ECOLOGY

# Invasive chameleons released from predation display more conspicuous colors

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**Conspicuous social and sexual signals are predicted to experience pronounced character release when natural selection via predation is relaxed. However, we have few good examples of this phenomenon in the wild and none in species with dynamic color change. Here, we show that Jackson's chameleons inadvertently introduced from Kenya to Hawaii (Oahu), where there are no coevolved, native lizard predators, experienced pronounced character release of color signals. Hawaiian chameleons displayed more conspicuous social color signals than Kenyan chameleons during male contests and courtship, were less cryptic in response to bird and snake predators, and showed greater change between display and antipredator color states. Hawaiian chameleon display colors were also more conspicuous in their local than ancestral habitats, consistent with local adaptation of social signals. These results demonstrate that relaxed predation pressure can result in character release of dynamic social signals in introduced species experiencing strong sexual selection.**

## INTRODUCTION

When species encounter previously uninhabited environments, such as during human-mediated introductions, phenotypic change through a combination of plasticity and evolution can occur remarkably rapidly (*1*–*4*). In particular, release from predation by native-range predators enables evolutionary exploration of entirely new regions of the phenotypic landscape (*4*, *5*). Such character release should be most evident for social and sexual signals; released from constraints on conspicuousness imposed by predation risk, signals could elaborate in many directions (*4*, *6*–*8*). However, we are only beginning to understand how social and sexual signals change in response to rapid environmental change, and there remain very few empirical examples from natural populations (*7*, *9*, *10*). This is especially true for species with dynamic color change, for which we are not aware of any examples. Here, we examine character release of a dynamic color signal by taking advantage of an unintended "evolutionary experiment": accidental translocation of chameleons to a previously uninhabited, low-predation environment.

In 1972, a shipment of Jackson's chameleons (*Trioceros jacksonii xantholophus*; Fig. 1 and fig. S1; thought to be 36 or fewer individuals) was sent from Kenya to the Hawaiian island of Oahu (fig. S2), destined for the pet trade. The animals arrived in poor condition and were left outdoors to recover, at which point they dispersed and became established on the island (*11*). Jackson's chameleons have a high intrinsic population growth rate [9- to 12-month generation time and live birth of up to 50 young (*12*)], enabling their rapid establishment and potentially rapid evolution (approximately 50 to 65 generations). On Oahu, there are few potential predators of chameleons (there are no snakes or lizard-eating raptors, and other potential bird predators are absent or rare; Supplementary Materials), while in Kenya, they are preyed upon by a wide range of bird, snake, and, occasionally, mammal predators (*13*). This accidental introduction enables us to test whether relaxed predation results in character release of a dynamic visual signal and whether this might be explained by local adaptation.

Chameleons are famous for their rapid color change (*14*–*16*). When presented with another chameleon, male Jackson's chameleons adopt a characteristic display posture and become intensely yellow-green during courtship or to signal dominance (Fig. 1 and figs. S1 and S3). At other times, they are a duller green or brown or become mottled green-brown, particularly in response to predators (Fig. 1 and fig. S4A). Their different color states vary in conspicuousness depending on the context, background, and viewer (e.g., conspecific or predator). If introduced chameleons in Hawaii experience character release from reduced predation, we expect males to have more conspicuous display colors in response to conspecifics and to be less cryptic in response to bird and snake predators (table S1). We should also see evidence of local adaptation: Color signals should be more conspicuous to conspecifics against the local than ancestral background.

To test these predictions, we presented wild-caught adult male chameleons in both Hawaii and the source population in Kenya with either a male chameleon, a female chameleon, a bird predator (stuffed African cuckoo-hawk, *Aviceda cuculoides*) or a snake predator (replica boomslang, *Dispholidus typus*) (fig. S4), or a control (stick). We measured the spectral reflectance of male color states during male-male display (dominant in male-male contests, $n = 34$ Hawaii and $n = 35$ Kenya) and courtship ($n = 25$ Hawaii and $n = 32$ Kenya) and in response to bird ($n = 56$ Hawaii and $n = 23$ Kenya) and snake ($n = 59$ Hawaii and $n = 38$ Kenya) predators (Fig. 2 and fig. S5). We then modeled how conspicuous these colors would appear to the relevant receiver's visual system (chameleon, bird, and snake), estimated as chromatic or luminance contrast [just noticeable differences (JNDs)] (*17*) against the background vegetation of each environment (Hawaii or Kenya; Supplementary Materials).

## RESULTS AND DISCUSSION

In support of predictions of character release, male display coloration of Hawaiian chameleons had higher luminance contrast against the

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**Fig. 1. Chameleon color signal change in response to different social stimuli. Male chameleons experience intense sexual selection.** During the breeding season, they change from dull green to a highly conspicuous bright yellow display signal. They also readily fight by locking horns and sometimes pierce their rival's skin with their horns. (**A**) A dominant male in display coloration. (**B**) A subordinate male that lost a contest and turned from bright yellow to brown. (**C**) Two males fighting, both are in display coloration and relatively evenly matched. (**D**) A courting male in full display color, while the female has turned to a contrasting color, rejecting the male. See the Supplementary Materials for additional photos, including in response to a snake. Photo credit: Martin J. Whiting, Macquarie University.

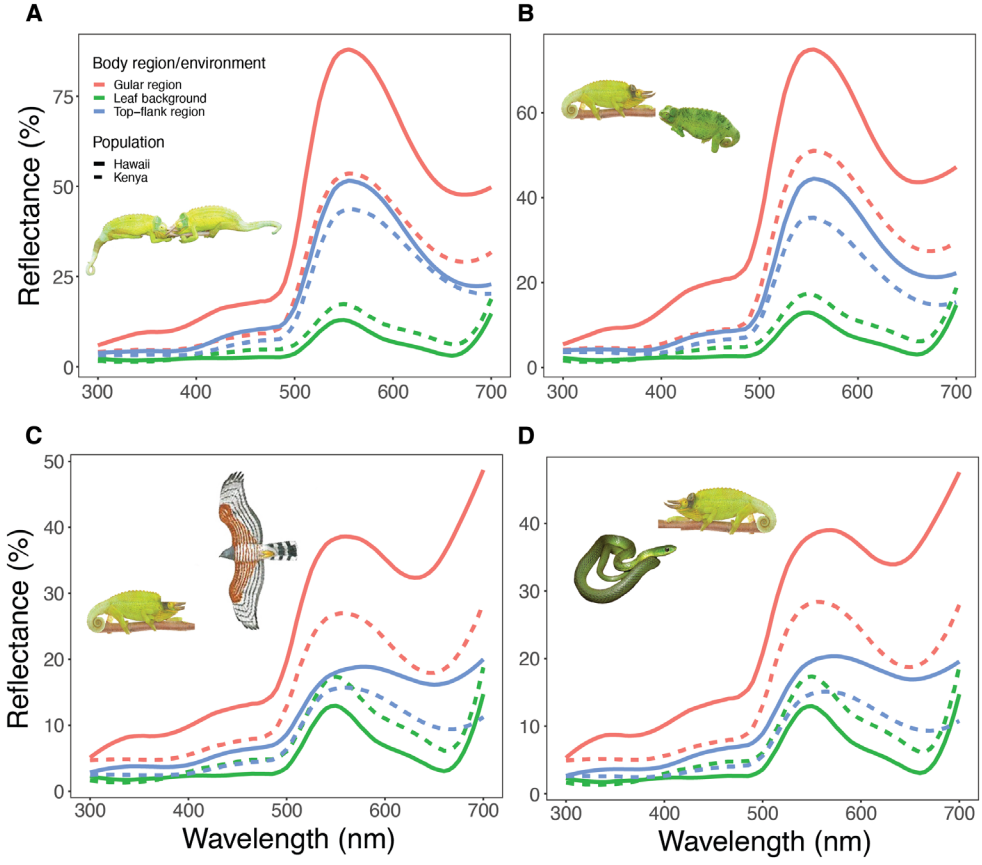


**Fig. 2. Mean spectral reflectance curves for male chameleons for representative body regions (gular and top flank) and background (leaves) for Hawaii and Kenya.** The context for measurement was (**A**) male contest displays, (**B**) courtship, (**C**) bird predator, and (**D**) snake predator. More details in the Supplementary Materials and fig. S5. Photo credit: Martin J. Whiting, Macquarie University.

local background than those of Kenyan chameleons [male-male contests: luminance contrast (*dl*); $F_{1,27.74} = 112.87$, $P$ from $< 0.0001$; courtship displays: luminance contrast (*dl*); $F_{1,27.58} = 51.31$, $P < 0.0001$] (Figs. 2 and 3A and table S2). This result was consistent for all body regions and was driven by the increased luminance of Hawaiian chameleons, rather than differences in the background environment. Hawaiian chameleons were more conspicuous against either Hawaiian or Kenyan environment [i.e., no population by background interaction effect for luminance: male-male contests: luminance contrast (*dl*); $F_{1,48.67} = 0.005$, $P = 0.94$; courtship displays: luminance contrast (*dl*); $F_{1,40.02} = 1.09$, $P = 0.30$; see also the Supplementary Materials and table S3]. However, Hawaiian chameleons did not differ from Kenyan chameleons in chromatic contrast of color signals (Fig. 3B and table S2, male-male contests: $F_{1,27.74} = 1.96$, $P = 0.17$; courtship: $F_{1,27.59} = 2.36$, $P = 0.14$).

The importance of luminance contrast is consistent with the nature and mechanism of rapid color change in individual chameleons, which usually involves much greater luminance than chromatic change (Fig. 3). Specifically, color change is caused by dispersion or concentration of melanin within melanophore pigment cells (*18*) or changes in the spacing of guanine crystals within iridophores (*16*), both of which strongly affect luminance. Accordingly, character release was observed in the overall luminance of signals rather than their hue.

Released from predation, Hawaiian chameleons were less cryptic than Kenyan chameleons when threatened by both bird and snake predators. Hawaiian chameleons had higher luminance contrast against the local background to the visual system of the corresponding predator (Fig. 3C; main effect of population; $F_{1,82.59} = 37.24$, $P < 0.0001$, no interaction between population and predator type, table S4). Differences between populations in chromatic contrast against the background, however, depended on the predator (significant predator by population interaction, table S4: $F_{1,45.34} = 55.34$, $P < 0.0001$). Hawaiian chameleons had higher chromatic contrast to

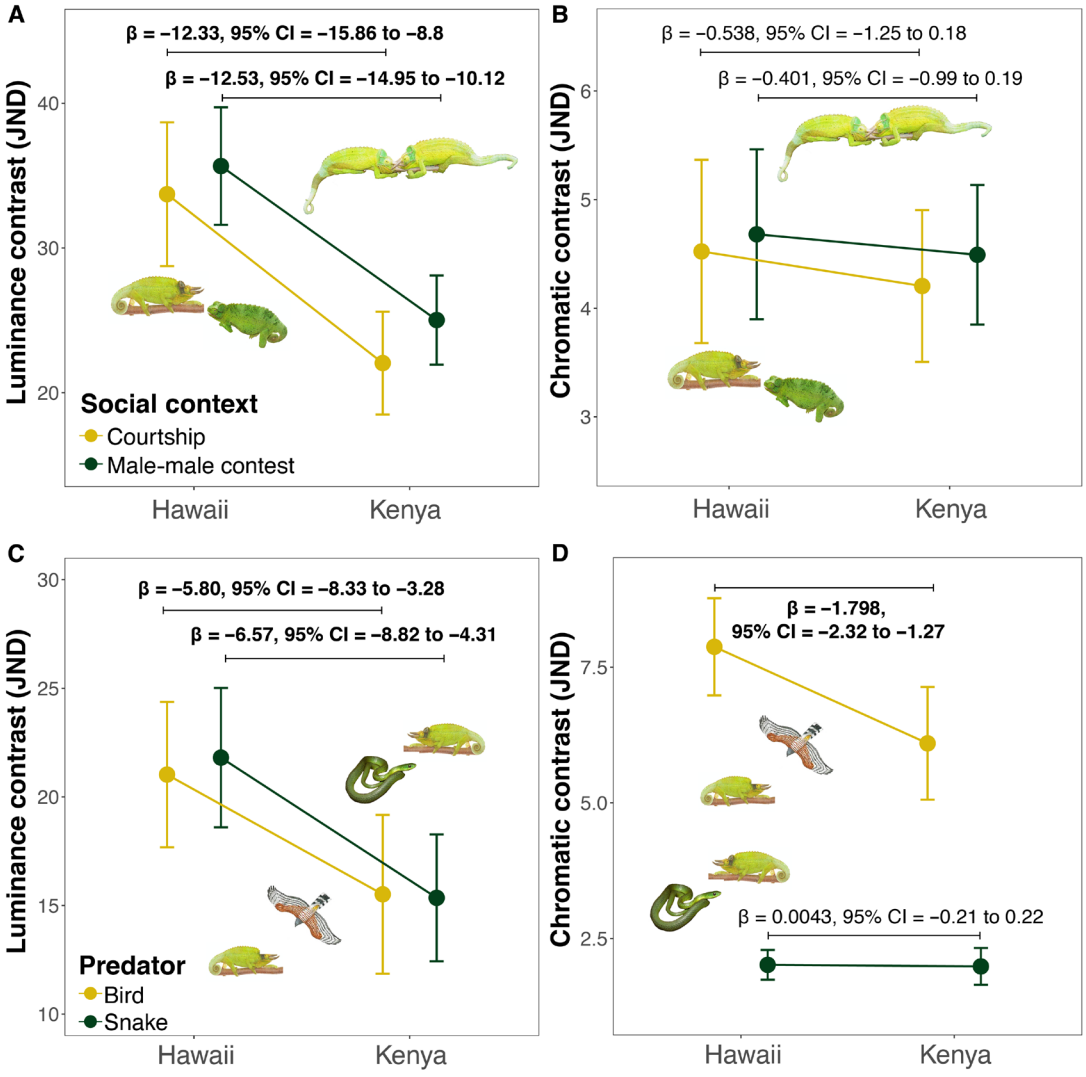


**Fig. 3. Luminance and chromatic contrast in JNDs of Hawaiian and Kenyan chameleons against their respective backgrounds (i.e., average environment of stems and leaves).** (**A**) Luminance and (**B**) chromatic contrast of male chameleons during male-male contests and female courtship. JNDs are calculated on the basis of the chameleon visual system. (**C**) Luminance and (**D**) chromatic contrast of male chameleons during snake and bird predator encounters. JNDs are calculated on the basis of the snake and bird visual systems. Contrasts between means, "beta," that are bold indicate significant effects.

