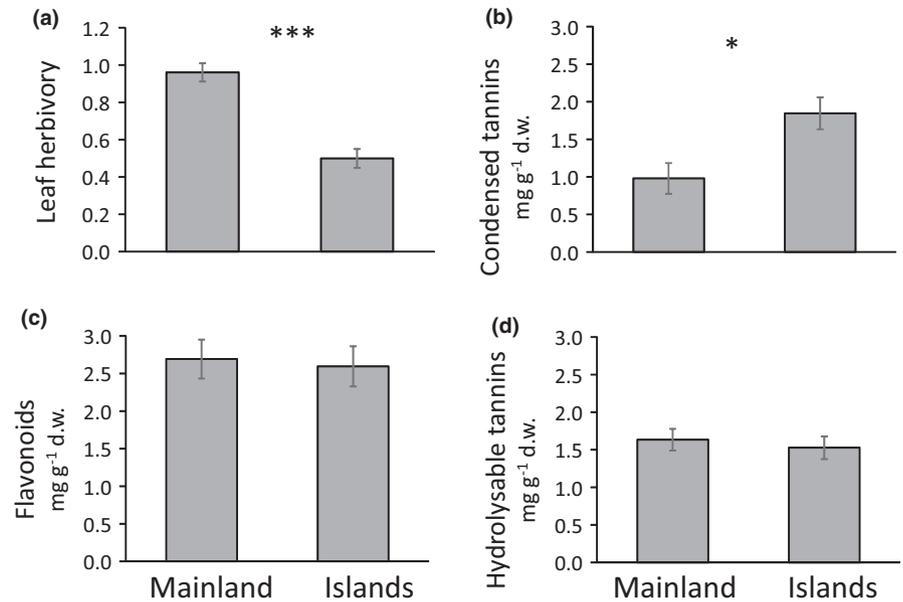
TABLE 1 Summary of results from general linear models testing for the effects of insularity (mainland vs. insular locations) and phylogeographic group (central Mediterranean, western Mediterranean and the Aegean region) on leaf herbivory by chewing insects and the concentration of leaf chemical defences (condensed tannins, flavonoids and hydrolysable tannins) for sampled holm oak (*Quercus ilex*) trees. We first ran models (a) including only the effect of insularity (without covariates), and then subsequently ran mechanistic models for herbivory (b) which additionally included defensive traits (condensed tannins, b1) or climatic variables (mean annual temperature and precipitation, b2) as covariates, whereas for defensive traits (phenolic compounds), the models included only climatic variables as covariates (c)

(a) Without covariates								
	Insularity		Phylogeography					
	$F_{1,54}$	<i>p</i>	$F_{2,54}$	<i>p</i>				
Herbivory	28.55	<0.001	1.83	0.170				
Condensed tannins	4.85	0.032	2.66	0.079				
Flavonoids	0.68	0.413	1.23	0.301				
Hydrolysable tannins	0.11	0.743	0.05	0.951				
(b) Herbivory mechanistic model								
(b1) Defence as a covariate	Insularity		Phylogeography		Condensed tannins			
	$F_{1,53}$	<i>p</i>	$F_{2,53}$	<i>p</i>	$F_{1,53}$	<i>p</i>		
Herbivory	26.06	<0.001	1.23	0.301	1.38	0.245		
(b2) Climate as a covariate	Insularity		Phylogeography		Temperature		Precipitation	
	$F_{1,52}$	<i>p</i>	$F_{2,52}$	<i>p</i>	$F_{1,52}$	<i>p</i>	$F_{1,52}$	<i>p</i>
Herbivory	30.35	<0.001	4.68	0.012	0.27	0.606	13.85	0.001
(c) Defence mechanistic model								
	Insularity		Phylogeography		Temperature		Precipitation	
	$F_{1,52}$	<i>p</i>	$F_{2,52}$	<i>p</i>	$F_{1,52}$	<i>p</i>	$F_{1,52}$	<i>p</i>
Condensed tannins	4.72	0.034	2.80	0.070	0.45	0.506	0.85	0.362

Note: By accounting for these covariates, we tested whether insularity effects were mediated by differences in leaf traits and/or climate (section 2.5.2 in the Methods). In all cases, we used mean values across plants within each population. *F*-values, degrees of freedom (in subscript) and associated significance levels are shown. Significant *p*-values ($p < 0.05$) are in bold.



