***In situ* sampling and common garden experiments reveal reductions in plant defense traits in the California Channel Islands**

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**Abstract**

Loss of defensive traits against herbivores is considered one of the most common transitions associated with plants living on oceanic islands, and this phenomenon is often invoked to explain why introduced herbivores can have devastating impacts on island flora. However, empirical evidence for reduced plant defenses on islands is somewhat mixed. In this paper, we present two studies aimed at evaluating differences in physical and chemical defenses between pairs of conspecific or congeneric taxa from the California Channel Islands—which only recently have been exposed to mammalian herbivores—and nearby mainland locations. In the first study, we focus on five taxonomic pairs of woody shrubs from three island and three mainland locations, as well as two common garden locations on the mainland. We find evidence for reductions in leaf area, marginal leaf spines, and concentrations of cyanogenic glycosides in island plants, although the magnitude of these effects varies substantially across islands, plant species, and for *in situ* versus common garden comparisons. In the second study, we conduct a common garden experiment with a perennial herb (*Stachys bullata*) collected from two island and four mainland locations. Compared to their mainland relatives, island genotypes show a ~100-fold reduction in volatile leaf secondary compounds, driven primarily by an almost complete loss of mono- and sesquiterpenes. Island genotypes had higher specific leaf area and grew more than mainland genotypes across two years of study, potentially reflecting a broader shift in growth habit. Our results provide support for reduced defenses in island plants and highlight the value of oceanic islands as systems for comparative research into the evolution of growth/defense tradeoffs in plants.

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

**Introduction**

Plant defenses against herbivory are thought to be energetically costly due to allocational tradeoffs with plant growth (Coley et al. 1985, Herms and Mattson 1992), leading to predictions that constitutive defenses should be proportional to the risk of attack by herbivores. One way to understand the evolution of plant defensive traits is to use naturally-occurring gradients of herbivory intensity to test for concomitant variation in plant defenses, either within (e.g. Pennings and Silliman 2005, Hahn et al. 2019) or across species (e.g. Levin 1976, Schemske et al. 2009). Examples of this approach include comparative studies that find reductions in defensive traits in plants at higher latitudes (Rasmann and Agrawal 2011) and higher elevations (Pellissier et al. 2014), concordant with the idea that herbivory is more intense in the tropics and at lower elevations. Studies that experimentally limit herbivory also often find evidence for reduced investment in defense traits, either as a consequence of phenotypic plasticity or natural selection. For example, Palmer et al. (2008) showed that excluding large mammalian herbivores led to the breakdown of ant-acacia defense mutualisms, and Agrawal et al. (2012) found that multi-year insecticide treatment was associated with evolution of increased competitive ability in evening primrose (*Oenothera biennis*).

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

A recent meta-analysis of studies comparing island and mainland plants found evidence for increased mammalian herbivore damage in island compared to mainland plants, supporting the idea that island plants may be more susceptible to mammalian herbivores (Moreira et al. 2020). However, perhaps surprisingly, this meta-analysis did not find consistent support for reductions in plant defensive traits on islands across a set of 173 cases of island/mainland comparisons. Thus, the degree to which island plants show reduced defensive traits remains unclear. Reasons for the lack of reduced defensive trait expression in island plants are numerous but might include (i) recent introduction of non-native mammalian herbivores that favor increased defensive traits, either through phenotypic plasticity or directional selection; (ii) an extended history of coevolution with native large herbivores (e.g. Bond and Silander 2007); (iii) selection by invertebrate herbivores on islands; (iv) ongoing gene flow from mainland populations that limits 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 and be maintained in island plants. 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 water balance and primary metabolism (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*: Lamiaceae) from two island and four mainland locations and grew them for two years in a mainland common garden to measure plant chemistry and growth.