birds than their Kenyan conspecifics [birds (Hawaii-Kenya): β = −1.8, 95% confidence interval (CI) = −2.32 to −1.27, $P$ < 0.0001; Fig. 3D and table S5]. By comparison, chameleons from both populations had very low and similar chromatic contrast to the visual system of snakes [snakes (Hawaii-Kenya): β = 0, 95% CI = −0.21 to 0.22, $P$ = 1; Fig. 3D and table S5]. The much lower chromatic contrast of chameleons to the visual system of snakes than birds reflects the much poorer chromatic discrimination of trichromatic snakes compared to tetrachromatic birds (*19*). The poor color discrimination of snakes may also explain why Hawaiian and Kenyan chameleons differ little in their chromatic response to snakes despite the absence of snakes in Hawaii.

Character release may reflect rapid evolutionary change, phenotypic plasticity, or a combination of these processes (*20*, *21*). Rapid evolutionary change results in local adaptation, whereby signals become more conspicuous to conspecifics in their local environment than in ancestral environments. Consistent with local adaptation, we found that Hawaiian chameleons were more conspicuous to other chameleons against their own (Hawaiian) background than against their ancestral Kenyan background, in both social contexts, although effect sizes for luminance were much larger than for chromatic contrast [male-male contests: luminance (*dl*), β = −8.19, 95% CI = −8.32 to −8.06, $F_{1,29.17}$ = 1641, $P$ < 0.0001; chromatic (*dS*), β = −0.75, 95% CI = −0.81 to −0.7, $F_{1,29.17}$ = 754.92, $P$ < 0.0001; courtship: luminance (*dl*), β = −8.16, 95% CI = −8.29 to −8.04, $F_{1,21.87}$ = 1811, $P$ < 0.0001; chromatic (*dS*), β = −0.69, 95% CI = −0.78 to −0.59, $F_{1,21.87}$ = 221.08, $P$ < 0.0001; Fig. 4 and tables S3 and S6]. These results are consistent with local adaptation, in which phenotypic plasticity, which can also evolve rapidly (*22*), may also play a role.

To gauge the extent of color plasticity, we examined individual change between display (male-male contest) and antipredator (bird) color states (absolute difference in JNDs between states; $n$ = 30 Hawaii and $n$ = 13 Kenya). While there was evidence for differences between body regions (Supplementary Materials, fig. S7, and tables S13 and S14), overall, Hawaiian chameleons showed greater chromatic but not luminance change compared to Kenyan chameleons (chromatic: $F_{1,18.96}$ = 10.51, $P$ < 0.01; luminance: $F_{1,18.8}$ = 2.64, $P$ = 0.12), although luminance change showed similar trends across body regions (fig. S7). Together, these results indicate that chameleons introduced to Hawaii only 50 years ago have evolved signals that are locally adapted, are more conspicuous to conspecifics, and show greater changes between display and antipredator color states. An alternative explanation is that differences might be due to a founder effect. Founder effects are expected to be random with respect to the environment unless sampling is deliberately biased, for example, selective collection of chameleons for the pet trade. The traits we quantified, and their observed differences, were all in the predicted direction of adaptive change, arguing against a random founder effect. The possibility that hunters selected the most brightly colored individuals is similarly unlikely because chameleons are almost always encountered in a camouflaged color state; they are solitary and only use display colors during relatively brief social encounters. Even if pet traders were selecting for larger animals, our analyses controlled for any relationship body size might have on color. Thus, a founder effect, due to random or deliberately biased

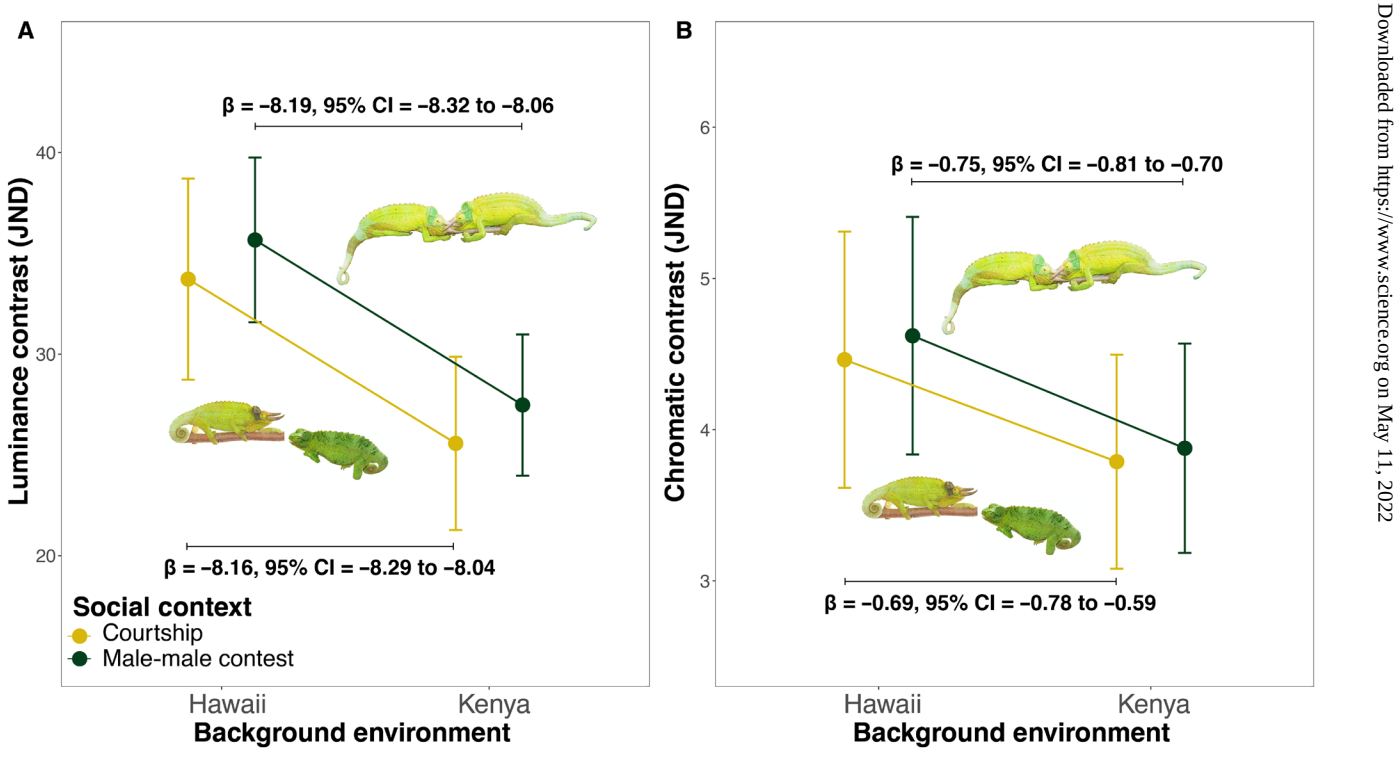


**Fig. 4. Luminance and chromatic contrast in JNDs of Hawaiian chameleon social signals against their own (Hawaii) background and that of Kenya.** (**A**) Luminance and (**B**) chromatic contrast of male chameleons during male-male contests and female courtship. For local adaptation, signals are predicted to be more conspicuous against their own background. In this case, Hawaiian chameleons were both significantly more chromatic and brighter (luminance contrast) against their own background compared to the Kenyan background, indicating increased local signal conspicuousness.

sampling, is unlikely to explain phenotypic differences consistent with character release in chameleons.

Differences in coloration between populations could be due to environmental effects such as diet or climatic variables (e.g., temperature and rainfall/humidity). However, the measured colors and color change represent a dynamic response to specific stimuli (conspecific and model predator), in contrast to the "fixed" color signals of many birds, fishes, and lizards that have been shown to be affected by diet and other environmental factors. In addition, the mechanisms underlying the rapid color response of chameleons are not linked to dietary pigments, and luminance change is partially structural (*16*). Therefore, environmental effects (other than predation) are unlikely to account for major differences in the color responses that we observed between populations. The degree to which color change capacity is plastic in chameleons and other organisms with dynamic color change is unknown. While we cannot exclude the role of phenotypic plasticity, plasticity can itself evolve and be an adaptive response (*22*). The population differences that we observed are likely an interaction between genetic and environmental factors, which are difficult to disentangle, even with common garden experiments (*23*). Regardless, we have demonstrated an unexpectedly strong effect of recent release from predation on a conspicuous, dynamic visual signal.

Oahu has no snakes or raptors that feed on lizards. The main threat for Oahu chameleons is domestic and feral cats, and cats have very limited color vision (*24*). In the absence of the usual suite of predators that they would experience in their native range of Kenya—a diverse community of snake and bird predators (*13*)—the conditions were highly favorable for character release of social signals. Jackson's chameleons likely experience strong sexual selection given their polygynous mating system, elaborate courtship displays, and intense male contest competition (Fig. 1) in which males lock and twist their three horns into rivals during intense bouts that sometimes result in physical injury. Consequently, a unique set of conditions set the stage for what we have demonstrated—character release of a dynamic social signal over a short ecological time scale. To the best of our knowledge, this is also the first example of character release in a dynamic color signal.

Rapid color change enables animals to be highly conspicuous during social interactions and highly cryptic at other times (*14*). Our results suggest that even for species capable of dynamic color change, there is an upper threshold to signal intensity that is constrained by natural selection. This novel finding raises the question of whether other animals that have convergently evolved dynamic color signals, such as cephalopods, frogs, other lizards, and fishes, may be likewise constrained. More generally, our study highlights the opportunities provided by invasions to study natural and sexual selection in the wild.

## MATERIALS AND METHODS

### Study area and collection of chameleons

We conducted experiments on chameleons in Hawaii and the likely source population in Kenya. In Hawaii, chameleons were collected from a single population in the forested Ko'olau Mountains north of Honolulu, between upper Makiki Valley and the summit of Tantalus Mountain, Oahu, accessible along Tantalus and Round Top drives at an elevation of ca. 400 to 600 m. The vegetation in this area consisted of closed-canopy, mid-elevation tropical rain forest, with extensive vines and undergrowth (fig. S2, C and D). Most of the plants in this area were non-native, and chameleons also occurred in hedgerows and stands of bamboo. The area experiences high annual rainfall (mean, ca. 1500 mm) (*25*), and the mean annual temperature is 22° to 24°C (*26*). In Kenya, fieldwork was conducted near the foothills of Mt. Kenya, close to the town of Runyenjes. This population is from the region (slopes of Mount Kenya) identified as the source population (*27*) for the introduction to the Hawaiian Islands. The area is partially cleared for agriculture, leaving small stands of forest. The vegetation was a mix of native trees and shrubs interspersed with exotic species (fig. S2, A and B). Mean annual temperature is 20.2°C (mean maximum, 24.8°C), and the mean annual rainfall is 121 mm (*28*).