FIGURE 2 Leaf herbivory by chewing insects (a), and the concentration (in mg/g d.w.) of leaf condensed tannins (b), flavonoids (c) and hydrolysable tannins (d) in holm oak (*Quercus ilex*) trees sampled on mainland and on islands ($N = 30$ and $N = 28$, respectively) in the Mediterranean Basin (from Spain to Greece). Leaf damage was visually estimated using an ordinal scale based on per cent leaf area removed. Bars are least square means \pm standard error from general linear models without covariates (see section 2.5.1 in the Methods). Asterisks indicate significant ($*p < 0.05$, $***p < 0.001$) differences between mainland and islands (see Table 1a)



(slope estimator [β] = 0.00049 \pm 0.00013), but no effect of temperature (Table 1b).

Results from the mechanistic model for plant defences indicated that the effect of insularity on condensed tannins remained significant after including temperature and precipitation as covariates (Table 1c), suggesting that differences in the amount of these phenolic compounds between island versus mainland populations were not explained by climatic conditions. In addition, we found no significant effects of temperature and precipitation on condensed tannins (Table 1c).

4 | DISCUSSION

Following predictions, insular populations of holm oak exhibited lower herbivory by leaf-chewing insects than their mainland counterparts. This finding is in accordance with previous studies showing that species richness and density of several important insects attacking *Q. ilex* such as *Lymantria dispar* and *Tortrix viridana* are notoriously higher in mainland areas than on the sampled Mediterranean islands (Blondel & Pradel, 1990; Dapporto & Dennis, 2009; Hausdorf & Hennig, 2005). These concordant patterns are consistent with the expectation of reduced herbivore pressure on islands. The observed pattern is particularly important in that most studies have focused on effects of insularity on mammalian herbivory (e.g., Bowen & Van Buren, 1997; Skaen & Arcese, 2018; Vourc'h et al., 2001), whereas studies reporting on patterns involving insects are much less common (but see Arnold & Asquith, 2002). To our knowledge, our work represents the first large-scale test of insularity effects on insect herbivory (and, more broadly, herbivory in general), and argues for increased consideration of such effects on insect herbivory for long-lived plants to improve our understanding of the factors driving the evolution of plant-herbivore interactions on islands.

Our assessment of underlying factors associated with herbivory indicated that leaf defences, specifically condensed tannins, were

significantly negatively associated with herbivory, suggesting that increasing amounts of these secondary compounds lowered insect leaf damage (Moreira, Abdala-Roberts, et al., 2018; Moreira, Castagneyrol, et al., 2018). Contrary to predictions, however, holm oak populations on islands had higher (rather than lower) leaf condensed tannins despite significantly lower herbivory at these sites. This finding, however, does stand alone as a recent study by Monroy and García-Verdugo (2019) similarly found that insular populations (Canary Islands, Cape Verde) of the Mediterranean shrub *Periploca laevigata* exhibited higher concentrations of leaf tannins than mainland populations (Monroy & García-Verdugo, 2019). However, the authors did not measure herbivory, which precluded a test of whether observed pattern in plant defences was underlain by concomitant differences in herbivore pressure. As an alternative, they argued that high levels of tannins found for insular populations could act as protectants against abiotic factors. In our case, findings suggest at first glance that higher levels of condensed tannins drive a concomitant reduction in insect herbivory on insular versus mainland sites. However, our analysis argues against this interpretation as accounting for condensed tannins in the statistical model for herbivory did not explain lower levels of leaf damage on islands. These inverse patterns of insect herbivory and plant defences in holm oak therefore appear to be seemingly unrelated.

Our analyses including climatic factors indicated that precipitation positively predicted herbivory, a pattern that agrees with previous work on plant-herbivore interactions along ecological gradients (e.g., elevational, latitudinal; Rasmann et al., 2014; Moreira, Abdala-Roberts, Parra-Tabla, & Mooney, 2015). In contrast, neither temperature nor precipitation were significantly associated with condensed tannins, suggesting a lack of climatic control over the expression of these compounds in holm oak (but see other oak studies such as Pearse & Hipp, 2012; Abdala-Roberts, Rasmann, et al., 2016; Moreira, Abdala-Roberts, et al., 2018). More importantly, however, insularity effects on both herbivory and condensed tannins remained significant after accounting for climatic factors, suggesting

that climate did not explain differences in leaf damage or chemical defences between insular and mainland populations. Therefore, observed patterns of herbivory and oak defences are presumably explained by other unmeasured (biotic or abiotic) factors or historical processes such as the history of holm oak colonization (see ahead).

