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Trade-offs direct the evolution of coloration in Galápagos land snails

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Increasingly, multiple selective factors are recognized as jointly contributing to the evolution of morphology. What is not clear is how these forces vary across communities to promote morphological diversification among related species. In this study of Galápagos endemic snails (genus *Naesiotus*), we test several hypotheses of colour evolution. We observe mockingbirds (genus *Mimus*) predating live snails and find that avian predation selects against conspicuous shells. The evolutionary outcome of this selection is a diversity of shell colours across snails of the archipelago, each closely matching local backgrounds. We also find that snails more regularly exposed to the hot, equatorial sun reflect more light than shells of species from shadier habitats, suggesting a role for thermoregulatory constraints directing colour evolution. The signature of thermoregulatory selection is most clear in comparatively young communities (on the youngest islands), while the signature of selection from predators is most evident in older communities (on the older islands). Together, our findings point to a scenario of shifting selective forces along island ontogeny and community maturity that lead to the distribution of snail coloration we observe in Galápagos. Complex selective regimes such as these may have more responsibility for morphological diversity than is currently recognized.

1. Introduction

Morphological diversity can be a spectacular outcome of selective forces. In the rare cases where such forces are singular and of large effect, the signature of selection is clearly written in the distribution of diversity across the landscape. In rock pocket mice (*Chaetodipus intermedius*), predation ensures that mice matching local backgrounds tend to survive and reproduce [1,2]. Thus, selection continually drives mice's phenotypes to match their backgrounds, and a diversity of backgrounds across the landscape leads to a diversity of mice phenotypes. Under these circumstances, trajectories of selection from biological agents are strongly directed by local factors like community composition (e.g. predators) and habitat (e.g. backgrounds). In other species, abiotic selective forces may drive or constrain evolution. For example, increased melanism is a common feature of ectothermic species living in colder environments where selection for increased thermoregulatory efficiency is presumably strong [3]. Increasingly, multiple selective factors are recognized as jointly contributing to the evolution of morphology (e.g. predators and solar radiation on *Cepaea nemoralis* land snails [4,5]; thermoregulation and crypsis in *Montivipera raddei* mountain vipers [6]; thermoregulation and aposematic warning signals in *Parasemia plantaginis* wood tiger moths [7]).

The above examples demonstrate how selective forces can generate or constrain variation within and across populations. A natural extension of these studies is to focus on morphological diversification among related species [8]. However, such studies can be hindered when the species in question are relatively old, long-time members of highly complex communities, which obfuscates when and how morphological divergence took place along their

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evolutionary history [9]. Consequently, groups of young species in relatively young communities may serve as powerful systems for the study of macroevolutionary morphological evolution. Volcanic islands with their young and relatively depauperate fauna are naturally ideal subjects for such research. A benefit of studies on volcanic islands is that differences between islands of varying ages allow for a comparison of species and communities at different stages of diversification and community assembly [10]. In such communities, we hypothesize that selective forces driving morphological diversification differ among islands of varying ages. For example, the studies described above outline two major forces driving colour evolution. The trajectory of abiotic selection in the form of thermoregulatory constraints (either for maximizing heat gain in cold environments or for avoiding overheating in hot environments) is likely to be similar for closely related species occupying similar environments [11]. Meanwhile, biotic selection (e.g. to reduce predation risk) may vary in intensity among communities, with species from increasingly older communities having had more consistent selection from a diversity of local predators [12] (a natural byproduct of this relationship is the 'enemy release hypothesis' in invasion ecology [13]). Furthermore, while the trajectory of evolutionary response to abiotic selection is likely to be uniform (increased solar reflectance in hot environments and decreased solar reflectance in cold environments), adaptation to biotic selection may be highly variable, dependent on local factors such as local backgrounds and predator communities [14].

Galápagos land snails of the genus *Naesiotus* are an exemplary group to study the adaptive diversification of morphology and the selective forces driving that evolution. With close to 70 named species radiating across an archipelago with islands younger than 4 million years, adaptations of species to their environments are likely to have arisen rapidly [15]. Within the group, morphology and ecology appear to have evolved in tandem. For example, shell size and shape are diverse and correlate with environmental variables [15]. Additionally, snail coloration was observed as being highly diverse by the very first malacological explorers in Galápagos, who were also the first to suggest that this diversity may be the result of several forms of selection [16].

In particular, at least two distinct selective forces may drive the evolution of colour variation in *Naesiotus* snails. First, predation by local birds (such as endemic mockingbirds, genus *Mimus*) may be an important biotic factor determining snail fitness, driving snail coloration to evolve to match local backgrounds to reduce rates of detection by predators [16]. While avian predation of *Naesiotus* snails has been suspected for over 100 years [16], we report some of the first direct observations of this predation (figure 1; electronic supplementary material, S1: video footage of a mockingbird eating a *Naesiotus* snail on Santa Cruz Island). Mockingbirds typically break snail shells at the end of the first body whorl (figure 1), and we observed fresh shells broken in this manner on several islands with mockingbirds. Second, abiotic selection in the form of thermoregulatory constraints may require snails to evolve increased reflectance to avoid overheating [4,17–20]. This may be especially relevant for species that spend a large proportion of their adult life directly exposed to the sun (i.e. arboreal species [17]). Importantly, different species of *Naesiotus* snails appear to be limited to a particular habitat (A.C.K. & C.E.P. 2014–2017,

personal observation), and species occurring in arboreal habitats experience significantly higher maximum temperatures as a result of direct solar radiation (electronic supplementary material, figure S1), including at sites where both arboreal and terrestrial habitats occur. Terrestrial and arboreal habitats are present on all islands, and for nearly every island in which we sampled more than one species, we found both terrestrial and arboreal species represented.

