Chameleon Color Change Communicates

Conquest and Capitulation

by

Russell Ligon

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved July 2015 by the
Graduate Supervisory Committee:

Kevin McGraw, Chair
Dale DeNardo
Kristopher Karsten
Ronald Rutowski
Pierre Deviche

ARIZONA STATE UNIVERSITY

August 2015

ABSTRACT

Sexual and social signals have long been thought to play an important role in speciation and diversity; hence, investigations of intraspecific communication may lead to important insights regarding key processes of evolution. Though we have learned much about the control, function, and evolution of animal communication by studying several very common signal types, investigating rare classes of signals may provide new information about how and why animals communicate. My dissertation research focused on rapid physiological color change, a rare signal-type used by relatively few taxa. To answer longstanding questions about this rare class of signals, I employed novel methods to measure rapid color change signals of male veiled chameleons *Chamaeleo calyptratus* in real-time as seen by the intended conspecific receivers, as well as the associated behaviors of signalers and receivers. In the context of agonistic male-male interactions, I found that the brightness achieved by individual males and the speed of color change were the best predictors of aggression and fighting ability. Conversely, I found that rapid skin darkening serves as a signal of submission for male chameleons, reducing aggression from winners when displayed by losers. Additionally, my research revealed that the timing of maximum skin brightness and speed of brightening were the best predictors of maximum bite force and circulating testosterone levels, respectively. Together, these results indicated that different aspects of color change can communicate information about contest strategy, physiology, and performance ability. Lastly, when I experimentally manipulated the external appearance of chameleons, I found that "dishonestly" signaling individuals (i.e. those whose behavior did not match their manipulated color) received higher aggression from unpainted opponents. The increased aggression received by dishonest signalers suggests that social costs play an important role in maintaining the honesty of rapid color change signals in veiled chameleons. Though the color change abilities of chameleons have interested humans since the time of Aristotle, little was previously known about the signal content of such changes. Documenting the behavioral contexts and information content of these signals has provided an important first step in understanding the current function, underlying control mechanisms, and evolutionary origins of this rare signal type.

DEDICATION

To Veronica, your love and support make me feel like I can do anything. You are everything.

To Theodore, you have enriched my life in ways you cannot yet understand, though I trust you will someday.

To Mom & Dad, thank you for showing me the world.

ACKNOWLEDGMENTS

I would like to thank my dissertation committee, Drs. Kris Karsten, Pierre Deviche, Ron Rutowski, Dale DeNardo, and Kevin McGraw, for the invaluable guidance they have provided me over the last 6 years. Their input, probing questions, and general skepticism have been instrumental in my scientific development. In particular, I would like to thank Dale DeNardo, whose steadfast and vocal support was integral to the process of initiating my independent, unfunded research program at ASU and whose intellectual contributions have greatly improved my research and writing. Additionally, I would like to thank Kevin McGraw for his committed mentorship and friendship during my time at ASU. Kevin gave me the freedom to develop intellectually but was always there to provide key insights and guidance when I needed them.

I would also like to thank the community of ASU graduate students that I had the privilege of working with and learning from. In particular, I thank Dr. Mike Butler, Dr. Matt Toomey, Brett Seymoure, Scott Davies, Karla Moeller, Melinda Weaver, and Rick Simpson for their numerous and varied contributions to my dissertation research and continued development as a scientist. Whether by providing training in new techniques, collaborating on side projects, or discussing new ideas, these individuals broadened my thinking and my knowledge. Additionally, I owe so very much to the dedicated undergraduate researchers who worked with me during my time at ASU. Specifically, I would like to thank Kristen McCartney, Sarah Bruemmer, Megan Best, and Brianna Bero-Buell for their patience, hard work, dedication, attention to detail, and friendship.

This work would not have been possible without the financial support of ASU’s Graduate and Professional Student Association, the School of Life Science’s Research and Training Initiatives Committee, David and Sandy Ligon, crowdfunding from users of the Rockethub website, the Animal Behavior Society, the American Society for Ichthyologists and Herpetologists, Sigma-Xi (ASU chapter), the American Society of Naturalists, the Graduate College at ASU, and the National Science Foundation. Additionally, this work would not have been technically possible without early assistance from Ellis Loew, Jim Bowmaker, Martin Stevens, and Thomas Pike. Thank you all for taking the time to help an unknown student understand and use the data, techniques, and methods you employ masterfully in your own research.

Lastly, I would like to thank my family for their love and support. Mom and Dad, thank you for showing me the wonder of nature and the wonder of wondering. Dan and Dave, thank you for always being able to make me laugh. Ronnie, thank you for your encouragement, compassion, and love. You make me feel like I can do anything. Teddy, thank you for bringing new focus to my life. Spending much of the last year with you was one of the best things I've ever done. I love you all.

TABLE OF CONTENTS

Page

CHAPTER

LIST OF TABLES

Table Page

LIST OF FIGURES

PREFACE

Overview & significance

Understanding adaptation and the processes that drive diversity are two of the central foci of evolutionary biology. In part due to the remarkable diversity of signals used throughout the animal kingdom, sexual and social signals have long been thought to play an important role in speciation and diversity. Hence, investigations of intraspecific signal processes may lead to important insights regarding key mechanisms of speciation (Edwards et al. 2005, Maia et al. 2013, Seehausen et al. 2008). We have learned a great deal about animal communication from studying common signal types (Bradbury and Vehrencamp 1998), though a powerful approach to better understand the control, function, and evolution of signals is to investigate rare signals, the study of which may elucidate evolutionary processes that generate diversity (e.g. Carlson et al. 2011). Though animals use a wide array of signals to communicate with one another, my dissertation research focuses on rapid physiological color change, a rare signal-type used by relatively few taxa.

Research on rare signals frequently begins by asking “How is this means of communication used?” and “Why is it so uncommon?” To explore these questions, my dissertation has relied on novel methods to measure rapid color change signals in real-time as seen by the intended, conspecific receivers, and how these signals are linked to relevant behaviors of signalers and receivers. In this preface, I provide background information on animal signals and rapid color change, then describe the guiding hypothesis of my dissertation concerning the function of physiological color change as a social signal in chameleons, a group that exhibits rapid, complex color and pattern changes during social interactions. Following the preface, I describe the research I have conducted on the use of rapid color change as a social signal in a colorful chameleon species, and the broader significance of my work.

Theoretical framework

Animals use many signals for communication, from postures and songs to elaborate dances and electrical impulses (Bradbury and Vehrencamp 1998, Maynard Smith and Harper 2003a, Searcy and Nowicki 2005a). Some signals are relatively static once formed, such as morphological structures (e.g. turtle shells, plumage), and can reveal information about individuals during the time the trait is developed (Buchanan et al. 2003, Tibbetts and Curtis 2007). Other signals are dynamic, including behaviors like songs, dances, and aggressive postures, and provide a real-time update of an individual's quality or intentions (Adamo and Hanlon 1996, Enquist et al. 1985, Wyman et al. 2008). Though we now have deep understandings of the control, function, and evolution of many different signal types, some rare traits blur the line between static and dynamic signals and could serve as excellent subjects for testing key tenets and limitations of signaling theory.

The rapidly changing color signals exhibited by some animals, like cephalopods or chameleons, are examples of such an intermediate signal (Stuart-Fox and Moussalli 2009). Unlike comparatively fixed ornamental colors (e.g. of hair or feathers), rapid physiological color changes allow individuals to display different colors and patterns in changing environmental and behavioral contexts (Adamo and Hanlon 1996, Cuadrado 1998, Stuart-Fox and Moussalli 2008, Umbers 2011). For example, body colors can change in response to predation threat (Allen et al. 2010, Stuart-fox et al. 2008), ambient temperature (Veron

1974), and humidity (Hinton and Jarman 1972). However, among animals capable of physiological color change (e.g., insects, Umbers 2011; crustaceans, Brown and Sandeen 1948; spiders, Wunderlin and Kropf 2013; amphibians, Filadelfi et al. 2005), only a few employ rapid color changes during social interactions (predominantly cephalopods, fish, and reptiles). Among the color changing members of these taxa, the plasticity of physiological color change theoretically allows individuals to display different color signals under different conditions, which suggests that certain color change signals may have more in common with behavioral displays than with static colors. For example, production costs are thought to be relatively low for some behavioral displays (Matsumasa et al. 2013, Oberweger and Goller 2001, Ward et al. 2004, Weiner et al. 2009), and the behavioral responses of signal receivers provide the selective pressure (e.g. policing) that maintains a tight match between displayed signals and the signaler's true intent/quality (Guilford and Dawkins 1995, Hurd and Enquist 2005). In such cases, weak animals displaying aggressive signals may win encounters with other weak individuals without physical contact ('bluffing'), but are expected to bear disproportionally large costs when displaying inaccurate signals to stronger competitors (i.e. punishment costs (Caryl 1982, Van Dyk and Evans 2008, Enquist 1985, Molles and Vehrencamp 2001, Moynihan 1982, Rohwer 1975, 1977; Tibbetts and Dale 2004, Tibbetts and Izzo 2010)). In contrast to the social costs of behavioral displays, however, there are usually significant physiological costs (e.g. nutrition, health) to obtaining many types of ornamental coloration (Kemp 2008, McGraw 2006). It is not currently known whether the honesty or reliability of social color-change signals, which seem to incorporate aspects of both dynamic and static ornaments, is maintained by physiological costs (Korzan et al. 2000), social costs (Tibbetts and Izzo 2010), or both.

Although knowledge of the physiological mechanisms underlying rapid color change (Fujii and Novales 1969, Fujii 2000, Nery and Castrucci 1997, Teyssier et al. 2015, Ligon and McCartney 2016) is integral for understanding its evolution across a wide variety of taxa, the first step in comprehending the costs of rapid physiological color change is to gain a better understanding of the behavioral and communication contexts in which color change occurs. To date, ecological and behavioral studies of complex physiological color change as a social signal have lagged far behind those focused on the cellular and sub-cellular mechanisms of these color shifts (Stuart-Fox and Moussalli 2009). The paucity of investigations undertaken to understand the signaling role of physiological color change may be due, in part, to the technological and methodological limitations associated with quantifying such a rapidly changing trait. Recently, however, advances in the photographic quantification of color (Pike 2011, Stevens et al. 2007) and physiological modeling of animal color vision (Bowmaker et al. 2005, Endler and Mielke 2005a, Hart and Vorobyev 2005) enabled me to non-invasively and quantitatively analyze the previously inaccessible chromatic signals used by color-changing animals during social interactions (Ligon and McGraw 2013, Ligon 2014).

Using newly developed photographic and analytical tools, I investigated the social use of dynamic color change in chameleons. Chameleons are the only terrestrial vertebrates that undergo elaborate physiological color changes that include multi-component chromatic and pattern-element alterations during social interactions. Through my dissertation work, I sought to answer the question: *How do chameleons use rapid* *physiological color change to communicate*? To address this question I evaluated the color changes used by male veiled chameleons *Chamaeleo calyptratus* during aggressive interactions. Because the rapid, complex color changes of male veiled chameleons occur only during intraspecific interactions (Nečas 1999), I hypothesized that these color changes represent an informative, multicomponent signal that modulates receiver behavior during aggressive interactions. Consequently, I predicted that colors or color changes would be differentially expressed in chameleons that escalate and win aggressive interactions relative to non-escalating, losing individuals. Additionally, I predicted that links between an individual's display coloration and behavior would be underlain by more direct links between a chameleon's display coloration, physiology, and physical performance. Lastly, I predicted that the honesty of color or color change signals used by veiled chameleons would be maintained, at least in part, by social costs directed towards dishonestly signaling individuals.

To test these predictions, I first measured the behavior and color changes of adult male veiled chameleons during agonistic, dyadic encounters. Specifically, I focused on the color attributes of chameleons that best predicted the likelihood that an individual would approach his opponent and win the contest (Appendix A). For my second dissertation chapter (Appendix B), I performed another series of agonistic trials that allowed me to investigate the opposite end of the color change spectrum − rapid darkening. Here, I evaluated the possibility that chameleons also use rapid color change as a signal of submission. After establishing links between rapid brightening, aggressive behavior, and likelihood of winning an aggressive interaction in my first chapter, I designed my third study (Chapter 1) to minimize the behavioral complications inherent in trials with two live animals and to facilitate an in-depth investigation of the specific information content of the rapid color changes used by veiled chameleons during agonistic encounters. In this study, I used a standardized robotic chameleon stimulus to elicit behavioral displays from live chameleons, then compared the color changes exhibited to this standardized stimulus to morphology, physiology, and physical performance of individual chameleons. Lastly, I performed an experiment in which I manipulated the external appearance of chameleons using customized paints designed to match live chameleon coloration (Chapter 2). By experimentally manipulating the appearance of chameleons and then allowing them to interact in agonistic trials, I was able to test the possibility that signal receivers facing dishonestly signaling opponents, those whose behavior did not match their external appearance, would receive higher levels of aggression. Higher aggression directed towards dishonestly signaling chameleons would indicate that social costs play a role in maintaining signal honesty.

CHAPTER 1

THE TIMING OF RAPID COLOR CHANGE SIGNALS DURING AGGRESSIVE INTERACTIONS COMMUNICATES INFORMATION ABOUT PHYSICAL PERFORMANCE

ABSTRACT

Animals use diverse signals to communicate with one another, but the function of dynamic color change as a social signal has only recently begun to be investigated. For example, male veiled chameleons *Chamaeleo calyptratus* use rapid brightening displays to communicate motivation and fighting ability during agonistic encounters. Because contest strategies and color signals are strongly influenced by opponent behavior, however, a standardized, experimentally-controlled chameleon stimulus could aid our understanding of rapid color change signals by minimizing confounding effects of opponent behavior. Here, I employ such an approach to better understand the mechanisms underlying the previously documented links between color change signals and contest behavior in chameleons. Specifically, I conducted behavioral trials between male veiled chameleons and standardized robotic chameleon models, then investigated links between display coloration and morphology, testosterone, and bite force. I found that smaller male veiled chameleons with narrow jaws had higher testosterone levels, while chameleons with wider casques (head ornaments) exhibited more powerful bites. Additionally, chameleons that brightened slowly had higher testosterone levels, and those reaching maximum stripe brightness earlier had stronger bites. I also found that chameleons with yellower stripes were more likely to approach robotic opponents, and chameleons with brighter stripes

were more aggressive. Overall, these relationships suggest that veiled chameleons gain more information about their opponent's testosterone levels, bite force, aggressive intent, and overall aggression from rapid color change signals than from morphological cues but should benefit by incorporating both sources of information into contest-specific strategies.

