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

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Key Words:	Condensed tannins, islands, Mediterranean Basin, phenolic compounds, plant-herbivore interactions, Quercus ilex

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3 43 **ABSTRACT**

4 44 **Aim:** Research on plant-herbivore interactions has shown that islands typically have low
5 45 abundances and diversity of herbivores because of barriers to dispersal, isolation, and reduced
6 46 land area. Islands commonly have lower levels of herbivory relative to mainland regions, and,
7 47 as a consequence, insular plants should exhibit lower levels of defences than their mainland
8 48 counterparts. Despite these predictions, there are significant gaps in our understanding of
9 49 insularity effects on plant-herbivore interactions. For instance, most work addressing the
10 50 effects of insularity on plant-herbivore interactions have compared one or a few islands with a
11 51 single mainland site. In addition, studies have measured herbivory or plant defences but not
12 52 both, and the influence of abiotic factors has been neglected.

13 53 **Location:** Mediterranean Basin (from Spain to Greece)

14 54 **Taxon:** *Quercus ilex* L.

15 55 **Methods:** We conducted a large-scale study to investigate whether insect leaf herbivory and
16 56 plant chemical defences in holm oak (*Quercus ilex* L.) differ between insular vs. mainland
17 57 populations. We further investigated mechanisms by which insularity effects on herbivory
18 58 may take place by assessing the influence of defences and climatic variables on leaf
19 59 herbivory.

20 60 **Results:** We found that insular populations exhibited lower herbivory and higher defences
21 61 (condensed tannins) than their mainland counterparts. Our analyses, however, suggest that
22 62 these concomitant patterns of insect herbivory and plant defences were seemingly unrelated as
23 63 island vs. mainland differences in defences did not account for the observed pattern in
24 64 herbivory. Furthermore, climatic factors did not explain insularity effects on either herbivory
25 65 or plant defences.

26 66 **Main conclusions:** Overall, this study provides one of the most robust assessments to date on
27 67 insularity effects on herbivory and builds towards a better understanding of the ecology and
28 68 evolution of plant-insect interactions in insular ecosystems.

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32 72 **Keywords:** Condensed tannins, islands, Mediterranean Basin, phenolic compounds, plant-
33 73 herbivore interactions, *Quercus ilex*
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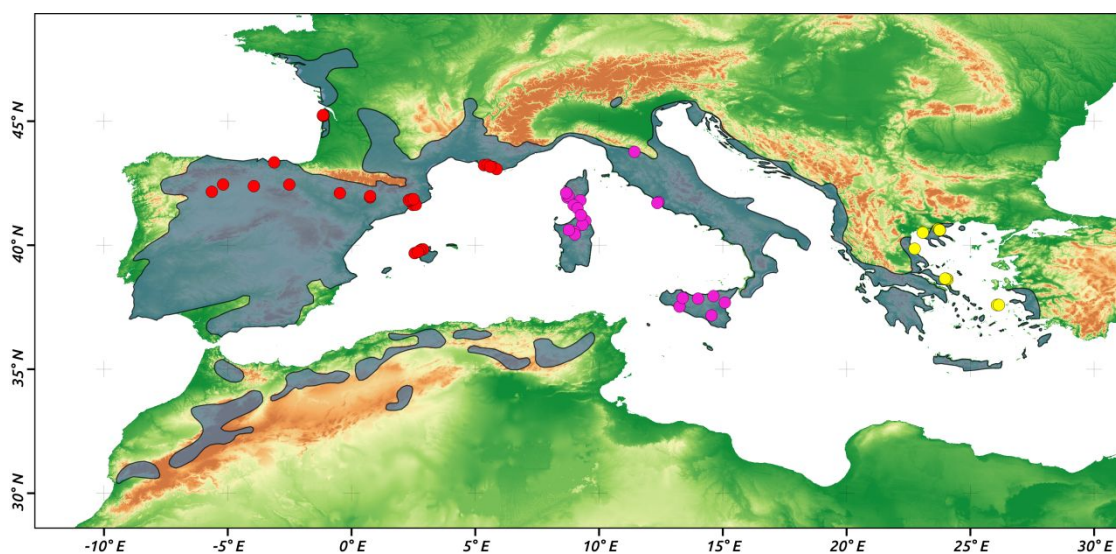
88 INTRODUCTION