We collected most chameleons (fig. S1) at night by spotlighting and a small proportion of individuals during the day. When chameleons were beyond reach in the forest canopy, we used lightweight 6-m extensible graphite composite poles (South Bend Kwik Stix 20′ Telescopic Fishing Pole), that chameleons could be encouraged to step on to, before lowering them to the ground. We then placed them in cotton bags with vegetation to cling to, before returning them to the laboratory. We measured snout-vent length (SVL), a standard measure of body size in lizards, from the tip of the snout to the posterior edge of the vent using a plastic ruler to the nearest 1 mm. Fieldwork was conducted in Hawaii during January to February 2006 and in Kenya during 5 to 13 April 2006, when chameleons are expected to show reproductive behavior. The timing was different because of seasonal/geographic differences between Kenya and Hawaii. The chameleons all exhibited very strong behavioral responses during courtship and male-male competition, consistent with reproductive behavior.

### Behavioral trials

To quantify display coloration, we staged encounters between conspecifics (male-male and male-female) and model predators (bird and snake) and measured their subsequent display coloration (details below). All chameleons were used in both predator and social trials, although in some cases we were unable to quantify all social signals for a particular individual. We conducted all conspecific-social trials first, in case antipredator trials unduly influenced their social interactions. Our analyses focused on comparing color responses of chameleons to conspecifics and predators to ensure that the color state corresponded to specific stimuli. We did not, and could not, measure a "neutral" color state because it is impossible to meaningfully gauge a neutral state in a color-changing organism such as a chameleon. However, for predator trials, we ensured that chameleons were specifically responding to the predators by conducting seven trials with just a branch with no predator attached as a control. Chameleons did not respond to the branch [see also (*19*)]. Furthermore, during trials with predators, chameleons focused on the predator itself and exhibited classic antipredator behavior (e.g., contrasting stress color, body inflation, and open-mouth threat).

Behavioral trials were conducted during the chameleons' natural activity period (0900 to 1600) beginning the day following capture and continuing over a 2- to 3-day period. We erected a frame consisting of branches from the chameleons' environment that were tied together in a horizontal triangular perch and that sat atop three vertical branches that formed a stand (fig. S3). This structure was about 1.5 m high, and each arm was approximately 60 cm. The

triangular setup allowed chameleons to display at a comfortable distance and, if necessary, to escape from aggressive interactions, particularly during male-male trials (fig. S3). All trials were conducted outdoors, but in the shade under the cover of a roof, surrounded by vegetation, to simulate their natural environment. The signaling environment was therefore the same for all animals.

We staged trials between males to elicit dominant and subordinate displays and between males and females to elicit courtship displays. This consisted of placing two chameleons at a comfortable distance at which point they typically responded to the conspecific's presence by rapidly changing color and behavior (fig. S1, A and B). In male-male trials, both individuals would adopt a lemon yellow display coloration and advance toward each other. This would be accompanied by head shakes and gapping, leading to horn-locking (fig. S1B) if males were not obviously asymmetric. Once dominance was settled, the subordinate would turn brown (figs. S1B and S3) and flee. In male-female (courtship) trials, males would turn the same lemon yellow and approach the female while head shaking and rocking (fig. S1C).

The day following social trials, we presented each chameleon with a snake (fig. S4A) and bird (fig. S4B) model predator model (separately). Lizards were drawn at random, and the order of presentation (snake or bird first) was random. Both trials were conducted on the same day but were separated by at least 3 hours. A trial began when a chameleon was removed from its cloth bag and placed on the triangular stand. In the case of the snake model, one of us slowly moved the model snake toward the chameleon from both above and below it, without ever contacting the chameleon, in a standardized fashion (fig. S4A). In the case of the bird trials, the bird was attached to a ca. 2-m wooden pole and was "flown" past the chameleon from above and below it (fig. S4B). Trials lasted no more than 2 min, and we scored whether the chameleon flipped to the opposite side of the branch, which is a classic antipredator behavior by chameleons. Once chameleons changed color, we quantified their spectral reflectance (details below). The snake model was molded from a dead boomslang (*D. typus*) and painted by a professional artist to resemble a typical green form of this species [previously used in (*19*, *29*)]. The bird was a mounted African Cuckoo-hawk (*A. cuculoides*).

## Color measurement

During all behavior trials, we measured the color of each of the four body regions: top flank, mid-flank, tail base, and gular region (fig. S5). We measured each body region once only because chameleons change color rapidly and we had to reduce the handling stress to the animal. The four body regions were measured in random sequence to avoid any bias of order. When a chameleon had completely changed color (to a human observer) and was also performing typical behavior associated with their display (e.g., head shakes, swaying, gaping, rapid approach, or flee), we lightly restrained it with one hand, without removing it from its perch, and measured spectral reflectance using a 1.2-m bifurcated probe connected to an Ocean Optics USB2000 spectrophotometer and a PX-2 light source. We used an Ocean Optics RPH-1 probe holder, which we placed in contact with the animal during measurement. The probe holder ensured that measurements were taken at a standard angle (45°) and distance (5 mm) for an area of 3 mm by 5 mm and excluded all ambient light. All measurements were relative to a dark and a certified 99% white reflectance standard (Labsphere, North Sutton, NH, USA). If chameleons started to change color in response to our handling, we returned them to the branch and allowed them to continue a social trial before measuring reflectance of the remaining body regions. In the case of a predator trial, we would once again present them with the predator model until they returned to their antipredator display. This system worked effectively because chameleons readily responded to social and antipredator stimuli and quickly returned to natural behavior following handling.

We obtained spectral reflectance measurements of male dominant color state in male-male contests for 34 individuals from Hawaii and 35 from Kenya, courtship color state for 25 individuals from Hawaii and 32 from Kenya, color state in response to the bird for 56 individuals from Hawaii and 23 from Kenya, and color state in response to the snake for 59 individuals from Hawaii and 38 from Kenya.

We quantified the background against which chameleons signal by measuring the spectral reflectance of vegetation in their signaling environment. We took 120 reflectance measures of the background at each location (Hawaii and Kenya), comprising 40 measurements of the top surface of leaves, 40 of the bottom surface of leaves, and 40 stems and branches of suitable width for chameleons. To reduce the number of backgrounds, we averaged the reflectance of the 20 darker measurements and 20 lighter measurements for each of the top leaf and bottom leaf measurements and averaged brown stems and green stems. This resulted in six average background spectra for each location, representing the range of background colors.

## Visual modeling

We visualized and quality-checked all spectra, adjusted very minor negative reflectance values caused by electrical noise by lifting the curves by the maximum negative value, and then smoothed all spectra with LOESS smoothing of 0.16 using the plotsmooth function in pavo version 1.0 (*30*). We also took the median reflectance for all leaves (top and bottom) and stems (two measures per stem) for each population (Hawaii and Kenya).

We modeled how conspicuous a chameleon would appear to both a conspecific receiver and a predator (bird and snake) using the receptor-noise limited (RNL) model (*17*, *31*) implemented in the R package pavo (*30*). This model assumes that color discrimination is limited only by photoreceptor noise and does not account for potential effects of neural processing. Color contrasts are expressed in units of JNDs, where 1 JND is the theoretical threshold of color discrimination for stimuli viewed simultaneously under ideal viewing conditions (*32*). Under natural conditions, discrimination thresholds are likely to be >1 and vary depending on the species and conditions (*33*–*35*). In addition, perceived conspicuousness may not scale linearly with color distance (JNDs) (*36*). Nevertheless, the models provide a reasonable approximation of relative conspicuousness that can be compared between populations and trial types (social and predator).

We calculated JNDs for chromatic and luminance contrast against each of the relevant backgrounds for each body region and color state based on the visual system of the relevant receiver (chameleon, bird, and snake; see below). We first calculated the quantum catch of each photoreceptor under standard daylight illumination (D65; Commission internationale de l'éclairage) using the "vismodel" function. We applied the von Kries chromatic adaptation (vonkries = TRUE) and used the average background reflectance of the relevant background (Hawaii or Kenya) as the adapting background. The signal

in each photoreceptor class was proportional to the natural logarithm of the quantum catch, in accordance with Fechner's law ("fi" in pavo). Next, we calculated the chromatic contrast between pairs of spectra using the function "coldist."

The visual models require information on the spectral sensitivities and photoreceptor noise (Weber fraction), which is a function of the relative density of each photoreceptor type within the retina, for the relevant receiver. Chameleons have four spectral classes of single cone enabling tetrachromatic vision (*37*). We used spectral sensitivities for the congeneric flap-necked chameleon (*Chamaeleo dilepis*) (*37*) and a standard Weber fraction of 0.1 (*32*) to account for the receptor noise of the LWS photoreceptor. We calculated the noise for the other photoreceptor classes using the relative photoreceptor ratio of 1:1.6:3.3:3.2 for the four single cones (UVS, SWS, MWS, and LWS, respectively). *C. dilepis* and other chameleons appear to have two populations of MWS cones ($\lambda_{max}$, 481 and 497 nm) and three populations of LWS cones ($\lambda_{max}$, 568, 584, and 605 nm), so we used a combined sensitivity function for the MWS and LWS cones (fig. S9).

For birds, we used an ultraviolet sensitive (UVS) visual system (*38*) characteristic of raptors and other birds of prey (*39*). A Weber fraction of 0.06 was used for the LWS photoreceptor based on the most recent empirical measurement (*40*). We used a relative photoreceptor ratio of 1:2:3.4:3 for the UVS, SWS, MWS, and LWS photoreceptors, respectively, which represent average values for UVS birds (fig. S9B) (*41*).

For snakes, we assumed a trichromatic visual system characteristic of active diurnal snake species, represented by the spectral sensitivities for the garter snake (*42*). We used a Weber fraction of 0.1 for the LWS photoreceptor and a relative photoreceptor ratio of 1:1.6:7.3 for the UVS, SWS, MWS, and LWS photoreceptors, respectively (fig. S9C) (*42*).

In each case, visual pigment absorbance curves were multiplied by the transmission spectra of ocular media (lens and cornea) and oil droplets associated with the corresponding photoreceptor class and normalized to equal area under the curve to satisfy the assumption of equal stimulation by white light (*38*).

Luminance contrast was calculated using the sensitivity function for the LWS photoreceptor (with transmission of the oil droplet associated with the double cone in chameleons and birds) for each visual system, assuming a Weber fraction of 0.05 because double cones used for luminance perception in birds and chameleons are by far the most abundant photoreceptor type in the retina (*37*, *43*).

### Statistical analysis

Chromatic and luminance contrast from visual models, measured in JNDs, were analyzed using linear mixed effects models in R using the lme4 package (*44*) in combination with the package clubSandwich (*45*). For each individual, measurements for each body region (top flank, mid-flank, tail base, and gular region) were contrasted against each of six average background spectra (i.e., average top and bottom of lighter leaves, average top and bottom of darker leaves, and average green and average brown stems). While we present sensitivity analyses for all body regions (see Supplementary Results), we were mainly interested in the overall signal differences displayed by chameleons. Hence, for our main analyses, we averaged estimates (marginalized) across these body regions and background environments given that, in many cases, body regions showed remarkably similar effects (see Supplementary Results). In all cases, our results and conclusions were not affected (see Supplementary Results). Deriving multiple contrasts for each individual body region and background, however, resulted in a substantial number of comparisons, each of which was not completely independent of each other. To ensure that nonindependence of data did not affect our inferences, we included individual ID as a random effect to account for repeated measures on each individual and used a robust variance estimator (using our final models to correct SEs) and a Satterthwaite degrees of freedom correction.

First, we tested whether Hawaiian chameleons were more conspicuous (as perceived by chameleons) in social contexts than Kenyan chameleons. We ran separate linear models with chromatic and luminance contrast of male display or courtship coloration against the local background as the response variable. We included population of origin and *z*-transformed SVL as fixed effects. To determine whether differences in conspicuousness were driven by differences in the color of chameleons or the background, we also fit models that compared the luminance and chromatic contrast of Hawaiian and Kenyan lizards against Hawaiian and Kenyan backgrounds and examined the interaction between population and background.

Second, we tested whether Kenyan chameleons were more cryptic (less conspicuous against the local background) in response to predators. For these models, chromatic or luminance contrast against the local background (as perceived by the corresponding predator type) was the response variable and predator type (i.e., snake or bird), population of origin (Hawaii or Kenya), and their interaction were fixed effects. For all models, interaction terms were first tested using Wald tests. If found to be nonsignificant ($P > 0.1$), we dropped the interaction and fitted a main effects model. We then tested the significance of the main effects using Wald tests with robust variance estimators and a Satterthwaite degrees of freedom correction.

Last, to evaluate evidence for local adaptation, we tested whether the chromatic or luminance contrast of Hawaiian chameleons during social interactions (male-male and male-female) varied depending on whether they were contrasted against a native Kenyan background or their current introduced habitat background in Hawaii. In other words, we parameterized these models to include the interaction between population and background such that each population (Kenya and Hawaii) was compared against each background (Kenyan and Hawaiian). Doing so decouples the confounding effect of lizard display from their background. We present full interaction models given that we were, a priori, interested in specific comparisons.

## SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abn2415

View/request a protocol for this paper from *Bio-protocol*.

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**Acknowledgments:** This research was approved by the Kenya Wildlife Service (R. Bagine, KWS 5001) and the Ministry of Science and Technology (Research Permit No. MOS&T 13/36C 208). We thank I. Farah and P. Malonza of the National Museums of Kenya for facilitating the research, J. Gathua for amazing field support, and J. Stipala for use of his field vehicle. We thank the Ditsong National Museum of Natural History (formerly Transvaal Museum) for the stuffed African Cuckoo Hawk. B. Phillips, R. Shine, and T. Uller provided feedback on earlier drafts of this manuscript. We thank F. Kar for help curating data and D. Allen for statistical advice. **Funding:** Financial support was provided by the University of the Witwatersrand (to M.J.W.) and the Australian National University (to J.S.K.). **Author contributions:** M.J.W. conceived the study. M.J.W. and D.S.-F. designed the study with help from J.S.K. and B.S.H. M.J.W., B.S.H., J.S.K., and D.S.-F. collected the data. M.J.W., D.S.-F., K.J.R., and D.W.A.N. performed data curation, visualization, and exploratory analyses. D.W.A.N. performed final analyses. M.J.W. wrote the first draft, with help from D.W.A.N. and D.S.-F. All authors contributed substantially to subsequent versions. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data and code needed to evaluate the conclusions in the paper can be found at the Open Science Framework: https://osf.io/vxyp5/?.

Submitted 12 November 2021
Accepted 25 March 2022
Published 11 May 2022
10.1126/sciadv.abn2415

Science Advances

# Invasive chameleons released from predation display more conspicuous colors

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*Sci. Adv.*, 8 (19), eabn2415. • DOI: 10.1126/sciadv.abn2415







*Science Advances* (ISSN ) is published by the American Association for the Advancement of Science. 1200 New York Avenue NW, Washington, DC 20005. The title *Science Advances* is a registered trademark of AAAS.

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# Supplementary Materials for

## Invasive chameleons released from predation display more conspicuous colors

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Published 11 May 2022, *Sci. Adv.* **8**, eabn2415 (2022)
DOI: 10.1126/sciadv.abn2415

**This PDF file includes:**

Supplementary Text
Figs. S1 to S9
Tables S1 to S14
References

## Expanded Hypotheses and Predictions

We provide an expanded table of the key hypothesis and associated predictions. We also identify the data needed to test these predictions.

**Table S1.** Hypothesis and associated predictions tested in this paper in relation to the population of interest, the receiver visual system used in visual modelling, and the background against which the contrast of the signal was calculated for chromatic and luminance contrast.

| Hypothesis | Prediction | Population | Visual System | Background |
|---|---|---|---|---|
| Relaxed predation pressure results in character (color signal) release | Display coloration used in male-male contests and courtship will be more conspicuous to a conspecific in Hawaii than Kenya. | Hawaii | chameleon | Hawaii |
| | | Kenya | chameleon | Kenya |
| | Kenya chameleons should be more camouflaged in response to bird and snake predator stimuli than Hawaiian chameleons because Kenya has more chameleon predators. Conversely, if Kenyan chameleons are more camouflaged, then Hawaiian chameleons will be more conspicuous. | Hawaii | snake/bird | Hawaii |
| | | Kenya | snake/bird | Kenya |

## Potential bird predators of chameleons on Oahu

We are not aware of any evidence of bird predation of chameleons on Oahu. We consulted with several ornithologists in Hawaii regarding feeding behavior of the few predatory bird species that consume vertebrate prey, and we examined the checklist of the birds of Oahu to determine if any species were potential predators of chameleons (*46,47*). Among the larger, predatory birds, two are owls (*Asio flammeus sandwichensis*, *Tyto alba*), which are extremely rare on Oahu. Both species are highly unlikely to feed on chameleons because they feed out in the open rather than in forest canopy where chameleons occur, and typically feed on rodents, birds and insects (*47*). Smaller to medium raptors include the Merlin (*Falco columbarius*) and the peregrine falcon (*Falco peregrinus*) which are both considered rare non-breeding vagrants in the Hawaiian Islands, and feed on small-medium birds in more open habitats (*47*). There are also eight species of egret and heron, which typically have generalist diets. Of these, six are considered rare or accidental (*47*), are associated with aquatic habitats, and are better known for eating fishes (*48*). The cattle egret (*Bubulcus ibis*) is a generalist predator that is mostly insectivorous but does eat lizards opportunistically (*49, 50*) However, it forages on the ground in more open habitats, often following domestic animals and agricultural or landscaping machinery (*50*) and therefore, is not found in forested habitats and is highly unlikely to encounter chameleons. Among other potential predators, the Hawaiian Hawk or ʻio (*Buteo solitarius*) is restricted to the Big Island, while the Hen Harrier (*Circus cyaneus*) is considered an accidental or rare visitor to the Hawaiian islands. Finally, among corvids, the Hawaiian Crow (*Corvus hawaiiensis*) is extinct in the wild while the Common Raven (*Corvus corax*) is extremely rare (*47*). In summary, we could not identify any bird species that could be regarded as a major predator of chameleons.

## Supplemental Materials and Methods


**Fig. S1. Additional examples of chameleon color signals and behavior in response to different social contexts**. **(A)** Two different males in conspicuous display color adopted at the start of a contest and retained by the dominant male. **(B)** Two examples of males engaged in horn locking. Males in the bottom panel are evenly matched at this stage and both are in full display color. In the top image, the male on the left is starting to change to a subordinate color. **(C)** Conspicuous yellow-green male courtship color. When females reject males, they develop a heavily contrasting pattern. Photo credit: Martin J. Whiting, Macquarie University.

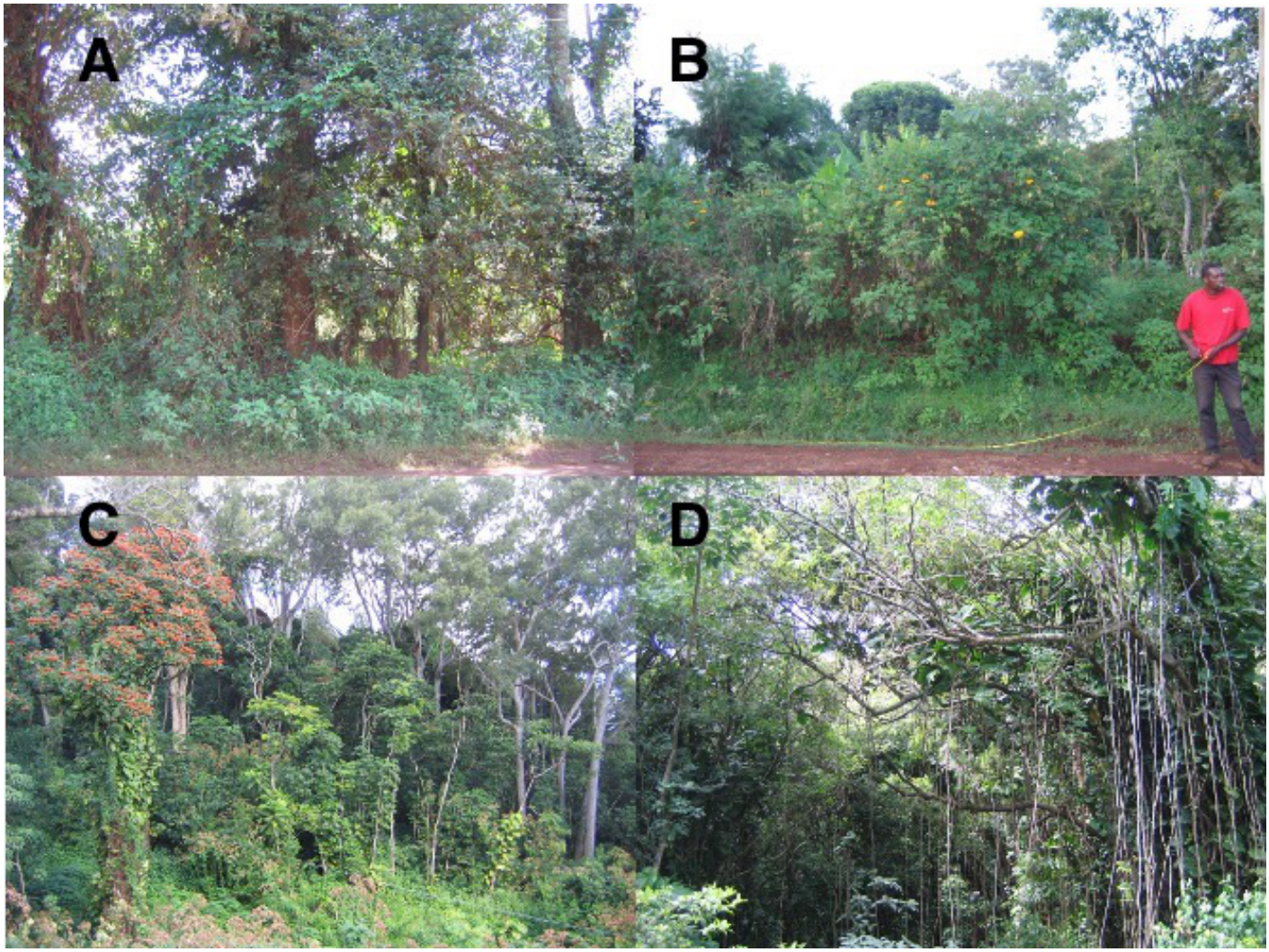


**Fig. S2. Representative photos of habitat in Kenya (A, B) and Hawaii (C, D)**. In both sites, chameleons favored well-vegetated trees and bushes, often with lots of vines. Photo credit: Martin J. Whiting, Macquarie University.

**Fig. S3. The experimental setup for behavioral trials**. We constructed a platform of three connected horizontal perches in a triangle, thereby always allowing an individual to escape a rival or if a female, a courting male. The male on the left won this contest and is in full display color while the male on the right has lost this contest and turned brown (subordinate). Photo credit: Martin J. Whiting, Macquarie University.


**Fig. S4. We presented chameleons with model snake and bird predators to elicit an anti-predator response, particularly, color change, to test the hypothesis that character release in Hawaii would also result in a signal more conspicuous to a predator**. **(A)** A model snake (*Dispholidus typus*) causing a characteristic contrasting color change and **(B)** a model bird (*Aviceda cuculoides*) which was flown over the top of the chameleon. Photo credit: Martin J. Whiting, Macquarie University.

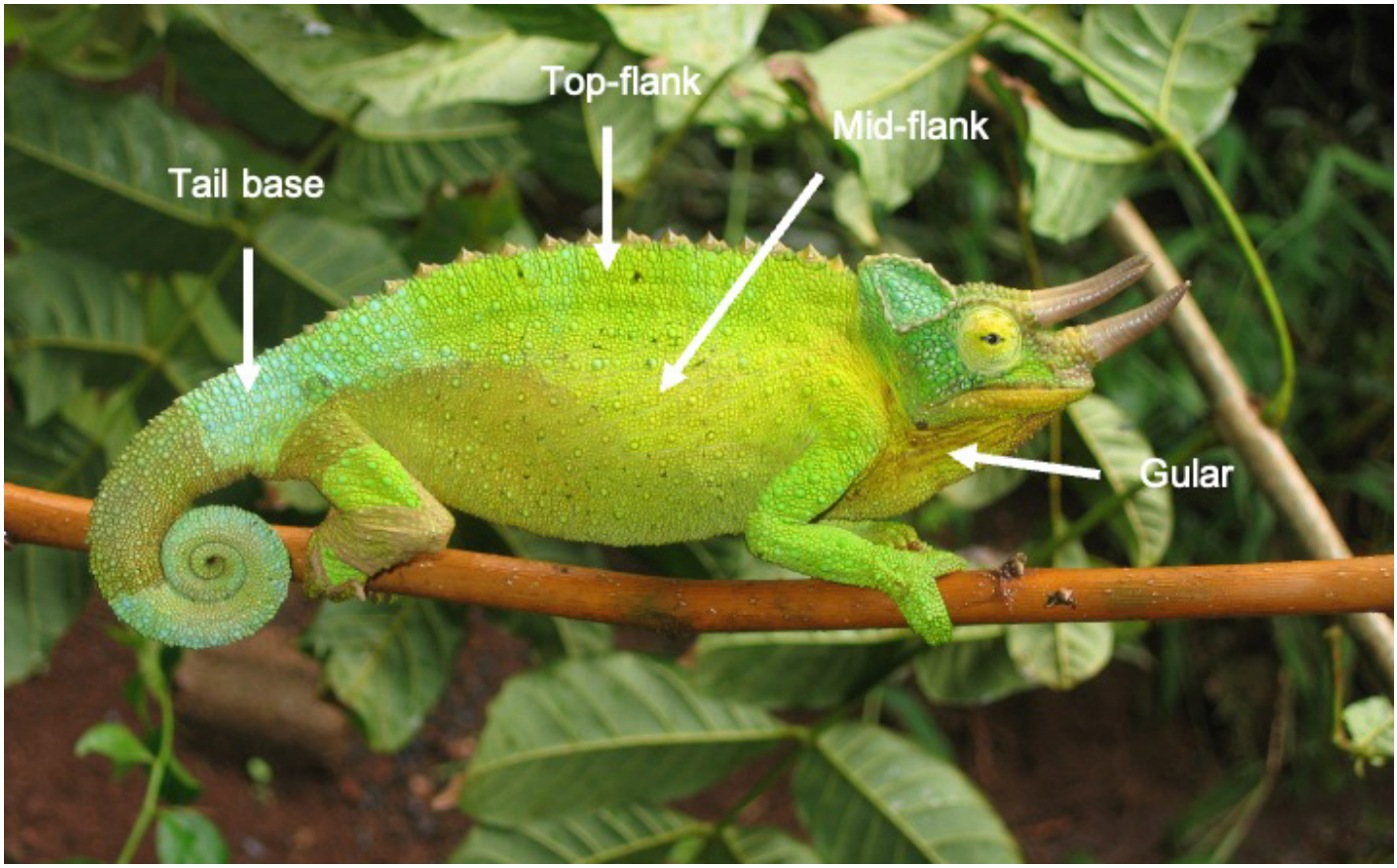


**Fig. S5. Location of body regions we measured for spectral reflectance**. See details in text. Photo credit: Martin J. Whiting, Macquarie University.