The selective forces directing colour evolution are likely to vary in trajectory and strength across the landscape depending on community age and structure. For example, the volcanic Galápagos Islands are formed by the Nazca Plate passing from west to east over a hot spot in the Pacific Ocean. The islands are thus arranged chronologically from east to west [21], resulting in a range of snail and predator community age and potential variation in the strength and trajectory of predator selection on coloration among these communities. Conversely, the radiating heat of a hot equatorial sun is present in nearly every terrestrial habitat in Galápagos (A.C.K. & C.E.P. 2014–2017, personal observation), probably making abiotic selection from solar radiation invariant in strength or trajectory (indeed, such selection tends to be largely unidirectional; e.g. [4,17]). Taken together, we hypothesize that biotic selection will play a relatively larger role in directing morphological evolution in older communities (i.e. on the older islands that are 700 thousand years or older), where predator communities have had more time to assemble and prey to evolve in response to increased predator abundance and diversity in their environment, while abiotic selection may be most influential in directing morphological evolution in the youngest communities (i.e. on the youngest islands that are less than 700 thousand years old).

From this hypothesis that selection differs among communities of varying ages, we make several predictions for biotic selection, abiotic selection and their combined outcome. From *biotic selection*, we make three testable predictions: (i) avian predators will attack snails that are conspicuous more often than snails that match their backgrounds, (ii) snail species will more closely match backgrounds they are found on than backgrounds other snails are found on from the perspective of avian predators and (iii) there will be a significant positive relationship between snails and their backgrounds on the basis of brightness and coloration. From *abiotic selection*, we predict that the disparity between snails and their backgrounds will be greater for arboreal species than terrestrial species. Finally, under the *hypothesis that selection varies among communities of varying ages*, we predict that (i) arboreal species from both young volcanoes and old islands will be brighter than the backgrounds they are found on, while only terrestrial species from young volcanoes will be brighter than their backgrounds and (ii) a negative relationship between island age and snail–background brightness disparity.

2. Material and methods

(a) Predator selection on snail shell coloration

In 2017, we collected 352 empty snail shells of the species *Naesiotus wolfi* from Santa Cruz. We used spray paint to uniformly colour them a shade of brown typical to living snails in Galápagos. We then distributed the shells in a paired manner

Figure 1. Image of a mockingbird (*Mimus parvulus*) using a rock to break open the shell of a Galápagos land snail (*Naesiotus* sp.). Note how it grasps the shell at the aperture (inset, bottom) to break the shell at the boundary between the end of the body whorl and the shell spire (inset, top). Also note the degree to which this snail species closely matches the local environment in overall coloration.

along a transect on Santa Cruz. Each pair was placed on a tree trunk that had two patches painted on it, with one patch being the same shade of brown as the shells, while the other patch was a contrasting grey colour typical of lichens from the trees found in that area of the island. These patches were typically located between 0.5 and 1 m apart on the same tree. A comparison of grey and brown reflectance spectra can be found in electronic supplementary material, figure S2; a description of reflectance spectra data collection can be found below. The end result was 176 pairs of shells, with each pair having a high-contrast and low-contrast shell. These shells were fixed to the tree using Sculpey III clay ('tan', which generally matches *Naesiotus* snail body coloration), which kept them in place as long as they were not attacked by a potential predator. After 48 h, we returned to the transect where we scored each shell as intact (unattacked) or displaced (attacked). We confirmed that the displaced shells were attacked by birds based on the beak marks that the birds left in the clay.

(b) Snail coloration data
(i) Snail and background coloration quantification
From August 2014 to February 2016, we surveyed 67 localities on Fernandina, Floreana, Isabela, Pinzón, Rábida, San Cristóbal, Santa Cruz, Santa Fé and Santiago islands in the Galápagos Archipelago. These localities vary with respect to island age (approx. 60 kyr to greater than 3 Myr [21]), environment (arid desert to tropical wet forest) and the presence of different habitats (categorized in this study as fully exposed arboreal and partly shaded terrestrial). We field-examined a total of 1555 adult snails representing 46 putative species (several of which are awaiting description; electronic supplementary material, table S1). Each species is represented by 4–142 individuals in our dataset (mean $n = 35$). We noted the background that each snail was found on, and at each locality, we collected 15 representative samples of each background class for colour quantification (e.g. arboreal green vegetation, arboreal trunk, terrestrial leaf litter and terrestrial underside of log; electronic supplementary material, table S2). As most *Naesiotus* snails from Galápagos are monochromatic, we measured spectral reflectance from the widest point on the body whorl of each snail shell using a portable JAZ-COMBO-2 spectrometer (OceanOptics, Dunedin, FL, USA) with a 25 μm entrance slit, a pulsed xenon lamp and a QR600-7-SR-125F reflectance probe. This probe was fitted with a tip that restricted the measured patch to a 2 mm diameter circle, maintained a constant distance (20 mm) between probe tip and measured patch, fixed the angle

between probe and shell surface at 45°, and excluded ambient light. We used a Spectralon white reflectance standard to correct for drift in lamp intensity every 10 measurements (e.g. [22]). We measured each spectrum at 1 nm intervals from 300 to 700 nm, which is the hypothesized range of ultraviolet-sensitive bird visual acuity [23].

(ii) Field irradiance measurements
While in the field we quantified the light environment via irradiance measurement from a separate module within the JAZ unit described above. This module was fitted with a CC-3-DA cosine corrector and was precalibrated for irradiance measurements with the OceanOptics SPEC-CAL-UV calibration service for UV-sensitive spectrometers. In total, 243 irradiance measurements were collected when birds were observed to be active (between 8.00 and 16.00) across a representative range of habitats within which we sampled snails ($n = 27$ localities). At each of these localities, we collected three sets of three irradiance measurements, for a total of nine irradiance measurements at each locality. We averaged each set of measurements to obtain a single measurement per sampled locality for a total of 27 irradiance measurements.

(iii) Brightness and multidimensional colour quantification
We first calculated snail and background brightness *sensu* Endler [24] by integrating across each spectrum from 300 to 700 nm (figures 2 and 3; y-axis for snail brightness). Snail–background brightness disparity scores were calculated by subtracting corresponding snail background brightness from each snail's shell brightness. We then calculated a multidimensional estimate of colour by first standardizing spectra with respect to brightness such that the area under each spectral curve summed to 1.0. We performed a principal component analysis on the standardized spectra to obtain a set of independent colour variables that together described variation in the shape of spectral curves and thus colour (figure 2; x-axis represents the first colour variable for snails, which contains 68.2% of the variation in colour among snails) [25].