89 Insular systems provide a valuable framework for elucidating the ecological mechanisms
90 shaping biodiversity (Wallace, 1880; Darwin, 1909; MacArthur & Wilson, 1967; Gillespie,
91 Claridge & Goodacre, 2008; Ricklefs & Bermingham, 2008), species interactions (Spiller &
92 Schoener, 1990; Traveset et al., 2013), and trait evolution (Grant & Grant, 1998). Research on
93 plant-herbivore interactions has shown that islands typically have low abundances and
94 diversity of vertebrate and invertebrate herbivores because of barriers to dispersal, isolation,
95 and reduced land area (MacArthur & Wilson, 1967; Carlquist, 1974; Burns, 2014). Islands are
96 therefore expected to have lower levels of herbivory relative to mainland regions, and, as a
97 consequence, insular plants should exhibit lower levels of physical (e.g. spines, thorns) and
98 chemical (e.g. secondary metabolites) defences than their mainland counterparts (Adersen &
99 Adersen, 1993; Bowen & Van Buren, 1997; Vourc'h, Martin, Duncan, Escarré & Clausen,
100 2001; Burns, 2014). However, to date there have been very few robust tests of insularity
101 effects on plant-herbivore interactions, and recent work has challenged expectations by
102 reporting higher (rather than lower) levels of physical and chemical traits associated with
103 herbivore resistance in insular plants (e.g. Hanse, Brimer & Mølgaard, 2003; Pardo, Cáceres
104 & Pulido, 2018; Monroy & García-Verdugo, 2019).

105 There are significant gaps in our understanding of insularity effects on plant-herbivore
106 interactions, some of which are common to other types of species interactions as well. First,
107 most studies suffer from low sample sizes and have been conducted at small spatial scales
108 comparing one or a few islands with an adjacent mainland site (e.g. Bowen & Van Buren,
109 1997; Vourc'h et al., 2001). These limitations have inevitably constrained our ability to reach
110 broader conclusions based on the observed patterns. Second, abiotic factors play a key role in
111 shaping spatial variation in plant-herbivore interactions (Rasmann, Pellissier, Defosse, Jactel
112 & Kunstler, 2014), but have not been explicitly accounted for in island *vs.* mainland
113 comparisons despite the fact that insular ecosystems frequently differ in their climatic
114 conditions relative to mainland counterparts (e.g. islands are usually cooler, wetter, and less
115 seasonal; Weigelt, Jetz & Krefta, 2013). [Explicitly accounting for these factors may help
116 elucidate the mechanisms of abiotic control over insularity effects on herbivory, with the
117 expectation that the degree of reduction in herbivore pressure on islands is contingent on the
118 difference in abiotic \(e.g. climatic\) conditions between insular and mainland ecosystems.](#)
119 Third, studies have usually measured herbivory or plant traits associated with herbivore
120 damage, but not both (e.g. Adersen & Adersen, 1993; Givnish, Sytsma, Smith & Hahn,
121 1994; Burns, 2014; Kavanagh, 2015), leading to an incomplete understanding of insularity
122 effects on plant-herbivore interactions. This aspect is also important in relation to the previous
123 point, since abiotic factors may concurrently influence herbivores and plant traits (Moreira et
124 al., 2018a). For example, climatic variables can directly influence herbivory by shaping
125 herbivore abundance or activity, but at the same time may also exert indirect effects on
126 herbivory by altering plant physical or chemical traits that affect herbivores (Huberty &
127 Denno, 2004; Jactel et al., 2012). A combined approach measuring herbivory, plant defences,
128 and abiotic factors is thus likely to explain the most variation in insularity effects on plant-
129 herbivore interactions. Finally, to date most studies have investigated the effect of insularity
130 on vertebrate (e.g. mammalian) herbivores (e.g. Bowen & Van Buren, 1997; Vourc'h et al.,
131 2001; Skaien & Arcese, 2018; Monroy & García-Verdugo, 2019), whereas research on insect
132 herbivory is less common but may be key for understanding insularity effects on plant
133 defences and herbivory (Cyr & Pace, 1993).

