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.

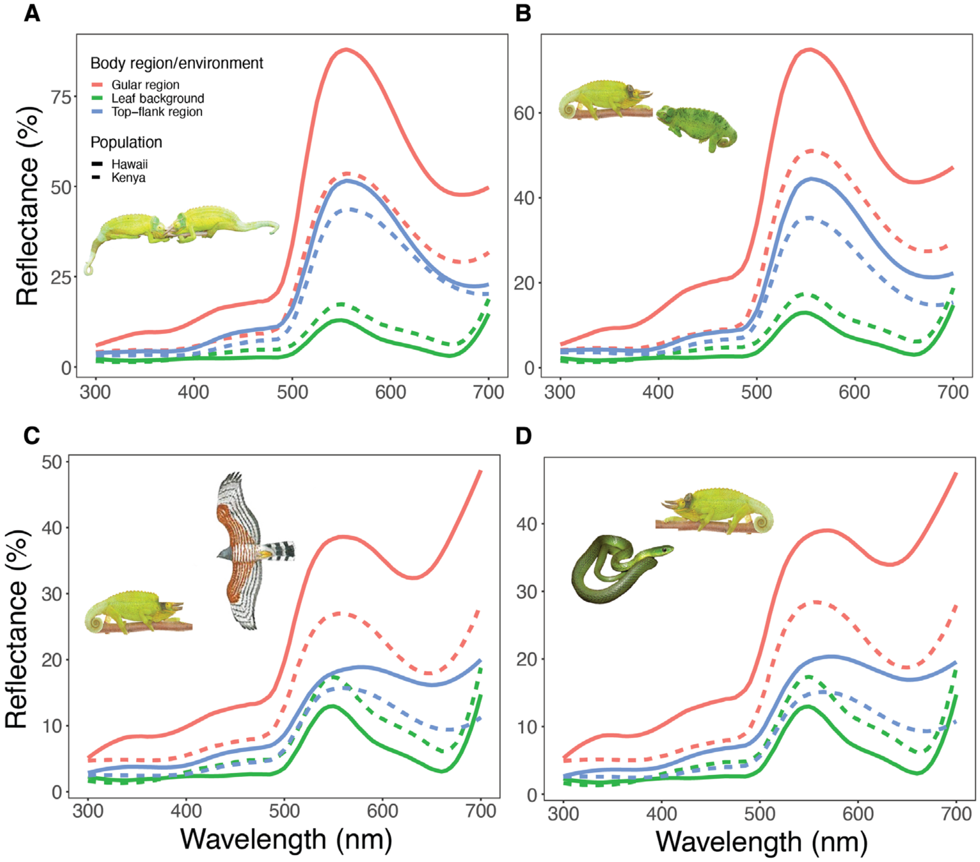

Fig. 2. Mean spectral reflectance curves for male chameleons for representative body regions (gular and top flank) and background (leaves) for Hawaii and Kenya. The context for measurement was (**A**) male contest displays, (**B**) courtship, (**C**) bird predator, and (**D**) snake predator. More details 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