(iv) Visual model
We estimated the ability of birds to discriminate between snails and the backgrounds they were found on with a visual model developed by Vorobyev *et al.* [26]. This analytical model is derived from the signal-to-noise ratio of predator photoreceptors and assumes that the ability of predators to discriminate between objects depends on this ratio [27]. The model can yield estimates of visual contrast between a target and a background [28–30], which are calculated for each of two visual channels, achromatic (ΔL) and chromatic (ΔS). In this analytical framework, the achromatic visual channel summarizes the aspects of visual stimuli pertaining to brightness, while the chromatic visual channel summarizes the aspects of visual stimuli pertaining to coloration. In each case, large contrast values correspond to clear and discriminable differences between targets and backgrounds from the perspective of the predator, while smaller values represent similar coloration between targets and backgrounds and are thus potentially indistinguishable. Contrast values of 1 or smaller are thought to describe pairs of spectra that are indistinguishable most of the time because photoreceptor processing of visual stimuli will result in more noise than original signal, while values greater than approximately 3 correspond to significantly more signal than noise [27]. Values between 1 and 3 may be somewhat distinguishable, depending on the light environment and other event-specific factors (e.g. distance between predator and prey).

As input data, the visual model requires reflectance measurements of the target and background, habitat irradiance and
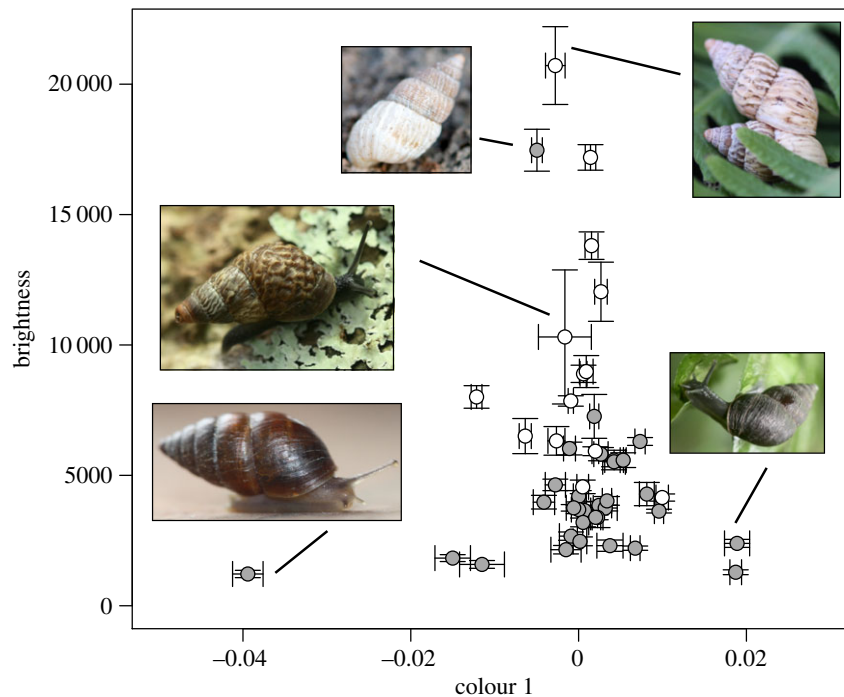
Figure 2. Illustration of colour and brightness variation among Galápagos *Naesiotus* species. The 'brightness' axis represents the sum of reflectance from 300 to 700 nm, while the 'colour 1' axis represents the first colour variable as described in the text. Filled circles denote terrestrial species while open circles denote arboreal species. Bars represent the standard error around each species estimate. (Online version in colour.)

photoreceptor sensitivities of the predator. We used spectral data obtained for each snail as target spectra, and we calculated the average spectrum of each background class at each site. We used three irradiance measurements to calculate snail ΔL and ΔS contrast scores (the most highly illuminated locality, the least illuminated locality and the average irradiance spectrum across all localities; electronic supplementary material, table S3). The relationships among contrast scores do not differ qualitatively between irradiance measurements, so we report only the results of the average irradiance spectrum here.

Mockingbirds, like most other passerine birds, are thought to have UV-sensitive, tetrachromatic visual acuity [23]. We thus used the known spectral sensitivities of another UV-sensitive tetrachromatic bird, the blue tit [31] in the visual model. A full description of the visual model calculations can be found in electronic supplementary material, supplementary analysis files.

(v) COI tree construction

We used an existing mitochondrial DNA (mtDNA) dataset [32] and expanded it by extracting, amplifying and sequencing a 500 base pair fragment of the mtDNA *COI* gene from specimens representing an additional 13 species. Total DNA was extracted from the foot of each specimen (approx. 10–15 mg) using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) as per the manufacturer's protocols. Sequences were amplified by PCR using the following primers: *1718* 5'-GGAG GATTTGGAAATTGATTAGTTCC-3' and *2191* 5'-CCCGGTA AAATTAAAATATAAACTTC-3'. Amplicons were electrophoresed in a 1% agarose gel to verify the amplifications and cleaned-up using the Qiaquick PCR cleanup kit (Qiagen, Valencia, CA, USA). DNA sequencing was carried out by Eurofins (eurofins-genomics.com) MWG Operon using the ABI Big Dye Terminator kit (v.3.1) and an automated DNA sequencer (model ABI 3730 XL). The sequences obtained were aligned using MAFFT online (http://www.ebi.ac.uk/Tools/msa/mafft/) and subsequently visually evaluated in MEGA6 [33] to ensure there were no indels or premature stop codons.

We used maximum-likelihood (ML) phylogeny estimation to identify the diversification branching pattern among sequences. We used the automodel command in PAUP* [34] to select a model of nucleotide sequence evolution for use in subsequent analyses, under the Bayesian information criterion and decision theory [35]. The GTR + I + Γ model was specified for the dataset, and an ML analysis was conducted with PAUP* with the chosen model using a heuristic search, TBR branch swapping and 10 replicate runs with random starting trees. Nodal support was assessed with 200 bootstrap replicates. We used the resulting ML phylogenies to test the assumption that the dataset has evolved in a clock-like fashion by testing for a global molecular clock in PAUP* using the likelihood-ratio test of Felsenstein [36].

