

1 **Field sampling and common gardens provide evidence for reductions in herbivore defense**
2 **traits in the California Channel Islands flora**

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11 **Abstract**

13 Reduced defense against large herbivores has been suggested to be part of the “island
14 syndrome” in plants. However, empirical evidence for this pattern is mixed. In this paper, we
15 present two studies that compare putative physical and chemical defense traits from plants on
16 the California Channel Islands and nearby mainland, based on sampling of both field and common
17 garden plants. In the first study, we focus on five pairs of woody shrubs from three island and
18 three mainland locations and find evidence for increased leaf area, decreased marginal leaf
19 spines, and decreased concentrations of cyanogenic glycosides in island plants. Insularity effect
20 sizes were similar between plants sampled *in situ* versus common gardens, suggesting that
21 observed reductions are not solely driven by environmental differences between island and
22 mainland sites. In the second study, we conducted a common garden experiment with a
23 perennial herb—*Stachys bullata* (Lamiaceae)—collected from two island and four mainland
24 locations. Compared to their mainland relatives, island genotypes show highly reduced glandular
25 trichomes and a nearly 100-fold reduction in mono- and sesquiterpene compounds from leaf
26 surfaces. Island genotypes also had significantly higher specific leaf area, somewhat lower rates
27 of gas exchange, and greater aboveground biomass than mainland genotypes across two years
28 of study, potentially reflecting a broader shift in growth habit. Together, our results provide
29 evidence for reduced expression of putative defense traits in island plants, though these results
30 may reflect adaptation to both biotic (i.e., historical absence of large herbivores) and climatic
31 conditions on islands.

33 **Keywords:** islands, plant defense, marginal spines, specific leaf area, cyanogenic glycosides,
34 *Stachys*, terpenes

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37 Plant defenses against herbivory are thought to be energetically costly due to allocational
38 tradeoffs with plant growth (Coley et al. 1985, Herms and Mattson 1992), leading to predictions
39 that constitutive defenses should be proportional to the risk of attack by herbivores. One way to
40 understand the evolution of plant defensive traits is to use naturally-occurring gradients of
41 herbivory intensity to test for concomitant variation in plant defenses, either within (e.g.
42 Pennings and Silliman 2005, Hahn et al. 2019) or across species (e.g. Levin 1976, Schemske et al.
43 2009). Examples of this approach include comparative studies that find reductions in defensive
44 traits in plants at higher latitudes (Rasmann and Agrawal 2011) and higher elevations (Pellissier
45 et al. 2014), concordant with the idea that herbivory is more intense in the tropics and at lower
46 elevations.

47 One commonly studied contrast in herbivory intensity is between plants from oceanic
48 islands and their mainland relatives. These contrasts are most informative when islands are
49 missing entire assemblages of herbivores—particularly large mammalian herbivores that are
50 poor overwater dispersers (Whittaker and Fernández-Palacios 2007)—and their constituent
51 floras have evolved in isolation for extended periods. Reduced defenses are often considered to
52 be part of the “island syndrome” in plants, which also involves reductions in dispersal ability,
53 increased woodiness, and increased reliance on clonal reproduction (Carlquist 1974, Burns et al.
54 2019, Ottaviani et al. 2020, Zizka et al. 2022). Numerous studies have used either conspecific or
55 congeneric comparisons of island and mainland taxa and found reduced expression of putative
56 defense traits on islands, including reductions in marginal leaf spines (Bowen and Van Vuren
57 1997, Burns 2016), prickles (Burns 2014), divaricate branching (Kavanagh 2015), root alkaloids
58 (Watts et al. 2011), and leaf tannins (Shimazaki and Miyashita 2002). Many studies and reports
59 have also noted that plants from oceanic islands are highly palatable to non-native mammalian
60 herbivores (Atkinson 1989, Bryant et al. 1989, Greenwood 1992, Bowen and Van Vuren 1997,
61 Cubas et al. 2019), which has been used as evidence of reduced defensive traits.

62 A recent meta-analysis of studies comparing island and mainland plants found evidence
63 for increased mammalian herbivore damage in island compared to mainland plants, supporting
64 the idea that island plants may be more susceptible to mammalian herbivores (Moreira et al.
65 2021). However, this meta-analysis did not find consistent support for reductions in plant
66 defensive traits on islands across a set of 173 island/mainland comparisons, and subsequent
67 comparative studies have also failed to support the idea of reduced defensive traits in island taxa
68 (Moreira et al. 2022). Thus, the degree to which island plants show reduced defensive traits
69 seems highly variable. Reasons for a lack of reduced defensive trait expression in island plants
70 are numerous (see Moreira and Abdala-Roberts 2022) but might include (i) recent introduction
71 of non-native mammalian herbivores that favor increased defensive traits, either through
72 phenotypic plasticity or directional selection; (ii) an extended history of coevolution with native

Introduction

73 large herbivores that are no longer present (e.g., Bond and Silander 2007); (iii) selection by
74 invertebrate herbivores on islands; (iv) ongoing gene flow from mainland populations that limits
75 the degree of differentiation in island populations.

Even in instances where studies do find evidence for reductions in plant defensive traits on islands, methodological drawbacks may limit the scope of these conclusions. First, many studies only include comparisons from a single conspecific or congeneric pair, and likewise, measurements may only contrast a single island and single mainland site. Thus, it is often unclear whether trait divergence between islands and mainland plants is the product of adaptive divergence or idiosyncrasy. Second, few studies have attempted to measure plant traits from island and mainland genotypes grown under common environmental conditions (but see Skaien and Arcese 2018, Monroy and García-Verdugo 2019), thereby raising the possibility that trait divergence is the result of differences in the abiotic environment between island and mainland sampling locations. Third, traits that are assumed to be defenses against herbivores may have alternative functions. For example, studies comparing island and mainland locations sometimes consider traits such as leaf area, leaf thickness, and condensed tannin concentrations as adaptations to herbivory, though these traits also have roles in primary metabolism and plant growth (Wright et al. 2004, Gourlay and Constabel 2019). Finally, and related to the third point, relatively few studies verify the importance of putative defensive traits using experiments with herbivores (but see Bryant et al. 1989, Bowen and Van Vuren 1997, Vourc'h et al. 2001, Watts et al. 2011, Salladay and Ramírez 2018).

In this paper, we present two studies comparing plant populations from the California Channel Islands and nearby mainland locations. In the first study, we used five taxonomic pairs of woody chaparral shrubs sampled across three island and three mainland sites, as well as two mainland common garden locations, to test for divergence in leaf area, specific leaf area, marginal leaf spinescence, and concentrations of cyanogenic glycosides. In the second study, we collected 44 genotypes of California hedgenettle (*Stachys bullata*) from two island and four mainland locations and grew them for two years in a mainland common garden to measure plant chemistry and growth-related traits.

Methods

Background – California Channel Islands

The California Channel Islands are a group of uplifted volcanic oceanic islands off the coast of southern California that arose over the past 5 million years (Pinter et al. 1998), ranging in size from 2.6 km² (Santa Barbara Island) to 249 km² (Santa Cruz Island) in land area (Figure 1A). The northern Channel Islands (including Santa Cruz and Santa Rosa) were periodically connected as a single landmass (Santa Rosae) during Pleistocene Ice Ages (Kennett et al. 2008), with as little as

111 10 km of separation between island and mainland. The southern Channel Islands (including Santa
112 Catalina) are generally more isolated from each other and the California mainland. The Channel
113 Islands flora has a high degree of endemism and features many examples of insular woodiness
114 and island gigantism (Junak et al. 1995, Guilliams et al. 2017).