INTRODUCTION

Throughout the animal kingdom, individuals engage in competitive interactions over indivisible resources (Hardy and Briffa 2013). The outcome of these interactions can have a strong influence on fitness, and optimal contest strategies depend on balancing individual-specific costs and benefits. In the context of competition, selection should favor the production and assessment of signals that convey information about both the relative value of contested resources (often referred to as motivation (Enquist 1985, Parker and Stuart 1976)) and resource holding potential (fighting ability (Parker 1974)) of contest participants because such signals can expedite aggressive interactions and reduce unnecessary costs associated with asymmetric conflicts (Enquist 1985, Maynard Smith and Harper 2003, Parker 1974, Searcy and Nowicki 2005). Specifically, signals that allow contest participants to recognize large asymmetries in motivation or fighting ability enable individuals to save time, reduce energetic expenditure, and resolve conflicts without the risk of physical violence (Rohwer 1982, Searcy and Nowicki 2005).

Rapid color change, which can serve as an agonistic signal, represents an interesting case study regarding links between signal design and information content because it possesses both static and dynamic attributes (Ligon and McCartney 2015).

Unlike fixed ornamental colors (e.g. of hair, feathers), physiological color change allows individuals to display different colors in changing environmental and behavioral contexts (Adamo and Hanlon 1996, Cuadrado 2000, Ligon 2014, Stuart-Fox and Moussalli 2008, Umbers 2011). This plasticity suggests that certain color change signals may have more in common with behavioral displays than with static colors. In contrast, the hues and patterns displayed by color changing organisms are influenced by previously created structural elements or previously deposited pigments (Cooper and Greenberg 1992, Ligon and McCartney 2015), which suggests that these signals also have a great deal in common with static color signals.

Despite the potential for complex color changes to contain different types of information, most of the intraspecific color change signals studied to date are somewhat simplified. For example, the information content of socially mediated color changes of cephalopods appears to be contained primarily within display pattern elements (Adamo and Hanlon 1996) rather than in chromatic cues. Numerous fish species rely on physiological color change to communicate during social interactions, though the best studied signals appear to be overall changes in brightness and darkness in salmonids (Eaton and Sloman 2011, Höglund et al. 2000, O'Connor et al. 1999) and the presence or absence of a single dark facial stripe in cichlids (Muske and Fernald 1987). Perhaps the best example of how rapid color change signal can influence social dynamics is the rapid 'eyespot' darkening of the lizard Anolis carolinensis. In this species, the speed with which a region of skin behind the eye darkens (modulated by adrenal catecholamines, (Goldman and Hadley 1969)) predicts social rank, whereby individuals that darken their eyespot more quickly than their opponents are dominant (Korzan et al. 2006, Summers and

Greenberg 1994). In spite of their taxonomic diversity, the common thread for these color changing animals appears to be a reliance on simplified on/off signals that indicate, or are correlated with, aggressive intent (or lack thereof). Though complex color displays could potentially provide more information than simple contest strategy, no subtle relationships have been uncovered between physiological color change and the various aspects of individual condition (e.g. fat reserves, body condition, strength) repeatedly discovered in taxa displaying fixed color signals.

Recently, I demonstrated that different aspects of dynamic color change signals in male veiled chameleons *Chamaeleo calyptratus* are correlated with the likelihood that a chameleon would approach his opponent and the likelihood that he would win an aggressive interaction (Ligon and McGraw 2013). However, we still do not know how particular aspects of these color change signals are linked to motivation and fighting ability. Identifying the underlying mechanisms connecting color change signals to contest behavior and outcome will inform our understanding of the processes ensuring signal honesty, as well as our interpretation of the evolutionary trajectories linking contest-relevant information to specific signals. To address these questions, I conducted experimental trials between adult male chameleons and standardized, artificial chameleon opponents. Because contest strategies are strongly influenced by opponent behavior, our standardized robotic chameleon models better enabled us to analyze the information content of rapid color change signals without the confounding effects of opponent behavior (Klein et al. 2012). I then used a model-averaging statistical approach to investigate potential links between display colorimetrics, morphology, physiology, and physical performance. Specifically, I investigated whether the rapid color change signals used by aggressively displaying chameleons served as signals of testosterone (Cox et al. 2008, Evans et al. 2000, Laucht and Dale 2012, McGlothlin et al. 2008, McGraw and Parker 2006, Whiting et al. 2006), bite force (Meyers et al. 2006, Plasman et al. 2015), or contest behavior (Ligon and McGraw 2013, Ligon 2014, Muske and Fernald 1987, Summers and Greenberg 1994).

I hypothesized that chameleon color changes during aggressive interactions would be linked to testosterone, because it is the major androgen in male lizards (Moore and Lindzey 1992) and underlies seasonal (Klukowski and Nelson 1998), sexual (Hews et al. 2012), and species-specific (Hews et al. 2012) differences in aggression among lizards. Though testosterone may be an important mediator of aggressive behavior, its variability over both short (Smith and John-Alder 1999) and long-term (Klukowski and Nelson 1998) time-scales makes it difficult to predict its specific influence on contests or color change. Additionally, because lizards primarily inflict damage upon rivals by biting, I hypothesized that chameleon color displays may communicate information about bite force. Strong positive links between bite force and dominance have been discovered for numerous lizard species (Husak et al. 2006, Huyghe et al. 2005, Lailvaux et al. 2004), and thus colorful signals that accurately convey information about individual bite force or testosterone levels should be particularly valuable for male chameleons engaged in aggressive contests. To test whether or not chameleon color changes during agonistic interactions serve as a signal of fighting ability or hormonal status, I conducted a series of staged agonistic encounters between veiled chameleons and remotely controlled chameleon models. I then compared display colorimetrics to testosterone, morphology, and bite force to more fully evaluate the information content of chameleon color change signals.

METHODS

Study species and housing

Veiled chameleons are territorial lizards native to the southwestern Arabian Peninsula (Nečas 1999). This species relies on rapid color changes to communicate during intraspecific interactions (Kelso and Verrell 2002, Ligon and McGraw 2013, Ligon 2014, Nečas 1999) and male veiled chameleons regularly exhibit high-levels of aggression towards conspecific males, likely because these behaviors are involved in territory or mate defense (Cuadrado 2001). Aggressive chameleons rapidly change colors and brightness, and typically undergo concomitant changes in body shape and orientation during these displays. Specifically, males compress their bodies laterally while simultaneously undergoing dorsal-ventral expansion: effectively turning their bodies into billboard signs. At any time during the interaction, either male can cease aggression and, if threatened, rapidly retreat. This submissive behavior is accompanied by rapid darkening, which serves as a signal of submission (Ligon 2014). If both chameleons continue to exhibit aggressive behavior, however, contests can escalate to physical fights that include lunging and biting.

The veiled chameleons in this study were obtained from a private breeder and a feral population, both located in Florida, USA. Our chameleons were housed individually in a temperature-controlled vivarium at Arizona State University. Each cage contained a mixture of live, dead, and artificial plants to provide climbing structure and shelter, and was misted four times per day to provide drinking water for the chameleons. Additionally, each cage was fitted with a UV light source (Zoo Med Reptisun 5.0 UVB Fluorescent Bulbs; Zoo Med Laboratories Inc., San Luis Obispo, CA, USA) and heat lamp (Zoo Med Repti-Basking Spot Lamp, 50 watt). Additional details regarding chameleon housing and basic husbandry can be found in (McCartney et al. 2014).

Behavioral Trials

Over the course of three days (30 June - 2 July 2013), I conducted aggression trials using 33 adult male veiled chameleons and life-like chameleon models (see Robochameleons section below). Each trial was conducted between one chameleon and one robochameleon. Prior to a given trial, I removed one male from his cage, measured his body mass using a digital scale (accurate to the nearest 1 g), and placed him on one end of a trial arena (183 x 53 x 81 cm) containing vertical and horizontal perches (Figure 1a). During the subsequent 5 minute acclimation period, the chameleon was visually isolated from the robotic chameleon model by a physical divider in the center of the arena. After acclimation, the divider was removed and the trial begun. Trials were recorded from behind a blind with a Panasonic HDC-TM 700 video camera (Osaka, Japan), which enabled us to take still photographs while recording video. Trials were stopped after 10 minutes or after the chameleon physically attacked (i.e. lunged at and bit) the model more than once.

Following my published methods (Ligon 2014), I quantified each of 11 aggressive behaviors exhibited by chameleons during aggression trials. In contrast to my previous work (Ligon 2014), I did not record instances of retreating or fleeing because these behaviors are associated with submission and our focus here was restricted to aggressive behaviors and color signals. I scored aggressive behaviors (Supplementary Table 1) based on the putative risk they posed to displaying chameleons (where riskier behaviors indicated higher aggression and were given higher weights) and used these scores, along with the frequency of the respective behaviors, to calculate an overall aggression score for each chameleon during each aggressive interaction.

Robochameleons

I modified three commercially available plastic male veiled chameleon models (Safari Ltd®, Miami Gardens, FL, USA) to create standardized, species-specific stimuli (Figure 1b) during trial presentations to live chameleons. Specifically, I removed the projecting tongue from each model and applied custom, non-toxic paints (Golden Artist Colors Inc, New Berlin, NY) created to mimic natural display coloration of veiled chameleons. These custom paints were measured with a reflectance spectrometer (Ocean Optics, Dunedin, FL) and, using visual models (Bowmaker et al. 2005, Vorobyev and Osorio 1998), compared to representative spectra collected from live, displaying veiled chameleons. Five of the six colors I used to paint model chameleons fell below the threshold of discriminability (measured in Just Noticeable Differences = JNDs;(Wyszecki and Stiles 1982), indicating that chameleons were unlikely to be able to detect differences between these artificial colors and real colors exhibited by veiled chameleons. Discriminability values greater than 1 JND indicate that the organism in question is capable of detecting a difference between the colors compared under ideal conditions, and the discriminability value for our sixth color, dark green, was marginally above this threshold (1.05 JNDs). However, this particular color made up a relatively small proportion of the overall body coloration of our models and our models still successfully elicited normal behavioral responses from live chameleons (see Results).

I animated our chameleon models by attaching them to a TrackerPod® (Eagletron Inc, Niagara Falls, New York) panning/tilting base designed for webcams. The TrackerPod® can be controlled via a USB cord attached to a computer and I used a small laptop placed near the trial arena to control the model during each trial. Additionally, I glued our TrackerPod® to a small, wheeled base and used a series of pulleys and string to control the forward/backward movement of the model during each trial. One individual (RAL) controlled all movements of each robochameleon during trials from behind a blind, observing the chameleon and robochameleon behavior via the LCD screen of one of our video cameras. Under the control of RAL, robochameleons began each trial facing away from the live chameleon, slowly rotated to mimic the body orientation that typically follows identification of a chameleon opponent, and then slowly advanced towards the live chameleon. To mimic the lateral display behaviors shown by real chameleons wherein individuals orient their bodies perpendicularly to the direction of their opponent and sway, robochameleons stopped approaching the live chameleons at short intervals to turn their bodies perpendicularly and sway, as live displaying chameleons do.

When using dynamic artificial stimuli to elicit behavioral responses from live animals, one experimental approach is to employ an identical sequence of stimulus behaviors for every focal animal. I did not use this approach. Instead, I employed a protocol where the intensity of robochameleon behavioral responses roughly matched those of the live chameleon to maximize the likelihood that a given chameleon would respond aggressively to the robochameleon model and undergo physiological color change. This approach resulted in 13 of 33 chameleons (39%) undergoing aggressive color change in response to the robochameleon models, a result consistent with earlier behavioral studies between two live chameleons (e.g. aggressive color change in 34 out of 79 trials the preceding summer = 43%).

Morphological measurements

To measure snout-vent length (SVL), one researcher used two hands to hold the chameleon in an outstretched position and a second researcher placed a flexible plastic ruler against the chameleon's body. Additionally, I collected seven measurements (Figure 1c,d) from the head region of each chameleon using digital calipers (accurate to the nearest 0.1 mm). Head measurements were chosen based on a previou investigations of the relationship between morphology and bite force in chameleons (Measey et al. 2009), as well as personal observations regarding a potential relationship between jaw and casque width and bite force. In total, I took morphometric measurements of head length (HL), head height (HH), casque height (CH), lower jaw length (LJL), head width (HW), casque width (CW), and jaw width (JW).

Bite force performance

To determine whether any links exist between bite force and the colors exhibited by chameleons to a standardized stimulus, as well as morphology, testosterone, or behavior, I measured the bite force of each of our 33 chameleons 3-5 days before the behavioral trials. Bite force was measured three times for each chameleon, and I used the maximum calculated bite force (see below) as a measure of individual biting power

(Anderson et al. 2008, Losos et al. 2002). Only vigorous bites were recorded and I discarded those with abnormally low readings (Losos et al. 2002, Vanhooydonck et al. 2010). To quantify bite force, I used a miniature, low-profile load cell (Transducer Techniques®, Temecula, CA, USA) fitted between custom bite plates coated with rubber (Figure 1d) to protect the chameleons' teeth when they bit down and to provide a compressible surface more similar to the biological matter (e.g. an opponent's flank) that they might typically bite.

I began each bite force measurement by placing a chameleon in front of the bite plates. Frequently, the chameleons would readily open their mouths as a threatening behavior (in response to being handled), and in these cases I simply placed the bite plates into their open mouths and waited for the animal to bite down. Other individuals opened their mouths when touched lightly around the head. Additionally, for others I had to manually open their mouths and place them onto the bite plates. To examine the influence of our bite measurement protocol, including bite order and the amount of stimulation required to elicit bites from each chameleon, I used a linear mixed model created with the "lme4" package (Bates et al. 2014) in the R computing environment (R Core Team 2014). Our model included stimulation (scored as "handling", "touching head", or "mouth physically opened"), bite order (first, second, or third), and their interaction as fixed effects, as well as chameleon identity as a random effect. I interpreted the results of this model using the "afex" package (Singmann and Bolker 2014), also in R. Neither stimulation ($F_{2,72.14} = 2.61$, $p = 0.08$), bite order ($F_{1,61.50} = 3.71$, $p = 0.06$), nor their interaction ($F_{2,69.30} = 1.71$, $p = 0.19$) had a significant influence on bite force, though there were non-significant tendencies for bite force to increase with bite order and with increased stimulation level.