We also conducted Bayesian phylogeny estimation with BEAST [37] to estimate the Bayesian posterior probability of nodes in the phylogeny estimate. As the strict clock model was rejected (2 ln LR = 30.34, d.f. = 38, 0.05 > p > 0.01), we used a relaxed lognormal molecular clock, the GTR + I + Γ substitution model and a birth–death speciation tree prior. The analysis consisted of 10 million generations with a sampling interval of 1000 and a burn-in of 25%. The BEAST output was analysed using TRACER v. 1.4 [38] to verify an effective sample size exceeding 200 for all parameters being estimated. The BEAST tool TreeAnnotator was used to produce a median branch length maximum clade credibility tree from the post-burn-in trees.

(c) Statistical analyses

(i) Biotic selection prediction 1: highly contrasting snails will be attacked at a higher rate than background matching snails

We built a generalized linear mixed model with binomial error distributions to test this prediction (*sensu* [39,40]). We used replica fate (attacked or not attacked) as our response variable, contrast as the predictor variable and pair as a random effect. By including pair as a random effect, we account for the possibility that an attack on one shell influenced the probability of attack on the other shell. We tested this prediction using the lme4 package [41] in R v. 3.5.0 [42].
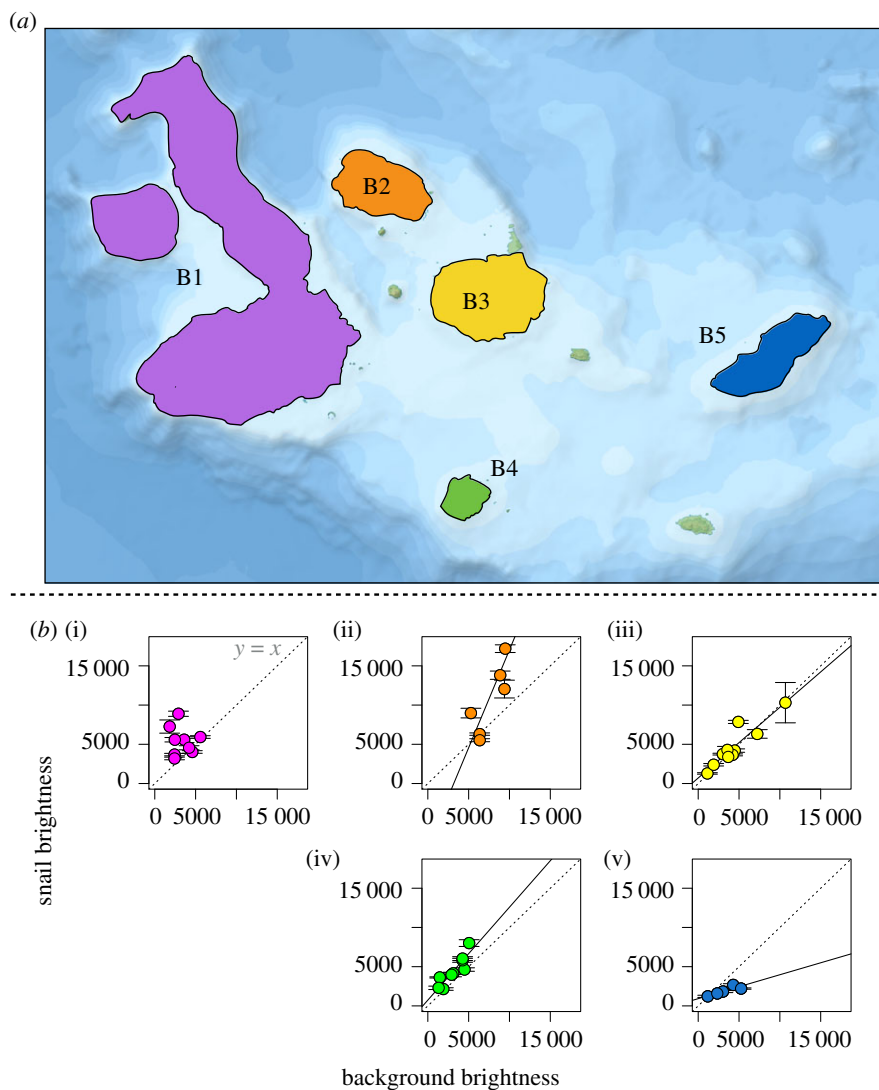
Figure 3. Summary of snail–background matching with respect to brightness. (*a*) Each island sampled is identified as B1–B5. Owing to the sparse sampling on the young islands of Fernandina and Isabela, they are grouped together under B1. Note how B1–B5 are arranged such that B1 contains the youngest two major islands, while B5 contains the oldest island sampled (San Cristóbal). (*b*) Each plot contains the species sampled for a particular major island. The *x*-axis represents the average background brightness a particular species is found on, while the *y*-axis represents the average shell brightness measured for that species. Error bars on the *y*-axis denote standard errors for species estimates of brightness. Dotted lines denote $y = x$, while solid lines denote the least-squares regression line for species-background comparisons for the island depicted. Note the lack of a significant relationship on the youngest islands (B1), while all other islands have a significant relationship between snails and their respective backgrounds with respect to brightness.

(ii) Biotic selection prediction 2: snails will more closely match local backgrounds than non-local backgrounds from the predator's perspective

To test this prediction, we first used the visual model to calculate chromatic and achromatic contrasts between snails and backgrounds using the following procedure. We first calculated contrast scores between each snail and a randomly chosen local background. These comparisons were made 100 times for each snail (with background replacement) and a mean contrast score was calculated. A global mean contrast score was then calculated across all snails, which was treated as the observed test statistic in this analysis (C.obs$_{Achromatic}$ and C.obs$_{Chromatic}$, respectively). This procedure was repeated with some important differences to create a sampling distribution under the null hypothesis. The pool of potential backgrounds for each snail was widened to include the entire background dataset. The resulting contrasts were recalculated 999 times, and each time a global permuted mean contrast was calculated. This resulted in a sampling distribution of 999 chromatic and achromatic mean contrasts under the null scenario (C.rand$_{Achromatic}$ and C.rand$_{Chromatic}$, respectively). We treated the proportion of randomly generated

contrast scores equal to or less than the observed scores as the significance of this test. Importantly, these tests statistically evaluate the degree to which snails match local backgrounds from a predator's perspective relative to non-local backgrounds. The distinct question of whether these differences are regularly perceived by predators under most natural circumstances [43] cannot be addressed with these data. Instead, experimental studies of predator behaviour (e.g. *biotic selection prediction 1*) directly address this question.