115 Large mammalian herbivores have historically been absent from the California Channel
116 Islands—with the notable exception of the pygmy mammoth (*Mammathus exilis*) (Agenbroad
117 2012)—but cattle, sheep, and pigs were introduced in the 1800s. In the last 50 years, concerted
118 eradication efforts have removed large mammals from Santa Cruz and Santa Rosa Island;
119 introduced mule deer (*Odocoileus hemionus*) and American bison (*Bison bison*) are still present
120 on Santa Catalina Island. The Channel Islands also lack gophers, squirrels, and other burrowing
121 mammals that are present on the mainland.

122

123 Study 1: Chaparral shrub sampling

124

125 We selected six pairs of taxa characteristic of the chaparral plant community that occur
126 on both the California Channel Islands and the nearby southern California mainland. Pairs were
127 chosen to match those in Bowen and Van Vuren (1997) and consisted of either congeners or
128 conspecifics (Figure 1B) from three plants families: Rosaceae (*Cercocarpus*, *Prunus*, *Heteromeles*),
129 Papaveraceae (*Dendromecon*), and Rhamnaceae (*Ceanothus*). One species pair (*Quercus pacifica*,
130 *Q. berberidifolia*) was collected but not included in subsequent analyses, since phylogenetic
131 evidence published after Bowen and Van Vuren (1997) has shown that these taxa are not sister
132 species and diverged in the Miocene (>5.3 mya) (Hipp et al. 2020). For the remaining five
133 taxonomic pairs, we took reconnaissance trips to each collection site in 2015 and noted the
134 location of focal taxa using a handheld Garmin GPS. We then returned to these locations in
135 February and March of 2016 to collect leaf tissue for use in morphological and chemical analysis.
136 In total, we sampled 291 individual plants from five taxonomic pairs across six sites (three island,
137 three mainland), for an average of approximately 10 plants per site (Figure 1B).

138 We collected leaf tissue for morphological analysis from focal plants by clipping branches
139 containing variable numbers of leaves. When possible, we collected a branch from both the lower
140 (<1 m in height) and the upper (>2 m in height) portion of the plant canopy to capture
141 morphological differences associated with accessibility to mammalian herbivores. For analysis of
142 cyanogenic glycosides, we collected individual leaves from the lower portion of the plant canopy
143 for three species (*Heteromeles*, *Prunus*, *Cercocarpus*), and when possible, included both fully
144 mature/expanded leaf tissue as well as young/actively expanding leaf tissue. Leaf chemistry
145 samples were immediately frozen on dry ice and were later transferred to a -80°C freezer until
146 processing. For each sampled plant, we recorded its GPS coordinates (see Figure S1), elevation,
147 and slope aspect (when relevant) using a handheld Garmin GPS device, and we also recorded the
148 approximate stem diameter at 0.25 m above the ground using a digital caliper.

149 For each sampled branch, leaves were removed and imaged using a flatbed scanner with
150 a scalebar. We recorded the following measurements from each leaf: total area (including
151 petiole), area and length (not including petiole), percent of leaf tissue missing due to herbivory,
152 and marginal leaf spinescence. All measurements were taken using ImageJ v. 1.51 (Schneider et
153 al. 2012). For a visual depiction of our measurement protocol, see Figure S2. Non-fully expanded
154 leaves ($n = 809$) were measured but were excluded from subsequent analyses. We also measured
155 specific leaf area (SLA) at the level of branches by taking the cumulative area of all fully expanded
156 leaves (in cm^2) and dividing this by their cumulative mass (in g).

157 To measure cyanogenic glycoside (CNglc) content, we followed a modified version of the
158 evolved hydrogen cyanide (HCN) protocol described in Experiment 2 of Gleadow et al. (2011).
159 We only collected tissue for species in the Rosaceae (*Cercocarpus*, *Heteromeles*, *Prunus*), which
160 are known to produce CNglcs. For a full description of methods used to quantify CNglc content,
161 see Supplemental Materials. In total, we generated 194 measurements of CNglc content from
162 108 individual plants.

163 We also sampled leaf tissue from two botanical gardens (Santa Barbara Botanic Garden
164 and Rancho Santa Ana Botanic Garden) on the mainland that featured island and mainland
165 genotypes of the species of interest (Figure 1B), grown from either seed or cuttings. All leaf tissue
166 collection, morphological analysis, and chemical analysis was conducted in the same way as
167 described above, although SLA was not measured for common garden plants. In total, we
168 sampled an additional 40 plants (18 island and 22 mainland genotypes) from these common
169 garden environments (Figure 1B).

170 We also took advantage of a series of herbivore exclosures on Santa Catalina Island (see
171 Ramírez et al. 2012, Dvorak and Catalano 2016)—which still has introduced deer and bison
172 present—to test for the potential effects of herbivore-mediated plasticity in plant traits. Because
173 of the relatively small number of intact exclosures available, our sampling across species was
174 somewhat uneven, though we were able to sample a total of 24 plants inside of exclosures and
175 35 plants outside of exclosures (Table S1).

176

Study 1: Climatic variation between sites

177

178 Island and mainland sites have generally similar climates, although island locations may
179 have more frequent nocturnal fog that reduces summertime evaporative water loss (Fischer et
180 al. 2016, Ramírez et al. 2020). To formally measure climatic differences between island and
181 mainland locations, we used recorded coordinates from each plant to extract bioclimatic
182 variables from the WorldClim2 database at 1 km resolution (Fick and Hijmans 2017) (Figure S3).
183 We used principal component analysis (PCA) to explore variation in climate data and found that
184 the first PC axis explained more than 83% of overall variation (Figure S4) and was dominated by
185 a single bioclimatic variable, temperature seasonality (bio4) (Figure S5). This axis separated island

187 sites from the two more inland mainland sites (Stunt Ranch, Santa Monica Mtns.), which have
188 higher temperature seasonality, while the third mainland site (Gaviota) had lower temperature
189 seasonality and was more akin to island sites. The second PC axis explained 13% of overall
190 variation and included loadings for precipitation-related variables; this axis separated the drier
191 Santa Catalina Island from all remaining sites (Figure S4).

192

Study 1: Statistical analyses

193

194

195 We analyzed our data using multilevel linear mixed models implemented in the lme4
196 package (Bates et al. 2015) in R version 4.1.3 (R Core Team) to account for the hierarchical nature
197 of our data. Response variables of interest were leaf area, specific leaf area (SLA), marginal leaf
198 spinescence, and leaf CNglc content. Leaf area and CNglc content were log transformed to ensure
199 that model-estimated confidence intervals were above 0; SLA and marginal leaf spinescence
200 were untransformed. For marginal leaf spinescence, we only included *Heteromeles* and *Prunus*,
201 since these were the only species with stiff rigid spines (Figure 1C). Likewise, because CNglc levels
202 in *Cercocarpus* were ~100x lower than in *Prunus* and *Heteromeles* (and often below our detection
203 limit), CNglc analysis was restricted to the latter two species. Covariates that were included in
204 each model included site of collection, canopy position (upper versus lower), north/south slope
205 aspect, and east/west slope aspect. We considered including elevation and stem diameter (as a
206 proxy for plant age) as covariates, though because of limited within-site and within-species
207 variation in these measures, we ultimately omitted them from analyses. Furthermore, we
208 attempted to include bioclimatic variables as covariates in these models, but because of the
209 relatively limited spatial scale of sampling across sites (Figure 1A) and within sites (Figure S1) and
210 the 1 km² resolution of the bioclim dataset, we captured relatively little overall climatic variability
211 for most bioclimatic variables (Figure S3, S6).