134 In this study, we investigated whether levels of insect leaf herbivory and leaf chemical
135 defences (phenolic compounds) in holm oak (*Quercus ilex* L.) populations found on islands
136 differ from those observed among their mainland counterparts. We additionally investigated
137 whether plant chemical defences and climatic variables (temperature and precipitation)

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3 138 underlie any such effect of insularity on herbivory. To this end, we sampled 28 populations
4 139 found on Mediterranean islands and 30 populations found on nearby mainland sites (Fig. 1).
5 140 Sampling sites were distributed from north-western Spain to Greece, covering most of the
6 141 latitudinal and elevational distribution of this species (Fig. 1). We predicted that oak trees
7 142 found on islands would have lower levels of insect leaf herbivory and **phenolic compounds**
8 143 than their mainland counterparts, and that such patterns would be underlain by differences in
9 144 climatic factors. **Phenolic compounds have been found to confer resistance against insect**
10 145 **herbivores in *Quercus* species (Feeny, 1970; Roslin & Salminen, 2008; Moreira et al.,**
11 146 **2018a,b), and were thus considered a suitable proxy for chemical defences in holm oak.**
12 147 Overall, this study contributes to better understand the ecological and evolutionary effects of
13 148 insularity on plant-herbivore interactions and its underlying drivers.
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38 153 **Figure 1.** Map including a spatial layout of holm oak (*Quercus ilex*) populations sampled
39 154 from Spain to Greece. Dots represent the location of each population (N = 58). Different
40 155 colours for dots represent the three phylogeographic groups inferred for *Q. ilex* in previous
41 156 studies (red for the western Mediterranean, pink for the central Mediterranean and yellow for
42 157 the Aegean region) (Vitelli et al., 2017). The blue shaded area represents the distribution
43 158 range of this species.
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46 160 MATERIAL AND METHODS

47 161 *Natural history*

48
49 162 Holm oak *Q. ilex* is a long-lived, evergreen tree native to the Mediterranean region, extending
50 163 from Portugal to Turkey, also found on most islands of the Mediterranean basin (Fig. 1).
51 164 Phylogeographical analyses have delimited three clearly differentiated genetic groups: the
52 165 western Mediterranean, the central Mediterranean, and the Aegean region (see Fig. 1), which
53 166 are the result of an east-to-west colonization pattern (Vitelli et al., 2017). The species grows
54 167 in pure stands or mixed forests at low (close to sea level) to moderate (up to 1200 m asl)
55 168 elevations, and in its native range is attacked by several specialist and generalist insect
56 169 herbivores. Leaf chewers are particularly important herbivores on this species, among which
57 170 the most common are caterpillars belonging to Noctuidae and Tortricidae (Southwood, Wint,
58 171 Kennedy, & Greenwood, 2005; Giffard, Jactel, Corcket, & Barbaro, 2012; Ruiz-Carbayo,
59 172 Bonal, Espelta, Hernández, & Pino, 2017).

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174 ***Field sampling and leaf herbivory measurements***

175 Approaching the end of the growing season (from late August to early September), we
176 surveyed 58 holm oak populations distributed from north-western Spain to Greece, of which
177 30 were found on mainland locations and 28 on insular locations (Fig. 1). The sampled sites
178 spanned most of the latitudinal (37° to 45°) and elevational (9 to 1133 m asl) ranges of this
179 species (Fig. 1), and varied substantially in climatic conditions including a 2.6-fold gradient in
180 mean annual precipitation (442 to 1155 mm) and 7.5°C variation in mean annual temperature
181 (10.4 to 17.9°C). We sampled all populations at the end of the growing season at each site;
182 this was late August in northernmost populations and early September in southernmost
183 populations. This approach was aimed at reducing phenological differences in herbivory and
184 plant defensive traits across sites (Moreira et al., 2018a). In addition, we restricted our
185 sampling to fully-expanded mature leaves located on branches of similar height (see ahead) to
186 minimize microhabitat effects on leaf chemistry and herbivory. We also took care in sampling
187 leaves on branches of a similar size and at a similar position within the branch to control for
188 within-plant allocation patterns influencing leaf chemistry. We opted to sample trees at the
189 end of the growing season because this provided an assessment of cumulative leaf herbivory
190 occurring over the entire growing season (Giffard et al., 2012).