(iii) Biotic selection prediction 3a: a positive relationship between snails and their backgrounds on the basis of brightness

In this analysis, we performed a linear regression of snail and background brightness scores, using the average brightness of each putative snail species as well as a weighted average of the backgrounds that that species was found on. To test whether any observed relationships between snail and background brightness were influenced by shared evolutionary history, we conducted a phylogenetic generalized least-squares analysis (PGLS) of snail and background brightness using the reduced dataset of 28 species that are represented in the COI trees.

(iv) Biotic selection prediction 3b: a positive relationship between snails and their backgrounds on the basis of coloration

To test this prediction, we performed a two-block partial least-squares analysis of snail and background colour scores (using the 'two.b.pls' function in the *geomorph* R package) [44], first calculating average colour variables for each species as well as a weighted average of the backgrounds that that species was found on. To test whether any observed relationships between snail and background coloration were influenced by shared evolutionary history, we conducted a phylogenetic partial least-squares analysis of snail and background coloration using the reduced dataset of 28 species that are represented in our COI trees (with the 'phylo.integration' function in *geomorph*).

(v) Abiotic selection prediction: the disparity between snails and their backgrounds will be greater for arboreal species than terrestrial species

To test this prediction, we performed a nested analysis of variance (ANOVA) using snail–background brightness disparity as the dependent variable, habitat as the main group factor and species as the subgroup factor.

(vi) Relationship between brightness, background matching and island age

First, to test whether island age and snail habitat could be used to predict the disparity of snail brightness against local backgrounds, we performed a generalized linear mixed model using snail brightness disparity as a dependent variable, island age and habitat (arboreal or terrestrial) as fixed effects, and background type as a random effect. Then, to clarify how these four groups of snails differed from one another, we performed a series of one-tailed, one-sample t-tests against the null hypothesis of zero difference (with a conservative Bonferroni correction).

All analyses were conducted in R v. 3.5.0 [42], and the code for these analyses can be found in electronic supplementary material.

3. Results

(a) Biotic selection prediction 1: highly contrasting snails will be attacked at a higher rate than background matching snails

We found that at the end of the experiment, 105 pairs of shells were not attacked, 27 were missing both shells, 16 were missing only the low-contrast shell and 25 were missing only the high-contrast shell. In total, 24.4% of the low-contrast shells were attacked, while 29.5% of the high contrast shells were attacked. The generalized linear mixed model was significant ($Z = 9.902$; $p < 0.001$), indicating that higher shell contrast results in higher attack rate.

(b) Biotic selection prediction 2: snails match the colour and brightness of local backgrounds from the predator's perspective

Using randomization, we found that snails matched local backgrounds significantly better than non-local backgrounds from the perspective of avian predators both in the achromatic (C.obs$_{Achromatic}$ = 2.830; 95% CI of C.rand$_{Achromatic}$ = 4.526–4.547; P.rand = 0.001) and chromatic (C.obs$_{Chromatic}$ = 1.917; 95% CI of C.rand$_{Chromatic}$ = 2.667–2.682; P.rand = 0.001) visual channels.

(c) Biotic selection prediction 3: the brightness and coloration of snails and their backgrounds covary

With linear regression, we found a significant, positive relationship between snail and background brightness (adjusted $R^2 = 0.57$; $F_{1,44} = 61.3$; $p < 0.001$). This relationship remained significant using PGLS and the reduced dataset of 28 species (using the likelihood-based phylogenetic covariance matrix: $R^2 = 0.45$; $F = 21.04$; $p = 0.001$, using the Bayesian phylogenetic covariance matrix: $R^2 = 0.37$; $F = 15.46$; $p = 0.002$). Using two-block partial least squares, we found a significant relationship between snail and background coloration among the 46 sampled species (PLS.corr = 0.713; $p = 0.001$). This relationship remained significant when an outlier species (*Naesiotus amastroides*) was removed from the analysis (PLS.corr = 0.509; $p = 0.01$). The phylogenetic partial least-squares analysis was not significant using the reduced dataset of 28 species (using the likelihood-based phylogenetic covariance matrix: PLS.corr = 0.39; $p = 0.415$; using the Bayesian phylogenetic covariance matrix: PLS.corr = 0.45; $p = 0.198$).

(d) Abiotic selection prediction: the disparity between snails and their backgrounds will be greater for arboreal species than terrestrial species

An ANOVA using snail–background brightness disparity as the dependent variable, habitat as the main group factor and species as the subgroup factor revealed that arboreal snails were significantly brighter than terrestrial snails (mean arboreal brightness disparity = 2759.23; mean terrestrial brightness disparity = 552.85; $F_{45,1509} = 52.61$; $p < 0.001$).

(e) Relationship between brightness, background matching and island age

Using ANOVA, we found that snail species on young volcanoes were poorer matches to their backgrounds than species on older islands, both with respect to achromatic contrasts (young volcano C.obs$_{Achromatic}$ = 2.478; older island C.obs$_{Achromatic}$ = 2.054; $F = 31.08$, $p < 0.001$) and chromatic contrasts (young volcano C.obs$_{Chromatic}$ = 1.872; older island C.obs$_{Chromatic}$ = 1.217; $F = 111.84$, $p < 0.001$). From the mixed-effects model, we found that island age and habitat were significant predictors of snail–background brightness disparity (table 1). Generally speaking, brightness disparity decreased as island age increased, though there was a significant interaction between island age and habitat. A series of *post hoc* one-sample t-tests revealed that arboreal snails from young volcanoes, terrestrial snails from young volcanoes and arboreal snails from older islands were brighter than their backgrounds, while terrestrial snails from older islands were not brighter than their backgrounds (table 1). We did not have the statistical power to conduct this analysis at the species level.