# Supplemental Results

## Body Size Comparisons between Hawaii and Kenya

Of the individuals used in social interactions (male-male display or male courtship), chameleons from Kenya were significantly larger than those from Hawaii (mean ± standard deviation Hawaii: 111.19± 11.77 mm, *n* = 36; Kenya 125.33± 14.12 mm, *n* = 27; t = -4.22, *P* <0.001). SVL did not significantly predict luminance contrast against the background (Table S2); thus the higher luminance contrast of Hawaiian chameleons during male-male contests and courtship cannot be explained by body size differences between the population. Snout-vent length was a weak predictor of chromatic contrast (Table S2): larger chameleons had slightly higher chromatic contrast against the background. However, the populations did not differ in chromatic contrast during male displays (courtship and contests).

## Supporting Supplementary Tables for Main Text

**Table S2.** Results of models comparing mean differences between Kenyan and Hawaiian lizards in luminance and chromatic contrast against the local background in two social contexts (male-male competition and courtship). Model estimates, standard errors (SE), Satterthwaite's degrees of freedom (df) and 95% confidence internals (95% CI). Standard errors are corrected by using robust variance estimators.

| Social Context | Visual Spectrum | Parameter | Estimate | SE | df | 95% CI lower | 95% CI upper |
|---|---|---|---|---|---|---|---|
| Male-Male Display | Luminance Contrast | Intercept | 36.416 | 0.8846 | 28 | 34.6027 | 38.230 |
| | | Snout-vent Length (SVL) | 0.122 | 0.0432 | 21 | 0.0327 | 0.212 |
| | | Population $_{Kenya}$ | -12.533 | 1.1797 | 28 | -14.9509 | -10.116 |
| | Chromatic Contrast | Intercept | 4.789 | 0.1829 | 28 | 4.4142 | 5.164 |
| | | Snout-vent Length (SVL) | 0.021 | 0.0092 | 21 | 0.0017 | 0.040 |
| | | Population $_{Kenya}$ | -0.401 | 0.2869 | 28 | -0.9894 | 0.187 |
| Courtship Display | Luminance Contrast | Intercept | 34.066 | 1.2758 | 21 | 31.4098 | 36.721 |
| | | Snout-vent Length (SVL) | 0.060 | 0.0712 | 19 | -0.0891 | 0.209 |
| | | Population $_{Kenya}$ | -12.328 | 1.7209 | 28 | -15.8552 | -8.800 |
| | Chromatic Contrast | Intercept | 4.590 | 0.2157 | 21 | 4.1415 | 5.039 |
| | | Snout-vent Length (SVL) | 0.018 | 0.0151 | 19 | -0.0139 | 0.049 |
| | | Population $_{Kenya}$ | -0.538 | 0.3498 | 28 | -1.2548 | 0.179 |

**Table S3.** Results of interaction models comparing luminance and chromatic contrast of Hawaiian and Kenyan lizards against a Hawaiian and Kenyan background (background = Kenya) in both social contexts (male-male competition and courtship). Model estimates (mean and contrasts), standard error (SE), degrees of freedom (df) and 95% confidence intervals (95% CI) for models were fitted using robust variance estimators to correct standard errors for non-independence, and we used Satterthwaite's degrees of freedom. Significant parameter estimates are those where the 95% CIs do not include zero. Relevant contrasts that compare Hawaiian and Kenyan populations both against the same Kenyan background are indicated by '*'.

| Social Context | Visual Spectrum | Parameter | Estimate | SE | df | 95% $CI_{lower}$ | 95% $CI_{upper}$ |
|---|---|---|---|---|---|---|---|
| Male-Male Display | Luminance Contrast | Intercept $_{Hawaii-Kenya}$ | 28.503 | 0.8574 | 28 | 26.7459 | 30.261 |
| | | Snout-vent length (SVL) | 0.122 | 0.0441 | 21 | 0.0304 | 0.214 |
| | | Population $_{Hawaii}$-Background $_{Hawaii}$ | 8.194 | 0.0639 | 29 | 8.0634 | 8.325 |
| | | Population $_{Kenya}$-Background $_{Hawaii}$ | 3.569 | 1.1655 | 30 | 1.1887 | 5.950 |
| | | * Population $_{Kenya}$-Background $_{Kenya}$ | -4.631 | 1.1574 | 30 | -6.9951 | -2.267 |
| | Chromatic Contrast | Intercept $_{Hawaii-Kenya}$ | 4.049 | 0.1825 | 28 | 3.6751 | 4.424 |
| | | Snout-vent length (SVL) | 0.021 | 0.0087 | 21 | 0.0027 | 0.039 |
| | | Population $_{Hawaii}$-Background $_{Hawaii}$ | 0.753 | 0.0274 | 29 | 0.6974 | 0.810 |
| | | Population $_{Kenya}$-Background $_{Hawaii}$ | 1.224 | 0.2708 | 30 | 0.6713 | 1.777 |
| | | * Population $_{Kenya}$-Background $_{Kenya}$ | 0.351 | 0.2825 | 30 | -0.2262 | 0.928 |
| Courtship Display | Luminance Contrast | Intercept $_{Hawaii-Kenya}$ | 25.988 | 1.2323 | 21 | 23.4262 | 28.550 |
| | | Snout-vent length (SVL) | 0.077 | 0.0704 | 19 | -0.0706 | 0.224 |
| | | Population $_{Hawaii}$-Background $_{Hawaii}$ | 8.162 | 0.0606 | 22 | 8.0358 | 8.287 |
| | | Population $_{Kenya}$-Background $_{Hawaii}$ | 4.010 | 1.7419 | 29 | 0.4482 | 7.571 |
| | | * Population $_{Kenya}$-Background $_{Kenya}$ | -3.921 | 1.6588 | 29 | -7.3121 | -0.529 |

| Social Context | Visual Spectrum | Parameter | Estimate | SE | df | 95% $CI_{lower}$ | 95% $CI_{upper}$ |
|---|---|---|---|---|---|---|---|
| | Chromatic Contrast | Intercept $_{Hawaii-Kenya}$ | 3.914 | 0.2041 | 21 | 3.4890 | 4.338 |
| | | Snout-vent length (SVL) | 0.020 | 0.0144 | 19 | -0.0105 | 0.050 |
| | | Population $_{Hawaii}$- Background $_{Hawaii}$ | 0.685 | 0.0461 | 22 | 0.5897 | 0.781 |
| | | Population $_{Kenya}$- Background $_{Hawaii}$ | 0.973 | 0.3283 | 29 | 0.3016 | 1.644 |
| | | * Population $_{Kenya}$- Background $_{Kenya}$ | 0.201 | 0.3359 | 29 | -0.4859 | 0.888 |

**Table S4.** Results of models comparing luminance and chromatic mean differences between Kenyan and Hawaiian lizards in response to snakes and birds. Model estimates, standard errors (SE), Satterthwaite's degrees of freedom (df) and 95% confidence internals (95% CI). Full models are provided and standard errors are corrected by using robust variance estimators.

| Visual Spectrum | Parameter | Estimate | SE | df | 95% CI lower | 95% CI upper |
|---|---|---|---|---|---|---|
| Luminance Contrast | Intercept | 21.61 | 0.92 | 60 | 19.8 | 23.4 |
| | Predator $_{Snake}$ | 0.33 | 0.74 | 53 | -1.2 | 1.8 |
| | Population $_{Kenya}$ | -5.80 | 1.27 | 72 | -8.3 | -3.3 |
| | Predator $_{Snake}$* Population $_{Kenya}$ | -0.76 | 1.20 | 40 | -3.2 | 1.7 |
| Chromatic Contrast | Intercept | 7.77 | 0.15 | 57 | 7.5 | 8.1 |
| | Predator $_{Snake}$ | -5.87 | 0.15 | 55 | -6.2 | -5.6 |
| | Population $_{Kenya}$ | -1.80 | 0.26 | 51 | -2.3 | -1.3 |
| | Predator $_{Snake}$* Population $_{Kenya}$ | 1.80 | 0.24 | 45 | 1.3 | 2.3 |

**Table S5.** Planned contrasts between mean luminance and chromatic contrast in Hawaii and Kenya for both predators (snakes and birds). Estimates of the mean difference ebtween Hawaii and Kenya populations are provided along with their 95% confidence intervals (95% CI lower and upper). Wald tests of contrast significance are provided (F, degrees of freedom (df) and associated p-value). We also applied a Bonferroni correction to p-values to control for multiple comparisons.

| Visual Spectrum | Contrast | Estimate | 95% CI $_{lower}$ | 95% CI $_{upper}$ | $F_{stat}$ | df | p | $p_{Bonferroni}$ |
|---|---|---|---|---|---|---|---|---|
| Chromatic Contrast | Snake (Hawaii-Kenya) | 0.0043 | -0.21 | 0.22 | 0.0016 | 1 | 0.97 | 1 |
| | Birds (Hawaii-Kenya) | -1.7980 | -2.32 | -1.27 | 47.1131 | 1 | < 0.0001 | < 0.0001 |
| Luminance Contrast | Snake (Hawaii-Kenya) | -6.5651 | -8.82 | -4.31 | 33.6418 | 1 | < 0.0001 | < 0.0001 |
| | Birds (Hawaii-Kenya) | -5.8045 | -8.33 | -3.28 | 21.0197 | 1 | < 0.0001 | < 0.001 |

**Table S6.** Results of models comparing luminance and chromatic contrast of Hawaiian lizards against their introduced Hawaiian background (intercept) and Hawaiian lizards against their native Kenyan background (background = Kenya) in both social contexts (male-male competition and courtship). Model estimates (mean and contrasts), standard error (SE), degrees of freedom (df) and 95% confidence intervals (95% CI) for models were fitted using robust variance estimators to correct standard errors for non-independence, and we used Satterthwaite's degrees of freedom. Significant parameter estimates are those where the 95% CIs do not include zero.

| Social Context | Visual Spectrum | Parameter | Estimate | SE | df | 95% CI lower | 95% CI upper |
|---|---|---|---|---|---|---|---|
| Male-Male Display | Luminance Contrast | Intercept | 35.829 | 0.779 | 30.0 | 34.2382 | 37.420 |
| | | Snout-vent length (SVL) | 0.180 | 0.062 | 12.4 | 0.0459 | 0.314 |
| | | Background $_{Kenya}$ | -8.194 | 0.064 | 29.2 | -8.3249 | -8.063 |
| | Chromatic Contrast | Intercept | 4.654 | 0.158 | 30.0 | 4.3309 | 4.977 |
| | | Snout-vent length (SVL) | 0.026 | 0.015 | 12.4 | -0.0067 | 0.058 |
| | | Background $_{Kenya}$ | -0.753 | 0.027 | 29.2 | -0.8096 | -0.697 |
| Courtship Display | Luminance Contrast | Intercept | 33.789 | 1.064 | 22.1 | 31.5822 | 35.995 |
| | | Snout-vent length (SVL) | -0.057 | 0.111 | 8.6 | -0.3093 | 0.196 |
| | | Background $_{Kenya}$ | -8.162 | 0.061 | 21.9 | -8.2873 | -8.036 |
| | Chromatic Contrast | Intercept | 4.505 | 0.181 | 22.1 | 4.1300 | 4.881 |
| | | Snout-vent length (SVL) | 0.014 | 0.023 | 8.6 | -0.0396 | 0.067 |
| | | Background $_{Kenya}$ | -0.685 | 0.046 | 21.9 | -0.7810 | -0.590 |

## Additional Supplemental Results

## Body regions are perceived differently by predators but exhibited similar effect sizes

Chromatic contrast of color signals against the background varied by predator type, population and body region (Fig. S6A and Table S7). Whereas all body regions in Kenyan chameleons showed a reduced contrast against the background for birds there were small differences among populations in how snakes perceived color signals (Fig. S6A). There was no significant interaction between predator type ('bird' vs 'snake') and population was not significant for luminance contrast against the background, but there was a marginally non-significant population and body region effect (Fig. S6B and Table S7). Effects did not vary by predator type (Table S7). Nonetheless, effect sizes across body regions were generally in the same direction (Fig. S6B and Table S8). Overall, there were significant differences between Kenya and Hawaii in luminance contrast of the gular and tailbase regions, but not for mid- and top-flank (Table S9). In contrast, no significant differences between Kenya and Hawaii existed across any body region for snakes, whereas significant differences existed between gular, midflank and tailbase for birds (Table S10).