**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) land area (Fig. 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 10 km of separation between island and mainland. The southern Channel Islands (including Santa Catalina) are generally more isolated from each other and the California mainland. Island and mainland sites have generally similar climates, although island locations show consistently lower interannual temperature variability (Fig. S1) and may have more frequent nocturnal fog that reduces summertime evaporative water loss (Fischer et al. 2016, Ramírez et al. 2020). The Channel Islands flora has a high degree of endemism and features many examples of insular woodiness and island gigantism (Junak et al. 1995, Guilliams et al. 2017).

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

Study 1: Chaparral shrub sampling

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

We collected leaf tissue for morphological analysis from focal plants by clipping branches containing variable numbers of leaves. When possible, we collected a branch from both the lower (<1 m in height) and the upper (>2 m in height) portion of the plant canopy to capture morphological differences associated with accessibility to mammalian herbivores. For analysis of cyanogenic glycosides, we collected individual leaves from the lower portion of the plant canopy for three species (*Heteromeles*, *Prunus*, *Cercocarpus*), and when possible, included both fully mature/expanded leaf tissue as well as young/actively expanding leaf tissue. Leaf chemistry samples were immediately frozen on dry ice and were later transferred to a -80°C freezer until processing. For each sampled plant, we recorded its GPS coordinates (see Fig. S2), elevation, and slope aspect (when relevant) using a handheld Garmin GPS device, and we also recorded the approximate stem diameter at 0.25 m above the ground using a digital caliper.

For each sampled branch, leaves were removed and imaged using a flatbed scanner with a scalebar. We recorded the following measurements from each leaf: total area (including petiole), area and length (not including petiole), percent of leaf tissue missing due to herbivory, and marginal leaf spinescence. All measurements were taken using ImageJ v. 1.51 (Schneider et al. 2012). For a visual depiction of our measurement protocol, see Fig. S3. Non-fully expanded leaves (n = 809) were measured but were excluded from subsequent analyses. We also measured specific leaf area (SLA) at the level of branches by taking the cumulative area of all fully expanded leaves (in cm2) and dividing this by their cumulative mass (in g).

To measure cyanogenic glycoside (CNglc) content, we followed a modified version of the evolved hydrogen cyanide (HCN) protocol described in Experiment 2 of Gleadow et al. (2011). We only collected tissue for species in the Rosaceae (*Cercocarpus*, *Heteromeles*, *Prunus*), which are known to produce CNglcs. A piece of frozen leaf tissue was massed, transferred to a 1.5 mL tube with magnetic grinding beads, added to liquid nitrogen, and pulverized in a tissue lyser (Qiagen TissueLyser LT) at 50 Hz for 30 seconds total. Next, we added 1 ml of 0.1M citrate buffer to each tube containing ground frozen tissue, which was then transferred into a larger 15 mL tube containing a separate 1.5 mL tube with 1 ml of 1M NaOH. This larger 15 mL tube was sealed, allowed to incubate at room temperature for 1 hour, and then transferred into a 37°C chamber to incubate for 12 hours overnight. The amount of NaCN captured in NaOH was measured via the pyridine-pyrazalone method using reagents purchased from Hanna Instruments (HI93714-01). The 1 mL of dissolved NaCN was added to 30 mL of deionized water and neutralized with 0.5 M acetic acid. We then added 1 mL of this solution to a separate container of 10 mL of deionized water and added reagents A, B, and C according to manufacturer instructions. The resulting color change (from clear to blue) was measured in a plastic cuvette inserted into a spectrometer (VWR V-1200), with absorbance was measured at 595 nm. This absorbance value was then compared to a calibration curve prepared using known concentrations of KCN (Fig. S4) to obtain a sample concentration. We also experimentally added 0.5 mg of β-glucosidase (Sigma Aldrich) to a small subset (n = 3) of tissue samples in citrate buffer to test whether endogenous enzyme activity was sufficient to hydrolyze all cyanogenic glycosides; this addition did not appreciably change the resulting absorbance values. In total, we generated 194 measurements of CNglc content from 108 individual plants.