4.1 | Future work

Our analyses indicated no effects of phylogeographic region on herbivory or oak defences, a proxy for regional variation in historical processes associated with this species. Likewise, we found no significant effects of island features such as size and isolation on these responses, despite previous work reporting on the influence of these factors on mainland-island biogeographical patterns (Arnold & Asquith, 2002; Pardo et al., 2018). Broad-scale patterns of herbivory and trait variation between island and mainland sites reported here may be inherently more complex than those documented previously at smaller spatial scales (Bowen & Van Buren, 1997; Vourc'h et al., 2001). This may warrant a more detailed investigation of regional processes within each holm oak phylogeographic area, including aspects such as the history of holm oak colonization and potential founder effects or genetic drift) and from there scale up to explain broader scale patterns in insularity effects on plant-insect herbivore interactions.

It should also be pointed out that observational studies such as the present work have been informative in assessing the influence of abiotic factors on herbivory and plant traits (Moreira, Abdala-Roberts, et al., 2018; Pearse & Hipp, 2012), but also impose limitations due to the correlative nature of the data. In this sense, we caution that a causality between plant defence-herbivory associations cannot be assigned, and that other (third party) variables not considered in our analyses could mediate the observed relationships between climatic factors and the response variables measured. Experimental approaches such as herbivore exclusions and bioassays (e.g., feeding trials with different insect and plant populations), as well as common garden and island-mainland reciprocal transplant experiments using different source populations are needed to understand the effects of insularity on the evolution of holm oak-insect herbivore interactions. In this sense, the present study provides a fundamental baseline for launching these subsequent experiments.

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REFERENCES

- Abdala-Roberts, L., Galmán, A., Petry, W. K., Covelo, F., de la Fuente, M., Glauser, G., & Moreira, X. (2018). Interspecific variation in leaf functional and defensive traits in oak species and its underlying climatic drivers. *PLoS ONE*, *13*, e0202548. <https://doi.org/10.1371/journal.pone.0202548>
- Abdala-Roberts, L., Moreira, X., Rasmann, S., Parra-Tabla, V., & Mooney, K. A. (2016). Test of biotic and abiotic correlates of latitudinal variation in defenses in the perennial herb *Ruellia nudiflora*. *Journal of Ecology*, *104*, 580–590. <https://doi.org/10.1111/1365-2745.12512>
- Abdala-Roberts, L., Rasmann, S., Berny-Mier y Terán, J. C., Covelo, F., Glauser, G., & Moreira, X. (2016). Biotic and abiotic factors associated with altitudinal variation in plant traits and herbivory in a dominant oak species. *American Journal of Botany*, *103*, 2070–2078. <https://doi.org/10.3732/ajb.1600310>
- Adersen, A. H., & Adersen, H. (1993). Cyanogenic plants in the Galapagos Islands: Ecological and evolutionary aspects. *Oikos*, *66*, 511–520. <https://doi.org/10.2307/3545363>
- Arnold, A. E., & Asquith, N. M. (2002). Herbivory in a fragmented tropical forest: Patterns from islands at Lago Gatun, Panama. *Biodiversity and Conservation*, *11*, 1663–1680. <https://doi.org/10.1023/A:1016888000369>
- Blondel, J., & Pradel, R. (1990). Is adult survival of the blue tit higher in a low fecundity insular population than in a high fecundity mainland one? *Population Biology of Passerine Birds*, *24*, 131–143. <https://doi.org/10.1007/978-3-642-75110-3>
- Bowen, L., & Van Buren, D. (1997). Insular endemic plants lack defences against herbivores. *Conservation Biology*, *11*, 1249–1254. <https://doi.org/10.1046/j.1523-1739.1997.96368.x>
- Burns, K. C. (2014). Are there general patterns in plant defence against megaherbivores? *Biological Journal of the Linnean Society*, *111*, 38–48. <https://doi.org/10.1111/bij.12181>
- Carlquist, S. (1974). *Island biology*. New York, NY: Columbia University Press. <https://doi.org/10.5962/bhl.title.63768>
- Cyr, H., & Pace, M. L. (1993). Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature*, *361*, 148–150. <https://doi.org/10.1038/361148a0>
- Dapporto, L., & Dennis, R. L. H. (2009). Conservation biogeography of large Mediterranean islands. Butterfly impoverishment, conservation priorities and inferences for an ecological “island paradigm”. *Ecography*, *32*, 169–179. <https://doi.org/10.1111/j.1600-0587.2008.05600.x>
- Darwin, C. R. (1909). *The voyage of the beagle*. New York, NY: P.F. Collier & son company. <https://doi.org/10.5962/bhl.title.98662>
- 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. <https://doi.org/10.2307/1934037>
- Giffard, B., Jactel, H., Corcket, E., & Barbaro, L. (2012). Influence of surrounding vegetation on insect herbivory: A matter of spatial scale and herbivore specialisation. *Basic and Applied Ecology*, *13*, 458–465. <https://doi.org/10.1016/j.baae.2012.08.004>
- Gillespie, R. G., Claridge, E. M., & Goodacre, S. L. (2008). Biogeography of the fauna of French Polynesia: Diversification within and between a series of hot spot archipelagos. *Philosophical Transactions of the Royal Society B*, *363*, 3335–3346. <https://doi.org/10.1098/rstb.2008.0124>
- Givnish, T. J., Sytsma, K. J., Smith, J. F., & Hahn, W. J. (1994). Thorn-like prickles and heterophylly in Cyanea: Adaptations to extinct avian browsers on Hawaii? *Proceedings of the National Academy of Sciences of the United States of America*, *91*, 2810–2814. <https://doi.org/10.1073/pnas.91.7.2810>
- Grant, B. R., & Grant, P. R. (1998). Hybridization and speciation in Darwin's finches: The role of sexual imprinting on a culturally transmitted trait. In D. J. Howard, & S. H. Berlocher (Eds.), *Endless forms: Species and speciation* (pp. 404–422). Oxford, UK: Oxford University Press.