Table 1. Results of a mixed-effects model with snail–background brightness disparity as an explanatory variable, island age and snail habitat as fixed effects, and background type as a random factor.

factor	estimate	s.e.	χ^2	*p*-value
island age	51.29	281.29	42.30	<0.001
habitat	−57.10	431.30	29.32	<0.001
island age × habitat	−1198.58	304.85	15.46	<0.001

4. Discussion

The spectacular diversity of animal coloration hints at a role for powerful selective forces, but evidence of these forces driving the evolution of variation above the species level is often weak. We use Galápagos snails of the genus *Naesiotus* to test several predictions of biotic selection, abiotic selection and a hypothesis that the signature of such selection varies among communities of different ages. We find that snails with shells that contrast local backgrounds are attacked by predators at a higher rate than those that are cryptic. As a result, snails match local backgrounds with respect to brightness, coloration and from the perspective of avian predators. We also find that arboreal species are brighter than terrestrial species, and the brightness disparity between snails and their backgrounds was greater for arboreal snails than terrestrial snails. Collectively, species found on young volcanoes were poorer matches to their backgrounds than those found on older islands, and nearly all of them were brighter than local backgrounds, while terrestrial species from older islands were better matches to their backgrounds.

The first major finding of this study is strong support for predation as a force directing colour evolution in this group. The first malacological explorers of Galápagos not only observed great colour diversity in the snail fauna they discovered, they also noted how snails tended to match the substrates they were typically found on [16]. We observed mockingbirds actively searching out and predating snails, and the evidence of regular predation litters the ground on many islands. We also demonstrate that poorly matching shells are attacked more often than cryptic shells. The evolutionary consequences of this are apparent in the viewer-independent covariation of both snail colour and brightness against local backgrounds. Perhaps most importantly, this background matching is apparent from the perspective of avian predators. It should be noted that while our comparisons tell us statistically how close the match tends to be between snails and their backgrounds from the perspective of predators, only our behavioural predation study demonstrates that a mismatch between snails and backgrounds is perceived by predators and effects predation rates. The hypothesis of predator-driven crypsis is well over 100 years old and apparent animal–background matching was one of the original arguments for the power of selection (*Biston bitularia* moths and the industrial revolution) [45]. Examples of predator selection driving colour diversification within species are common [1,5,7], and although the same forces are assumed to play a role in generating morphological diversity among species, evidence of this is scant (but see e.g. [46]). Our findings indicate that predation across a variable landscape of backgrounds has selected for diverse snail shell colours, including some shells that are velvety black, others that are brilliant white and many shades in between (figure 2).

We also find a potential conflict between thermoregulatory and predation-driven selection on brightness. Species that spend a majority of their time exposed to direct sunlight (arboreal species) are relatively poorer matches to their backgrounds than species that spend less time exposed (terrestrial species). Importantly, these arboreal species are significantly more reflective (brighter) than their local backgrounds, concordant with the prediction that thermoregulatory constraints require arboreal species to be brighter than their backgrounds. It is important to note here that the strength of both biotic and abiotic selection on these species may be seasonal, much like that observed on beak shape in Darwin's finches (reviewed in [47]). During the wet season, individuals of a single species of *Naesiotus* can be found foraging in many microhabitats. During the dry season, however, resting microhabitats appear to become highly stereotyped, with species restricted to specific microhabitats such as under rocks, on tree trunks and on vegetated substrates. The diversity of microhabitats and consequently backgrounds experienced by snails during the wet season may lead to variation in the degree of background matching among species. Concordant with this hypothesis, we find that humid zone species are poorer matches to their backgrounds than their arid zone counterparts (electronic supplementary material, figure S3). This is relevant because individuals in the humid zone likely experience a smaller proportion of their lives resting in a single microhabitat, instead ranging widely (from the perspective of a snail) among various backgrounds as they forage (A.C.K. & C.E.P. 2014–2017, personal observation). Thus, differences among regions (like the humid and arid zones) may also contribute to the impressive diversity of coloration we see among Galápagos endemic snails.

Interestingly, we find variation in the signature of selection among islands. The hypothesis of biotic selection is supported only on older islands, while we find support for abiotic selection on all islands. From the snail's perspective, microhabitat light environments vary little among islands. For example, we observed no consistent differences in irradiance within particular vegetation zones among islands. Therefore, abiotic selection in the form of solar radiation is unlikely to vary among islands. However, the predator community and the backgrounds snails are found on are likely to differ greatly from island to island. Directly quantifying predation across islands and habitats is beyond the scope of this paper and will be the topic of future work. Through this study we have focused on establishing a pattern consistent with the idea that selection on species in particular communities is similar, while selection pressure among islands differs. From the results of this study, we propose a scenario of shifting selective forces along island ontogeny that leads to the distribution of snail colour and brightness we observe in Galápagos. First, snails on the youngest volcanoes are recent arrivals that have had little time to respond to local selection from predators relative to most species from older communities (i.e. species on older islands). Therefore, uniform abiotic selection may either ensure that only the brightest colonists are able to initially colonize and become established, the first colonist snails are selected for brighter coloration after colonization or, most likely, a combination of these

scenarios. In either case, as islands age predator–prey communities become more established and snails evolve not only in response to abiotic selective forces but also biotic selection. This shift may explain why species from young volcanoes tend to be uniformly bright, while species from older islands are more variable in coloration, as abiotic selection in this system is generally unidirectional while the trajectory of biotic selection from predators is determined by local factors like the predator community and snail microhabitat. Future research efforts on this system will focus directly on the predator communities and how they affect selection on *Naesiotus* snails.