Because most of our leaf tissue sampling was conducted *in situ* (i.e. from plants growing in their native environment), it is therefore difficult to know whether any potential phenotypic differentiation between island and mainland plants is environmentally or genetically determined.Thus, we also sampled leaf tissue from two botanical gardens (Santa Barbara Botanic Garden and Rancho Santa Ana Botanic Garden) on the mainland that featured island and mainland genotypes of the species of interest (Fig. S5), grown from either seed or cuttings. All leaf tissue collection, morphological analysis, and chemical analysis was conducted in the same way as described above, although SLA was not measured for common garden plants. In total, we sampled an additional 40 plants (18 island and 22 mainland genotypes) from these common garden environments (Fig. S5).

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

Study 1: Chaparral shrubs – statistical analyses

We analyzed our data using multilevel linear mixed models implemented in the lme4 package (Bates et al. 2015) in R version 3.6.3 (R Core Team 2020) to account for the hierarchical nature of our data. Response variables of interest were leaf area, specific leaf area, marginal leaf spinescence, and leaf CNglc content. In all cases, response variables were analyzed as untransformed values with a multivariate normal error distribution. For marginal leaf spinescence, we only included *Heteromeles* and *Prunus*, since these were the only species with stiff rigid spines (Fig. 1C). Likewise, because CNglc levels in *Cercocarpus* were ~100x lower than in *Prunus* and *Heteromeles* (and often below our detection limit), CNglc analysis was restricted to the latter two species. Fixed covariates that were included in each model included site of collection, canopy position (upper versus lower), north/south slope aspect, and east/west slope aspect. We considered including elevation and stem diameter (as a proxy for plant age) as covariates, though because of limited within-site and within-species variation in these measures, we ultimately omitted them from analyses.

For each response variable, we started by fitting an overall model that included all samples collected *in situ* across all species (n = 4096 leaves from 291 plants). These models were of the form (in lme4 syntax):

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

where IM corresponds to whether samples came from an island or mainland site. Plant species is included as a random intercept, with a random slope for island vs. mainland status to allow for variation in the magnitude of the island/mainland contrast across species. Collection site was included as a random intercept, with plant ID nested within site, and branch ID nested within plant ID. Since specific leaf area was calculated by pooling leaves from within branches, the SLA model does not include a branch ID term. In these overall models, the primary contrast of interest is the fixed effect of island/mainland, which reflects the average of the five species-level island/mainland contrasts.

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

Next, to understand variation among species in the degree of island/mainland trait divergence, we ran separate models for each species that were identical to the models specified above, but without the (IM|Species) term. These models allowed us to generate species-specific estimates of the island/mainland effect, which are of interest in part due to the heterogeneity in degree of evolutionary divergence among our taxonomic pairs. We report both the overall models and the species-level models together for each response variable for comparison.

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

***Response variable ~ Source.IM + (Source.IM|Species) + covariates + (1|Plant.ID/Branch.ID)***

where Source.IM refers to whether the plants’ original provenance was an island or mainland location. As above, we also separately generated species-specific estimates of the island/mainland effect for samples collected from common garden locations. To compare the magnitude of insularity effects across plants sampled *in situ* versus from common gardens, we calculated effect sizes (Cohen’s D) for each trait *x* species combination using the effectsize package (Ben-Schahar et al. 2020).

Finally, to test for the effect of access to introduced herbivores on Catalina Island, we analyzed all trait data from Catalina and included a term to account for whether samples came from inside versus outside of an herbivore exclosure.

For all models evaluated, their summary output was generated using the lmerTest package (Kuznetsova et al. 2017), which gives approximate degree of freedom and p-value calculations. Comparisons across factor levels were generated using emmeans version 1.5.3 (Lenth 2020). Raw data were plotted using ggplot2 (Wickham 2016).

Study 2: *Stachys bullata* – background

*Stachys bullata* (Lamiaceae) is a perennial herbaceous plant that occurs in coastal California from approximately Orange County to the San Francisco Bay Area, with populations present on Santa Cruz, Santa Rosa, and Anacapa Islands. It reproduces both clonally via rhizomes and sexually and is described as being glandular, with aromatic foliage that is characteristic of many plants in the Lamiaceae. However, island populations have been noted to have non-aromatic foliage as well as larger leaves and flowers than their mainland relatives (Junak et al. 1995), and densities of glandular trichomes appear to be much lower on island plants (Fig. 2C).