212 For each response variable, we fit an overall model that included all samples collected *in*
213 *situ* across all species ($n = 4,096$ leaves from 291 plants). These models were of the form (in lme4
214 syntax):

215

216 ***Response variable ~ IM*Species + Covariates + (1|Site/Plant.ID/Branch.ID)***

217

218 where IM corresponds to whether samples came from an island or mainland site. Plant species
219 interacts with island vs. mainland status to allow for variation in the magnitude of island vs.
220 mainland contrasts across species. Collection site was included as a random intercept, with plant
221 ID nested within site, and branch ID nested within plant ID. Since specific leaf area was calculated
222 by pooling leaves from within branches, the SLA model does not include a branch ID term. To
223 assess within-species differences in trait expression between islands and mainland locations, we
224 used the emmeans package (Lenth 2023).

225 For two of the response variables (marginal spinescence, CNglc content), we included
226 additional parameters based on *a priori* hypotheses. In the model considering marginal
227 spinescence, we included an interaction between island/mainland status and canopy position to
228 allow for the degree of spinescence heteroblasty to vary across locations (e.g. Burns 2014). In the
229 model considering CNglc content, we included a term for leaf age (old vs. young) based on our
230 sampling scheme and predictions from optimal plant defense theory that younger leaf tissue
231 should be more heavily defended against herbivores (e.g., Herms and Mattson 1992).

232 To test for genetically based differences in trait values, we analyzed samples collected
233 from common garden samples in a separate set of linear mixed models. These models were
234 similar to those described above and were of the form:

235

236 ***Response variable ~ Source_IM*Species + Covariates + (1/Plant.ID/Branch.ID)***

237

238 where Source_IM refers to whether the plants' original provenance was an island or mainland
239 location. As above, we also estimated within-species differences between island and mainland
240 locations.

241 To compare insularity effects across studies (our field sampling, our common garden
242 sampling, and the field sampling from Bowen and Van Vuren [1997]), we calculated standardized
243 effect sizes (Cohen's d_s) for each measured trait. Since Bowen and Van Vuren (1997) only report
244 t statistics and sample sizes, we used the following formula for Cohen's d_s :

245

$$d_s = t \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

247

248 where t corresponds to the mean of their reported t statistics, and n_1 and n_2 correspond to
249 sample sizes from island and mainland locations. To generate effect size estimates and
250 corresponding confidence intervals from our in situ and common garden sampling, we used the
251 effectsize package (Ben-Shachar et al. 2020).

252

253 Finally, to test for effects of introduced herbivores on plant traits on Santa Catalina Island,
254 we separately analyzed all trait data from Santa Catalina and included a term to account for
255 whether samples came from inside versus outside of an herbivore enclosure.

256

Study 2: *Stachys bullata* – background

257

258 *Stachys bullata* (Lamiaceae) is a perennial herbaceous plant that occurs in coastal
259 California from approximately Orange County to the San Francisco Bay Area, with populations
260 present on Santa Cruz, Santa Rosa, and Anacapa Islands. It reproduces both clonally via rhizomes

261 and sexually and is described as being glandular, with aromatic foliage that is characteristic of
262 many plants in the Lamiaceae. However, island populations have been noted to have non-
263 aromatic foliage as well as larger leaves and flowers than their mainland relatives (Junak 1995),
264 and densities of glandular trichomes appear to be much lower on island plants (Figure 2C).

265

266 Study 2: *Stachys bullata* common garden experiment

267

268 To determine whether observed trait variation between island and mainland *S. bullata*
269 populations is environmentally or genetically determined, we set up a multi-year common
270 garden experiment in which we grew island and mainland *S. bullata* genotypes together at the
271 Santa Barbara Botanic Garden (SBBG). Plants were collected in the field in late 2015 from two
272 island (Santa Cruz, Santa Rosa) and four mainland locations (Figure 2A) as rhizomes, which were
273 transported to UC Davis and shallowly planted in potting mix. Plants were grown in 1 gallon pots
274 for approximately three months and were then split into clonal replicates that were grown in
275 their own 1 gallon pots. In total, we collected 44 *S. bullata* genotypes that were separated into
276 112 individual plants (Figure 2B).

277 In February 2016, we set up a common garden plot at the SBBG (Figure 2D). The plot was
278 located on an east-facing slope that received partial or full sun throughout the year. Plants were
279 spaced at a distance 1 m apart from each other in a gridded pattern. The plot was surrounded by
280 a 2 m fence to prevent browsing by deer, and each plant was enclosed in a cage made from
281 hardware cloth to limit root herbivory by pocket gophers (*Thomomys bottae*), which were
282 common at the site. We installed a drip irrigation system to assist with initial plant establishment.
283 Plants were outplanted randomly with respect to island/mainland status in late February and
284 early March of 2016 and received approximately 2L of water from a drip irrigation system at 1
285 week intervals between March-August 2016. In late August 2016, we ceased supplemental
286 watering, and plants subsequently only received water from ambient precipitation. Plants
287 became dormant in October 2016, and then subsequently began to regrow naturally in early
288 February 2017. In addition, we set up a smaller common garden at the Santa Cruz Island Reserve,
289 although due to concerns over introduction of non-native genotypes, this common garden
290 consisted of only genotypes from Santa Cruz Island.

291 We generated four categories of data from common garden *S. bullata* plants. Three
292 measures (biomass, SLA, gas exchange) were related to plant growth, while one measure (leaf
293 surface chemistry) was related to plant defense. For biomass measurements, we collected all
294 above-ground biomass at the end of each growing season (in 2016 and 2017) and recorded its
295 dry mass. For SLA, we collected leaf tissue from each plant in April of 2017 and measured leaf
296 area and dry mass from fully expanded *S. bullata* leaves. In April 2017 we used a Li-6800 portable
297 photosynthesis system (LI-COR Biosciences Inc., Lincoln, NE) to measure gas exchange rates on

298 the most recent mature leaves that were sun exposed. For details on gas exchange
299 measurements, see Supplemental Materials.

300 For leaf chemistry, we focused on volatile organic compounds (VOCs) present on leaf
301 surfaces and in glandular trichomes. We used a modified version of the protocol described in
302 Pratt et al. (2014) for measuring terpenes in *Artemisia californica* (Asteraceae). Briefly, in April of
303 2017, we used a hole punch to collect six leaf discs, each from a different leaf, from approximately
304 75 *Stachys* plants across all genotypes. Leaf surface chemistry was quantified using gas
305 chromatography-mass spectrometry (GCMS). For a full description of chemical methods, see
306 Supplementary Materials.

307

308 Study 2: *Stachys* data analysis

309

310 We analyzed aboveground biomass using a linear-mixed effects model of the form:

311

312 ***Aboveground biomass ~ IM + Year + (1/Source.Pop/Genotype) + (1/Column) + (1/Row)***

313

314 where IM refers to whether a given plant originated from an island or mainland site and column
315 and row refer to the location of plants within the common garden grid. To analyze gas exchange
316 measurements, because of our smaller sample, we used a simple linear model with net carbon
317 assimilation (A_{net}) as the response variable and provenance (island vs. mainland).

318 To analyze plant chemistry, we divided each integrated peak area by its corresponding
319 internal standard peak area to standardize all values. We added all peaks together to get a
320 cumulative compound abundance measure and also separated compounds based on their
321 biochemical pathway (e.g., monoterpenes, sesquiterpenes, aromatic compounds). We used non-
322 metric multidimensional scaling to visualize compositional differences between sites.