To correct for differences in mechanical advantage (i.e. force amplification), and thus measured output of bite force, arising from differences in the specific location where chameleons bit down on the bite plates, I used a high-definition video camera to record each series of bites in profile (Figure 1d). Video recordings included a metric ruler placed in the same plane as the bite plates, which allowed us to make measurements of the chameleon's head and bite location from still frames extracted from the videos of each bite event. Using the ruler within the extracted image to calibrate distance measurements, I was then able to measure the distance from the quadrate-articular jaw joint to the bite point (first point of contact between teeth and bite plate) using ImageJ (Schneider et al. 2012) which allowed us to calculate the true force applied by a chameleon's jaws using second order lever calculations (*cf.* Lappin et al. 2006b).

Testosterone measurement

I measured circulating testosterone levels of chameleons following agonistic interactions with robochameleons using blood samples collected immediately (< 5 min) after each trial. Blood samples were collected following contests to minimize pre-trial stressors that might influence contest behavior. I collected blood samples from the caudal vein of each chameleon immediately after each behavioral trial using heparanized, 0.5 ml syringes. Blood samples were stored on ice until centrifugation, after which I froze plasma samples at -80° C until analysis.

I measured plasma testosterone using commercially available enzyme-linked immunoassay (ELISA) kits (Enzo Life Sciences, Farmingdale, NY, USA) in accordance with manufacturer's instructions. All samples for this study were run on a single ELISA plate that included positive and negative controls and standards to create a standard curve. Plasma T levels were calculated for each chameleon from absorbance values. All standards and samples were run in duplicate (mean intra-sample coefficient of variation = 6.37). Additionally, the slopes of a plasma dilution curve created by serially diluting chameleon plasma (10x – 100x) and that of the standard curve were statistically indistinguishable ($F_{1,12} = 0.026$, $p = 0.89$).

Color measurement

I used digital photography to collect color and brightness data from chameleons during agonistic interactions with robochameleons following our previously published methods (Ligon and McGraw 2013). First, I analyzed video recordings of each aggression trial to determine visually the timing of rapid, agonistic color change bouts. I used photographs taken at approximately 4 second intervals during these color change bouts to quantify all color and color change variables. Second, I standardized photographs (ensuring equalization and linearization (Pike 2011, Stevens et al. 2007)) using a specialized color standard (ColourChecker Passport, X-Rite Photo) and a software plug-in (PictoColour® inCamera™, PictoColour Software, Burnsville, MN) for Adobe Photoshop (Bergman and Beehner 2008). Third, I used specialized mapping functions (Pike 2011) to convert RGB (red, green, blue) values from standardized photographs to relative stimulation values of the chameleon photoreceptors (Bowmaker et al. 2005).

Fourth, I plotted each color in chameleon-specific colorspace designed to preserve perceptual distances (Pike 2012). For full details, see Supplementary Materials in (Ligon and McGraw 2013).

I focused my analyses on four color patches on the head and lateral stripes of each chameleon during agonistic interactions with robochameleons. Specifically, I chose two color patches on the vertical yellow body stripes and two locations on the heads (Supplementary Figure 1). These patches were chosen because their brightness and speed of color change were highly correlated with composite principal component (PC) scores collected from many, previously measured, locations within the same general body regions (Ligon and McGraw 2013) that predicted approach likelihood and fighting ability. For each color patch, I quantified the maximum brightness achieved (stimulation of chameleon double cones (Osorio and Vorobyev 2005)), the maximum speed of brightening, and the time it took (in sec) to achieve maximum brightness from the beginning of the trial. I also measured color change, as the distance between the start and end color during brightening bouts calculated within chameleon color space (in units of Just Noticeable Differences or JNDs), and the rate of color change (JND/sec). Furthermore, I quantified maximum chroma for each color patch, and the hue at the point of maximum chromaticity. Maximum chroma was determined for each patch as the farthest point a given color travelled from the achromatic center of chameleon colorspace and hue was calculated as the angle of the vector connecting the achromatic center and a given color's location within colorspace at the time of maximum chromaticity (Endler and Mielke 2005, Stoddard and Prum 2008).

To reduce the number of variables in our analyses I standardized each variable ($\bar{x} = 0$, SD = 1) and averaged the colorimetric data for each body region (i.e. for the stripe region and for the head region). Thus, I created average values for brightness, maximum brightening speed, time to reach maximum brightness, color change (chromatic distance traveled), speed of color change (i.e. movement through chameleon color space), maximum chroma, and hue at maximum chroma for both the stripe and head regions.

Statistical analyses

Data preparation and investigation

Following prior recommendations (Zuur et al. 2010), I first evaluated the data for possible outliers using Cleveland plots. I then checked our data for homogeneity of variance using plots of residuals vs. fitted values, and assessed normality of residuals via visual inspection of Q-Q plots. Next, all variables were standardized to a mean of zero and a standard deviation of one (Schielzeth 2010) to facilitate direct comparisons of parameter estimates.

Information-theoretic model averaging approach

I used Akaike's information criterion corrected for small sample size (AICc) to evaluate all statistical models (Burnham and Anderson 2002). Specifically, I modeled relationships examining continuous variables (testosterone, bite force, aggression scores) using linear models and relationships examining approach likelihood using generalized linear models (approach as a binary response variable, binomial error structures). I evaluated our complete dataset (n = 33) usingnmodels with three or fewer predictor variables (following rule of thumb described in (Bolker et al. 2009, Harrell Jr. 2001)). Because not all chameleons underwent color change displays during aggressive interactions (n = 13), thereby reducing our sample size, I limited analysis of color change models to those with two or fewer predictor variables.

Though multiple models may be well-supported within an information-theoretic framework, evaluating the relative importance of specific variables is still possible using model averaging approaches (Burnham and Anderson 2002, Burnham et al. 2010). Model averaging allows researchers to incorporate parameter estimates from multiple models, each weighted by the support for that model (Burnham and Anderson 2002). Thus, parameter estimates from well-supported models will contribute relatively more to multi-model parameter estimates. Information-theoretic approaches can generally provide accurate parameter estimates when confronted with collinearity among predictor variables, but I omitted additive models that included highly correlated variables ($r > 0.7$) because of the increased variance among parameter estimates when models include highly correlated predictors (Freckleton 2010).

The use of multiple models also allowed us to calculate relative importance (RI) values for each predictor variable within a given model set. Specifically, I calculated RI values by summing the Akaike weights (wi) for all models in which that variable appeared. Akaike weights for a given set of models sum to 1, so RI values range from 0 to 1 (where RI values near 0 indicate variables that occur infrequently or in poorly-supported models and RI values near 1 indicate variables frequently represented in well-supported models). RI values were calculated from 95% confidence sets, for which the cumulative Akaike weight was ~0.95 indicating a 95% probability that the best model was within this set, or from the models that had lower AICc scores than the null model containing no predictor variables.

All statistical analyses were conducted within the R computing environment (R Core Team 2014). Additionally, model selection was performed using the MuMIn package in R (Barton 2013), and forest plots of parameter estimates and 95% confidence intervals using the Gmisc package in R (Gordon 2014).

RESULTS

Of the 33 veiled chameleons I allowed to interact with robotic chameleon models, 13 individuals engaged in rapid brightening displays directed toward the robochameleon (39%). There was a strong association between brightening and the likelihood of approaching the robotic opponent (GLMM with binomial error distribution and chameleon ID included as a random effect; $z = 2.84$, $p = 0.004$, odds ratio=14.40), indicating that brightening chameleons were 14 times more likely to approach the robochameleon than individuals that did not brighten. In the analyses described below, I evaluated relationships between morphology, testosterone, bite force, and behavior for all chameleons, but were forced to restrict colorimetric analyses to the subset of chameleons ($n = 13$) that displayed active color change directed at the standardized robotic chameleon.

(A) All chameleons

(i) Morphological predictors of testosterone & bite force

Preliminary analysis of collinearity between morphological variables revealed high degrees of correlation between multiple traits (Supplementary Table 2). However, our multiple model investigation omitted any models containing highly correlated ($r > 0.7$) traits to minimize the effect that multicollinearity might have on the variance of parameter estimates. Evaluation of this set of models including only uncorrelated morphological predictor variables yielded no clear model as the best predictor of post-trial circulating testosterone concentration (Supplementary Table 3). However, multi-model averaging uncovered jaw width as the best predictor of circulating testosterone levels (RI = 0.63), with body mass (RI = 0.37) also being somewhat important (Fig 2a). Specifically, chameleons with narrower jaws ($F_{1,31} = 12.08$, $p = 0.001$, $R^2 = 0.28$; Figure 2b) and smaller body masses ($F_{1,31} = 10.23$, $p = 0.003$, $R^2 = 0.25$) had higher plasma testosterone levels.

Our analyses of the morphological predictors associated with maximum bite force, again using multiple model inference, yielded no clear best model (Supplementary Table 4). However, every model within our 95% confidence set contained casque width as a predictor of bite force. Hence, casque width (RI = 1.0) was by far the best predictor of the maximum bite force of male veiled chameleons (Figure 2c); chameleons with wider casques had more forceful bites ($F_{1,30} = 15.16$, $p = 0.0005$, $R^2 = 0.34$; Figure 2d).

(ii) Phenotypic characters (morphological variables, bite force, testosterone) and i) likelihood of approach and ii) peak aggression

Only a single model exploring the importance of phenotypic characters on the likelihood of approaching the robotic chameleon performed better than the null model (Supplementary Table 5). This model had SVL as the single predictor of approach likelihood, with longer chameleons exhibiting a non-significant tendency towards being more likely to approach the robotic chameleon (Figure 3a; $z = 1.74$, $p = 0.08$, odds ratio = 2.29).

Our multiple model approach examining the relationship between phenotypic characters and peak aggression revealed six models that performed better than the null model (Supplementary Table 6). Evaluating only these models, I found that SVL was present in every model giving it the highest possible relative importance (RI = 1.0; Figure 3b). Specifically, chameleons that were longer exhibited higher peak aggression scores towards robotic chameleons ($F_{1,30} = 4.23$, $p = 0.048$, $R^2 = 0.12$).

(B) Brightening chameleons

(i) Colorimetric predictors of testosterone & bite force

Though several color metrics were highly correlated (Supplementary Table 7), I restricted our models to those containing only uncorrelated variables. Two models linking color change and circulating testosterone performed better than the null model, both containing the maximum brightening speed as an explanatory variable (maximum stripe brightening speed RI = 1.0; Figure 4a, Supplementary Table 8). Chameleons that brightened more quickly had lower testosterone levels ($F_{1,11} = 6.42$, $p = 0.028$, $R^2 = 0.37$; Figure 4b).

Analyzing the relationship between color metrics and bite force, I found that all 14 models that performed better than the null model contained the time to reach maximum stripe brightness as an explanatory variable (Supplementary Table 9; time to maximum stripe brightness RI = 1.0; Figure 4c); chameleons that reached maximum stripe brightness more quickly exhibited greater bite forces ($F_{1,11} = 13.53$, $p = 0.004$, $R^2 = 0.55$; Figure 4d).

(ii) Relative value of colorimetic and phenotypic (testosterone, bite force, and morphology) variables in predicting i) likelihood of approach and ii) peak aggression

In an attempt to determine the relative importance of all color change and phenotypic variables in predicting the aggressive behavior of chameleons during agnostic trials with a robotic chameleon stimulus, I again used a multiple model averaging approach.

Our multimodel approach revealed that 25 models performed better than the null model in predicting the likelihood of a chameleon approaching the robotic chameleon (Supplementary Table 10), and the variable with the highest relative importance was stripe hue (RI = 0.83; Figure 5a). This variable had a marginally significant influence on the likelihood that a chameleon would approach the robotic chameleon (Figure 5b; $z = 1.91$, $p = 0.056$, odds ratio = 6.53), such that chameleons with larger stripe hue values (more yellow, less orange) were more likely to approach the robotic chameleon.

Additionally, our multimodel approach revealed that only three models performed better than the null model in predicting the peak aggression scores displayed by chameleons towards the robotic chameleons (Supplementary Table 11). Within these models, the variable with the highest relative importance was maximum stripe brightness (RI = 0.60; Figure 5c); chameleons with brighter stripes exhibited more aggression ($F_{1,11} = 6.54$, $p = 0.027$, $R^2 = 0.37$; Figure 5d).

DISCUSSION

In this study, I uncovered links among morphology, physiology, performance, and contest behavior (discussed below), though stronger, more statistically robust links were revealed between display colorimetrics and these same values. Specifically, the proportion of variance in testosterone, bite force, approach behavior, and overall aggression explained by colorimetric variables was, in every case, higher than that explained by morphological cues. Thus, a veiled chameleon in an aggressive interaction with a conspecific can get an excellent idea of his opponent's potential for inflicting injury and winning the aggressive interaction by paying attention to the rapid color change signals produced by that opponent.

Physiological color change signals

Latency to maximum brightness signals biting…and fighting?

The strongest correlate of maximum chameleon bite force, and thus the ability to inflict serious damage in an aggressive interaction, was the time it took for a chameleon to reach maximum brightness (Figure 4c,d), which explained 55 percent of the variation in bite force. Latency to reach maximum stripe brightness could be interpreted as a measure of motivation, where more-highly motivated chameleons initiated color change more quickly during agonistic encounters, reaching maximum brightness values earlier. Is it possible then, that motivation underlies the relationship between brightness latency and bite force, with highly aggressive/motivated chameleons changing color more quickly and bite force simply reflecting aggression rather than performance capability? If so, we should expect differences in the bite force between the chameleons that brightened towards the robochameleon and those that did not. However, such a difference did not exist (two sample t-test, $t_{30.03} = -1.18$, $p = 0.245$) suggesting that, while chameleons displaying earlier in a behavioral interaction do indeed bite harder than those that wait, these differences in bite force reflect real variation in performance. Because bite-force is known to have strong links to fighting ability and dominance in other lizards (Husak et al. 2006, Lailvaux and Irschick 2007), it seems likely that variation in bite force is also related to fighting ability in veiled chameleons. We were not able to test this possibility in the current study because it simply does not make sense to evaluate fighting ability against an artificial chameleon model. However, if hard-biting chameleons fared better in previous aggressive interactions as a consequence of their biting ability, these winning experiences could partially account for the observed differences in latency to maximum brightness because prior success decreases display and attack latency in a number of other species (Adamo and Hoy 1995, Martinez et al. 20AD, Oyegbile and Marler 2005).