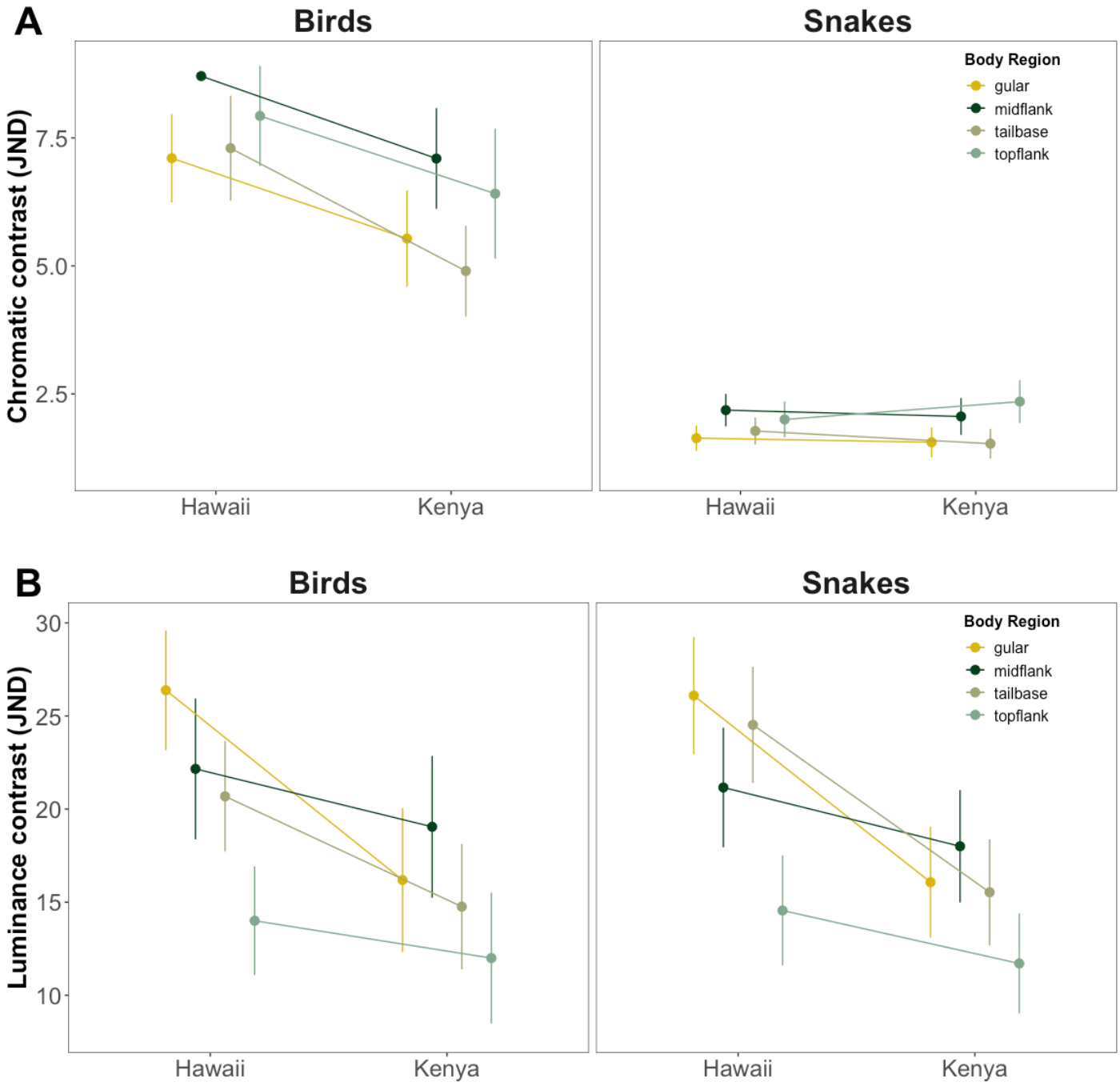


**Fig. S6.** All four body regions (gular, mid-flank, tailbase and topflank) and their luminance (B) and chromatic contrast (A) to Hawaiian and Kenyan backgrounds (each population against their own background) are shown averaged across stems and leaves found in their habitat. Just noticable differences (JNDs) were calculated for both bird and snake predators. Mean JNDs are provided along with 95% confidence intervals.

**Table S7.** Wald F tests comparing the significance of model parameters for predator models. Wald F tests were computed using robust variance estimators with a Satterthwaite correction to the degrees of freedom. Interactions were first tested, and models refitted, to test the significance of main effects.

| Visual Spectrum | Variable | F | df num | df denom | p |
|---|---|---|---|---|---|
| Luminance Contrast | Body Region | 33.715 | 3 | 90 | < 0.0001 |
| | Population | 37.310 | 1 | 83 | < 0.0001 |
| | Predator | 0.051 | 1 | 76 | 0.82 |
| | Predator* BodyRegion | 2.682 | 3 | 88 | 0.05 |
| | Predator* Population | 0.374 | 1 | 40 | 0.54 |
| Chromatc Contrast | Body Region | 20.408 | 3 | 90 | < 0.0001 |
| | Population | 20.585 | 1 | 80 | < 0.0001 |
| | Predator | 1,232.257 | 1 | 79 | < 0.0001 |
| | Predator* BodyRegion | 7.728 | 3 | 88 | < 0.001 |
| | Predator* Population | 55.626 | 1 | 45 | < 0.0001 |

**Table S8.** Estimated model parameters, standard errors (SE), degrees of freedom (df) along with 95% confidence interval for each parameter from linear mixed effects models. Standard errors were corrected using robust variance estimators and Satterthwaite's degrees of freedom correction was used for calculated significance.

| Visual Spectrum | Parameter | Estimate | SE | $T_{statistic}$ | df | p |
|---|---|---|---|---|---|---|
| Luminance Contrast | Intercept | 25.246 | 1.15 | 21.949 | 67 | < 0.0001 |
| | $Predator_{Snake}$ | -0.714 | 1.00 | -0.711 | 80 | 0.48 |
| | $Population_{Kenya}$ | -5.614 | 1.23 | -4.579 | 72 | < 0.0001 |
| | $Body\ Region_{midflank}$ | -2.050 | 1.48 | -1.388 | 76 | 0.17 |
| | $Body\ Region_{tailbase}$ | -4.317 | 1.17 | -3.689 | 76 | < 0.001 |
| | $Body\ Region_{topflank}$ | -9.035 | 1.25 | -7.218 | 73 | < 0.0001 |
| | $Predator_{Snake}$* $BodyRegion_{midflank}$ | -0.119 | 1.54 | -0.077 | 90 | 0.94 |
| | $Predator_{Snake}$* $BodyRegion_{tailbase}$ | 3.254 | 1.34 | 2.433 | 90 | 0.02 |
| | $Predator_{Snake}$* $BodyRegion_{topflank}$ | 1.120 | 1.34 | 0.837 | 88 | 0.41 |
| | $Predator_{Snake}$* $Population_{Kenya}$ | -0.729 | 1.19 | -0.611 | 40 | 0.54 |
| Chromatic Contrast | Intercept | 7.182 | 0.20 | 36.809 | 70 | < 0.0001 |
| | $Predator_{Snake}$ | -5.556 | 0.20 | -27.814 | 81 | < 0.0001 |
| | $Population_{Kenya}$ | -1.798 | 0.26 | -6.889 | 52 | < 0.0001 |
| | $Body\ Region_{midflank}$ | 1.595 | 0.27 | 5.816 | 76 | < 0.0001 |
| | $Body\ Region_{tailbase}$ | -0.035 | 0.20 | -0.177 | 76 | 0.86 |
| | $Body\ Region_{topflank}$ | 0.824 | 0.28 | 2.952 | 74 | < 0.01 |
| | $Predator_{Snake}$* $BodyRegion_{midflank}$ | -1.063 | 0.28 | -3.857 | 90 | < 0.001 |
| | $Predator_{Snake}$* $BodyRegion_{tailbase}$ | 0.108 | 0.20 | 0.541 | 90 | 0.59 |
| | $Predator_{Snake}$* $BodyRegion_{topflank}$ | -0.293 | 0.29 | -1.005 | 88 | 0.32 |
| | $Predator_{Snake}$* $Population_{Kenya}$ | 1.798 | 0.24 | 7.458 | 45 | < 0.0001 |

**Table S9.** Pairwise comparisons of luminance contrast between Kenyan and Hawaiian chameleon displays to a snake and bird visual system. Four different body regions ('gular', 'midflank', 'tailbase', topflank') are provided along with pairwise Wald tests for contrast significance. The Wald test uses a robust variance correction on the standard errors.

| Predator | Contrast | F | DF | p | $p_{Bonferroni}$ |
|---|---|---|---|---|---|
| Snake | $Kenya_{gular}$ - $Hawaii_{gular}$ | 32.7 | 82 | < 0.0001 | < 0.0001 |
| | $Kenya_{midflank}$ - $Hawaii_{midflank}$ | 2.6 | 82 | 0.11 | 0.91 |
| | $Kenya_{tailbase}$ - $Hawaii_{tailbase}$ | 27.9 | 82 | < 0.0001 | < 0.0001 |
| | $Kenya_{topflank}$ - $Hawaii_{topflank}$ | 5.6 | 83 | 0.02 | 0.16 |
| Bird | $Kenya_{gular}$ - $Hawaii_{gular}$ | 26.4 | 59 | < 0.0001 | < 0.0001 |
| | $Kenya_{midflank}$ - $Hawaii_{midflank}$ | 2.2 | 58 | 0.15 | 1 |
| | $Kenya_{tailbase}$ - $Hawaii_{tailbase}$ | 14.0 | 58 | < 0.001 | < 0.01 |
| | $Kenya_{topflank}$ - $Hawaii_{topflank}$ | 2.5 | 59 | 0.12 | 0.95 |

**Table S10.** Pairwise comparisons of chromatic contrast between Kenyan and Hawaiian chameleon displays to a snake and bird visual system. Four different body regions ('gular', 'midflank', 'tailbase', topflank') are provided along with a pairwise Wald test for contrast significance. The Wald test uses a robust variance correction on the standard errors.

| Predator | Contrast | F | DF | p | $p_{Bonferroni}$ |
|---|---|---|---|---|---|
| Snake | $Kenya_{gular}$ - $Hawaii_{gular}$ | 0.073 | 80 | 0.79 | 1 |
| | $Kenya_{midflank}$ - $Hawaii_{midflank}$ | 0.250 | 79 | 0.62 | 1 |
| | $Kenya_{tailbase}$ - $Hawaii_{tailbase}$ | 2.360 | 79 | 0.13 | 1 |
| | $Kenya_{topflank}$ - $Hawaii_{topflank}$ | 2.847 | 81 | 0.1 | 0.76 |
| Bird | $Kenya_{gular}$ - $Hawaii_{gular}$ | 17.319 | 43 | < 0.001 | < 0.01 |
| | $Kenya_{midflank}$ - $Hawaii_{midflank}$ | 10.193 | 43 | < 0.01 | 0.02 |
| | $Kenya_{tailbase}$ - $Hawaii_{tailbase}$ | 84.064 | 43 | < 0.0001 | < 0.0001 |
| | $Kenya_{topflank}$ - $Hawaii_{topflank}$ | 5.804 | 45 | 0.02 | 0.16 |

## All body regions exhibit similar contrasts between Kenyan and Hawaiian backgrounds suggesting local adaptation of entire visual signal

We tested whether different body regions of Hawaiian chameleons exhibited different contrasts between Hawaiian habitat background compared to their ancestral, Kenyan background. We started off testing models comparing the interaction between background environment and body region for both chromatic and luminance contrast. However, for luminance contrast, these models had poor convergence. Based on the raw data (Fig. S7B and S7D) there was also weak evidence that body regions differed in how they changed when viewed by conspecifics against the Hawaiian and Kenyan backgrounds. As such, we simplified models and only present the main effects of body region and background environment for luminance contrast. However, inspection of the raw data (Fig. S7A and S7C) suggested that body regions may differ in how chromatically contrasting they were against the background environment of Hawaii and Kenya. In addition, our main results suggested that predators may deferentially drive chromatic signal conspicuousness, and given different body regions maybe more exposed to bird compared to snake, predators, we fitted an interaction and main effects model for chromatic contrast.