323

324 Results

325

326 Chaparral shrubs

327

328 We found evidence for increased leaf area and specific leaf area and decreased marginal
329 spines and cyanogenic glycosides in island plants, with the magnitude of these effects differing
330 across species and for field vs. common garden sampling. For field sampled plants, leaf area was
331 significantly higher on islands for *Ceanothus* ($t = 5.058$, $p < 0.001$), *Cercocarpus* ($t = 2.558$, $p =$
332 0.018), *Dendromecon* ($t = 6.123$, $p < 0.001$), and *Prunus* ($t = 7.817$, $p < 0.001$), but not for
333 *Heteromeles* ($t = 1.793$, $p = 0.095$) (Figure 3A, Figure S7A). Overall leaf area patterns were similar
334 for common garden plants, though only *Prunus* was significantly larger from islands ($t = 3.616$, p
335 = 0.001) (Figure S8A). For field sampled plants, specific leaf area (SLA) was significantly higher for

336 *Ceanothus* ($t = 2.554$, $p = 0.015$) and *Prunus* ($t = 3.403$, $p = 0.002$) (Figure 3B, Figure S7B); SLA was
337 not measured for common garden plants. Overall, leaves sampled from the upper canopy were
338 significantly smaller than leaves sampled from the lower canopy ($t = -2.877$, $p = 0.004$). SLA was
339 also lower for leaves from the upper canopy ($t = -6.694$, $p < 0.001$), and SLA was higher for plants
340 growing on north-facing ($t = 3.369$, $p < 0.001$) and west-facing aspects ($t = 3.110$, $p = 0.002$) (Table
341 S2).

342 Among traits with clearer links to defense against herbivores, we found significantly
343 reduced marginal leaf spines in field sampled plants for island *Prunus* ($t = -8.484$, $p < 0.001$) and
344 marginally reduced leaf spines in island *Heteromeles* ($t = -2.406$, $p = 0.059$) (Figure 3C, Figure
345 S10). These differences were similar for common garden plants, with significantly reduced
346 marginal spines in island *Prunus* ($t = -6.568$, $p < 0.001$) but not in *Heteromeles* ($t = -1.422$, $p =$
347 0.180) (Figure S8B). Marginal spines were less prevalent on leaves from the upper canopy (Figure
348 S9), and spinescence heteroblasty was more pronounced in mainland plants, with larger
349 differences in marginal spines between upper and lower canopy positions than on islands ($t =$
350 2.689, $p = 0.008$) (Figure S9). Similar patterns were observed in common garden plants, with more
351 pronounced spinescence heteroblasty in mainland plants ($t = 3.187$, $p = 0.006$).

352 Field sampled plants had significantly reduced levels of cyanogenic glycosides (CNglcs) for
353 *Heteromeles* ($t = -2.635$, $p = 0.037$) and modestly reduced levels for *Prunus* ($t = -1.796$, $p = 0.122$)
354 (Figure 3D, Figure S7D). For common garden plants, CNglcs were marginally reduced in both
355 *Prunus* ($t = -2.000$, $p = 0.058$) and *Heteromeles* ($t = -1.841$, $p = 0.085$) (Figure S8C). Overall,
356 younger leaf tissue contained significantly higher concentrations of CNglcs ($t = 7.689$, $p < 0.001$),
357 and the magnitude of the island/mainland difference in CNglcs was more pronounced for older
358 leaf tissue ($t = 3.532$, $p < 0.001$) (Figure S10).

359 Overall insularity effect sizes were comparable between our field and common garden
360 sampling, but lower than estimates from an earlier study in the same system (Bowen and Van
361 Vuren 1997) (Figure 4). The strongest observed differences between island and mainland
362 locations were for marginal spinescence, where overall effect sizes ranged from 1.61 (common
363 garden plants) to 1.88 (field plants) to 2.14 (Bowen and Van Vuren).

364 We did not find any significant differences for any measured traits inside versus outside
365 of herbivore exclosures on Catalina Island (Table S3).

366

Stachys common garden

368

369 Of the 112 plants originally transplanted in 2016, 108 survived through the first year, and
370 103 survived through the second year. Plants had significantly higher biomass in 2016 when they
371 received supplemental water ($t = 12.094$, $p < 0.001$). Overall, island genotypes grew modestly
372 larger than their mainland relatives ($t = 3.303$, $p = 0.067$) (Figure 5A); the absolute difference in
373 biomass across years was identical, with island plants supporting an average of 54.9g of

374 additional biomass in each year (Figure 5A). Island plants had significantly higher SLA than their
375 mainland relatives ($t = 3.073$, $p = 0.042$) (Figure 5B). Island plants generally had higher rates of
376 gas exchange than mainland plants (Figure 5C), although this difference was not significant ($t =$
377 1.717 , $p = 0.099$). Plants with higher values of SLA had lower rates of carbon assimilation ($t = -$
378 2.389 , $p = 0.026$) (Figure 5D).

379 Consistent with observations from *Stachys* growing in situ, island and mainland genotypes
380 sampled from the common garden had markedly different chemical compositions, both in terms
381 of absolute abundance and the presence/absence of compounds (Figure 6B) (PERMANOVA: F_{site}
382 = 38.71 , $p < 0.001$). The most pronounced chemical difference between island and mainland
383 genotypes was for mono- and sesquiterpenes, with island genotypes showing an approximate
384 100-fold reduction in the abundance of these compounds (Figure 6C). Santa Cruz Island
385 genotypes did not differ in leaf chemistry based on whether they were grown on Santa Cruz Island
386 versus the mainland (Figure S11).

387

388

Discussion

389

390 We found general support for divergence in plant traits between islands and mainland
391 sites, including a reduction in putative plant defense traits (marginal spines, cyanogenic
392 glycosides, terpenes) and an increase in traits associated with growth in island plants (leaf area,
393 specific leaf area) (Wright et al. 2004). These results were consistent across both field-sampled
394 and common garden plants and match the predictions of the island syndrome. However, the
395 magnitude of insularity effects varied widely across our sampled taxa, and we did not directly
396 assess whether the traits we measured actually deterred mammalian herbivory. Furthermore,
397 our results reflect some degree of ascertainment bias: the shrub species that we measured were
398 already noted to have reduced levels of putative defenses in a previous study (Bowen and Van
399 Vuren 1997), and *Stachys bullata* was chosen for study specifically because of its pronounced
400 phenotypic differentiation between island and mainland locations (Junak 1995). Thus, although
401 we find evidence for reduced putative defenses in island flora, our results do not invalidate recent
402 analyses that challenge the generality of reduced defenses as part of the island syndrome (e.g.,
403 Moreira et al. 2022).

404 One of the clearest traits that showed reduced expression in island taxa was marginal leaf
405 spinescence (Figure 1B), consistent with two previous studies of chaparral shrubs from the
406 Channel Islands (Bowen and Van Vuren 1997, Salladay and Ramirez 2018). Spinescence in the
407 broad sense (e.g., prickles, thorns, and leaf spines) has been suggested as a defensive adaptation
408 for deterring mammalian herbivores (Charles-Dominique et al. 2016); spinescence may also have
409 ecophysiological functions (e.g., Barton 2014), although the physiological role of leaf margin
410 morphology (including marginal spines) has rarely been evaluated (but see Givnish and Kriebel
411 2017). In a recent survey of spinescence in island flora, Barton et al. (2024) highlighted many

412 examples of island taxa that remain spinescent, potentially due to past selection by now-extinct
413 megafauna. By contrast, with the exception of the pygmy mammoth (which has been extinct
414 since the Late Pleistocene) and the very recent introduction of grazing animals, the Channel
415 Islands flora is thought to have evolved without any large mammalian herbivores present.