Testosterone and maximum speed of brightening

Among chameleons that exhibited aggressive color changes towards the robochameleon, those with higher testosterone levels brightened more slowly (on their heads) than chameleons with lower testosterone levels (Figure 4a, b). Because circulating testosterone levels of lizards do not necessarily change following agonistic encounters (McEvoy et al. 2015, Moore 1987), this correlation hints at an 'organizational' effect of testosterone (e.g. where dermal chromatophores respond differently based on pre-existing differences in testosterone) rather than an 'activational' one (e.g. where chameleons rely on testosterone to brighten more quickly, temporarily depleting testosterone levels).

Perhaps testosterone impedes brightening due a similar mechanism underlying the darkening induced in *Rana pipiens* skin when exposed to testosterone (Himes and Hadley 1971). Regardless of the specific mechanisms linking brightening rate and testosterone, interpreting a signaling function of this correlation is difficult and, at this point, I have no straightforward explanation for the negative relationship between brightening speed and testosterone.

Aggressive behavior and stripe colorimetrics

Veiled chameleons whose stripes became brighter (higher maximum brightness values) were more aggressive towards robotic chameleon models, a finding largely consistent with, though not identical to, our earlier work. Previously, I found that chameleons displaying brighter stripes were more likely to approach their opponents during agonistic encounters (Ligon and McGraw 2013), though I did not quantify a composite metric of overall aggression as in the current experiment. Hence, the fact that I uncovered similar results linking stripe brightness to aggression in an experiment where the influence of receiver responsiveness was minimized suggests that stripe brightness contains information linked to the displaying chameleon independent of the particular social context in which these stripes are displayed. Bright display colors have been linked to aggression and dominance in numerous other taxa (e.g. (Crothers et al. 2011, Martín and López 2009, Penteriani et al. 2007)), though the signals explored to date have not been as temporally flexible as those displayed by chameleons. Brightness contrasts improve general conspicuousness and motion detection (Kelber et al. 2003, Persons et al. 1999), so one advantage of using a brightness-based signal may be to increase visibility and detectability – which can in-turn influence success in male-male competitions (Marchetti 1993). Bright skin may also signal aggression, at least in part, because it is the exact opposite of the dark appearance used by males of this species to signal submission (Ligon 2014).

I also found a relationship between stripe hue and the likelihood that a chameleon would approach the robotic chameleon model, suggesting that there is information contained within the specific color of the stripes (in addition to the brightness and timing of maximum brightness). Competitive ability has been linked to specific colors and hues in diverse taxa (e.g. (Martín and López 2009, Pryke and Andersson 2003, Siefferman and Hill 2005, Steffen and Guyer 2014)), though the specific color attributes that signal competitive ability are highly variable among species. The diversity of color signals associated with agonistic signaling is not surprising, however, because each species' life-history, environment, and perceptual abilities will influence signal design and conspicuousness (e.g. (Stuart-Fox and Moussalli 2008, Stuart-Fox et al. 2007)). Perhaps yellow signals, associated an increased likelihood of approaching the robochameleon in the present study, provide optimal detection probabilities in the natural habitats of veiled chameleons and have consequently been favored by selection. Additionally, it is also possible that the physiological machinery or pigments associated with yellower stripes are linked mechanistically to aggression. A better understanding of the biochemical and structural mechanisms of these colorful stripes will undoubtedly lead to new insights regarding the information content of chameleon color signals (Teyssier et al. 2015).

Morphology, physiology, performance, and behavior

Casque morphology and bite force

Casque height is positively correlated with fighting success in Labord's chameleons *Furcifer labordi* (Karsten et al. 2009) and Cape dwarf chameleons *Bradypodion pumilum* (Stuart-Fox et al. 2006). Additionally, casque height has been linked to bite force in *B. pumilum* (Measey et al. 2009), suggesting functional, performance-based benefits associated with taller casques. However, I found no links between casque height and bite force in veiled chameleons and instead uncovered a strong link between casque width and maximum biting strength. This relationship makes sense when you consider that jaw musculature influences bite force (Lappin et al. 2006a) and casque width in veiled chameleons is directly linked to lateral jaw adductor musculature (R. Fisher, *pers. comm.*), which should enable greater bite force. Differences in casque morphology (e.g. shape, relative height, muscle attachment points (Rieppel 1981)) are likely responsible for the observed differences between dwarf and veiled chameleons with respect to the relationship between casque height and biting ability.

Given the absence of a link between casque height and bite force in veiled chameleons, why does this species exhibit the tallest casque (Hillenius 1966) of any chameleon species? I suggest that social selection has favored extreme casques in male veiled chameleons because such casques present a larger surface area for signaling via rapid color change. I have previously demonstrated that head-specific color changes are linked with fighting success in this species (Ligon and McGraw 2013), and larger casques may therefore provide more efficient or reliable means of communicating this information (I did not measure fighting success in the present study). Consistent with the idea that social selection pressures may favor the exaggeration of casque height as a social signal in chameleons, rather than as a means of increasing bite force, casque size in male warty chameleons *Furcifer verrucosus* is four times more important for predicting mating success than fighting success (Karsten et al. 2009).

Aggression and body length

Differences in size, motivation, or fighting ability are predicted to influence contest behavior (Archer 1988, Austad 1983, Parker 1974). Thus, the direction of our results, where longer chameleons showed a trend towards being more likely to approach the robochameleon and were slightly more aggressive towards it, are not surprising. However, the weakness of the relationships between morphology and aggression in our study was somewhat unexpected. Body size can influence the likelihood of winning aggressive interactions for some lizard species (Aragón et al. 2005, Sacchi et al. 2009, Umbers et al. 2012), including two species of Madagascan chameleons (Karsten et al. 2009), and larger combatants are expected to incur reduced costs in agonistic encounters with smaller opponents (Austad 1983). Yet, for neither veiled chameleons (Ligon and McGraw 2013) nor Cape dwarf chameleons (Stuart-Fox et al. 2006) does body mass appear to be an important predictor of contest success. Bite force is not related to SVL ($F_{1,30} = 0.23$, $p = 0.64$) or body mass ($F_{1,30} = 0.22$, $p = 0.64$) in veiled chameleons, hence selection is likely acting primarily on weapon performance (Lappin and Husak 2005) and signaling efficacy (Stuart-Fox and Moussalli 2008) rather than body size in this system.

Jaw width, body mass, and testosterone

Chameleons that were less massive and had narrower jaws circulated higher levels of testosterone after agonistic encounters. Because body mass and jaw width are highly correlated (Supplementary Table 2), it is likely that the same causal factor(s) underlie both relationships. Given the positive links frequently documented between testosterone and muscle development in numerous vertebrates (Herbst and Bhasin 2004, Norris 2007), these results were unexpected (though this relationship is not universal, see (Husak and Irschick 2009)). However, I measured testosterone and morphology in adult chameleons, putatively after the majority of growth had been completed. Consequently, the relationships I uncovered may have arisen because of the influence of testosterone on adult animals, specifically with respect to metabolism and activity levels. Experimental testosterone implants can cause significant mass losses in male mountain spiny lizards *Sceloporus jarrovi* (Klukowski et al. 2004), northern fence lizards *Sceloporus undulatus hyacinthinus* (Klukowski and Nelson 2001), and sand lizards *Lacerta agilis* (Olsson et al. 2000). In at least the case of mountain spiny lizards, these experimental manipulations also resulted in increased activity levels for implanted males. Though I do not have the data to evaluate whether activity levels or time budget differed among chameleons in our captive study population, this is at least one potential mechanism mediating the observed negative relationship between testosterone and body mass/jaw width.

Conclusions

Color signals have evolved as efficient means of communicating information about developmental conditions (Walker et al. 2013), foraging ability (Senar and Escobar 2002), health (Martín and López 2009), testosterone (Laucht et al. 2010, McGraw et al. 2006), and fighting ability (Whiting et al. 2006), and their value as informative signals, while dependent upon their pigmentary or structural basis (Hill and McGraw 2006, Teyssier et al. 2015), is a consequence of the numerous and varied factors that influence their expression (Grether et al. 2004). Here, I provide new evidence that the information-content and complexity of the information conveyed via ornamental colors increases when rapid color change, brought about by the dynamic reorganization of pigmentary or structural components within the dermal chromatophore (Teyssier et al. 2015), takes place within the time-frame of a given social interaction. Specifically, I document that the speed of brightening, timing of maximum brightness, and the brightness and hue of colorful patches used by displaying chameleons to mediate competitive interactions communicate information about physiological status, physical performance, and aggression. The complexity and potential information content of such signals increases markedly when organisms can display rapid, context-specific variation in colorful ornaments and the study of rapid color change signals is therefore a ripe field for new explorations into the functions, mechanisms, and evolutionary origins of multi-component signal types.

.

LITERATURE CITED

Adamo, S. A., and R. T. Hanlon. 1996. Do cuttlefish (Cephalopoda) signal their intentions to conspecifics during agonistic encounters? Animal Behaviour 52:73–81.

Adamo, S. A., and R. R. Hoy. 1995. Agonistic behaviour in male and female field crickets, Gryllus bimaculatus, and how behavioural context influences its expression. Animal Behaviour 49:1491–1501.

Anderson, R. A., L. D. Mcbrayer, and A. Herrel. 2008. Bite force in vertebrates: Opportunities and caveats for use of a nonpareil whole-animal performance measure. Biological Journal of the Linnean Society 93:709–720.

Aragón, P., P. López, and J. Martín. 2005. Roles of male residence and relative size in the social behavior of Iberian rock lizards, Lacerta monticola. Behavioral Ecology and Sociobiology 59:762–769.

Archer, J. 1988. The behavioural biology of aggression. Cambridge University Press, New York.

Austad, S. N. 1983. A game theoretical interpretation of male combat in the bowl and doily spider (Frontinella pyramitela). Animal behaviour 31:59–73.

Barton, K. 2013. MuMIn: Multi-model inference. R package version 1.9.13.:http://CRAN.R–project.org/package=MuMIn.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7:http://cran.r–project.org/package=lme4.

Bergman, T. J., and J. C. Beehner. 2008. A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (Theropithecus gelada). Biological Journal of the Linnean Society 94:231–240.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in ecology & evolution 24:127–35.

Bowmaker, J. K., E. R. Loew, and M. Ott. 2005. The cone photoreceptors and visual pigments of chameleons. Journal of Comparative Physiology A 191:925–32.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York.

Burnham, K. P., D. R. Anderson, and K. P. Huyvaert. 2010. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65:23–35.

Cooper, W. E., and N. Greenberg. 1992. Reptilian Coloration and Behavior. Pages 298–422 in Biology of the Reptilia, Vol. 18: Hormones, Brain, and Behavior (C. Gans, Ed.). University of Chicago Press, Chicago.

Cox, R. M., V. Zilberman, and H. B. John-Alder. 2008. Testosterone stimulates the expression of a social color signal in Yarrow's spiny lizard, Sceloporus jarrovii. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 309:505–514.

Crothers, L., E. Gering, and M. Cummings. 2011. Aposematic signal variation predicts male-male interactions in a polymorphic poison frog. Evolution 65:599–605.

Cuadrado, M. 2000. Body Colors Indicate the Reproductive Status of Female Common Chameleons: Experimental Evidence for the Intersex Communication Function. Ethology 106:79–91.

Cuadrado, M. 2001. Mate guarding and social mating system in male common chameleons (Chamaeleo chamaeleon). Journal of Zoology 255:425–435.

Eaton, L., and K. A. Sloman. 2011. Subordinate brown trout exaggerate social signalling in turbid conditions. Animal Behaviour 81:603–608. Elsevier Ltd.

Endler, J. A., and P. W. J. Mielke. 2005. Comparing entire colour patterns as birds see them. Biological Journal of the Linnean Society 86:405–431.

Enquist, M. 1985. Communication during aggressive interactions with particular reference to variation in choice of behaviour. Animal Behaviour 33:1152–1161.

Evans, M. R., A. R. Goldsmith, and S. R. A. Norris. 2000. The effects of testosterone on antibody production and plumage coloration in male house sparrows (Passer domesticus). Behavioral Ecology and Sociobiology 47:156–163.

Freckleton, R. P. 2010. Dealing with collinearity in behavioural and ecological data: model averaging and the problems of measurement error. Behavioral Ecology and Sociobiology 65:91–101.

Goldman, J. M. J. M., and M. E. M. E. Hadley. 1969. In vitro demonstration of adrenergic receptors controlling melanophore responses of the lizard, Anolis carolinensis. Journal of Pharmacology and Experimental Therapeutics 166:1–7.

Gordon, M. 2014. Gmisc: A few handy misc functions for plots, tables, and more. R package version 0.6.4.:http://CRAN.R–project.org/package=Gmisc.

Grether, G. F., G. R. Kolluru, and K. Nersissian. 2004. Individual colour patches as multicomponent signals. Biological Reviews 79:583–610.

Hardy, I. C. W., and M. Briffa. 2013. Animal Contests. Cambridge University Press, New York.

Harrell Jr., F. E. 2001. Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis. Springer, New York, NY.

Herbst, K. L., and S. Bhasin. 2004. Testosterone action on skeletal muscle. Current opinion in clinical nutrition and metabolic care 7:271–277.

Hews, D. K., E. Hara, and M. C. Anderson. 2012. Sex and species differences in plasma testosterone and in counts of androgen receptor-positive cells in key brain regions of Sceloporus lizard species that differ in aggression. General and Comparative Endocrinology 176:493–499. Elsevier Inc.

Hill, G. E., and K. J. McGraw. 2006. Bird Coloration. Volume I. Mechanisms And Measurements. Harvard University Press, Cambridge, MA, MA.

Hillenius, D. 1966. Notes on chameleons III. The chameleons of southern Arabia. Beaufortia 13:91–108.

Himes, P. J., and M. E. Hadley. 1971. In vitro effects of steroid hormones on frog melanophores. The Journal of investigative dermatology. .

Höglund, E., P. H. Balm, and S. Winberg. 2000. Skin darkening, a potential social signal in subordinate arctic charr (Salvelinus alpinus): the regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides. The Journal of Experimental Biology 203:1711–1721.

Husak, J. F., and D. J. Irschick. 2009. Steroid use and human performance: Lessons for integrative biologists. Integrative and Comparative Biology 49:354–364.

Husak, J. F., A. K. Lappin, S. F. Fox, and J. A. Lemos-Espinal. 2006. Bite-Force Performance Predicts Dominance in Male Venerable Collared Lizards (Crotaphytus antiquus). Copeia 2006:301–306.