Even though we find that island age is a significant predictor of snail–background brightness disparity, strange patterns remain, such as the remarkable brightness of snails from Santiago, the youngest 'older island' of the archipelago at about 700 kyr [21]. On this island, even though we observe a significant snail–background relationship, we find that as a group these snails are among the brightest in the archipelago (and are typically far brighter than the backgrounds they are found on). This is particularly interesting because mockingbirds are present on this island (though we encountered few in the habitats where snails were most common). We propose that there may be a lag in adaptation with respect to brightness. When considered at the island scale, it appears that snails tend to gain brightness for the first million years of the island's ontogeny, then selection from predators begins to draw down snail brightness as the islands age. While this overall pattern makes intuitive sense, the details of this pattern are somewhat puzzling. For example, the inflection point from increasing brightness to decreasing brightness occurs surprisingly late in island ontogeny (between 700 kyr and 1.5 Myr). This is particularly surprising because mockingbird predators are present on most islands regardless of island age [48]. From our observations of snails and mockingbirds in the field, we propose an additional scenario that may better explain this pattern. First, snail populations on young islands are typically found at elevations much higher than the maximum elevations of older islands. Second, we observe few mockingbirds in snail habitats on young islands, though they are typically common on older islands (except for Floreana, where mockingbirds went extinct in modern history). Therefore, even though snail and mockingbird ranges overlap at the island scale across much of Galápagos, mockingbirds may not be a strong selective force on snails until their ranges overlap at local scales, which may not occur for the

first several hundred thousand years of island ontogeny. However, we have not completed a systematic survey of snail predator diversity or density. Although beyond the scope of the present study, such a study focused on avian predatory behaviour and local-scale distribution is needed to test this hypothesis.

The diversity of snail brightness and coloration across the Galápagos Archipelago is concordant with the predictions of selection by visually oriented predators and solar radiation over millions of years. However, surprisingly few details of snail ecology are known. While we have observed mockingbirds hunting snails, the potential effects of predation from other species, like Darwin's finches remain unknown (though they have been observed feeding on snails) [49]. Furthermore, no studies have directly quantified the relationship between shell brightness and thermoregulation in *Naesiotus*, though this is well established in other gastropods [4,17–20]. Clearly, our understanding of the forces generating shell colour diversity in this remarkable lineage would benefit from further research into the evolutionary ecology of coloration at finer scales. A focus on the evolutionary history and contemporary ecological dynamics of the brilliantly coloured snails of Santiago Island may help to further clarify the evolutionary importance of coloration in this group.

References

1. Dice LR, Blossom PM. 1937 *Studies of mammalian ecology in southwestern North America: with special attention to the colors of desert mammals* (no. 485). Washington, DC: Carnegie Institution of Washington.

2. Dice LR. 1947 *Effectiveness of selection by owls of deer-mice (Peromyscus maniculatus) which contrast in color with their background.* Ann Arbor, MI: University of Michigan.

3. Pinkert S, Brandl R, Zeuss D. 2017 Colour lightness of dragonfly assemblages across North America and Europe. *Ecography* **40**, 1110–1117. (doi:10.1111/ecog.02578)

4. Heath DJ. 1975 Colour, sunlight and internal temperatures in the land-snail *Cepaea nemoralis* (L.). *Oecologia* **19**, 29–38. (doi:10.1007/BF00377587)

5. Cain AJ, Sheppard PM. 1954 Natural selection in Cepaea. *Genetics* **39**, 89.

6. Rajabizadeh M, Adriaens D, Kaboli M, Sarafraz J, Ahmadi M. 2015 Dorsal colour pattern variation inEurasian mountain vipers (genus *Montivipera*): a trade-off between thermoregulation and crypsis. *Zool. Anz.* **257**, 1–9. (doi:10.1016/j.jcz.2015.03.006)

7. Hegna RH, Nokelainen O, Hegna JR, Mappes J. 2013 To quiver or to shiver: increased melanization benefits thermoregulation, but reduces warning signal efficacy in the wood tiger moth. *Proc. R. Soc. B* **280**, 20122812. (doi:10.1098/rspb.2012.2812)

8. Morii Y, Porozorova L, Chiba S. 2016 Parallel evolution of passive and active defense in land snails. *Sci. Rep.* **6**, 35600. (doi:10.1038/srep35600)

9. Gould SJ, Vrba ES. 1982 Exaptation—a missing term in the science of form. *Paleobiology* **8**, 4–15. (doi:10.1017/S0094837300004310)

10. Losos JB, Ricklefs RE (eds). 2009 *The theory of island biogeography revisited*. Princeton, NJ: Princeton University Press.

11. Clusella-Trullas S, Terblanche JS, Blackburn TM, Chown SL. 2008 Testing the thermal melanism hypothesis: a macrophysiological approach. *Funct. Ecol.* **22**, 232–238. (doi:10.1111/j.1365-2435.2007.01377.x)

12. Borregaard MK *et al.* 2017 Oceanic island biogeography through the lens of the general dynamic model: assessment and prospect. *Biol. Rev.* **92**, 830–853. (doi:10.1111/brv.12256)

13. Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ. 2004 Is invasion success explained by the enemy release hypothesis?. *Ecol. Lett.* **7**, 721–733. (doi:10.1111/j.1461-0248.2004.00616.x)

14. Schluter D, Grant PR. 1984 Determinants of morphological patterns in communities of Darwin's finches. *Am. Nat.* **123**, 175–196. (doi:10.1086/284196)

15. Parent CE, Crespi BJ. 2009 Ecological opportunity in adaptive radiation of Galapagos endemic land snails. *Am. Nat.* **174**, 898–905. (doi:10.1086/646604)

16. Ochsner WH. 1905 *Washington H. Ochsner Galápagos expedition journal of conchology*. San Francisco, CA: Washington Henry Ochsner Papers.

17. Cain AJ. 1968 Sand-dune populations of *Cepaea nemoralis* (L.). *Phil. Trans. Roy. Soc. B* **253**, 499–517. (doi:10.1098/rstb.1968.0009)

18. Jones JS. 1973 Ecological genetics and natural selection in molluscs. *Science* **182**, 546–552. (doi:10.1126/science.182.4112.546)

19. Jones JS, Leith BH, Rawlings P. 1977 Polymorphism in Cepaea: a problem with too many solutions? *Ann. Rev. Ecol. Syst.* **8**, 109–143. (doi:10.1146/annurev.es.08.110177.000545)

20. Heller J. 1981 Visual versus climatic selection in the land snail *Theba pisana* in Israel. *J. Zool.* **194**, 85–101. (doi:10.1111/j.1469-7998.1981.tb04580.x)

21. Geist D, Snell H, Snell H, Goddard C, Kurz MD. 2014 A paleogeographic model of the Galápagos Islands and biogeographical and evolutionary implications. In *The Galápagos: a natural laboratory for the earth sciences*, pp. 145–166. Washington, DC: American Geophysical Union.