416 Consistent with previous research on spinescence across plant development, we found
417 support for a pattern of spinescence heteroblasty, with leaves lower in the canopy showing
418 greater spinescence (e.g. Clark and Burns 2015). This pattern was more pronounced for mainland
419 plants, mirroring the results of Burns (2014), who also found a weaker vertical gradient in leaf
420 spinescence within *Drypetes deplanchei* from Lord Howe Island compared to mainland Australia.
421 Taken together, our results suggest that the reduced marginal leaf spines in the Channel Islands
422 flora may reflect reduced selection by mammalian herbivores, though we cannot rule out
423 alternative ecophysiological explanations.

424 We also found evidence for reduced cyanogenic glycosides (CNGlcS) on islands in the two
425 genera that we surveyed (*Prunus* and *Heteromeles*), although the magnitude of this reduction
426 was less pronounced than for marginal spinescence. Leaf CNGlc content was lower for island
427 compared to mainland plants grown in common gardens, suggesting a genetic basis for this
428 reduction. CNGlcS are acutely toxic to many vertebrate herbivores and are generally thought to
429 have evolved as defenses as herbivores (Gleadow and Woodrow 2002 and references therein).
430 Many other studies have found no difference (Vourc'h et al. 2001) or even increased chemical
431 defenses for island plants (Moreira et al. 2019), and at least one study found evidence for
432 increased levels of CNGlcS in relict island populations of *Prunus lusitanica* (Pardo et al. 2016). One
433 potential explanation for differences in CNGlc content relates to climate and freeze susceptibility:
434 because CNGlcS are spontaneously converted to HCN with freeze/thaw cycles, CNGlc production
435 may be lower in evergreen plants subject to freezing temperatures (e.g., Santangelo et al. 2022).
436 However, our island sites showed *less* temperature seasonality than mainland sites (Figures S3-
437 S5), which suggests that climatic differences between sites are unlikely to be the reason for
438 reduced CNGlcS in island *Heteromeles* and *Prunus*. Finally, we also found strong evidence for
439 ontogenetic decreases in CNGlc concentrations in older leaf tissue, a pattern previously shown in
440 *Heteromeles* (Dement and Mooney 1974) and other cyanogenic species (e.g. Goodger et al. 2006)
441 and concordant with optimal plant defense theory. Thus, our observed reductions in CNGlc
442 content are also consistent with relaxed selection from mammalian herbivores in the Channel
443 Islands.

444 Although we did not directly assess the efficacy of putative leaf defenses against
445 herbivores, two studies using plants from the Channel Islands have shown that mammalian
446 herbivores perceive differences between island and mainland plants. Bowen and Van Vuren
447 (1997) showed that sheep preferentially consumed leaf tissue from plants collected on Santa
448 Cruz Island compared to a mainland location, and Salladay and Ramírez (2018) likewise showed
449 the same pattern with goats and plant tissue from Santa Catalina Island. Thus, the reductions in

450 spinescence and CNGIcs that we measured (or potentially other correlated traits) seem to be
451 reasonable proxies for increased palatability to mammalian herbivores.

452 Because we sampled the same taxa as Bowen and Van Vuren (1997) (and at the same
453 time of year), we can directly compare our data on leaf area and marginal spines from Santa Cruz
454 Island to theirs. Insularity effects are in the same direction (Figure 4), although the magnitude of
455 island/mainland differences reported in Bowen and Van Vuren is larger. One intriguing
456 comparison would be to focus on the chrono-sequence of introduced sheep removal and
457 subsequent vegetation recovery from Santa Cruz Island (see Beltran et al. 2014). Sheep
458 eradication efforts involved sequentially fencing off sections of the island, with eradication on
459 the west end around 1980, but not on the east end until 2001 (Faulkner and Kessler 2011). One
460 might therefore predict stronger insularity effects on the western end of the island, where sheep
461 were eradicated first.

462 In our common garden study of *S. bullata*, we found unambiguous evidence for reductions
463 in leaf chemical compounds from island genotypes. These patterns were most pronounced for
464 monoterpenes and sesquiterpenes, a diverse group of plant secondary metabolites thought to
465 be involved in defense against herbivores and pathogens, plant communication, and modulating
466 thermal and oxidative stress (Loreto and Schnitzler 2010, Pichersky and Raguso 2018). Because
467 of the varied ecological functions of terpenes, it is difficult to ascribe their loss in island *S. bullata*
468 strictly to the absence of vertebrate herbivores, though their reduction is certainly consistent
469 with strong divergent selection between island and mainland environments. The reduction in leaf
470 secondary compounds was also accompanied by a strong reduction in leaf and stem trichomes
471 (Figure 2C), although we did not formally quantify their abundance.

472 Island *S. bullata* genotypes had significantly thinner and larger leaves and grew more than
473 mainland genotypes across both study years. In addition to accumulating more biomass, island
474 *S. bullata* populations also appeared to be taller, with woodier and more upright stems, as well
475 as increased branching on terminal inflorescences (Figure S12), consistent with evidence for
476 increased woodiness among island flora (Lens et al. 2013, Zizka et al. 2022). The smaller stature
477 of mainland *S. bullata* is reminiscent of a recent study that found that *Plectritis congesta*
478 populations from islands without deer grow to be 2.6 times taller than populations from nearby
479 islands with deer present (Skaien and Arcese 2018). In addition to having higher SLA (Figure 5B)
480 and accumulating more aboveground biomass than their mainland relatives, island *S. bullata* also
481 had marginally higher rates of carbon assimilation (Figure 5C); however, overall rates of carbon
482 assimilation were negatively correlated with SLA (Figure 5D). This result is consistent with a
483 recent analysis of Macaronesian island flora (Garcia-Verdugo et al. 2024), which hypothesized
484 that reduced temperature seasonality on islands (see Figures S3-S5) drives increases in leaf size
485 (as in Wright et al. 2017) but concomitant decreases in photosynthetic rates.

486 It may at first seem counterintuitive that island *S. bullata* genotypes outperformed their
487 mainland relatives when grown in a mainland common garden. However, it is important to note

488 that our experimental setup precluded herbivory by mainland deer and gophers (see fencing and
489 cages in Figure 3D), which may have disproportionately benefited island plants. Furthermore,
490 common garden plants were exposed to relatively mesic conditions that may have favored island
491 genotypes: in 2016 plants received supplemental water during their growing season, and the
492 2017 water year at the Santa Barbara Botanic Garden featured 118% of average annual rainfall
493 (63.3 cm, average = 53.4 cm).

494 An intriguing parallel to the reduced aromaticity and increased stature of island *Stachys*
495 can be seen in the Hawaiian mint *Haplostachys haplostachya*, which is part of an adaptive
496 radiation of more than species of 50 species of Hawaiian mints (also including *Phyllostegia* and
497 *Stenogyne*) derived from temperate North American *Stachys* (Lindqvist and Albert 2002, Roy et
498 al. 2015). The Hawaiian mints include numerous examples of derived viny and sub-shrub growth
499 forms (e.g. Wagner et al. 1999), and *H. haplostachya* is noted for its lack of leaf scent (Native
500 Plants of Hawaii Database). More generally, the species native to the Hawaiian Islands have been
501 noted to produce fewer mono- and sesquiterpenes than species recently introduced there
502 (Sardans et al. 2010), suggesting that reductions in terpene production may be common in island
503 plants.