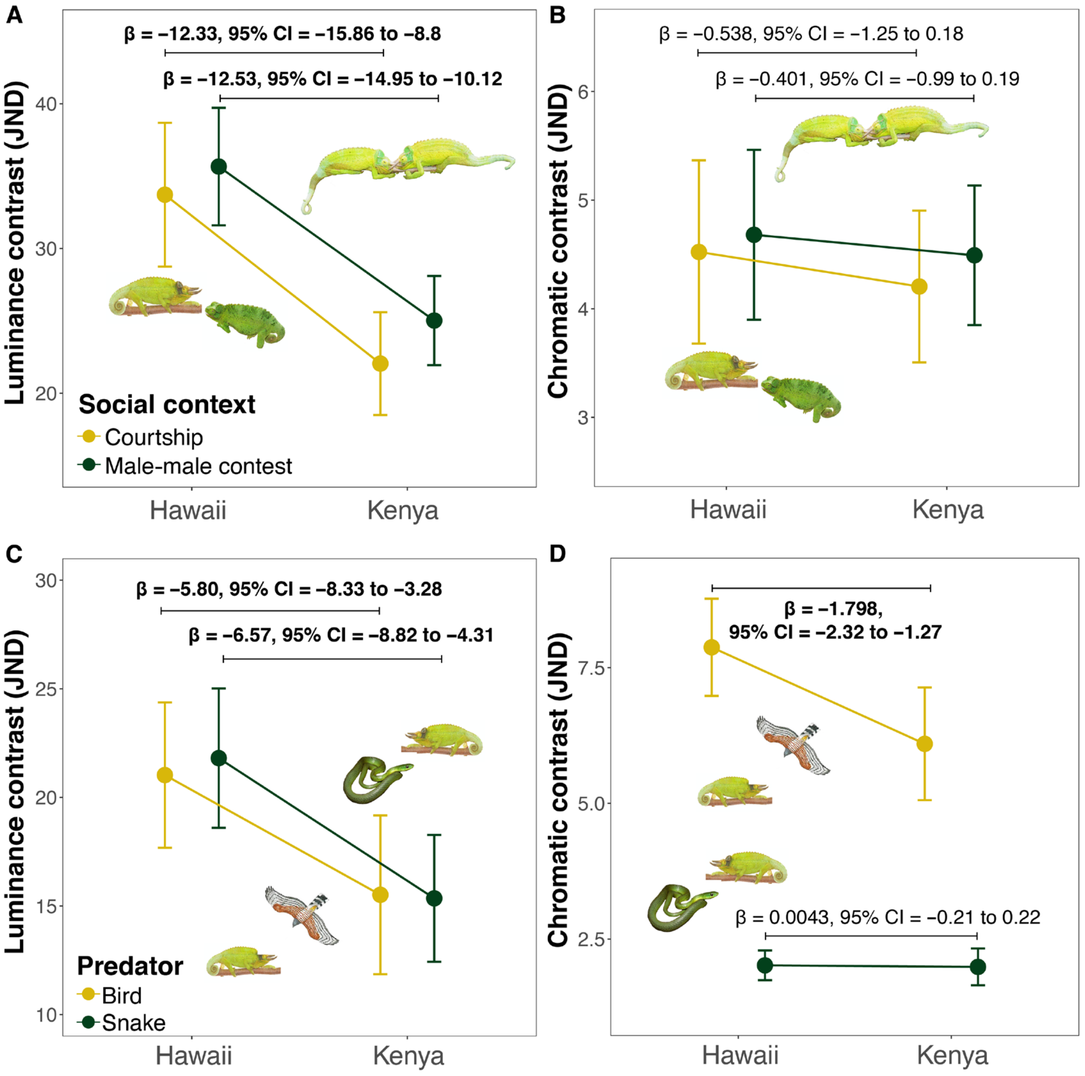

Fig. 3. Luminance and chromatic contrast in JNDs of Hawaiian and Kenyan chameleons against their respective backgrounds (i.e., average environment of stems and leaves). (**A**) Luminance and (**B**) chromatic contrast of male chameleons during male-male contests and female courtship. JNDs are calculated 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

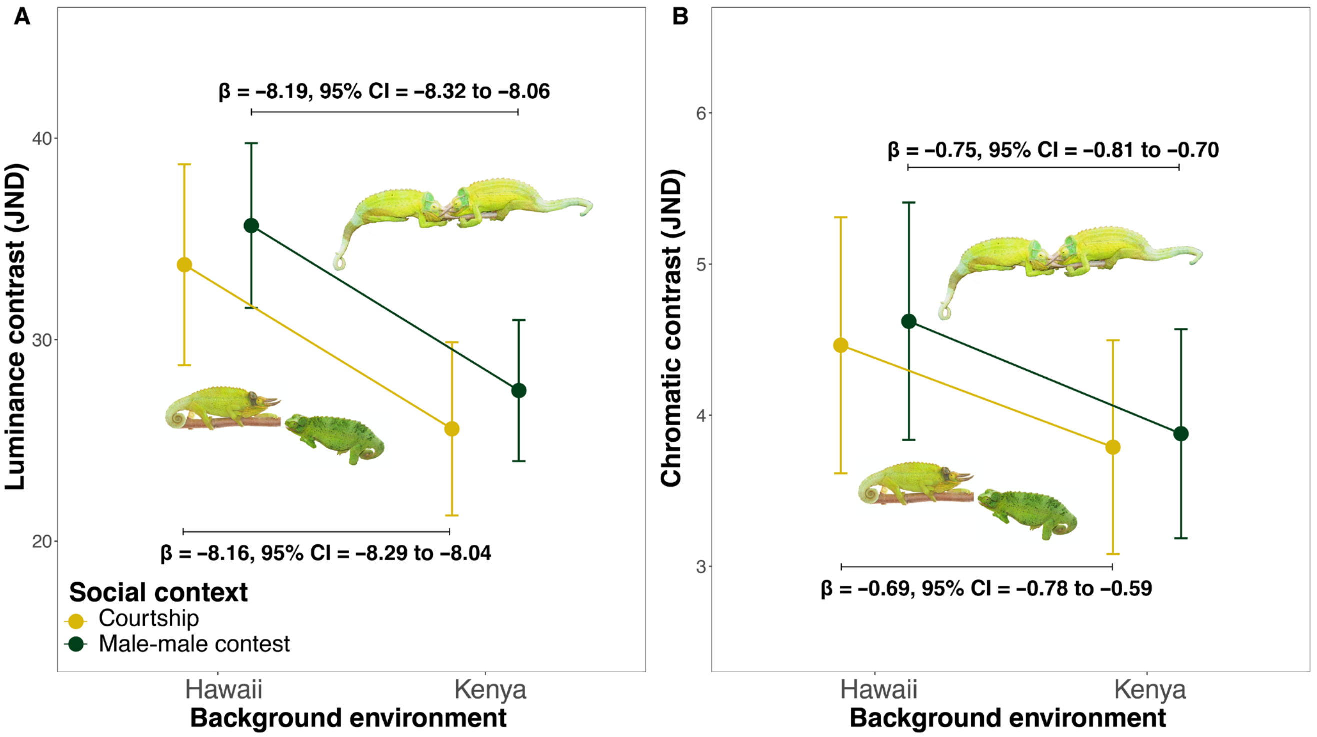

Fig. 4. Luminance and chromatic contrast in JNDs of Hawaiian chameleon social signals against their own (Hawaii) background and that of Kenya. (**A**) Luminance and (**B**) chromatic contrast of male chameleons during male-male contests and female courtship. For local adaptation, signals are predicted 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 (λ_{max}, 481 and 497 nm) and three populations of LWS cones (λ_{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*.

REFERENCES AND NOTES

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