Study 2: *Stachys bullata* common garden experiment

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

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

We collected three categories of data from common garden *S. bullata*. First, we measured annual growth by collecting all above-ground biomass at the end of the growing season and recording its mass. Biomass measurements were collected in both 2016 and 2017. Second, we collected leaves to measured SLA in April of 2017. Third, we measured plant secondary chemistry—with an emphasis on volatile organic compounds present on leaf surfaces and in glandular trichomes—using a modified version of the protocol described in Pratt et al. (2014) for measuring terpenes in *Artemisia californica*. Briefly, in April of 2017, we used a hole punch to collect six leaf discs, each from a different leaf, from approximately 75 *Stachys* plants across all genotypes. This plant tissue was added to 2 mL glass vials containing 500 μL of dichloromethane and 5 μL of a 90 ng/ml tetralin internal standard and was stored at 4°C until processing. Vials were sonicated for 10 minutes, and then 200 μL of the eluent was filtered through a modified capillary tube containing powdered silica into a GC vial insert. Samples were injected onto an Agilent 7890B gas chromatograph fitted with a 30 m × 0.25 mm × 0.25 um HP‐5 Ultra Inert column coupled to an Agilent 5977A mass spectrometer (Agilent Technologies) using a 5:1 split ratio, a 1 μL injection volume, and an inlet temperature of 250°C. The initial oven temperature was 40°C, held for 3 minutes, followed by a temperature ramp of 5°C/min up to 210°C, followed by a subsequent ramp of 20°C/min to 300°C, followed by a final hold at 300°C for one minute. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. Electron impact mass spectra were obtained by scanning between 30-550 *m/z*.

GC/MS data were processed using MassHunter GC/MS Acquisition software version B.07.00 and MSD ChemStation Enhanced Data Analysis Software version F.01.00 (Agilent). Peaks were initially called automatically using the RTE integrator and a detection threshold limit of 1.0% of the largest peak. Chromatograms were manually annotated to include peaks that were visible but fell below this detection threshold. Peak alignment was based on retention times, and we assigned identifications to compounds by comparing mass spectra and retention times to published databases (Adams 2007, NIST mass spectral library). In total, our dataset included 79 peaks.

Study 2: *Stachys* data analysis

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

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

where IM refers to whether a given plant originated from an island or mainland site and column and row refer to the location of plants within the common garden grid.

To analyze plant chemistry, we divided each integrated peak area by its corresponding internal standard peak area to standardize all values. We added all peaks together to get a cumulative compound abundance measure and also separated compounds based on their biochemical basis (i.e. fatty acid derivatives, mono- and sesquiterpenes, aromatics). To visualize multivariate disparity among populations in chemical composition, we used non-metric multidimensional scaling (nMDS) implemented in the vegan package version 2.5-7 (Oksanen et al. 2020), specifying Bray-Curtis distances, k = 8 dimensions, and 1000 random starts in the metaMDS function; group-level differences between genotypes from island versus mainland sites were assessed using perMANOVA implemented via the adonis2 function in vegan (Oksanen et al. 2020). We also separately tested for multivariate disparity using only genotypes collected from mainland sites, as well as Santa Cruz Island genotypes grown on Santa Cruz Island versus the mainland.

**Results**

Chaparral shrubs

In our overall models of island/mainland differentiation, none of the traits we measured resulted in significant community-level differences between island and mainland locations. That said, all effect sizes were in the predicted direction: overall, island plants had larger leaves (t = 2.398, p = 0.073), lower specific leaf area (t = -1.794, p = 0.135), reduced marginal spinescence (t = 1.933, p = 0.266), and lower concentrations of CNglcs (t = -2.392, p = 0.154) (Fig. 3, Fig. 4).