504 In conclusion, we found evidence for reduced defensive traits in both chaparral shrubs
505 and a perennial herbaceous plant from the California Channel Islands. Future research comparing
506 the flora of the Channel Islands and the California mainland would benefit from a number of
507 approaches (also highlighted more broadly in Moreira and Abdala-Roberts 2022). First, studies
508 that focus on traits with unambiguous roles in plant defense against herbivores (e.g., latex
509 exudation, alkaloids, cardenolides) may be more insightful for understanding selection imposed
510 by herbivores than studies that focus on traits with multiple potential functions (e.g., phenolic
511 compounds and leaf area). Second, comparisons that involve broad sampling from multiple island
512 and mainland locations and from a wide range of taxa chosen without a priori knowledge of
513 putative defense trait expression will provide the most robust inferences as to the degree of
514 island/mainland divergence. Finally, for most taxa from the Channel Islands, there is no clear
515 phylogenetic hypothesis for explaining relatedness between island and mainland populations,
516 including estimates of divergence times and contemporary gene flow, and future studies would
517 greatly benefit from exploring this evolutionary history.

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520

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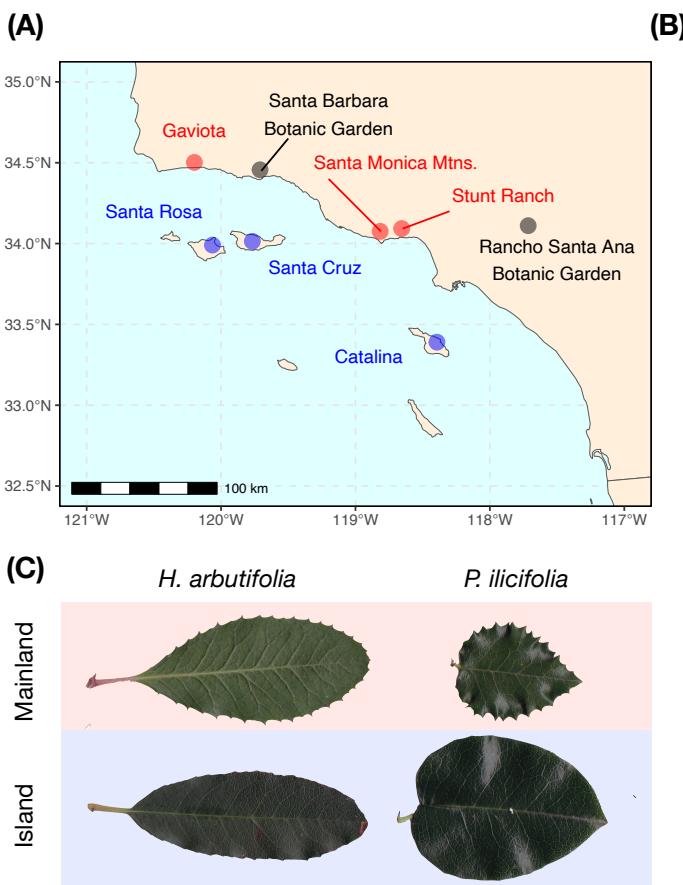
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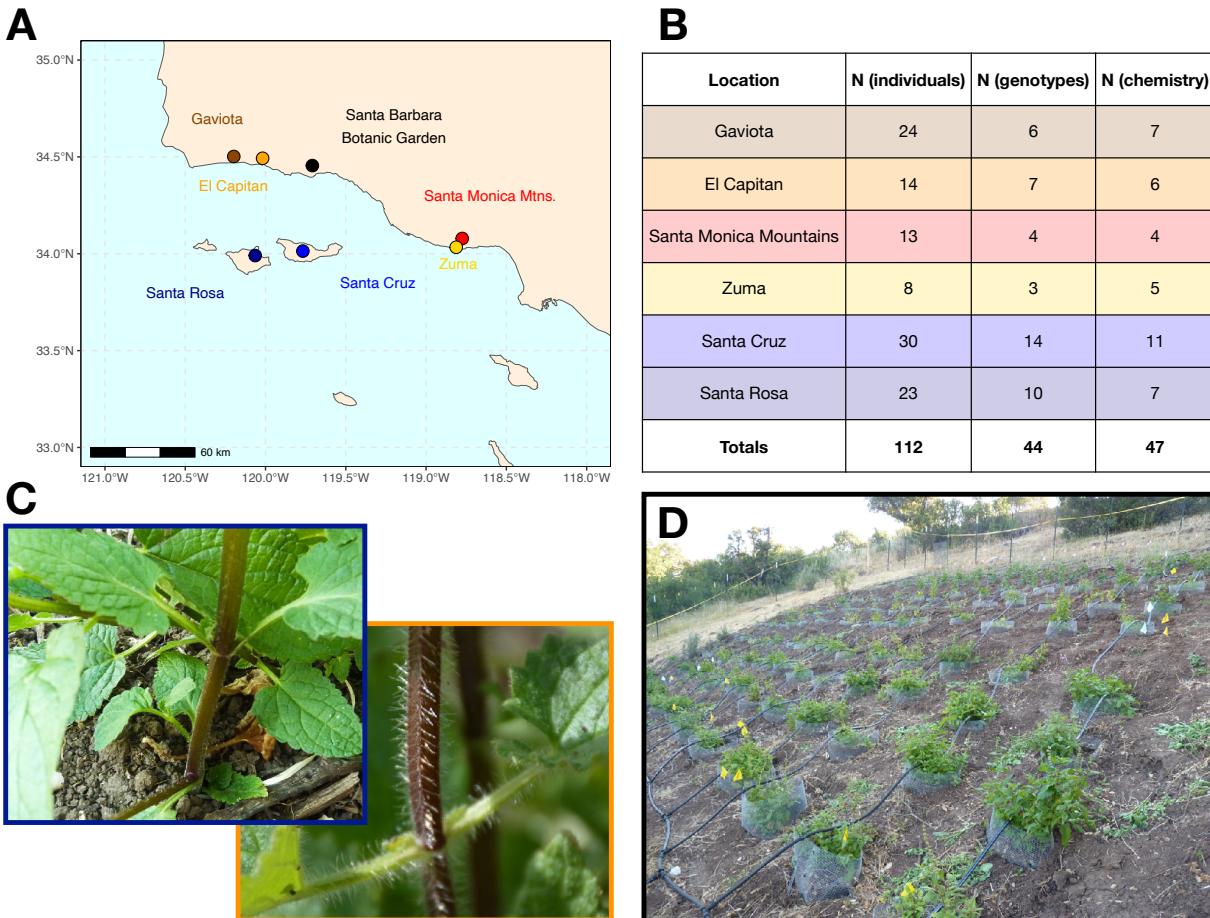
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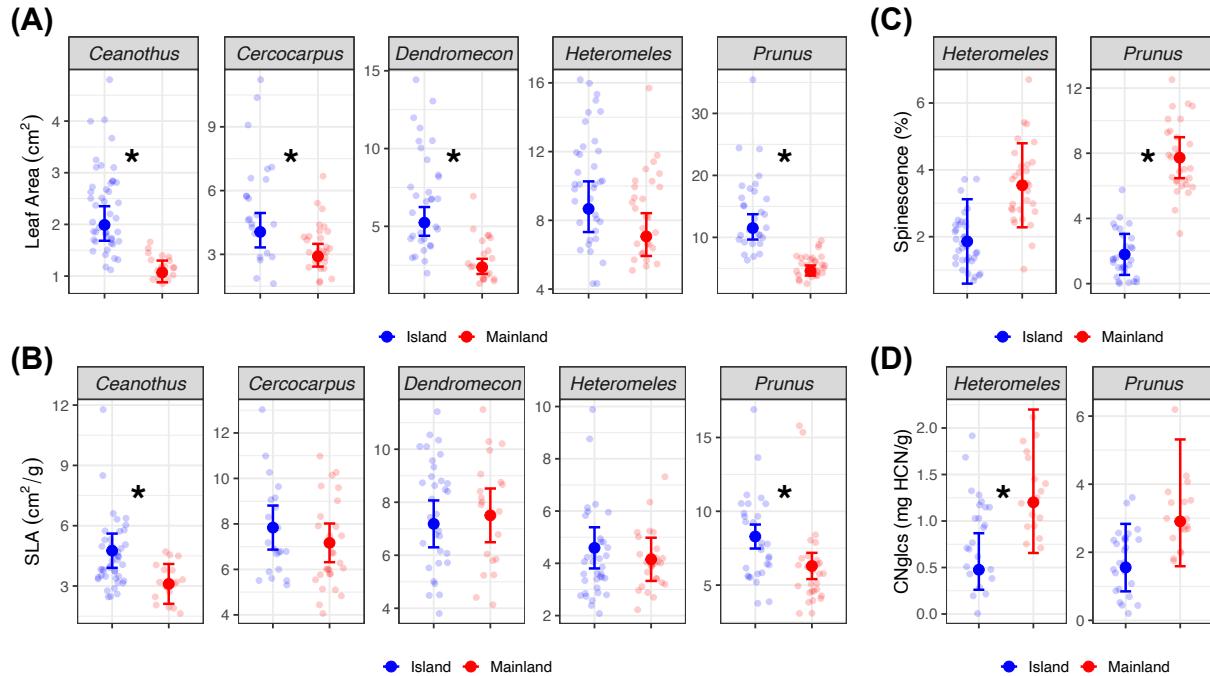