191 Each population included at least 15 mature, reproductive individuals. We randomly
192 selected five trees per population (mean diameter at breast height = 50.33 ± 2.33 [\pm SE] cm).
193 For each tree, we randomly selected two low-hanging branches (2-3 m above the ground) and
194 collected 25 leaves from each of them. Leaves measured at our study sites were mostly
195 damaged by insect chewers, whereas leaf miners and gall formers were rare (< 2% of the
196 leaves; results not shown). Because branches were sampled above the browsing height of
197 ungulates, we did not find signs of leaf herbivory by mammals (X. Moreira, personal
198 observation). For each leaf, we visually estimated percent leaf area removed by insect leaf
199 chewers using the following scale: 0 = undamaged; 1 = 1-5% damaged; 2 = 6-10% damaged;
200 3 = 11-25% damaged; 4 = 26-50% damaged; 5 = 51-75% damaged; 6 = >75% damaged)
201 (“leaf herbivory” hereafter) (Moreira et al., 2019), and averaged values across leaves to obtain
202 a mean value per branch. We then averaged values across these two branches to obtain a mean
203 value per individual for statistical analyses. To avoid biases in our herbivory estimates, the
204 same person (XM) scored all the leaves.

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

217

218 ***Quantification of phenolic compounds***

219 Phenolic compounds were extracted from 20 mg of dry leaf tissue with 1 mL of 70%
220 methanol in an ultrasonic bath for 15 min, followed by centrifugation (Moreira et al., 2014).
221 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 et al., 2018a). 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⁻¹ 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 et al., 2018b). We achieved quantification of these phenolic compounds by external calibration using calibration curves at 0.25, 0.5, 1, 2 and 5 µg mL⁻¹. We quantified all compounds using a wavelength of 330 nm. We expressed phenolic compound concentrations in mg g⁻¹ tissue on a dry weight basis.

Geographic and climatic variables

We obtained geographic coordinates of each holm oak population using a Global Positioning System device (Garmin, Kansas, USA). 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 second 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 et al., 2018a).

Statistical analyses

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, Cary NC) (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 in Appendix S1).

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

271 negatively correlated with leaf damage (see *Results*), suggesting that defences drove
272 herbivory (see Abdala-Roberts et al., 2016a). Specifically, we ran separate models including
273 either climatic variables (temperature and precipitation) or plant defences, because running a
274 single model with both types of predictors would not permit to tease apart the relative
275 influences of climate *vs.* leaf traits underlying the effect of insularity on herbivory. To assess
276 whether these predictors mediate the effect of insularity on leaf herbivory, we compared the
277 significance of the insularity effect in each of these models relative to the initial model
278 without covariates to interpret whether such effect was associated (and potentially underlain)
279 by leaf traits or climate. If climate or plant defences mediate the effect of insularity on
280 herbivory, then a significant effect of insularity in the model without covariates should turn
281 non-significant after including these predictors in the mechanistic model (Abdala-Roberts et
282 al., 2016a; Moreira et al., 2018a). Rather than including all groups of phenolic compounds in
283 the GLM with leaf defences, we selected the most relevant traits to include in this model by
284 running a separate multiple regression including all three groups of phenolics as predictors of
285 herbivory (PROC REG in SAS 9.4). Results indicated that condensed tannins was the only
286 significant predictor of herbivory (Table S2 in Appendix S1), with higher amounts of these
287 compounds being associated with lower herbivory. We therefore only included this group of
288 phenolic compounds in the above GLM assessing the influence of plant defences on
289 herbivory.

290 Finally, we also tested whether the effect of insularity on leaf chemical defences was
291 mediated by climate conditions by performing GLMs including the effect of insularity,
292 phylogeographic group, and climatic variables as covariates for each group of phenolic
293 compounds. We followed the same approach as above by comparing the significance of the
294 insularity effect in models without *vs.* with covariates (climatic variables). We ran
295 mechanistic models with covariates (and thus performed the model comparison) only for
296 phenolic compounds (i.e., condensed tannins) for which the effect of insularity was
297 significant in the initial model without covariates.

298

299 RESULTS

300 *Effect of insularity on leaf herbivory and plant chemical defences*

301 We found a significant effect of insularity on insect herbivory (Table 1a). In accordance with
302 predictions, leaf damage was 1.9-fold lower on holm oaks found on islands (percent leaf
303 damage score: 0.50 ± 0.05) than on mainland sites (0.96 ± 0.05) (Fig. 2a). We also found a
304 significant effect of insularity on leaf condensed tannins (Table 1a), but in an inverse manner
305 to herbivory as the concentration of these compounds was 1.9-fold greater for holm oak trees
306 on islands ($1.84 \pm 0.21 \text{ mg g}^{-1} \text{ d.w.}$) than on mainland locations ($0.98 \pm 0.20 \text{ mg g}^{-1} \text{ d.w.}$) (Fig.
307 2b). There was no effect of insularity on flavonoids (Table 1a, Fig. 2c) or hydrolysable
308 tannins (Table 1a, Fig. 2d). In all cases, we found no effect of phylogeographic group on
309 herbivory or plant chemical defences (Table 1a).