In the overall model for leaf area, island plants had leaves that were, on average, 44.5% larger than their mainland relatives (x̄island = 7.66, x̄mainland = 4.25) (Fig. 3A). Leaves from the upper canopy were modestly smaller than leaves from lower in the canopy (t =  -1.711, p = 0.088). For within-species comparisons of leaf area, two species pairs had significantly larger leaves on islands: *Ceanothus* (t = 3.853, p = 0.013) and *Prunus* (t = 4.126, p = 0.015) (Table S2). Among common garden plants, island genotypes had leaves that were, on average, 36.6% larger than their mainland relatives (x̄isl = 10.72, x̄main = 6.79) (Fig. 4A), although the island/mainland contrast was not significant in the overall model (t = 3.925, p = 0.239) (Table S2). For within-species comparisons of common garden leaf area, *Prunus* genotypes from islands had significantly larger leaves than mainland genotypes (t = 4.880, p = 0.001) (Table S2, Fig. 4A).

In the overall model for specific leaf area, island plants had SLA that was, on average, 13.5% higher than for mainland plants (x̄isl = 6.53, x̄main = 5.65) (Fig. 3B), indicating thinner leaves for island genotypes, although the island/mainland difference was not significant in the overall model for plants sampled in situ (t = 1.794, p = 0.135) (Table S3). Leaves from the upper canopy had consistently lower SLA (t = -6.661, p < 0.001), as did leaves from plants with northward (t = 3.248, p = 0.001) and westward facing aspects (t = 3.076, p = 0.002) (Table S3). None of the species-level island/mainland contrasts resulted in significant island/mainland contrasts (Table S3). SLA was not measured for plants from common gardens.

In the overall model for marginal leaf spinescence, island plants had spines that were, on average, reduced by 67.4% compared to mainland plants (x̄isl = 10.72, x̄main = 6.79) (Fig. 3C), but the overall island/mainland difference was not significant (t = 1.887, 0.279) due to the small number of species (n = 2) included in the analysis (Table S4). Leaves from the upper canopy had consistently reduced spinescence (i.e. spinescence heteroblasty) (t = -2.028, p = 0.045) (Fig. 3D), and there was a significant interaction between island/mainland status and canopy position (t = 2.826, p = 0.006) (Table S4) that reflects more pronounced spinescence heteroblasty in mainland plants. Within species, marginal spines were significantly reduced on islands for both Heteromeles (t = -3.063, p = 0.034) and Prunus (t = 8.008, p < 0.001) (Fig. 3C). Among common garden plants, within species comparisons of marginal spines were similar to those from plants measured in situ, with modestly reduced spines in island Heteromeles (t = -1.952, p = 0.094) and significantly reduced spines in island Prunus (t = 8.597, p < 0.001) (Table S4, Fig. 4C).

In the overall model for CNglc content, island plants had, on average, 40.2% lower CNglc concentrations than their mainland relatives (x̄isl = 1.31, x̄main = 2.19) (Fig. 3D), though the overall island/mainland difference was not significant (t = 2.392, p = 0.154) (Table S5) because only two species were sampled for this analysis. Younger leaf tissue had consistently higher CNglc content than older, fully expanded leaf tissue, regardless of plant provenance (t = 5.364, p < 0.001) (Fig. 3F). Within species, CNglc concentrations were lower for island Heteromeles (t = -2.430, p = 0.055) and significantly lower for island Prunus (t = -3.353, p = 0.015) (Fig. 3D, Table S5). Among common garden plants, within species comparisons of CNglc concentrations were similar to those from plants measured *in situ* but with a smaller island/mainland effect, with modestly reduced concentrations in island Heteromeles (t = -1.316, p = 0.213) and significantly reduced concentrations in island Prunus (t = -2.389, p = 0.036) (Fig. 4D, Table S5).

We did not find any significant differences for any measured traits inside versus outside of herbivore exclosures on Catalina Island (Table S6).