- Hanse, I., Brimer, L., & Mølgaard, P. (2003). Herbivore-deterrent secondary compounds in heterophyllous woody species of the Mascarene Islands. *Perspectives in Plant Ecology, Evolution and Systematics*, 6, 187–203. <https://doi.org/10.1078/1433-8319-00077>
- Hausdorf, B., & Hennig, C. (2005). The influence of recent geography, palaeogeography and climate on the composition of the fauna of the central Aegean Islands. *Biological Journal of the Linnean Society*, 84, 785–795. <https://doi.org/10.1111/j.1095-8312.2005.00467.x>
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978. [https://doi.org/10.1002/\(ISSN\)1097-0088](https://doi.org/10.1002/(ISSN)1097-0088)
- Huberty, A. F., & Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: A new synthesis. *Ecology*, 85, 1383–1398. <https://doi.org/10.1890/03-0352>
- Jactel, H., Petit, J., Desprez-Loustau, M.-L., Delzon, S., Piou, D., Battisti, A., & Koricheva, J. (2012). Drought effects on damage by forest insects and pathogens: A meta-analysis. *Global Change Biology*, 18, 267–276. <https://doi.org/10.1111/j.1365-2486.2011.02512.x>
- Kavanagh, P. H. (2015). Herbivory and the evolution of divaricate plants: Structural defences lost on an offshore island. *Austral Ecology*, 40, 206–211. <https://doi.org/10.1111/aec.12196>
- Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R., & Schabenberger, O. (2006). *SAS System for mixed models* (2nd ed.). Cary, NC: Cary.
- MacArthur, R. H., & Wilson, E. O. (1967). *The theory of island biogeography*. Princeton, NJ: Princeton University Press.
- Monroy, P., & García-Verdugo, C. (2019). Testing the hypothesis of loss of defences on islands across a wide latitudinal gradient of *Periploca laevigata* (Apocynaceae) populations. *American Journal of Botany*, 106, 303–312. <https://doi.org/10.1002/ajb2.1232>
- Moreira, X., Abdala-Roberts, L., Berny Mier y Teran, J. C., Covelo, F., de la Mata, R., Francisco, M., Hardwick, B., ... Tack, A. J. M. (2019). Impacts of urbanization on insect herbivory and plant defences in oak trees. *Oikos*, 128, 113–123.
- Moreira, X., Abdala-Roberts, L., Galmán, A., Francisco, M., de la Fuente, M., Butrón, A., & Rasmann, S. (2018). Assessing the influence of biogeographical region and phylogenetic history on chemical defences and herbivory in *Quercus* species. *Phytochemistry*, 153, 64–73. <https://doi.org/10.1016/j.phytochem.2018.06.002>
- Moreira, X., Abdala-Roberts, L., Parra-Tabla, V., & Mooney, K. A. (2015). Latitudinal variation in herbivory: Influences of climatic drivers, herbivore identity, and natural enemies. *Oikos*, 124, 1444–1452. <https://doi.org/10.1111/oik.02040>
- Moreira, X., Castagnyrol, B., Abdala-Roberts, L., Berny-Mier y Terán, J. C., Timmermans, B. G. H., Bruun, H. H., Covelo, F., ... Tack, A. J. M. (2018). Latitudinal variation in plant chemical defenses drives latitudinal patterns of leaf herbivory. *Ecography*, 41, 1124–1134. <https://doi.org/10.1111/ecog.03326>
- Moreira, X., Mooney, K. A., Rasmann, S., Petry, W. K., Carrillo-Gavilán, A., Zas, R., & Sampedro, L. (2014). Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. *Ecology Letters*, 17, 537–546. <https://doi.org/10.1111/ele.12253>
- Pardo, A., Cáceres, Y., & Pulido, F. (2018). Intraspecific variation in heritable secondary metabolites and defensive strategies in a relict tree. *Journal of Plant Ecology*, 11, 256–265.