310 *Underlying predictors associated with insularity effects on herbivory and leaf defences*

311 Results from the mechanistic model for herbivory indicated that the effect of insularity on leaf
312 herbivory remained significant after including condensed tannins on the one hand, or
313 temperature and precipitation on the other (Table 1b), suggesting that neither chemical
314 defences nor climate accounted for lower levels of insect herbivory on islands relative to
315 mainland. Results from the herbivory model including condensed tannins indicated no
316 significant effect of this predictor (Table 1b), whereas the model including climatic factors
317 indicated a significant positive effect of precipitation on herbivory (slope estimator $[\beta] =$
318 0.00049 ± 0.00013), but no effect of temperature (Table 1b).

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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 vs. mainland populations were not explained by climatic conditions. In addition, we found no significant effects of temperature and precipitation on condensed tannins (Table 1c).

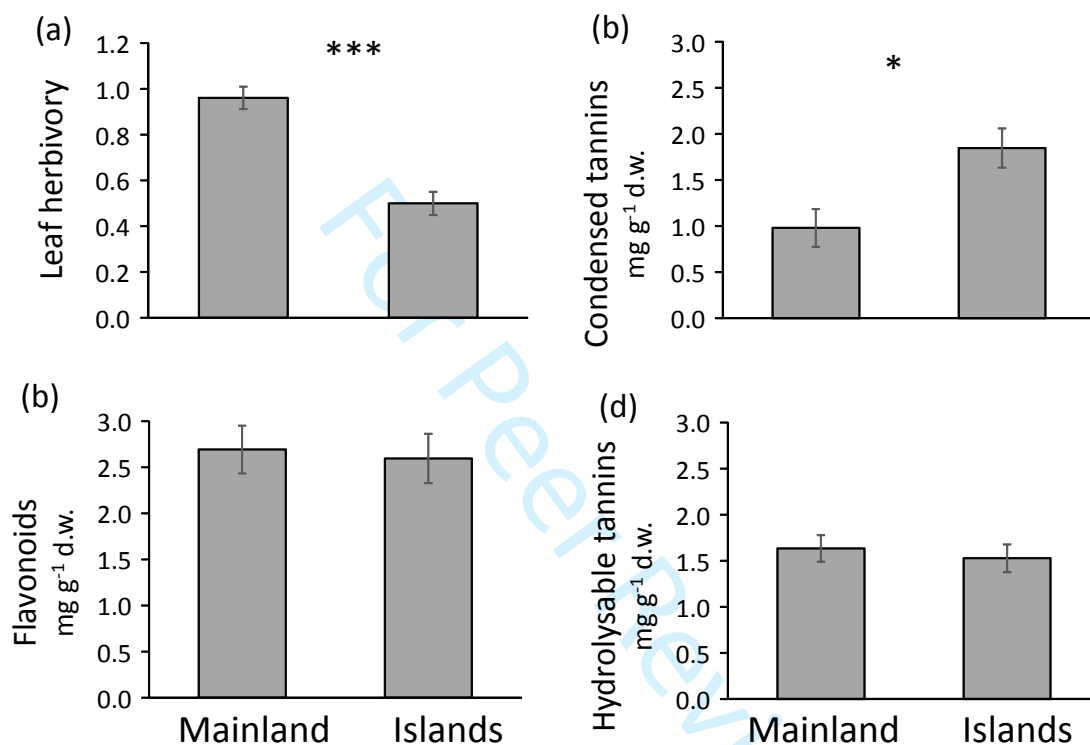


Figure 2. Leaf herbivory by chewing insects (a), and the concentration (in mg g⁻¹ d.w.) of leaf condensed tannins (b), flavonoids (c), and hydrolysable tannins (d) in holm oak (*Quercus ilex*) trees sampled on mainland and on islands (N = 30 and N = 28, respectively) in the Mediterranean Basin (from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

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). By accounting for these covariates, we tested whether insularity effects were mediated by differences in leaf traits and/or climate (see *statistical analyses* 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.