*Stachys* common garden

Of the 112 plants originally transplanted in 2016, 108 survived through the first year, and 103 survived through the second year. Plants had significantly higher biomass in 2016 when they received supplemental water (t = 12.094, p < 0.001). Overall, island genotypes grew modestly larger than their mainland relatives (t = 3.303, p = 0.067) (Fig. 5A); the absolute difference in biomass across years was identical, with island plants supporting an average of 54.9 g of additional biomass in each year (Fig. 5A). Island plants had significantly higher SLA than their mainland relatives (t = 3.073, p = 0.042) (Fig. 5B).

Consistent with observations from *Stachys* growing in situ, island and mainland genotypes sampled from the common garden had markedly different chemical compositions, both in terms of absolute abundance and the presence/absence of compounds (Fig. 6B) (PERMANOVA: Fsite = 38.71, p < 0.001). The most pronounced chemical difference between island and mainland genotypes was for mono- and sesquiterpenes, with island genotypes showing an approximate 100-fold reduction in the abundance of these compounds (Fig. 6C). Santa Cruz Island genotypes did not differ in leaf chemistry based on whether they were grown on Santa Cruz Island versus the mainland (Fig. S6).

**Discussion**

We found general support for divergence in plant traits between islands and mainland sites, including a reduction in putative plant defense traits (marginal spines, cyanogenic glycosides, terpenes) and an increase in traits associated with resource acquisition in island plants (leaf area, specific leaf area) (Wright et al. 2004). These results are consistent with broader hypotheses about tradeoffs between plant growth and defense as well as predictions related to the “island syndrome” in plants: in the absence of vertebrate herbivores, island plants may reallocate resources from secondary metabolism (i.e. defense) towards primary metabolism (i.e. growth). However, the magnitude of insularity effects varied widely across our sampled taxa, and further work is needed to formally demonstrate that growth/defense tradeoffs are operating in this system.

Overall statistical models did not suggest significant differences in plant traits between island and mainland plants (Tables S2-S5), though it is important to note that these models included species pair as a random intercept term to reflect that our chosen species represent a small subset of a broader plant community. In practice, this model formulation reduced the effective degrees of freedom for the island/mainland contrast to n-1, where n is the number of species pairs included in any given comparison (n = 5 for leaf area and SLA; n = 2 for marginal spines and CNglcs). By comparison, contrasts between island and mainland traits were more robust in species-specific models, since degree of freedom calculations in these models were based on the number of sampled plants within each species pair. The distinction between the results of overall and species-specific models of insularity effects may seem mostly philosophical, though this pattern highlights the need to carefully consider which species are chosen for island/mainland comparisons (and why) and whether the inferences drawn from single comparisons are broadly translatable. For example, we chose to study *S. bullata* based on the *a priori* observation of reduced aromaticity (Junak et al. 1995), but this pattern may not be reflective of broader trends within the Channel Islands flora.

For chaparral shrubs, the magnitude of defense trait reduction was generally greater for plants sampled *in situ* compared to those from common gardens, although effect sizes for insularity were still negative for plant defenses measured from common garden locations (Fig. 4). This result suggests that abiotic differences between island and mainland locations may contribute to some, but not all, of observed *in situ* differentiation. Notably, marginal leaf spines and CNglc content were both significantly lower in *P. ilicifolia lyonii* from common garden locations, indicating genetically based differences in these traits between island and mainland genotypes. Defense reductions were less pronounced in *H. arbutifolia*, perhaps reflecting ongoing gene flow with mainland populations: *H. arbutifolia* has small fruits dispersed by birds that are easily capable of traversing the Santa Barbara Channel.

In addition to finding reductions in marginal spines of island *H. arbutifolia* and *P. ilicifolia* (consistent with Bowen and Van Vuren 1997, Burns 2014, Salladay and Ramírez 2018), we also found evidence for decreased spinescence heteroblasty in island plants. All sampled plants, regardless of island or mainland origin, had reduced spinescence in their upper compared to lower branches, though this pattern was more pronounced for mainland plants (Fig. 3E). This result mirrors some of the patterns presented by Burns (2014), who also found a weaker vertical gradient in leaf spinescence within *Drypetes deplanchei* from Lord Howe Island compared to mainland Australia.