Huyghe, K., B. Vanhooydonck, H. Scheers, M. Molina-Borja, and R. Van Damme. 2005. Morphology, performance and fighting capacity in male lizards, Gallotia galloti. Functional Ecology 19:800–807.

Karsten, K. B., L. N. Andriamandimbiarisoa, S. F. Fox, and C. J. Raxworthy. 2009. Sexual selection on body size and secondary sexual characters in 2 closely related, sympatric chameleons in Madagascar. Behavioral Ecology 20:1079–1088.

Kelber, A., M. Vorobyev, and D. Osorio. 2003. Animal colour vision – behavioural tests and physiological concepts. Biological Reviews of the Cambridge Philosophical Society 78:81–118.

Kelso, E. C., and P. A. Verrell. 2002. Do Male Veiled Chameleons, Chamaeleo calyptratus, Adjust their Courtship Displays in Response to Female Reproductive Status? Ethology 512:495–512.

Klein, B. A., J. Stein, and R. C. Taylor. 2012. Robots in the service of animal behavior. Communicative and Integrative Biology 5:466–472.

Klukowski, M., B. Ackerson, and C. E. Nelson. 2004. Testosterone and daily activity period in laboratory-housed mountain spiny lizards, Sceloporus jarrovi. Journal of Herpetology 38:120–124.

Klukowski, M., and C. E. Nelson. 1998. The challenge hypothesis and seasonal changes in aggression and steroids in male northern fence lizards (Sceloporus undulatus hyacinthinus). Hormones and behavior 33:197–204.

Klukowski, M., and C. E. Nelson. 2001. Ectoparasite loads in free-ranging northern fence lizards, Sceloporus undulatus hyacinthinus: effects of testosterone and sex. Behavioral Ecology and Sociobiology 49:289–295.

Korzan, W. J., Ø. Øverli, and C. H. Summers. 2006. Future social rank: forecasting status in the green anole (Anolis carolinensis). Acta Ethologica 9:48–57.

Lailvaux, S. P., A. Herrel, B. Vanhooydonck, J. J. Meyers, and D. J. Irschick. 2004. Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (Anolis carolinensis). Proceedings of the Royal Society B 271:2501–2508.

Lailvaux, S. P., and D. J. Irschick. 2007. The Evolution of Performance-Based Male Fighting Ability in Caribbean Anolis Lizards. Evolution 170:573–586.

Lappin, A. K., Y. Brandt, J. F. Husak, J. M. Macedonia, and D. J. Kemp. 2006a. Gaping Displays Reveal and Amplify a Mechanically Based Index of Weapon Performance. The American Naturalist 168:100–113.

Lappin, A. K., P. S. Hamilton, and B. K. Sullivan. 2006b. Bite-force performance and head shape in a sexually dimorphic crevice-dwelling lizard , the common chuckwalla [Sauromalus ater (= obesus)]. Biological Journal of the Linnean Society 88:215–222.

Lappin, A. K., and J. F. Husak. 2005. Weapon Performance, Not Size , Determines Mating Success and Potential Reproductive Output in the Collared Lizard (Crotaphytus collaris). American Naturalist 166:426–436.

Laucht, S., and J. Dale. 2012. Correlations of condition, testosterone, and age with multiple ornaments in male house sparrows: patterns and implications. The Condor 114:865–873.

Laucht, S., B. Kempenaers, and J. Dale. 2010. Bill color, not badge size, indicates testosterone-related information in house sparrows. Behavioral ecology and sociobiology 64:1461–1471.

Ligon, R. A. 2014. Defeated chameleons darken dynamically during dyadic disputes to decrease danger from dominants. Behavioral Ecology and Sociobiology 68:1007–1017.

Ligon, R. A., and K. L. McCartney. 2015. Biochemical regulation of pigment motility in vertebrate chromatophores: A review of physiological color change mechanisms. Current Zoology.

Ligon, R. A., and K. J. McGraw. 2013. Chameleons communicate with complex colour changes during contests: different body regions convey different information. Biology Letters 9:20130892.

Losos, J. B., D. a Creer, and J. a Schulte. 2002. Cautionary comments on the measurement of maximum locomotor capabilities. Journal of Zoology 258:57–61.

Marchetti, K. 1993. Dark habitats and bright birds illustrate the role of the environment in species divergence. Nature 362:149–152.

Martín, J., and P. López. 2009. Multiple color signals may reveal multiple messages in male Schreiber’s green lizards, Lacerta schreiberi. Behavioral Ecology and Sociobiology 63:1743–1755.

Martinez, M., A. Salvador, and V. M. Simon. 20AD. Behavioral Changes Over Several Successful Agonistic Encounters Between Male Mice : Effects of Type of “ Standard Opponent .” Aggressive Behavior 20:441–51.

Maynard Smith, J., and D. Harper. 2003. Animal Signals. Oxford University Press, New York.

McCartney, K. L., R. A. Ligon, M. W. Butler, D. F. Denardo, and K. J. McGraw. 2014. The effect of carotenoid supplementation on immune system development in juvenile male veiled chameleons (Chamaeleo calyptratus). Frontiers in zoology 11:26. Frontiers in Zoology.

McEvoy, J., G. M. While, S. M. Jones, and E. Wapstra. 2015. Examining the Role of Testosterone in Mediating Short-Term Aggressive Responses to Social Stimuli in a Lizard. Plos One 10:e0125015.

McGlothlin, J. W., J. M. Jawor, T. J. Greives, J. M. Casto, J. L. Phillips, and E. D. Ketterson. 2008. Hormones and honest signals: Males with larger ornaments elevate testosterone more when challenged. Journal of Evolutionary Biology 21:39–48.

McGraw, K. J., S. M. Correa, and E. Adkins-Regan. 2006. Testosterone upregulates lipoprotein status to control sexual attractiveness in a colorful songbird. Behavioral Ecology and Sociobiology 60:117–122.

McGraw, K. J., and R. S. Parker. 2006. A novel lipoprotein-mediated mechanism controlling sexual attractiveness in a colorful songbird. Physiology & behavior 87:103–8.

Measey, G. J., K. Hopkins, and K. A. Tolley. 2009. Morphology, ornaments and performance in two chameleon ecomorphs: is the casque bigger than the bite? Zoology 112:217–226.

Meyers, J. J., D. J. Irschick, B. Vanhooydonck, and a. Herrel. 2006. Divergent roles for multiple sexual signals in a polygynous lizard. Functional Ecology 20:709–716.

Moore, M. C. 1987. Circulating steroid hormones during rapid aggressive responses of territorial male mountain spiny lizards, Sceloporus jarrovi. Hormones and behavior 21:511–521.

Moore, M. C., and J. Lindzey. 1992. The physiological basis of sexual behavior in male reptiles. Pages 70–113 in Biology of the Reptilia. Physiology E: Hormones, Brain and Behavior, vol. 18 (C. Gans and D. Crews, Eds.). University of Chicago Press, Chicago.

Muske, L. E., and R. D. Fernald. 1987. Control of a teleost social signal. I. Neural basis for differential expression of a color pattern. Journal of Comparative Physiology A 160:89–97.

Nečas, P. 1999. Chameleons: Nature’s hidden jewels. Chimaira, Frankfurt.

Norris, D. O. 2007. Vertebrate Endocrinology. 4th editio. Elsevier, Burlington, MA.

O’Connor, K. I., N. B. Metcalfe, and A. C. Taylor. 1999. Does darkening signal submission in territorial contests between juvenile Atlantic salmon, Salmo salar? Animal behaviour 58:1269–1276.

Olsson, M., E. Wapstra, T. Madsen, and B. Silverin. 2000. Testosterone, ticks and travels: a test of the immunocompetence-handicap hypothesis in free-ranging male sand lizards. Proceedings of the Royal Society B 267:2339–2243.

Osorio, D., and M. Vorobyev. 2005. Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. Proceedings of the Royal Society B 272:1745–1752.

Oyegbile, T. O., and C. a. Marler. 2005. Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. Hormones and Behavior 48:259–267.

Parker, G. A. 1974. Assessment strategy and the evolution of fighting behaviour. Journal of Theoretical Biology 47:223–243.

Parker, G. A., and R. A. Stuart. 1976. Animal behavior as a strategy optimizer: Evolution of resource assessment strategies and optimal emigration thresholds. American Naturalist 110:1055–1076.

Penteriani, V., M. del Mar Delgado, C. Alonso-Alvarez, and F. Sergio. 2007. The importance of visual cues for nocturnal species: eagle owls signal by badge brightness. Behavioral Ecology 18:143–147.

Persons, M. H., L. J. Fleishman, M. A. Frye, and M. E. Stimphil. 1999. Sensory response patterns and the evolution of visual signal design in anoline lizards. Journal of Comparative Physiology A 184:585–607.

Pike, T. W. 2011. Using digital cameras to investigate animal colouration: estimating sensor sensitivity functions. Behavioral Ecology and Sociobiology 65:849–858.

Pike, T. W. 2012. Preserving perceptual distances in chromaticity diagrams. Behavioral Ecology 23:723–728.

Plasman, M., V. H. Reynoso, L. Nicolás, and R. Torres. 2015. Multiple colour traits signal performance and immune response in the Dickerson’s collared lizard <i>Crotaphytus dickersonae. Behavioral Ecology and Sociobiology 69:765–775.

Pryke, S. R., and S. Andersson. 2003. Carotenoid-based epaulettes reveal male competitive ability: experiments with resident and floater red-shouldered widowbirds. Animal Behaviour 66:217–224.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria:http://www.R–project.org/.

Rieppel, O. 1981. The skull and jaw adductor musculature in chameleons. Revue Suisse de Zoologie 88:433–445.

Rohwer, S. 1982. The Evolution of Reliable and Unreliable Badges of Fighting Ability. American Zoologist 22:531–546.

Sacchi, R., F. Pupin, A. Gentilli, D. Rubolini, S. Scali, M. Fasola, and P. Galeotti. 2009. Male-male combats in a polymorphic lizard: residency and size, but not color, affect fighting rules and contest outcome. Aggressive Behavior 35:274–283.

Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. Methods in Ecology and Evolution 1:103–113.

Schneider, C. a, W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675. Nature Publishing Group.

Searcy, W. A., and S. Nowicki. 2005. The Evolution of Animal Communication. Princeton University Press, New Jersey.

Senar, J. C., and D. Escobar. 2002. Carotenoid derived plumage coloration in the siskin Carduelis spinus is related to foraging ability. Avian Science 2:19–24.

Siefferman, L., and G. E. Hill. 2005. UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. Animal Behaviour 69:67–72.

Singmann, H., and B. Bolker. 2014. afex: Analysis of Factorial Experiments. R package version 0.11-131:http://CRAN.R–project.org/package=afex.

Smith, L. C., and H. B. John-Alder. 1999. Seasonal specificity of hormonal, behavioral, and coloration responses to within- and between-sex encounters in male lizards (Sceloporus undulatus). Hormones and Behaviorehavior 36:39–52.

Steffen, J. E., and C. C. Guyer. 2014. Display behaviour and dewlap colour as predictors of contest success in brown anoles. Biological Journal of the Linnean Society 111:646–655.

Stevens, M., C. A. Párraga, I. C. Cuthill, J. C. Partridge, and T. S. Troscianko. 2007. Using digital photography to study animal coloration. Biological Journal of the Linnean Society 90:211–237.

Stoddard, M. C., and R. O. Prum. 2008. Evolution of Avian Plumage Color in a Tetrahedral Color Space: A Phylogenetic Analysis of New World Buntings. The American Naturalist 171:755–776.

Stuart-Fox, D. M., D. Firth, A. Moussalli, and M. J. Whiting. 2006. Multiple signals in chameleon contests: designing and analysing animal contests as a tournament. Animal Behaviour 71:1263–1271.

Stuart-Fox, D., and A. Moussalli. 2008. Selection for social signalling drives the evolution of chameleon colour change. PLoS biology 6:e25. doi:10.1371/journal.pbio.0060025.

Stuart-Fox, D., A. Moussalli, and M. J. Whiting. 2007. Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. The American Naturalist 170:916–30.

Summers, C. H., and N. Greenberg. 1994. Somatic Correlates of Adrenergic Activity during Aggression in the Lizard, Anolis carolinensis. Hormones and Behavior 28:29–40.

Teyssier, J., S. V. Saenko, D. van der Marel, and M. C. Milinkovitch. 2015. Photonic crystals cause active colour change in chameleons. Nature Communications 6:6368.

Umbers, K. 2011. Turn the temperature to turquoise: Cues for colour change in the male chameleon grasshopper (Kosciuscola tristis) (Orthoptera: Acrididae). Journal of Insect Physiology 57:1198–1204. Elsevier Ltd.

Umbers, K. D. L., L. Osborne, and J. S. Keogh. 2012. The effects of residency and body size on contest initiation and outcome in the territorial dragon, Ctenophorus decresii. PloS ONE 7:e47143.

Vanhooydonck, B., F. B. Cruz, C. S. Abdala, D. L. M. Azócar, M. F. Bonino, and A. Herrel. 2010. Sex-specific evolution of bite performance in Liolaemus lizards (Iguania: Liolaemidae): The battle of the sexes. Biological Journal of the Linnean Society 101:461–475.

Vorobyev, M., and D. Osorio. 1998. Receptor noise as a determinant of colour thresholds. Proceedings of the Royal Society B-Biological Sciences 265:351–8.

Walker, L. K., M. Stevens, F. Karadas, R. M. Kilner, and J. G. Ewen. 2013. A window on the past: male ornamental plumage reveals the quality of their early-life environment. Proceedings of the Royal Society B 280:20122852.

Whiting, M. J., D. M. Stuart-Fox, D. O’Connor, D. Firth, N. C. Bennett, and S. P. Blomberg. 2006. Ultraviolet signals ultra-aggression in a lizard. Animal Behaviour 72:353–363.

Wyszecki, G., and W. S. Stiles. 1982. Color Science: Concepts and Methods, Quantitative Data and Formulae. 2nd Ed. Wiley, New York.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3–14.