The magnitude of chromatic contrast differences between Hawaiian chameleons against their own environment and a Kenyan enviroment did depend on the body region (Male-male display: $F_{3,26.92}$ = 5.86, p = < 0.01; Courtship Display: $F_{3,19.7}$ = 14.79, *P* = < 0.0001, Fig. S7A, S7C and Table S11). However, in all cases there was a tendency for all body regions of Hawaiian populations to decrease in average contrast when viewed by a conspecific against a Hawaiian versus Kenyan background (Fig. S7A and S7C). Tailbase was the only body region that showed a non-significant change (Table S12).

The magnitude of luminance contrast showed a significant overall decrease when viewed by conspecifics against a Kenyan background (Overall Background Effect: Male-male display, $F_{1,29.17}$ = 1.6410^{4}, *P* = < 0.0001, Courtship Display, $F_{1,21.87}$ = 1.8110^{4}, *P* = < 0.0001, Fig. S7B, S7D and Supplementary Table S11), and there were significant differences among body regions (Overall Body Region Effect: Male-male display, $F_{3,26.67}$ = 47.94, *P* = < 0.0001, Courtship Display, $F_{3,19.52}$ = 16.58, *P* = < 0.0001, Fig. S7B, S7D and Table S11).

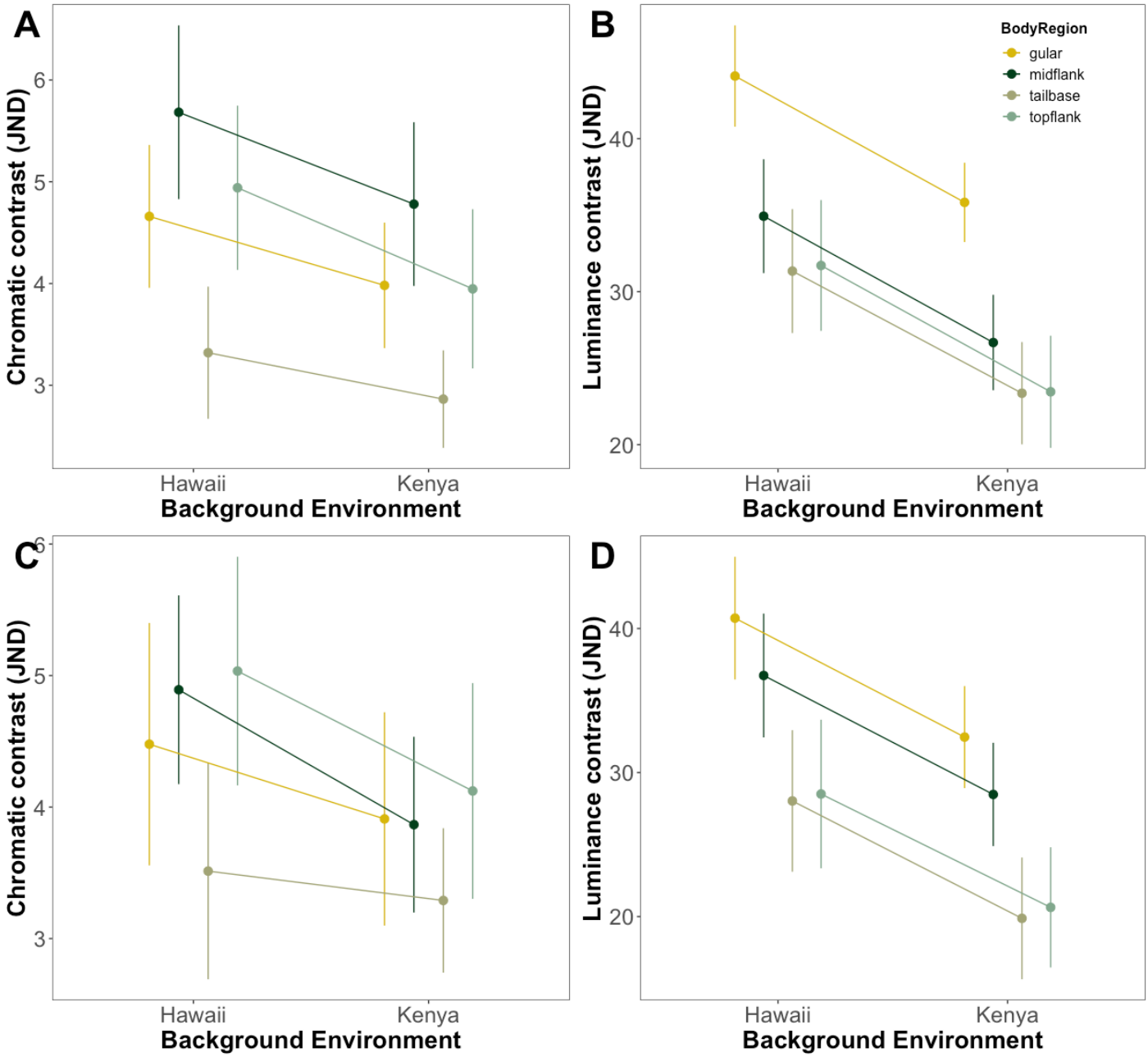


**Fig. S7.** Local adaptation. All four body regions (gular, mid-flank, tailbase and topflank) are shown averaged across stems and leaves found in their habitat. Mean JNDs are provided along with 95% confidence intervals. **(A & B)** represent male-male contest displays whereas **(C & D)** represent courtship displays.

**Table S11.** Estimated model parameters, standard errors (SE), degrees of freedom (df), and 95% confidence intervals for each parameter. Main effects and interaction models are presented. Only main effect models are presented for luminance contrast (see text for details), whereas interaction models are presented for chromatic contrast. Pairwise contrasts are presented for chromatic contrast models for ease of interpretation.

| Social Context | Visual Spectrum | Parameter | Estimate | SE | df | 95% $CI_{lower}$ | 95% $CI_{upper}$ |
|---|---|---|---|---|---|---|---|
| Male-Male Display | Luminance Contrast | Intercept | 44.132 | 0.671 | 30.0 | 42.7630 | 45.502 |
| | | Snout-vent length (SVL) | 0.179 | 0.061 | 12.4 | 0.0467 | 0.311 |
| | | Background Population $_{Kenya}$ | -8.194 | 0.064 | 29.2 | -8.3249 | -8.063 |
| | | Body Region $_{midflank}$ | -9.249 | 0.997 | 29.5 | -11.2862 | -7.211 |
| | | Body Region $_{tailbase}$ | -12.220 | 1.251 | 28.2 | -14.7815 | -9.658 |
| | | Body Region $_{topflank}$ | -12.307 | 1.106 | 27.8 | -14.5748 | -10.040 |
| | Chromatic Contrast | Intercept | 4.688 | 0.247 | 29.8 | 4.1839 | 5.192 |
| | | Snout-vent length (SVL) | 0.026 | 0.015 | 12.4 | -0.0061 | 0.059 |
| | | Background Population $_{Kenya}$ | -0.690 | 0.055 | 27.9 | -0.8027 | -0.576 |
| | | Body Region $_{midflank}$ | 1.065 | 0.411 | 29.5 | 0.2253 | 1.904 |
| | | Body Region $_{tailbase}$ | -1.365 | 0.259 | 28.2 | -1.8960 | -0.835 |
| | | Body Region $_{topflank}$ | 0.255 | 0.417 | 28.3 | -0.5992 | 1.109 |
| | | Background Population $_{Kenya}$* BodyRegion $_{midflank}$ | -0.217 | 0.086 | 29.6 | -0.3922 | -0.043 |
| | | Background Population $_{Kenya}$* BodyRegion $_{tailbase}$ | 0.213 | 0.102 | 28.2 | 0.0042 | 0.423 |
| | | Background Population $_{Kenya}$* BodyRegion $_{topflank}$ | -0.299 | 0.085 | 28.7 | -0.4739 | -0.125 |
| Courtship Display | Luminance Contrast | Intercept | 40.459 | 1.296 | 22.3 | 37.7740 | 43.144 |
| | | Snout-vent length (SVL) | -0.059 | 0.105 | 8.6 | -0.2992 | 0.181 |
| | | Background Population $_{Kenya}$ | -8.162 | 0.061 | 21.9 | -8.2873 | -8.036 |
| | | Body Region $_{midflank}$ | -4.235 | 1.504 | 22.1 | -7.3530 | -1.116 |
| | | Body Region $_{tailbase}$ | -12.035 | 2.125 | 22.1 | -16.4420 | -7.628 |
| | | Body Region $_{topflank}$ | -11.363 | 2.048 | 20.5 | -15.6283 | -7.099 |

| Social Context | Visual Spectrum | Parameter | Estimate | SE | df | 95% $CI_{lower}$ | 95% $CI_{upper}$ |
|---|---|---|---|---|---|---|---|
| | Chromatic Contrast | Intercept | 4.594 | 0.367 | 22.3 | 3.8337 | 5.354 |
| | | Snout-vent length (SVL) | 0.014 | 0.024 | 8.6 | -0.0411 | 0.069 |
| | | Background Population $_{Kenya}$ | -0.584 | 0.085 | 21.5 | -0.7605 | -0.407 |
| | | Body Region $_{midflank}$ | 0.233 | 0.400 | 22.1 | -0.5969 | 1.064 |
| | | Body Region $_{tailbase}$ | -1.067 | 0.349 | 22.1 | -1.7912 | -0.343 |
| | | Body Region $_{topflank}$ | 0.580 | 0.404 | 20.7 | -0.2605 | 1.420 |
| | | Background Population $_{Kenya}$* BodyRegion $_{midflank}$ | -0.453 | 0.088 | 22.0 | -0.6347 | -0.271 |
| | | Background Population $_{Kenya}$* BodyRegion $_{tailbase}$ | 0.335 | 0.101 | 22.0 | 0.1254 | 0.545 |
| | | Background Population $_{Kenya}$* BodyRegion $_{topflank}$ | -0.319 | 0.124 | 20.9 | -0.5756 | -0.062 |

**Table S12.** Pairwise Wald F tests comparing changes in chromatic contrast between different body regions for Hawaiian lizards against both Hawaiian and Kenyan backgrounds during male-male and courtship displays. F statistic, degrees of freedom (DF) and p-values are provided (p). A Bonferroni correction to p-values is also provided to control for multiple testing.

| Context | Contrast | F | DF | p | $p_{Bonferroni}$ |
|---|---|---|---|---|---|
| Male-Male Display | Kenyan Background $_{gular}$ - Hawaiian Background $_{gular}$ | 155.9 | 28 | < 0.0001 | < 0.0001 |
| | Kenyan Background $_{midflank}$ - Hawaiian Background $_{midflank}$ | 387.4 | 30 | < 0.0001 | < 0.0001 |
| | Kenyan Background $_{tailbase}$ - Hawaiian Background $_{tailbase}$ | 28.4 | 28 | < 0.0001 | < 0.001 |
| | Kenyan Background $_{topflank}$ - Hawaiian Background $_{topflank}$ | 235.9 | 26 | < 0.0001 | < 0.0001 |
| Courtship Display | Kenyan Background $_{gular}$ - Hawaiian Background $_{gular}$ | 47.1 | 22 | < 0.0001 | < 0.0001 |
| | Kenyan Background $_{midflank}$ - Hawaiian Background $_{midflank}$ | 828.0 | 22 | < 0.0001 | < 0.0001 |
| | Kenyan Background $_{tailbase}$ - Hawaiian Background $_{tailbase}$ | 4.9 | 22 | 0.04 | 1 |
| | Kenyan Background $_{topflank}$ - Hawaiian Background $_{topflank}$ | 152.9 | 19 | < 0.0001 | < 0.0001 |

## Hawaiian chameleons show greater individual change between display and bird color states than Kenyan chameleons

To gauge the extent of individual color change, we calculated the absolute difference in JNDs between for each individual that had both display (male-male contest) and bird response data (*n* = 30 individuals from Hawaii, *n* = 13 Kenya). We chose these two color states to maximize sample size and because chameleons and birds have similar tetrachromatic visual systems. We tested whether the absolute difference in luminance or chromatic contrast between these two color states differed between populations and body regions. We included SVL, population, body region and the interaction between population and body region as fixed effects. ID was included as a random effect becasue we had measures for more than one body region per individual.