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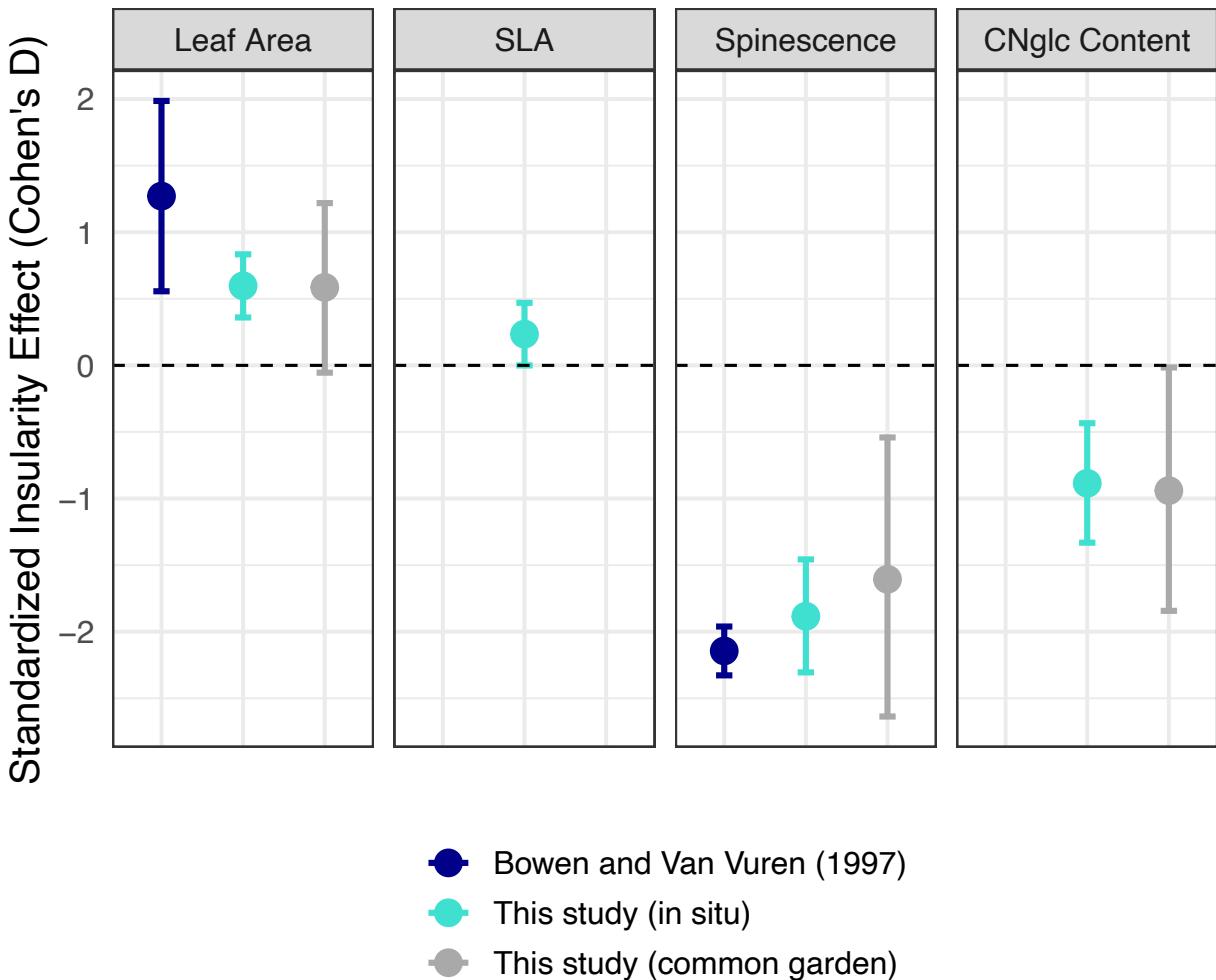
727 **Figure 1 – (A)** Map of sampling locations for field and common garden leaf collection of chaparral
 728 shrubs. Island locations are shown in blue, mainland locations are in red, and common garden
 729 locations are in grey. **(B)** Table showing the number of plants sampled across each combination
 730 of species x site. All species were sampled from all sites, with the exception of *C. betuloides* from
 731 Santa Rosa Island. Parenthetical values refer to sampling from Rancho Santa Ana Botanic Garden
 732 and the Santa Barbara Botanic Garden, respectively. **(C)** Example of leaves from island and
 733 mainland populations of *Heteromeles arbutifolia* and *Prunus ilicifolia*. Note reductions in
 734 marginal spines in both species.



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 736 **Figure 2 – (A)** Map of collection locations for *Stachys bullata* grown in common garden
 737 experiment. The common garden was located at the Santa Barbara Botanic Garden (black dot).
 738 **(B)** Table showing the number of plants from each population grown in the common garden.
 739 Genotypes refer to the number of rhizomes originally propagated from discrete plant patches
 740 collected from each location. These plants were then separated to create clones within most
 741 genotypes. **(C)** Example of stem trichome density in *S. bullata* from Santa Rosa Island (top left)
 742 and El Capitan (bottom right). **(D)** Layout of the common garden plot. Photo taken in April 2016,
 743 approximately one month after transplanting.