CHAPTER 2
SOCIAL COSTS OF DYNAMIC COLOR SIGNALS

ABSTRACT

Animal signals must contain reliable information to remain evolutionarily stable, and the costs associated with the production, maintenance, or display of different signals prevent individuals from signaling dishonestly (i.e. 'bluffing'). In contrast to performance, handicap, or indicator signals, conventional signals have low production costs and are thought to be maintained primarily by social enforcement. Using an experimental manipulation of external color, we tested the idea that the honesty of chameleon display coloration is maintained by social costs. In concordance with this hypothesis, we found that dishonestly signaling chameleons, those whose behavior did not match their externally manipulated appearance, received higher levels of aggression than their honestly signaling counterparts. Interestingly, the stress hormone corticosterone was lower in chameleons when facing dishonest opponents than when facing honestly signaling individuals, suggesting a potential link between hormones and trial behavior. This is the first demonstration that the honesty of rapid physiological color change signals are maintained by differentially high levels of aggression directed towards dishonestly signaling individuals.

INTRODUCTION

Animal signals are wildly diverse, yet all signals must contain reliable information to remain evolutionarily stable (Bradbury and Vehrencamp 1998, Enquist 1985, Grafen 1990, Johnstone 1997, Maynard Smith and Harper 2003, Searcy and Nowicki 2005a). When the interests of signalers and receivers are aligned, as in the case of related individuals, minimal enforcement mechanisms are required to ensure signal honesty (Searcy and Nowicki 2005b). However, when animals with different interests rely on signals to mediate social interactions, costs and constraints of signal production, maintenance, or display are required to preserve signal reliability (Grafen 1990, Maynard Smith and Harper 2003, Zahavi 1975). Though the specific costs vary with different classes of signals, costs should generally prevent low-quality individuals from dishonestly signaling high-quality, or "bluffing" (Grafen 1990, Maynard Smith and Harper 1988).

Conventional signals are commonly used by animals to minimize the costs associated with competition over limited resources. Unlike performance, handicap, and indicator signals (Kodric-Brown and Brown 1984, Zahavi 1975), conventional signals usually have low production costs and are arbitrarily linked to the signaled quality (Guilford and Dawkins 1995, Hurd and Enquist 2005, Senar 1999). The absence of a direct cost limiting the production of conventional signals would leave them open to invasion by dishonest 'cheaters', unless there was some other means of ensuring signal honesty (Owens and Hartley 1991). A key hypothesis regarding the function, evolution, and maintenance of conventional signals is that the costs that keep these signals honest come in the form of social policing, whereby conspecifics impose significant punishment costs on dishonest individuals (Caryl 1982, Molles and Vehrencamp 2001, Moynihan 1982, Rohwer 1975, 1977; Tibbetts and Dale 2004, Tibbetts and Izzo 2010). The physiological mechanisms underlying punishment of cheaters have yet to be investigated in any taxon.

When signal production is well-understood, concrete predictions can be made regarding the processes maintaining signal honesty (Bradbury and Vehrencamp 1998, Hurd and Enquist 2005, Maynard Smith and Harper 2003, Searcy and Nowicki 2005a). Some signals, however, are extremely complex and regulated by multiple processes, such that the costs maintaining their honesty are unclear. Dynamic color changes represent one such class of signals, where the flexibility of rapid color change allows individuals to display different color signals under different conditions (Muske and Fernald 1987, O'Connor et al. 1999, Summers and Greenberg 1994). This plasticity suggests that color change signals may have low production costs, as do a number of behavioral displays (Matsumasa et al. 2013, Oberweger and Goller 2001, Ward et al. 2004, Weiner et al. 2009) (but see (Brandt 2003, Kotiaho et al. 1998, Matsumasa and Murai 2005, Ryan 1988)). In contrast, the colors revealed during dynamic color change displays are a product of pigmentary and structural elements (Cooper and Greenberg 1992, Teyssier et al. 2015), which may be physiologically costly to obtain or produce (e.g. (Kemp 2008, McGraw 2006)). Because of the dual nature of physiological color change signals, it is currently unknown whether the honesty and reliability of rapid color-change signals are maintained by production costs, social costs, or some combination of the two.

Here, we test the hypothesis that social costs are an important mechanism maintaining the honesty of the dynamic color change signals used by chameleons to mediate social interactions. Widely known for cryptic color changes (Stuart-fox et al. 2006), many species of chameleon (Squamata: Chameleonidae) exhibit dramatic chromatic shifts during conspecific displays (Nečas 1999), and comparative evidence suggests that selection for conspicuous signals has driven the evolution of display coloration in some chameleon groups (Stuart-Fox and Moussalli 2008, Stuart-Fox et al. 2007). Additionally, recent investigations incorporating photographic and analytical tools (Bergman and Beehner 2008, Pike 2011, 2012; Stevens et al. 2007) with chameleon-specific visual models (Bowmaker et al. 2005) have begun to shed light on the specific signaling functions of dynamic color changes used by male veiled chameleons *Chamaeleo calyptratus* during agonistic interactions. Generally speaking, bright display coloration serves as a signal of aggression and fighting ability (Ligon and McGraw 2013), whereas dark coloration serves as a signal of submission (Ligon 2014).

In this study, we experimentally uncoupled chameleon color expression from the typically associated behavioral displays by painting individuals to mimic the two ends of the aggression-submission color spectrum used by veiled chameleons during agonistic displays (Ligon and McGraw 2013, Ligon 2014). Specifically, we manipulated the external coloration of male chameleons using customized paints, staged dyadic competitions between painted individuals and unfamiliar chameleons, and recorded behavioral responses and hormone levels of the unfamiliar, unpainted opponents. Manipulated chameleons were painted with either the i) bright colors used by aggressively displaying individuals or ii) the dark colors exhibited by submissive chameleons, then allowed to interact with their unpainted opponents. Because individuals who under-report or exaggerate ('Trojans' (Owens and Hartley 1991) and 'bluffers' (Gardner and Morris 1989), respectively) their aggression or fighting ability relative to their signal expression are expected to experience greater social costs during competitive interactions than honestly signaling individuals (Enquist 1985, Rohwer 1977), we predicted that 'dishonest' (bright-submissive and dark-aggressive) chameleons would experience more aggression than 'honest' (bright-aggressive and dark-submissive) chameleons. Additionally, we predicted that the hormonal responses of unpainted chameleons would mirror any differential aggression exhibited towards mismatched opponents and that facing such opponents would result in increased corticosterone levels.

METHODS

Study species and husbandry

Veiled chameleons are territorial, arboreal lizards native to southwestern Arabia (Nečas 1999). Veiled chameleons use rapid color changes to communicate during intraspecific interactions (Kelso and Verrell 2002, Ligon and McGraw 2013, Ligon 2014, Nečas 1999), and males typically display aggressive behaviors toward one another when they come into contact. In addition to behavioral and morphological changes, aggression is conveyed by rapid brightening (Ligon and McGraw 2013) and submission is conveyed by rapid darkening during male-male contests (Ligon 2014).

Our chameleons, obtained from feral populations and a private breeder in Florida, USA, were housed individually in opaque-walled cages containing a variety of perches and climbing substrates. All cages were located in a temperature-controlled vivarium at Arizona State University, and each cage was equipped with a UV light source and heat lamp. Additional housing and husbandry details can be found in (McCartney et al. 2014).

Aggression trials

To attempt to better understand the relative importance of display coloration and behavior in modulating intraspecific aggression, we staged a series of aggressive, dyadic encounters using 36 adult male veiled chameleons. In each trial, one chameleon was painted (see *Chameleon color manipulation* below) to appear either i) brightly colored (aggressive) or ii) darkly colored (submissive) and one chameleon was unmanipulated. Each painted chameleon participated in two encounters as the experimentally manipulated participant, one in which they were painted bright to appear aggressive and one in which they were painted dark to appear submissive. Trials in which a given chameleon participated as the painted individual were separated by 2-7 days. The order of paint treatment was balanced such that half of the painted chameleons were painted bright first and half of the chameleons painted dark first. Each of the two fights in which a painted chameleon participated was against a size-matched, novel, unpainted opponent.

Overall, we conducted 54 aggression trials using 27 painted chameleons. The 54 total contests were conducted in two rounds that were 2.5 months apart to allow time for chameleons painted in the first round to complete ecdysis and serve, if necessary, as unmanipulated chameleons in the second round. The first round consisted of 36 contests, in which 18 chameleons served as painted chameleons, and the second round consisted of 18 trials, in which 9 previously unpainted chameleons served as painted chameleons (i.e. painted chameleons experienced both treatments within a single round).

Agonistic trials were conducted similarly to those previously conducted (Ligon and McGraw 2013, Ligon 2014). Briefly, we measured the body mass of each chameleon before placing them on opposite, visually-isolated sides of the trial arena, where they were allowed to acclimate for 5 minutes before we removed a central divider and began the trial. Trials were recorded using Panasonic HDC-TM 700 video cameras (Osaka, Japan), which we also used to take still photographs of each chameleon throughout the trials (concurrent with video recording). Trials were conducted for 10 minutes or until the losing chameleon retreated from his opponent twice. Additionally, we stopped one trial because chameleon combatants were in a precarious position that, if left unattended, may have increased the likelihood of injury.

Chameleon color manipulation

We used six colors of non-toxic acrylic paint (Golden Artist Colors Inc, New Berlin, NY) to mimic natural chameleon display coloration (Figure 7). Five of the colors we used were custom mixtures of paint designed to match naturally occurring colors, and one color (white) was unmixed (i.e. straight from the bottle). Each paint mixture was measured with a UV-Vis reflectance spectrometer (Ocean Optics, Dunedin, FL) and compared to a series of representative spectra taken from displaying chameleons using chameleon-specific visual models. To compare the chromatic match between real and artificial colors, the spectral sensitivity of four classes of chameleon photoreceptors (Bowmaker et al. 2005) were incorporated into visual models (Vorobyev and Osorio 1998) and discriminability was calculated in units of Just Noticeable Differences (JNDs). Additionally, we calculated achromatic discriminability following Siddiqi et al. (2004). Five of our six paints 'matched' chameleon coloration with chromatic contrasts less than 1.0 JND, the theoretical threshold for discrimination, but the dark green paint mixture had lower fidelity to real chameleon green. Specifically, this paint was 1.11 JNDs from natural chameleon green and therefore had the potential to be noticeably different to a chameleon under ideal conditions. All six colors had achromatic contrasts less than 1.0 JND.

To facilitate detailed paint application to the intricate stripes and patches of chameleon body color, each to-be-painted chameleon was temporarily anesthetized using inhaled isoflurane. Each paint was applied to the relevant body regions of a given chameleon in an attempt to manipulate only the coloration displayed while leaving individual-specific body patterning unchanged (Figure 8). We applied enough paint to anesthetized chameleons that the painted surfaces were opaque and not, therefore, influenced by changes in underlying skin color. Additionally, we did not paint legs or the areas around the mouths, nostrils, and eyes. The entire painting process took 30-45 min per chameleon, and chameleons were returned to their visually isolated home cages following the procedure to prevent any social feedback based on their appearance prior to behavioral trials. Painted chameleons always had at least 24 hours to recover from painting prior to participation in a contest.

Behavioral quantification

Two trained observers used a customized version of the open-source behavior logging software CowLog (Hänninen and Pastell 2009) to record chameleon behaviors during aggressive interactions. Observers quantified numerous behaviors (Supplementary Table 15), which allowed us to calculate inter-observer repeatability (Lessells and Boag 1987) for 16 quantified behavioral metrics (Supplementary Table 15). Repeatability of the quantified behavioral metrics among the two observers was high (mean = 0.85, median = 0.92), so we used averaged behavior values in all subsequent analyses.

For each trial, we determined whether unpainted chameleons approached their painted opponents and whether they exhibited any class of attack (any instance of fighting, biting, lunging, attacking, or knocking; Supplementary Table 15). Additionally, we calculated a total aggression score for each painted chameleon based on the frequency and associated weights of behaviors (*sensu* (Karsten et al. 2009, Ligon 2014)). We assigned weights to each behavior based on presumed costliness, with more costly/aggressive behaviors receiving higher values (Supplementary Table 15). Because we were interested in the social costs inflicted by unpainted chameleons on painted recipients, and not in determining an overall metric of all contest behaviors, we did not assign negative weights to submissive behaviors when determining overall aggression scores (Ligon 2014). Lastly, we used the behaviors exhibited by trial participants to qualify the 'winners' and 'losers' of each trial. Losing chameleons were those that retreated (exhibiting directed movement away from their opponent) at some point during the trial, and not every trial had a definitive winner and loser. In exactly half of the trials we were able to assign a winner and loser, and all subsequent analyses were conducted on this subset of definitive trials (n = 27).

Hormonal analyses

To measure circulating testosterone and corticosterone levels of unpainted chameleons following agonistic interactions, we collected blood samples from the caudal vein immediately (<5 min) after each behavioral trial using heparanized, 0.5 ml syringes. Blood samples were stored on ice until centrifugation, after which we froze plasma samples at -80° C until analysis.

We measured plasma testosterone and corticosterone using commercially available enzyme-linked immunoassay (ELISA) kits (Enzo Life Sciences, Farmingdale, NY, USA) in accordance with manufacturer's instructions. Standard curves were obtained for each assay using standards of known concentration, and chameleon hormone levels were calculated from absorbance values. All standards and samples were run in duplicate. We previously documented the efficacy of these kits for measuring testosterone in chameleon blood samples (Chapter 1) and, in the present study, we validated the corticosterone kits as well. Specifically, we found that the slope of the dilution curve that we created by serially diluting chameleon plasma and measuring corticosterone concentrations was statistically indistinguishable from that of the standard curve ($F_{1,10} = 0$, $p = 0.99$). Additionally, we calculated the intra-sample coefficient of variation using the Bd/Bo ratio for both testosterone and corticosterone and found these values to be 3.40 and 3.08, respectively.

Statistical analyses

All analyses were conducted in the R computing environment (R Core Team 2014). We employed linear mixed models to analyze the factors that influenced continuous response variables (i.e. aggression received, hormone levels) and generalized linear mixed models to analyze the factors that influenced binomial response variables (i.e. was the painted chameleon ever approached, was the painted chameleon ever attacked). For both model types we included chameleon identity as a random effect. Additionally, we used the “mixed” function in the afex package (Singmann and Bolker 2014) to fit mixed models and calculate p-values. We also used the "r.squaredGLMM" function in the MuMIn package (Barton 2013) to estimate both marginal and conditional R^2 values (Nakagawa and Schielzeth 2013). Marginal R^2 (R_m^2) values represent variance explained by fixed factors, while conditional R^2 (R_c^2) values provide information about the variance explained by the complete model (both fixed and random factors; Nakagawa and Schielzeth 2013).