We found evidence for reduced CNglc content in island *Heteromeles* and especially *Prunus*, representing one of the clearest examples to date of a reduced chemical defense associated with insularity. This pattern persisted even for common garden genotypes of *P. ilicifolia*. Many other studies have found no difference (Vourc’h et al. 2001) or even increased chemical defenses for island plants (Moreira et al. 2019), and at least one study found evidence for increased levels of CNglcs in relict island populations of *Prunus lusitanica* (Pardo et al. 2016). We also found strong evidence for ontogenetic decreases in CNglc concentrations in older leaf tissue, a pattern previously shown in *Heteromeles* (Dement and Mooney 1974) and other cyanogenic species (e.g. Goodger et al. 2006). While CNglcs are acutely toxic to many vertebrate herbivores and are generally thought to have evolved as defenses as herbivores (Gleadow and Woodrow 2002 and references therein), it is important to note that CNglcs can be recycled within plants as sources of nitrogen (Pičmanová et al. 2015), which challenges the typical notion of an allocational tradeoff between growth and defense.

Although we did not directly assess the efficacy of putative leaf defenses against herbivores, two studies using plants from the Channel Islands have shown that mammalian herbivores perceive differences between island and mainland plants. Bowen and Van Vuren (1997) showed that sheep preferentially consumed leaf tissue from plants collected on Santa Cruz Island compared to a mainland location, and Salladay and Ramírez (2018) likewise showed the same pattern with goats and plant tissue from Catalina Island. Thus, the reductions in spinescence and CNglcs that we measured (or potentially other correlated traits) seem to be reasonable proxies for increased palatability to mammalian herbivores.

Because we sampled the same taxa as Bowen and Van Vuren (1997) (and at the same time of year), we can directly compare our data on leaf area and marginal spines from Santa Cruz Island to theirs. In general, comparisons are the same in direction (Fig. S7), although the magnitude of island/mainland differences reported in Bowen and Van Vuren tends to be larger. Another intriguing comparison would be to focus on the chrono-sequence of sheep removal and subsequent vegetation recovery from Santa Cruz Island (see Beltran et al. 2014). Sheep eradication efforts involved sequentially fencing off sections of the island, with eradication on the west end around 1980, but not on the east end until 2001 (Faulkner and Kessler 2011). One might therefore predict stronger insularity effects on the western end of the island, where sheep were eradicated first.

In contrast to chaparral shrubs, for which reductions in defense traits were present but variable in magnitude, *S. bullata* showed unambiguous evidence for insular reductions in leaf chemical compounds. These patterns were most pronounced for monoterpenes and sesquiterpenes, a diverse group of plant secondary compounds thought to be involved in defense against herbivores and pathogens, plant communication, and modulating thermal and oxidative stress (Loreto and Schnitzler 2010, Pichersky and Raguso 2018). Because of the varied ecological functions of terpenes, it is difficult to ascribe their loss in island *S. bullata* to the absence of vertebrate herbivores, though their reduction is certainly consistent with strong divergent selection between island and mainland environments. Although we did not quantify their loss, the reduction in leaf secondary compounds was also accompanied by a strong reduction in leaf and stem trichomes (Fig. 2C).

In accordance with a growth/defense tradeoff, island plants had significantly thinner and larger leaves and grew more than their mainland relatives across both study years. Formally demonstrating within-plant allocational tradeoffs is notoriously difficult and is best accomplished using transgenic modification to inhibit defense production (e.g. Guo et al. 2018). That said, terpene compounds are noted for being especially carbon-intensive relative to other classes of plant defenses (Schultz et al. 2013), and it therefore seems conceivable that their near complete absence in island *S. bullata* is associated with additional carbon available for growth. In addition to accumulating more biomass, island *S. bullata* populations also appeared to be taller, with woodier and more upright stems, as well as increased branching on terminal inflorescences (Fig. S8). The smaller stature of mainland *S. bullata* is consistent with a recent study that found that *Plectritis congesta* populations from islands without deer grow to be 2.6 times taller than populations from nearby islands with deer present (Skaien and Arcese 2018).