- Pearse, I. S., & Hipp, A. L. (2012). Global patterns of leaf defenses in oak species. *Evolution*, 66, 2272–2286. <https://doi.org/10.1111/j.1558-5646.2012.01591.x>
- Rasmann, S., Pellissier, L., Defosse, E., Jactel, H., & Kunstler, G. (2014). Climate-driven change in plant–insect interactions along elevation gradients. *Functional Ecology*, 28, 46–54. <https://doi.org/10.1111/1365-2435.12135>
- Ricklefs, R., & Bermingham, E. (2008). The West Indies as a laboratory of biogeography and evolution. *Philosophical Transactions of the Royal Society B*, 363, 2393–2413. <https://doi.org/10.1098/rstb.2007.2068>
- Roslin, T., & Salminen, J. P. (2008). Specialization pays off: Contrasting effects of two types of tannins on oak specialist and generalist moth species. *Oikos*, 117, 1560–1568. <https://doi.org/10.1111/j.0030-1299.2008.16725.x>
- Ruiz-Carbayo, H., Bonal, R., Espelta, J. M., Hernández, M., & Pino, J. (2017). Community assembly in time and space: The case of Lepidoptera in a *Quercus ilex* L. savannah-like landscape. *Insect Conservation and Diversity*, 10, 21–31. <https://doi.org/10.1111/icad.12184>
- Skaïen, C. L., & Arcese, P. (2018). Spatial variation in herbivory, climate and isolation predicts plant height and fruit phenotype in *Plectritis congesta* populations on islands. *Journal of Ecology*, 106, 2344–2352. <https://doi.org/10.1111/1365-2745.12982>
- Southwood, T. R. E., Wint, G. R. W., Kennedy, C. E. J., & Greenwood, S. R. (2005). The composition of the arthropod fauna of the canopies of some species of oak (*Quercus*). *European Journal of Entomology*, 102, 65–72. <https://doi.org/10.14411/eje.2005.009>
- Spiller, D. A., & Schoener, T. W. (1990). A terrestrial field experiment showing the impact of eliminating top predators on foliage damage. *Nature*, 347, 469–472. <https://doi.org/10.1038/347469a0>
- Traveset, A., Heleno, R., Chamorro, S., Vargas, P., McMullen, C. K., Castro-Urgal, R., Nogales, M., ... Olesen, J. M. (2013). Invaders of pollination networks in the Galápagos Islands: Emergence of novel communities. *Proceedings of the Royal Society B*, 280, 20123040.
- Vitelli, M., Vessella, F., Cardoni, S., Pollegioni, P., Denk, T., Grimm, G. W., & Simeone, M. C. (2017). Phylogeographic structuring of plastome diversity in Mediterranean oaks (*Quercus* Group Ilex, Fagaceae). *Tree Genetics & Genomes*, 13, 3. <https://doi.org/10.1007/s11295-016-1086-8>
- Vourc'h, G., Martin, J. L., Duncan, P., Escarré, J., & Clausen, T. P. (2001). Defensive adaptations of *Thuja plicata* to ungulate browsing: A comparative study between mainland and island populations. *Oecologia*, 126, 84–93.
- Wallace, A. R. (1880). *Island life: Or, the phenomena and causes of insular faunas and floras, including a revision and attempted solution of the problem of geological climates*. London, UK: Macmillan & Co.
- Weigelt, P., Jetz, W., & Krefta, H. (2013). Bioclimatic and physical characterization of the world's islands. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 15307–15312.

BIOSKETCH

Xoaquín Moreira is interested in the ecological and evolutionary processes that occur among different trophic levels (plants, herbivores and natural enemies), and predict how the future global change might influence not only each species individually but also the various interactions as a whole.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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