RESULTS

Aggressive behavior

In the 27 contests with a definitive outcome, the model that best explained the likelihood that an unpainted chameleon approached his painted opponent included the painted chameleon's treatment (bright or dark), whether or not the painted chameleon approached the unpainted chameleon, and their interaction (Table 12; $R_m^2 = 0.68$, $R_c^2 = 1.00$). When behavior and appearance were mismatched for painted chameleons, the likelihood that they would be approached by unpainted chameleons significantly increased (Figure 9a). Similarly, the model including the interaction between opponent treatment and approach behavior best explained the likelihood that an unpainted chameleon would attack his painted opponent (Table 12), though this model had extremely low predictive power when not accounting for painted chameleon identity ($R_m^2 = 0.01$, $R_c^2 = 1.00$). Again, chameleons whose color treatment and aggressive behavior were mismatched (i.e. colorful males who were subordinate or drab males who were dominant) were more likely to be attacked than chameleons whose manipulated color matched their behavior (Figure 9b).

In trials with a definitive outcome, the overall level of aggression that a chameleon received from his opponent was strongly influenced by the interaction between his paint treatment (painted bright or dark) and approach behavior (Table 13). Specifically, bright, non-approaching chameleons and dark, approaching chameleons received a higher level of aggression than their painted counterparts whose painted coloration matched their behavior (Figure 10). In fact, the most aggression received by any painted chameleon was directed at a dark painted, approaching chameleon (opponent aggression = 71.5). We kept this data point in the analysis because our investigation specifically set out to uncover all costs associated with signal mismatches, however, we also ran the analysis excluding this point. When this high-aggression data point was removed from the analysis, only the influence of painted chameleon approach behavior remained significant (Supplementary Table 16). Overall, the combination of paint treatment and approach behavior explained 15% of the variation in opponent aggression, a figure that rose to 76% when individual identity was included as a random effect ($R_m^2 = 0.15$, $R_c^2 = 0.76$).

Did opponent behavior and paint treatment influence steroid hormone levels?

To maximize the likelihood of detecting patterns relating unpainted chameleon hormone levels, we restricted our analyses to unpainted chameleons that participated in trials with a definitive outcome. We found that the testosterone levels of unpainted chameleons were influenced by opponent paint treatment but not by approach behavior or by the interaction between these two variables (Table 14). Specifically, testosterone levels were higher in chameleons facing dark painted opponents. Although there was a significant influence of paint treatment on chameleon testosterone, with chameleons facing dark painted opponents exhibiting higher testosterone levels, the amount of variation explained by this fixed effect alone was quite small ($R_m^2 = 0.05$). However, when accounting for chameleon identity, the full model explained 98% of the variation in testosterone levels ($R_c^2 = 0.98$).

Post-contest corticosterone levels in unpainted chameleons were significantly influenced by the interaction between opponent paint treatment and approach behavior, but not by either of these variables independently (Table 14). Unpainted chameleons facing mismatched opponents exhibited significantly lower corticosterone levels than when facing painted opponents whose color treatment matched their competitive behavior (Figure 11). The variation explained by the fixed effects of opponent treatment and approach behavior was considerable ($R_m^2 = 0.24$), and did not change when accounting for individual identity ($R_m^2 = 0.24$).

DISCUSSION

Here we show that the honesty of rapid color change signals used by veiled chameleons in male-male contests appears to be maintained, at least in part, by social costs. Dishonest chameleons (i.e. those that exhibited colorful signals that did not match their competitive behavior) were more likely to be approached by their opponents, more likely to be attacked, and received higher overall aggression relative to honestly signaling individuals (i.e. those who were colorful and aggressive or were drab and submissive). Additionally, chameleons facing dishonestly signaling opponents had lower circulating corticosterone levels than those facing opponents whose signals matched their behaviors. Together these results identify, for the first time, social costs ensuring signal honesty of a dynamic color signal and a physiological metric correlated with the punishment of dishonest signalers.

Biologists have been interested in the possibility that signal receivers may punish bluffing opponents for a long time (Rohwer and Rohwer 1978, Rohwer 1977), but the physiological mechanisms regulating opponent aggression towards dishonestly signaling individuals have never been investigated. In direct opposition to our prediction that facing dishonestly signaling opponents would be inherently stressful, we found that chameleons facing 'dishonest' opponents (i.e. with discordant colors and competitive behaviors) exhibited lower circulating levels of corticosterone. Corticosterone levels did not differ between winners and losers ($F_{1,12.30} = 2.21$, $p = 0.16$), suggesting that this physiological difference arose as a result of interacting with dishonest signalers rather than of winning or losing per se. Without measuring pre- and post-trial hormone levels and performing manipulative studies, however, our ability to interpret the overall importance of these hormonal differences and whether these differences are involved in the differential aggression directed towards honest/dishonest opponents is limited at best.

Chameleons can recognize individuals with whom they have previously interacted (RAL *unpublished data*), vary contest strategy depending on context (present study), and can detect discordance between colorful signals and aggressive behavior in their opponents. Hence, chameleon life-history appears to be well-suited for a signaling system where honesty is maintained largely by social costs. However, signals may need only be honest 'on average' to remain evolutionarily stable (Johnstone and Grafen 1993). Thus, bluffing is expected to exist within signaling populations (and is seen among veiled chameleons at low frequency, *pers obs*), either with a mixture of exclusively honest and exclusively deceptive individuals, or with individuals adopting different signaling strategies over time and context (Adams and Mesterton-Gibbons 1995, Dey et al. 2014, Searcy and Nowicki 2005c, Wilson and Angilletta Jr. 2015, Wilson et al. 2007). Investigating flexible signals (e.g. songs, behavioral postures, and dynamic color changes) in natural populations across time and context could therefore provide new insights into the conditions associated with when, why, and how dishonest signaling naturally occurs (*sensu* Bywater and Wilson 2012).

Though a number of investigations have explored social policing as a mechanism ensuring signal honesty for conventional or low-cost signals, experimental design issues have prevented unambiguous interpretation of the results (see Slotow et al. 1993, Tibbetts 2013). However, work on *Polistes dominulus* paper wasps has unequivocally demonstrated that this species uses facial patterns to signal status (Tibbetts and Dale 2004, Tibbetts and Lindsay 2008), that these signals reflect nutrition during development (Tibbetts and Curtis 2007), and that experimentally manipulated, dishonest individuals experience high social costs (Tibbetts and Dale 2004, Tibbetts and Izzo 2010). By conducting trials between unfamiliar opponents of the same age and sex, allowing painted chameleon behavior to vary naturally, and demonstrating that differential aggression directed towards mismatched individuals arose as a result of incongruence between colorful signals and contest behavior, we have established that social control is involved in maintaining the honesty of aggressive color change signals for veiled chameleons. Likewise, reduced stress hormones among receivers facing dishonestly signaling opponents suggests a potential endocrine role in permitting increased aggression directed towards these opponents, promoting signal honesty and evolutionary stability of conventional signaling systems.

LITERATURE CITED

Adams, E. S., and M. Mesterton-Gibbons. 1995. The Cost of Threat Displays and the Stability of Deceptive Communication. Journal of Theoretical Biology 175:405–421.

Barton, K. 2013. MuMIn: Multi-model inference. R package version 1.9.13.:http://CRAN.R–project.org/package=MuMIn.

Bergman, T. J., and J. C. Beehner. 2008. A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (*Theropithecus gelada*). Biological Journal of the Linnean Society 94:231–240.

Bowmaker, J. K., E. R. Loew, and M. Ott. 2005. The cone photoreceptors and visual pigments of chameleons. Journal of Comparative Physiology A 191:925–32.

Bradbury, J., and S. Vehrencamp. 1998. Principles of Animal Communication. Sinauer, Massachusetts.

Brandt, Y. 2003. Lizard threat display handicaps endurance. Proceedings of the Royal Society B 270:1061–1068.

Bywater, C. L., and R. S. Wilson. 2012. Is honesty the best policy? Testing signal reliability in fiddler crabs when receiver-dependent costs are high. Functional Ecology 26:804–811.

Caryl, P. G. 1982. Telling the truth about intentions. Journal of Theoretical Biology 97:679–689.

Cooper, W. E., and N. Greenberg. 1992. Reptilian Coloration and Behavior. Pages 298–422 *in* Biology of the Reptilia, Vol. 18: Hormones, Brain, and Behavior (C. Gans, Ed.). University of Chicago Press, Chicago.

Dey, C. J., J. Dale, and J. S. Quinn. 2014. Manipulating the appearance of a badge of status causes changes in true badge expression. Proceedings of the Royal Society B 281:20132680.

Enquist, M. 1985. Communication during aggressive interactions with particular reference to variation in choice of behaviour. Animal Behaviour 33:1152–1161.

Gardner, R., and M. R. Morris. 1989. The evolution of bluffing in animal contests: an ESS approach. Journal of Theoretical Biology 137:235–243.

Grafen, A. 1990. Biological Signals as Handicaps. Journal of Theoretical Biology 144:517–546.

Guilford, T., and M. S. Dawkins. 1995. What are conventional signals? Animal Behaviour 49:1689–1695.

Hänninen, L., and M. Pastell. 2009. CowLog: Open source software for coding behaviors from digital video. Behavior Research Methods 41:472–476.

Hurd, P. L., and M. Enquist. 2005. A strategic taxonomy of biological communication. Animal Behaviour 70:1155–1170.

Johnstone, R. A. 1997. The evolution of animal signals. Pages 155–178 *in* Behavioural Ecology (J. R. Krebs and N. B. Davies, Eds.). Blackwell Science, Oxford.

Johnstone, R. A., and A. Grafen. 1993. Dishonesty and the handicap principle. *Animal Behaviour*. .

Karsten, K. B., L. N. Andriamandimbiarisoa, S. F. Fox, and C. J. Raxworthy. 2009. Sexual selection on body size and secondary sexual characters in 2 closely related, sympatric chameleons in Madagascar. Behavioral Ecology 20:1079–1088.

Kelso, E. C., and P. A. Verrell. 2002. Do Male Veiled Chameleons, *Chamaeleo calyptratus*, Adjust their Courtship Displays in Response to Female Reproductive Status? Ethology 512:495–512.

Kemp, D. J. 2008. Resource-mediated condition dependence in sexually dichromatic butterfly wing coloration. Evolution 62:2346–2358.

Kodric-Brown, A., and J. H. Brown. 1984. Truth in advertising: The kinds of traits favored by sexual selection. American Naturalist 124:309–323.

Kotiaho, J. S., R. V Alatalo, J. Mappes, M. G. Nielsen, S. Parri, and A. Rivero. 1998. Energetic costs of size and sexual signalling in a wolf spider. Proceedings of the Royal Society B 265:2203.

Lessells, C. M., and P. T. Boag. 1987. Unrepeatable Repeatabilities: A Common Mistake. The Auk 104:116–121.

Ligon, R. A. 2014. Defeated chameleons darken dynamically during dyadic disputes to decrease danger from dominants. Behavioral Ecology and Sociobiology 68:1007–1017.

Ligon, R. A., and K. J. McGraw. 2013. Chameleons communicate with complex colour changes during contests: different body regions convey different information. Biology Letters 9:20130892.

Matsumasa, M., and M. Murai. 2005. Changes in blood glucose and lactate levels of male fiddler crabs: Effects of aggression and claw waving. Animal Behaviour 69:569–577.

Matsumasa, M., M. Murai, and J. H. Christy. 2013. A low-cost sexual ornament reliably signals male condition in the fiddler crab *Uca beebei*. Animal Behaviour 85:1335–1341. Elsevier Ltd.

Maynard Smith, J., and D. Harper. 2003. Animal Signals. Oxford University Press, New York.

Maynard Smith, J., and D. G. Harper. 1988. The evolution of aggression: can selection generate variability? Philosophical Transactions of the Royal Society B 319:557–570.

McCartney, K. L., R. A. Ligon, M. W. Butler, D. F. Denardo, and K. J. McGraw. 2014. The effect of carotenoid supplementation on immune system development in juvenile male veiled chameleons (Chamaeleo calyptratus). Frontiers in zoology 11:26. Frontiers in Zoology.

McGraw, K. 2006. Mechanics of carotenoid-based coloration. Pages 177–242 *in* editors. Bird Coloration: Volume I, Mechanisms and Measurements. (G. Hill and K. McGraw, Eds.). Harvard University Press, Cambridge, Mass.

Molles, L. E., and S. L. Vehrencamp. 2001. Songbird cheaters pay a retaliation cost: evidence for auditory conventional signals. Proceedings of the Royal Society B 268:2013–2019.

Moynihan, M. 1982. Why is lying about intentions rare during some kinds of contests? Journal of Theoretical Biology 97:7–12.

Muske, L. E., and R. D. Fernald. 1987. Control of a teleost social signal. I. Neural basis for differential expression of a color pattern. Journal of Comparative Physiology A 160:89–97.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4:133–142.

Nečas, P. 1999. Chameleons: Nature's hidden jewels. Chimaira, Frankfurt.

O'Connor, K. I., N. B. Metcalfe, and A. C. Taylor. 1999. Does darkening signal submission in territorial contests between juvenile Atlantic salmon, Salmo salar? Animal behaviour 58:1269–1276.

Oberweger, K., and F. Goller. 2001. The metabolic cost of birdsong production. The Journal of experimental biology 204:3379–3388.

Owens, I. P. F., and I. R. Hartley. 1991. "Trojan Sparrows": Evolutionary consequences of dishonest invasion for the badges-of-status model. American Naturalist 138:1187–1205.

Pike, T. W. 2011. Using digital cameras to investigate animal colouration: estimating sensor sensitivity functions. Behavioral Ecology and Sociobiology 65:849–858.

Pike, T. W. 2012. Preserving perceptual distances in chromaticity diagrams. Behavioral Ecology 23:723–728.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria:http://www.R–project.org/.

Rohwer, S. 1975. The social significance of avian winter plumage variability. Evolution 29:593–610.

Rohwer, S. 1977. Status signaling in Harris sparrows: Some experiments in deception. Behaviour 61:107–129.

Rohwer, S., and F. C. Rohwer. 1978. Status signalling in harris sparrows: Experimental deceptions achieved. Animal Behaviour 26:1012–1022.

Ryan, M. J. 1988. Energy, Calling, and Selection. American Zoologist 28:885–898.

Searcy, W. A., and S. Nowicki. 2005a. The Evolution of Animal Communication. Princeton University Press, New Jersey.

Searcy, W. A., and S. Nowicki. 2005b. Signaling when interest overlap. Pages 24–77 *in* The Evolution of Animal Communication. Princeton University Press, New Jersey.