It may at first seem counterintuitive that island *S. bullata* genotypes outperformed their mainland relatives when grown in a mainland common garden. However, it is important to note that our experimental setup precluded herbivory by mainland deer and gophers (see fencing and cages in Fig. 3D), which may have favored island plants. Furthermore, common garden plants were exposed to relatively mesic conditions that may have favored island genotypes: in 2016 plants received supplemental water during the growing season, and in 2017 there was above-average precipitation at the SBBG.

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

We make three suggestions for future research into contrasts between island and mainland plant defenses. First, no studies to date have used phylogenetic comparative methods to evaluate reductions in plant defenses on islands, and this would represent an advance over studies that treat congeneric and conspecific pairs equivalently. Second, although nearly all defensive traits have may have ecological functions outside of deterring herbivores, some traits are more unambiguously defensive than others (e.g. alkaloids, glucosinolates, latex, thorns) and should be given greater emphasis in future comparisons. Third, more studies should use common garden experiments, ideally in multiple locations, to limit environmental influences on putative defense traits. These studies remain rare, even for annual and other short-lived species.

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**Figure 1** – **(A)** Map of sampling locations for in situ leaf collection of chaparral shrubs. Island locations are shown in blue, mainland in red. **(B)** Table showing the number of plants sampled across each combination of species *x* site. All species were sampled from all sites, with the exception of C. betuloides from Santa Rosa Island. **(C)** Example of leaves from island and mainland populations of *Heteromeles arbutifolia* and *Prunus ilicifolia*. Note reductions in marginal spines in both species. Maps generated using ggmap (Kahle and Wickham 2013).

**Figure 2 –** (**A**) Map of collection locations for *Stachys bullata* grown in common garden experiment. The common garden was located at the Santa Barbara Botanic Garden (black dot). (**B**) Table showing the number of plants from each population grown in the common garden. Genotypes refer to the number of rhizomes originally propagated from discrete plant patches collected from each location. These plants were then separated to create clones within most genotypes. (**C**) Example of stem trichome density in *S. bullata* from Santa Rosa Island (top left) and El Capitan (bottom right). (**D**) Layout of the common garden plot. Photo taken in April 2017, approximately one month after transplanting.

**Figure 3** – Boxplots showing trait values for each species across island and mainland locations. Each dot corresponds to a single plant-level mean, which is itself the mean of leaf traits from branches in the upper and lower canopy. Measured leaf traits were (**A**) leaf area (**B**) specific leaf area (**C**) marginal leaf spinescence (*Heteromeles* and *Prunus* only) and (**E**) concentrations of cyanogenic glycosides (*Heteromeles* and *Prunus* only). Panel (**D**) separates marginal leaf spinescence based on canopy position; the steeper slope in mainland plants corresponds to stronger spinescence heteroblasty. Panel (**F**) separates CNglc concentrations based on tissue age. Younger tissues consistently had higher concentrations, regardless of island versus mainland origin. Panels (**D**) and (**F**) reflect estimated marginal means and associated standard errors.

**Figure 4** – Effect sizes (Cohen’s D) and 95% confidence intervals for each island/mainland contrast across traits and species. Estimates are separated and colored based on whether measurements came from *in situ* (IS) plants or common gardens (CG). Numbers in parentheses refer to the number of plants (island + mainland) used to generate effect sizes. Positive values in panels (**A**) and (**B**) 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 panels (**C**) and (**D**) correspond to reduced leaf spinescence and CNglc concentration in island plants.

**Figure 5** – (**A**) Aboveground biomass across 2016 and 2017 for S. bullata populations grown at the SBBG common garden. (**B**) Specific leaf area for the same populations in 2017.

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