<i>(a) Without covariates</i>								
	Insularity		Phylogeography					
	<i>F</i> _{1,54}	<i>P</i>	<i>F</i> _{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				
<i>(b) Herbivory mechanistic model</i>								
<i>(b1) Defence as a covariate</i>	Insularity		Phylogeography		Condensed tannins			
	<i>F</i> _{1,53}	<i>P</i>	<i>F</i> _{2,53}	<i>P</i>	<i>F</i> _{1,53}	<i>P</i>		
Herbivory	26.06	<0.001	1.23	0.301	1.38	0.245		
<i>(b2) Climate as a covariate</i>	Insularity		Phylogeography		Temperature		Precipitation	
	<i>F</i> _{1,52}	<i>P</i>	<i>F</i> _{2,52}	<i>P</i>	<i>F</i> _{1,52}	<i>P</i>	<i>F</i> _{1,52}	<i>P</i>
Herbivory	30.35	<0.001	4.68	0.012	0.27	0.606	13.85	0.001
<i>(c) Defence mechanistic model</i>								
Condensed tannins	Insularity		Phylogeography		Temperature		Precipitation	
	<i>F</i> _{1,52}	<i>P</i>	<i>F</i> _{2,52}	<i>P</i>	<i>F</i> _{1,52}	<i>P</i>	<i>F</i> _{1,52}	<i>P</i>
Condensed tannins	4.72	0.034	2.80	0.070	0.45	0.506	0.85	0.362

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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; Hausdorf & Hennig, 2005; Dapporto & Dennis, 2009). 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; Vourc'h et al., 2001; Skaïen & Arcese, 2018), 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 et al., 2018a,b). 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 since a recent study by Monroy & García-Verdugo (2019) similarly found that insular populations (Canary Islands, Cape Verde) of the Mediterranean shrub *Periploca laevis* 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 vs. mainland sites. However, our analysis argues against this interpretation since 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 et al., 2016b; Moreira et al., 2018a). 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).

Future work

Our analyses indicated no effects on herbivory or oak defences of phylogeographic region, 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 (Pearse & Hipp, 2012; Moreira et al., 2018a), 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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30 565 BIOSKETCH

31 566 **Xoaquín Moreira is interested in the** ecological and evolutionary processes that occur
32 567 among different trophic levels (plants, herbivores and natural enemies), and predict how the
33 568 future global change might influence not only each species individually, but the various
34 569 interactions as a whole.

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Journal of Biogeography

SUPPORTING INFORMATION

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

Xoaquín Moreira, Bastien Castagneyrol, Raúl de la Mata, Nikolaos M. Fyllas, Andrea
Galmán, Carlos García-Verdugo, Asier R. Larrinaga, and Luis Abdala-Roberts

For Peer Review

Table S1. Summary of results from linear mixed models testing for the effects of insularity (two levels: mainland vs. insular) and phylogeographic group (three levels: the central and the western Mediterranean and the Aegean region) on leaf herbivory by chewing insects and the concentration of leaf chemical defences (flavonoids, condensed tannins, and hydrolysable tannins) in holm oak (*Quercus ilex*) populations (N = 30 mainland, N = 28 insular) using the individual tree as replicate and including population as a random factor. F-values with degrees of freedom (numerator, denominator) and associated significance levels are shown. Significant *P*-values ($P < 0.05$) are in bold.

	Insularity		Phylogeography	
	F _{1,54}	<i>P</i>	F _{2,232}	<i>P</i>
Herbivory	27.60	<0.001	1.58	0.207
Condensed tannins	5.38	0.024	3.33	0.038
Flavonoids	0.49	0.485	1.09	0.339
Hydrolysable tannins	0.04	0.852	0.02	0.983

Table S2. Summary of results from a multiple regression model testing for the effects of the concentration of leaf condensed tannins, flavonoids, and hydrolysable tannins on leaf herbivory in holm oak (*Quercus ilex*) trees growing in populations from mainland and insular locations (N = 30 populations from mainland locations and N = 28 populations from insular locations). In all cases, we used mean values across plants within each location. β = slope estimator, r^2 = partial correlation coefficient. Significant ($P < 0.05$). P -values are typed in bold.

	Leaf herbivory		
	β	r^2	P -value
Condensed tannins	-0.099	0.116	0.010
Flavonoids	-0.005	0.0001	0.941
Hydrolysable tannins	-0.041	0.0022	0.731