Body regions varied in their overall effects but mainly showed a decrease in absolute JND difference between display and antipredator color states in Kenya compared to Hawaii (Fig. S8). While there was some evidence for an interaction between population and body region for luminance change (albeit weak) this was not the case for chromatic change (population by body region interaction: chromatic: $F_{3,21.24}$ = 1.64, *P* = 0.21; luminance: $F_{3,21.23}$ = 2.61, *P* = 0.08) (Fig. S8 and Table S13). However, the degree to which body regions varied within poplations was more apparent for luminance than for chromatic change (main effect of body region: chromatic: $F_{3,38}$ = 1.02, *P* = 0.4; luminance: $F_{3,37.76}$ = 7.17, *P* = < 0.001) (Fig. S8; Table S13 and Table S14).

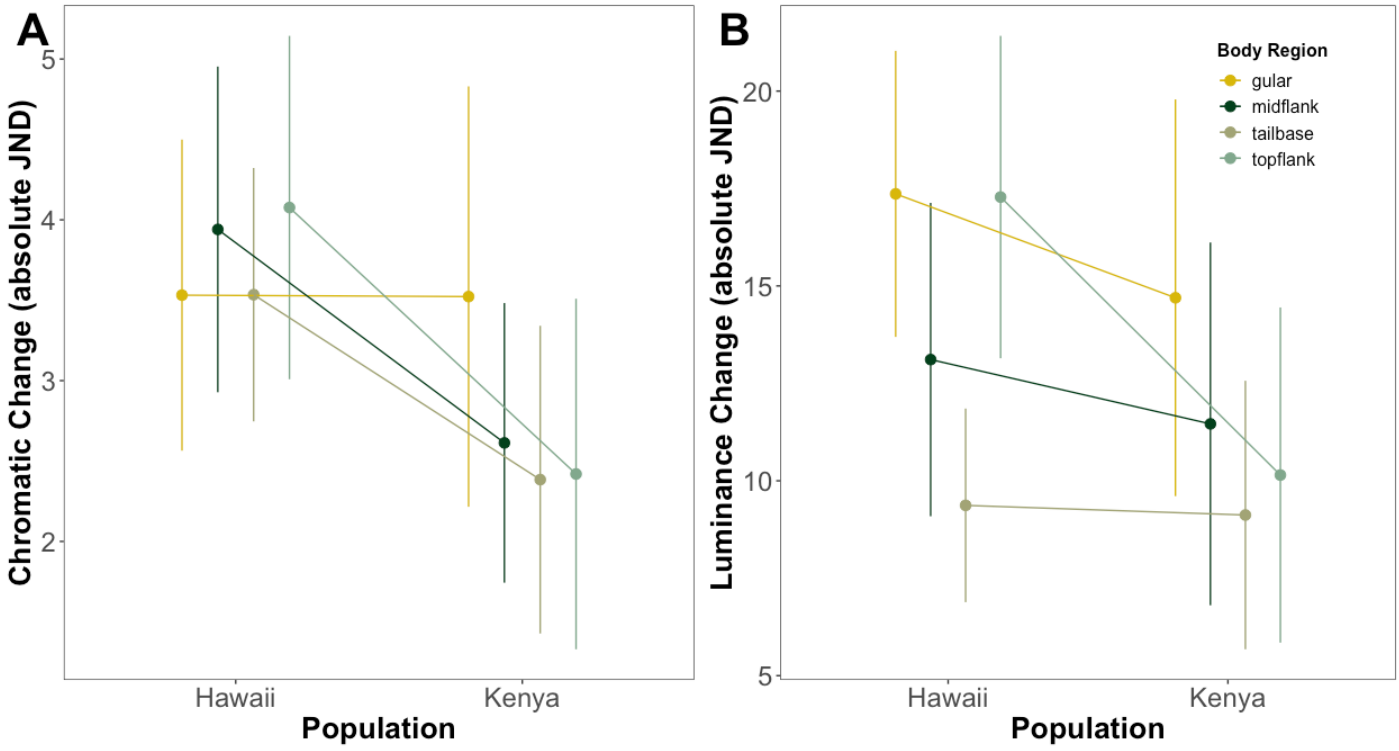


**Fig. S8.** Extent of color plasticity between display (male-male contest) and anti-predator (bird) color states (absolute difference in JNDs between states) for Hawaiian (*n* = 30) and Kenyan (*n* = 13) chameleons. **(A)** Average chromatic and **(B)** luminance contrast are provided for each body region

**Table S13.** Estimated model parameters, standard errors (SE), degrees of freedom (df) along with 95% confidence interval for each parameter. Full interaction models are presented for chromatic and luminance change (absolute JND).

| Visual Spectrum | Parameter | Estimate | SE | df | 95% $CI_{lower}$ | 95% $CI_{upper}$ |
|---|---|---|---|---|---|---|
| Luminance Contrast | Intercept | 18.0594 | 1.8536 | 27 | 14.255 | 21.8637 |
| | Snout-vent Length (SVL) | 0.0746 | 0.0769 | 17 | -0.088 | 0.2367 |
| | $Population_{Kenya}$ | -3.6220 | 2.7668 | 20 | -9.390 | 2.1458 |
| | $Body\ Region_{midflank}$ | -4.6925 | 2.2397 | 28 | -9.280 | -0.1052 |
| | $Body\ Region_{tailbase}$ | -8.4340 | 2.2205 | 28 | -12.982 | -3.8860 |
| | $Body\ Region_{topflank}$ | -0.4708 | 2.1608 | 25 | -4.918 | 3.9764 |
| | $Population_{Kenya}$* $BodyRegion_{midflank}$ | 1.1457 | 3.2192 | 24 | -5.505 | 7.7969 |
| | $Population_{Kenya}$* $BodyRegion_{tailbase}$ | 3.0209 | 2.8816 | 22 | -2.953 | 8.9952 |
| | $Population_{Kenya}$* $BodyRegion_{topflank}$ | -4.2845 | 2.8570 | 25 | -10.166 | 1.5970 |
| Chromatic Contrast | Intercept | 3.4719 | 0.2775 | 27 | 2.903 | 4.0412 |
| | Snout-vent Length (SVL) | -0.0198 | 0.0081 | 17 | -0.037 | -0.0027 |
| | $Population_{Kenya}$ | 0.1655 | 0.5342 | 23 | -0.939 | 1.2705 |
| | $Body\ Region_{midflank}$ | 0.4006 | 0.4724 | 28 | -0.567 | 1.3680 |
| | $Body\ Region_{tailbase}$ | -0.0052 | 0.3413 | 28 | -0.704 | 0.6937 |
| | $Body\ Region_{topflank}$ | 0.5485 | 0.4824 | 26 | -0.443 | 1.5400 |
| | $Population_{Kenya}$* $BodyRegion_{midflank}$ | -1.2834 | 0.6826 | 23 | -2.694 | 0.1271 |
| | $Population_{Kenya}$* $BodyRegion_{tailbase}$ | -1.0468 | 0.7044 | 22 | -2.507 | 0.4131 |
| | $Population_{Kenya}$* $BodyRegion_{topflank}$ | -1.5966 | 0.7199 | 25 | -3.079 | -0.1140 |

**Table S14.** Pairwise Wald F tests for chromatic and luminance change between different body regions for color plasticity between display (male-male contest) and anti-predator (bird) color states (absolute difference in JNDs between states) for Hawaiian (*n* = 30) and Kenyan (*n* = 13) chameleons. F statistic, degrees of freedom (DF) and p-values are provided (p). A Bonferroni correction to p-values is also provided to control for multiple testing.

| Visual Spectrum | Contrast | F | DF | p | p Bonferroni |
|---|---|---|---|---|---|
| Luminance Change | Kenyan $_{gular}$ - Hawaiian $_{gular}$ | 1.714 | 20 | 0.2052 | 1.00 |
| | Kenyan $_{midflank}$ - Hawaiian $_{midflank}$ | 0.707 | 20 | 0.4105 | 1.00 |
| | Kenyan $_{tailbase}$ - Hawaiian $_{tailbase}$ | 0.069 | 20 | 0.7951 | 1.00 |
| | Kenyan $_{topflank}$ - Hawaiian $_{topflank}$ | 9.361 | 21 | 0.0060 | 0.17 |
| Chromatic Change | Kenyan $_{gular}$ - Hawaiian $_{gular}$ | 0.096 | 23 | 0.7594 | 1.00 |
| | Kenyan $_{midflank}$ - Hawaiian $_{midflank}$ | 8.270 | 22 | 0.0087 | 0.24 |
| | Kenyan $_{tailbase}$ - Hawaiian $_{tailbase}$ | 4.798 | 21 | 0.0401 | 1.00 |
| | Kenyan $_{topflank}$ - Hawaiian $_{topflank}$ | 7.833 | 25 | 0.0098 | 0.27 |

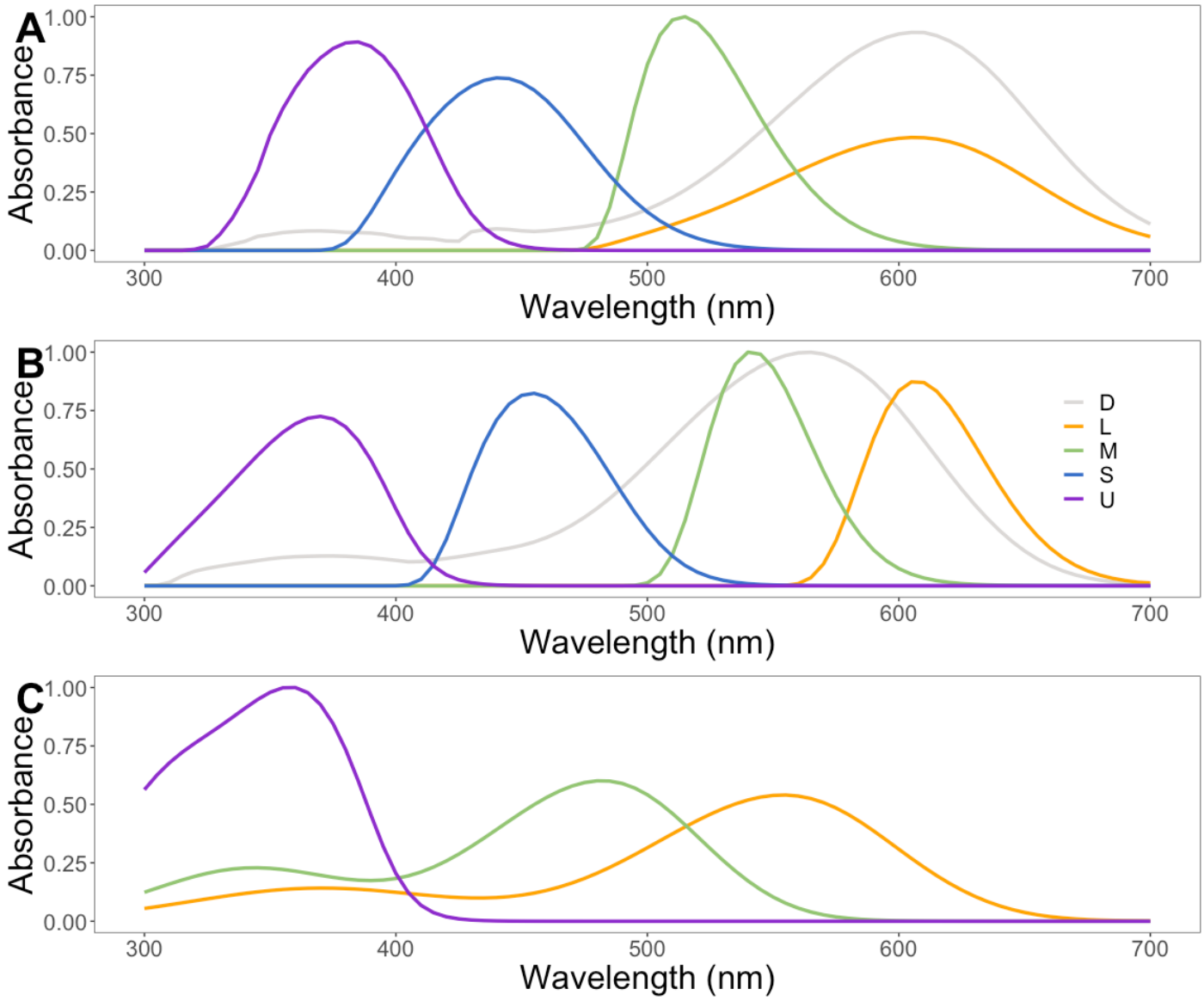


**Fig. S9. Spectral sensitivities of chameleons (A), bird (B) and snake (C) used for visual models (details in text)**.

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