22. Kraemer AC, Kissner J, Adams DC. 2012 Morphological color-change in the red-backed salamander (*Plethodon cinereus*) while kept in captivity. *Copeia* **2012**, 748–755. (doi:10.1643/CH-12-006)

23. Aidala Z *et al.* 2012 Ultraviolet visual sensitivity in three avian lineages: paleognaths, parrots, and passerines. *J. Comp. Phys. A* **198**, 495–510. (doi:10.1007/s00359-012-0724-3)

24. Endler JA. 1990 On the measurement and classification of colour in studies of animal colour patterns. *Biol. J. Linn. Soc.* **41**, 315–352. (doi:10.1111/j.1095-8312.1990.tb00839.x)

25. Grill CP, Rush VN. 2000 Analysing spectral data: comparison and application of two techniques. *Biol. J. Linn. Soc.* **69**, 121–138. (doi:10.1111/j.1095-8312.2000.tb01194.x)

26. Vorobyev M, Brandt R, Peitsch D, Laughlin SB, Menzel R. 2001 Colour thresholds and receptor noise: behaviour and physiology compared. *Vision. Res.* **41**, 639–653. (doi:10.1016/S0042-6989(00)00288-1)

27. Vorobyev M, Osorio D, Bennett ATD, Marshall NJ, Cuthill IC. 1998 Tetrachromacy, oil droplets and bird plumage colours. *J. Comp. Physiol. A* **183**, 621–633. (doi:10.1007/s003590050286)

28. Maan ME, Cummings ME. 2012 Poison frog colors are honest signals of toxicity, particularly for bird predators. *Am. Nat.* **179**, E1–E14. (doi:10.1086/663197)

29. Kraemer AC, Adams DC. 2014 Predator perception of Batesian mimicry and conspicuousness in a salamander. *Evolution* **68**, 1197–1206. (doi:10.1111/evo.12325)

30. Kraemer AC, Serb JM, Adams DC. 2015 Batesian mimics influence the evolution of conspicuousness in an aposematic salamander. *J. Evol. Biol.* **28**, 1016–1023. (doi:10.1111/jeb.12622)

31. Hart NS, Partridge JC, Cuthill IC, Bennett ATD. 2000 Visual pigments, oil droplets, ocular media and cone photo-receptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *J. Comp. Physiol. A* **186**, 375–387. (doi:10.1007/s003590050437)

32. Parent CE, Crespi BJ. 2006 Sequential colonization and diversification of Galápagos endemic land snails genus *Bulimulus* (Gastropoda, Stylommatophora). *Evolution* **60**, 2311–2328.

33. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729. (doi:10.1093/molbev/mst197)

34. Swofford DL. 2003 *PAUP*: phylogenetic analysis using parsimony, version 4.0 b10*. Sunderland, MA: Sinauer Associates.

35. Minin V, Abdo Z, Joyce P, Sullivan J. 2003 Performance-based selection of likelihood models for phylogeny estimation. *Syst. Biol.* **52**, 674–683. (doi:10.1080/10635150390235494)

36. Felsenstein J. 1988 Phylogenies from molecular sequences: inference and reliability. *Ann. Rev. Gen* **22**, 521–565. (doi:10.1146/annurev.ge.22.120188.002513)

37. Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012 Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969–1973. (doi:10.1093/molbev/mss075)

38. Rambaut A, Drummond AJ. 2009 Tracer v1.4. (2007). See http://beast.bio.ed.ac.uk/Tracer.

39. Kikuchi DW, Pfennig DW. 2009 High model abundance may permit the gradual evolution of Batesian mimicry: an experimental test. *Proc. R. Soc B* **277**, 1041–1048. (doi:10.1098/rspb.2009.2000)

40. Kikuchi DW, Pfennig DW. 2010 Predator cognition permits imperfect coral snake mimicry. *Am. Nat.* **176**, 830–834. (doi:10.1086/657041)

41. Bates D. 2005 Fitting linear mixed models in R. *R News* **5**, 27–30.

42. R Core Development Team. 2017 *R: a language and environment for statistical computing*. Vienna, Austria: Foundation for Statistical Computing.

43. Maia R, White TE. 2018 Comparing colors using visual models. *Behav. Ecol.* **29**, 649–659. (doi:10.1093/beheco/ary017)

44. Adams DC, Otarola-Castillo E. 2013 geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Meth. Ecol. Evol.* **4**, 393–399. (doi:10.1111/2041-210X.12035)

45. Kettlewell HD. 1958 A survey of the frequencies of *Biston betularia* (L.) (Lep.) and its melanic forms in Great Britain. *Heredity* **12**, 51–72. (doi:10.1038/hdy.1958.4)

46. Mallet J. 2010 Shift happens! Shifting balance and the evolution of diversity in warning colour and mimicry. *Ecol. Ent.* **35**, 90–104. (doi:10.1111/j.1365-2311.2009.01137.x)

47. Grant PR, Grant BR. 2014 *40 years of evolution: Darwin's finches on daphne major island*. Princeton, NJ: Princeton University Press.

48. Bungartz F, Herrera HW, Jaramillo P, Tirado N, Jiménez-Uzcátegui G, Ruiz D, Guézou A, Ziemmeck F (eds). 2009 *Charles Darwin Foundation Galapagos Species Checklist—Lista de Especies de Galápagos de la Fundación Charles Darwin*. Puerto Ayora, Galapagos: Charles Darwin Foundation/Fundación Charles Darwin. See http://www.darwinfoundation.org/datazone/checklists/ (last updated 31 October 2016).

49. Schluter D. 1982 Distributions of Galapagos ground finches along an altitudinal gradient: the importance of food supply. *Ecology* **63**, 1504–1517. (doi:10.2307/1938876)