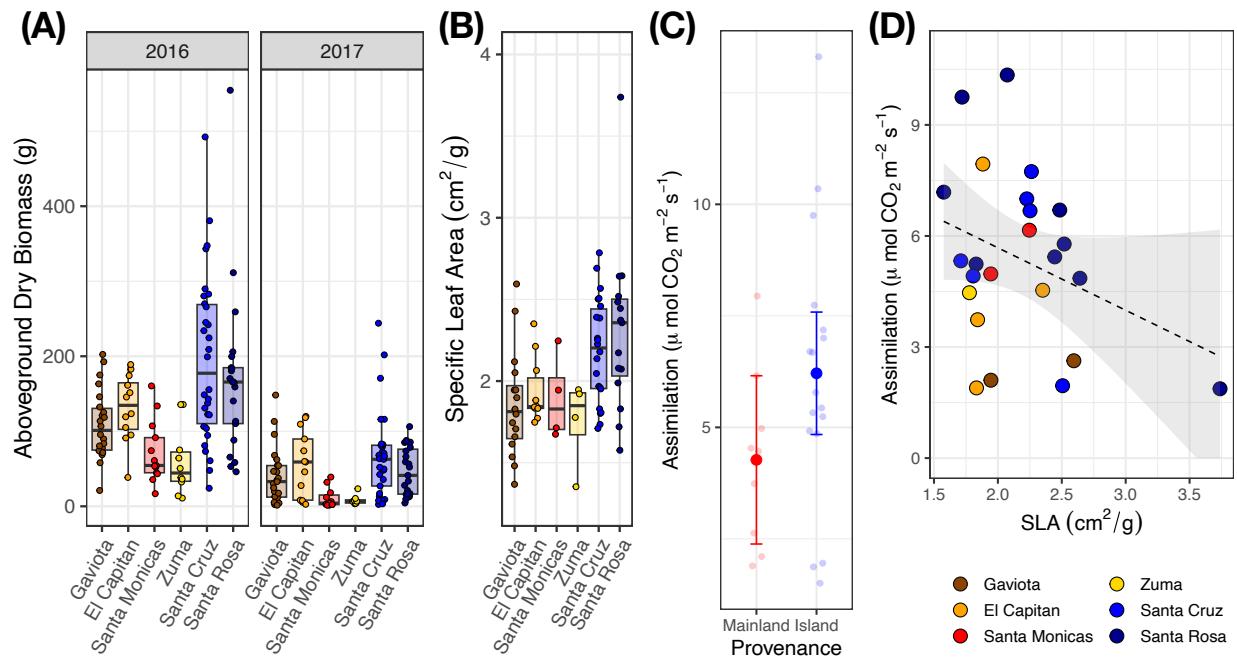


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745 **Figure 3** – Trait values for each species across island and mainland locations, based on field
746 sampling. Model-estimated marginal means and 95% confidence intervals are shown with solid
747 points and lines. Each pale dot corresponds to a single plant-level mean, which is itself the mean
748 of leaf traits from branches in the upper and lower canopy. Measured leaf traits were (A) leaf
749 area (B) specific leaf area (C) marginal leaf spinescence (*Heteromeles* and *Prunus* only) and (E)
750 concentrations of cyanogenic glycosides (*Heteromeles* and *Prunus* only). Asterisks correspond to
751 significant ($p < 0.05$) differences between island and mainland plants within each species x trait
752 combination.

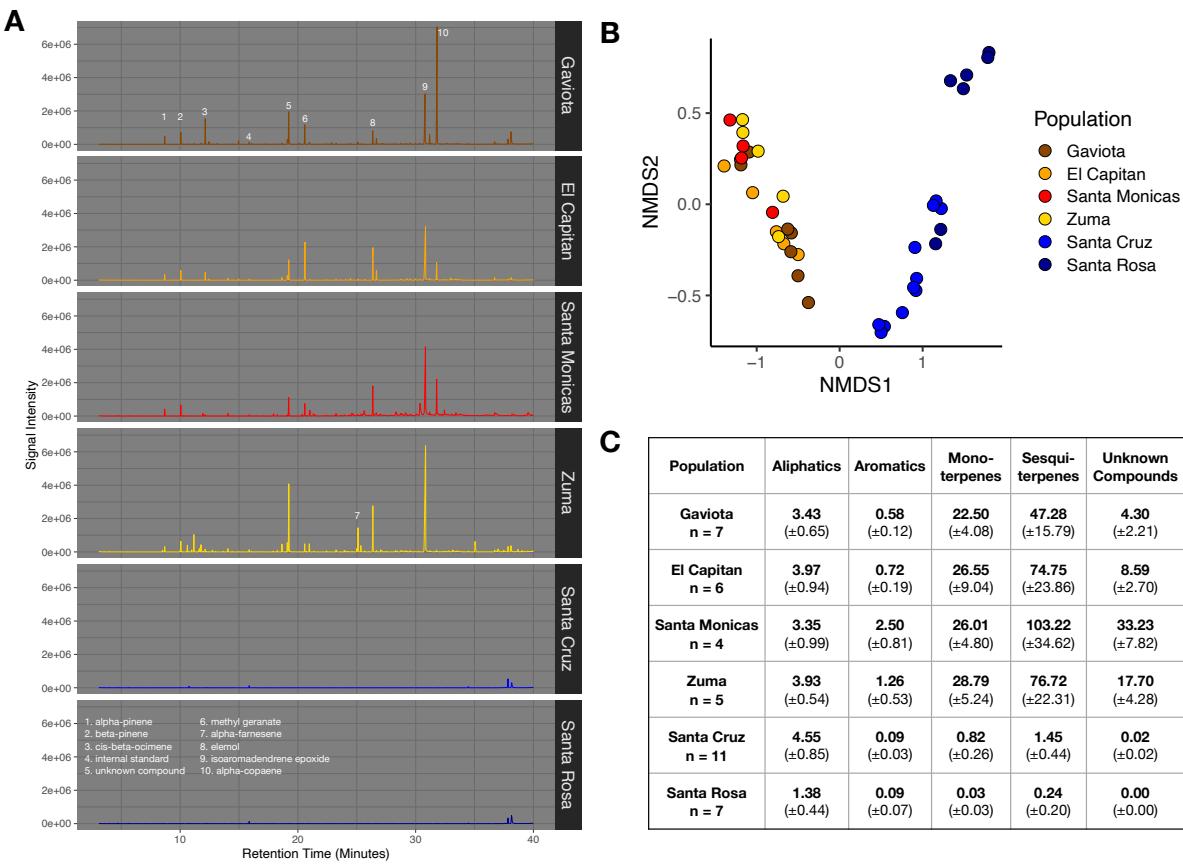


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Figure 4 – Effect sizes (Cohen's D) and 95% confidence intervals for each island/mainland contrast across traits. Estimates are separated and colored based on the comparison. Positive values in the first two panels correspond to increased leaf area and specific leaf area for island plants. Note that SLA was not measured for common garden plants. Negative values in the second two panels correspond to reduced leaf spinescence and CNglc concentration in island plants.



759 **Figure 5 – (A)** Aboveground biomass across 2016 and 2017 for *S. bullata* populations grown at
760 the SBBG common garden. **(B)** Specific leaf area for the same populations in 2017. **(C)** Net carbon
761 assimilation (A_{net}), measured for a subset of 26 *S. bullata* plants. **(D)** Negative correlation between
762 carbon assimilation and specific leaf area across sampled plants.



765 **Figure 6 – (A)** Representative chromatograms for *S. bullata* from each of the six collection
 766 locations. Tentative identity of numbered peaks is shown in the bottom panel. **(B)** NMDS plot
 767 with samples grouped based on collection location. **(C)** Average concentration (in tetralin
 768 equivalents) for major compound classes detected in samples. Values represent mean ± standard
 769 errors.