Searcy, W. A., and S. Nowicki. 2005c. Signaling when interests oppose. Pages 134–180 *in* The Evolution of Animal Communication. Princeton University Press, New Jersey.

Senar, J. C. 1999. Plumage coloration as a signal of social status. Proceedings of the International Ornithological Congress 22:1669–1686.

Siddiqi, A., T. W. Cronin, E. R. Loew, M. Vorobyev, and K. Summers. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog Dendrobates pumilio. The Journal of Experimental Biology 207:2471–2485.

Singmann, H., and B. Bolker. 2014. afex: Analysis of Factorial Experiments. R package version 0.11-131:http://CRAN.R–project.org/package=afex.

Slotow, R., J. Alcock, and S. I. Rothstein. 1993. Social status signalling in white-crowned sparrows: an experimental test of the social control hypothesis. Animal Behaviour 46:977–989.

Stevens, M., C. A. Párraga, I. C. Cuthill, J. C. Partridge, and T. S. Troscianko. 2007. Using digital photography to study animal coloration. Biological Journal of the Linnean Society 90:211–237.

Stuart-Fox, D., and A. Moussalli. 2008. Selection for social signalling drives the evolution of chameleon colour change. PLoS biology 6:e25. doi:10.1371/journal.pbio.0060025.

Stuart-Fox, D., A. Moussalli, and M. J. Whiting. 2007. Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. The American Naturalist 170:916–30.

Stuart-fox, D., M. J. Whiting, and A. Moussalli. 2006. Camouflage and colour change: antipredator responses to bird and snake predators across multiple populations in a dwarf chameleon. Biological Journal of the Linnean Society 88:437–446.

Summers, C. H., and N. Greenberg. 1994. Somatic Correlates of Adrenergic Activity during Aggression in the Lizard, Anolis carolinensis. Hormones and Behavior 28:29–40.

Teyssier, J., S. V. Saenko, D. van der Marel, and M. C. Milinkovitch. 2015. Photonic crystals cause active colour change in chameleons. Nature Communications 6:6368.

Tibbetts, E. A. 2013. The function, development, and evolutionary stability of conventional signals of fighting ability. *Advances in the Study of Behavior*. .

Tibbetts, E. A., and T. R. Curtis. 2007. Rearing conditions influence quality signals but not individual identity signals in *Polistes* wasps. Behavioral Ecology 18:602–607.

Tibbetts, E. A., and J. Dale. 2004. A socially enforced signal of quality in a paper wasp. Nature 432:218–222.

Tibbetts, E. A., and A. Izzo. 2010. Social Punishment of Dishonest Signalers Caused by Mismatch between Signal and Behavior. Current Biology 20:1637–1640. Elsevier Ltd.

Tibbetts, E. A., and R. Lindsay. 2008. Visual signals of status and rival assessment in Polistes dominulus paper wasps. Biology letters 4:237–239.

Vorobyev, M., and D. Osorio. 1998. Receptor noise as a determinant of colour thresholds. Proceedings of the Royal Society B-Biological Sciences 265:351–8.

Ward, S., H. M. Lampe, and P. J. Slater. 2004. Singing is not energetically demanding for pied flycatchers, *Ficedula hypoleuca*. Behavioral Ecology 15:477–484.

Weiner, S. A., W. A. Woods, and P. T. Starks. 2009. The energetic costs of stereotyped behavior in the paper wasp, *Polistes dominulus*. Naturwissenschaften 96:297–302.

Wilson, R. S., and M. J. Angilletta Jr. 2015. Dishonest signaling during aggressive interactions: Theory and empirical evidence. Pages 205–228 *in* Animal Signaling and Function: An Integrative Approach (D. J. Irschick, M. Briffa and J. Podos, Eds.). Wiley-Blackwell, Hoboken, New Jersey.

Wilson, R. S., M. J. A. Jr, R. S. James, C. Navas, and F. Seebacher. 2007. Dishonest Signals of Strength in Male Slender Crayfish (*Cherax dispar*) during Agonistic Encounters. The American Naturalist 170:284–291.

Zahavi, A. 1975. Mate selection-a selection for a handicap. Journal of Theoretical Biology 53:205–214.

CHAPTER 3
CONCLUDING REMARKS

When I began my PhD research almost six years ago, the information content of rapid, complex color change signals used during intraspecific interactions was not known for chameleons or any other animals. Descriptive reports of rapid color change used by chameleons in social contexts certainly suggested that chameleons relied on physiological color change to communicate with one another during conspecific encounters (Brain 1961, Burrage 1973, Bustard 1965, 1967; Kästle 1967, Kelso and Verrell 2002, Nečas 1999, Parcher 1974, Singh et al. 1984, Stuart-Fox and Moussalli 2008, Stuart-Fox et al. 2006, 2007; Trench 1912), but we did not know which particular aspects of color change were likely serving as informative signals used to mediate intraspecific interactions. What matters to a chameleon observing a conspecific counterpart changing skin color? Is it the pattern attained during these interactions? The final display coloration? The speed of the change? The difference between start and end coloration? Though there are undoubtedly additional, undiscovered elements of chameleon color change signals that matter to chameleons, my dissertation research strongly suggests that the act of brightening or darkening, the maximum display brightness and color, and the speed of color and brightness changes represent the most informative components of the colorful displays used by male veiled chameleons to mediate aggressive interactions. These elements of the color change signals used by veiled chameleons reliably predict contest behavior (e.g. the likelihood of approaching an opponent, submission) and physical performance (i.e. bite force and fighting ability). Hence, a male veiled chameleon should attend to these aspects of their opponent's color change signals because they can inform him about what his opponent is going to do and how well he is going to do it.

Skin brightening, irrespective of maximum brightness or speed of color change, indicates the likelihood that a chameleon will approach his opponent. Across four different studies involving 41 chameleons and 100 dyadic interactions with definitive outcomes, we found a strong association between brightening and the likelihood of approaching an opponent (GLMM with binomial error distribution; $z = 5.48$, $p < 0.0001$, odds ratio=9.67). This odds ratio means that brightening chameleons were almost 10 times more likely to approach their opponents than individuals that did not brighten. In this respect, brightening seems to have a great deal in common with the postural displays exhibited by aggressive individuals in a wide-variety of species (e.g. fish, Baerends and Baerends-Van Roon 1950; mammals, Feddersen-Petersen 1991; birds, Daanje 1950, Hurd and Enquist 2001; lizards, Van Dyk and Evans 2008; crabs, Crane 1966), wherein a particular behavior or posture reliably indicates the signaler's subsequent behavior. Like many of the postural signals observed in other taxa, rapid brightening appears to be (at least partially) a conventional signal of aggression for veiled chameleons. Conventional signals are characterized by low production costs and an arbitrary connection between signal form and function (Guilford and Dawkins 1995, Hurd and Enquist 2005, Senar 1999). There is no immediately apparent reason why rapid brightening should be linked to aggression while rapid darkening is linked to submission (hence, an arbitrary connection). Likewise, no significant metabolic costs of rapid color change have yet been identified, though the mechanisms underlying rapid color change rely on ATP consumption indicating *some* energetic cost (e.g. Rodgers et al. 2013). Rather, the honesty of rapid brightening as a signal of aggression in chameleons appears to be maintained by social costs (*sensu* Molles and Vehrencamp 2001, Rohwer 1977, Tibbetts and Dale 2004). Specifically, dishonestly signaling chameleons (i.e. those individuals with manipulated appearances that did not match their trial behavior) received higher levels of aggression than their honestly signaling counterparts. Social costs ensuring the honesty of dynamic color change signaling strategies have never before been documented and highlight the parallels between the flexible colors and behavioral displays of animals.

Interestingly, physiological color change of veiled chameleons appears to play an important role in *both* escalating and de-escalating agonistic encounters. In contrast to the aggressive individuals who rapidly brighten during agonistic encounters, males who terminate aggression or who lose physical encounters (i.e. retreat from opponents) tend to darken all over. Darkening as a signal of submission has also been found in Atlantic salmon *Salmo salar* (O'Connor et al. 1999) and Arctic charr *Salvelinus alpinus* (Höglund et al. 2000) and, consistent with true signals of submission, rapid darkening by an individual (whether fish or chameleon) is accompanied by a marked reduction in his own aggression, as well as that of his opponent. Social costs are likely involved in keeping signals of submission honest for chameleons, as evidenced by the high levels of aggression received by those individuals who were painted dark but did not behave submissively. Further, the particular 'form' of the submission signal employed by veiled chameleons (darkening) may be favored because it is essentially opposite of that used by aggressive chameleons (brightening), as suggested by Charles Darwin's principle of antithesis (Darwin 1872, Hurd et al. 1995). Alternatively, darkening of submissive individuals may favor the antithetical response of brightening in aggressive chameleon. Regardless, the fact that male veiled chameleons can use physiological color change to signal opposing competitive trajectories highlights the flexibility of this signaling approach.

In addition to the divergent information conveyed when chameleons brighten or darken, variation in color signals among aggressively displaying chameleons was correlated with specific individual attributes. Maximum stripe brightness was linked with approach behavior such that if two chameleons brighten towards one another during an aggressive interaction, the one that attained brighter stripes was more likely to approach his opponent. Similarly, head brightness and speed of color change were linked to fighting success; contests between two aggressively displaying chameleons undergoing rapid color change were likely to be won by the individual whose head got brighter and changed color faster. Though ornamental coloration has previously been linked to contest success and the likelihood of winning aggressive interactions (Martín and López 2009, Santos et al. 2011, Senar 2006, Whiting et al. 2006), my findings represent the first demonstration of the signaling role of the dynamic aspects of color change. To test whether the differences that underlie variation in stripe and head coloration were linked to variation in motivation, physiology, or physical performance, I conducted behavioral trials between live chameleons and standardized robotic models. Interestingly, the color attributes that were linked to approach behavior and fight success in my first study (between live chameleons) were not correlated with circulating testosterone levels or bite force when displaying to a robotic chameleon model. However, chameleons with stronger bites reached maximum brightness much earlier, suggesting that the timing of color changes is also an informative component of these signals. Both bite force and the timing of brightening are likely influenced by motivation in chameleons, suggesting that perhaps the context-dependent variability of the brightening signal may convey information about the context-dependent variability of bite force. In contrast, relatively static signals of bite force such as *Anolis* lizard dewlap size (Vanhooydonck et al. 2005) may reflect relatively static components of bite force, perhaps related to morphology rather than motivation. Confusingly, plasma testosterone concentration was negatively tied to the speed of head brightening, though testosterone itself was not tied to the expression of any aggressive behaviors or performance metric. A lack of relationship between individual contest behavior and circulating testosterone is not uncommon among squamates (e.g. Knapp and Moore 1995), hence, the general importance of intraspecific variation in testosterone and its link to brightening speed within a given trial is unclear.

Overall, my dissertation research has revealed that rapid brightening and color change as a signal of aggression in male veiled chameleons is multifaceted and complex, with different aspects of color change and display coloration signaling different bits of information. In an attempt to provide a conceptual overview of the different signaling components of color change signals in chameleons, I now draw an analogy between chameleon color change and a light switch possessing dimmer functionality (Figure 13). First, a chameleon engaged in an aggressive encounter with a conspecific has the option to brighten, indicating aggression, or darken, indicating submission. This decision is analogous to the decision to turn the light switch "on" or "off." The timing of this decision is important in itself, as chameleons with greater bite force (and putatively greater fighting ability) 'flip the switch' and brighten earlier than those with weaker bites. Among chameleons that brighten (switched turned 'on'), there is significant variation in the maximum brightness and speed of color change, which provide additional information about the likelihood of approaching one's opponent and winning aggressive interactions. This variability may be a consequence of the developmental conditions experienced by chameleons (e.g. pigment acquisition during ontogeny), current health status (Cook et al. 2013, Molnár et al. 2013), or motivation (Ligon and McGraw 2013). Similarly, variability among light switches may result in some dimmers not sliding as quickly or producing equally bright light, potentially as a consequence of the way the switches were built, how much power they can currently draw, and the motivation of the individual controlling the speed of the dimmer.

In addition to gathering data that would facilitate a better understanding of the current function and use of rapid color change as a social signal, one of my primary aims was to obtain insight regarding how dynamic color signals fit into existing signaling paradigms. Because some aspects of physiological color change exhibit similarities to i) behavioral signals that are highly flexible and not particularly expensive to produce, and ii) static color signals for which the production and acquisition of color-producing pigments and structures is energetically or physiologically costly, it was not clear where rapid color change would fall on this spectrum and how color change signals fit within the existing signaling framework. Over the course of my dissertation, I found that the elements of color change signals that are superficially similar to behavioral displays convey similar information to these displays and likely share the same mechanisms maintaining their honesty. Specifically, the honesty of the strategic signal of brightening/darkening for chameleons is maintained by social costs, as has been shown for behavioral signals (e.g. Molles and Vehrencamp 2001). Conversely, the elements of chameleon color signals that are most comparable to static color signals (e.g. brightness, hue) appear to contain information related to overall aggression and fighting ability, as has been shown for other static color ornaments (Chaine and Lyon 2008, Martín and López 2009, Santos et al. 2011, Senar 2006, Whiting et al. 2006). As proposed and experimentally confirmed (Pryke and Andersson 2003, Tibbetts 2010), production costs of these types of signals likely ensure their honesty and maintain the reliability of their information content, though such production costs have not yet been shown for any aspects of chameleon color displays.

As with any successful research project, my dissertation generated many more questions about color change signals than it answered. However, documenting the behavioral contexts in which color change signals occur, as well as correlations between color, behavior, morphology, physiology, and performance, have provided an important first step for understanding the current function of this rare signal type. Additionally, incorporating organism-specific sensory models into the assessment of the signals used by animals during behavioral interactions, as I have done in this dissertation, may provide novel insights into the potential factors favoring the use of a particular signal type and aid in a more general understanding of the processes linking communication efficacy to signal structure and, potentially, diversity.

REFERENCES

Adamo, S. A., and R. T. Hanlon. 1996. Do cuttlefish (Cephalopoda) signal their intentions to conspecifics during agonistic encounters? Animal Behaviour 52:73–81.

Adamo, S. A., and R. R. Hoy. 1995. Agonistic behaviour in male and female field crickets, *Gryllus bimaculatus*, and how behavioural context influences its expression. Animal Behaviour 49:1491–1501.

Adams, E. S., and M. Mesterton-Gibbons. 1995. The Cost of Threat Displays and the Stability of Deceptive Communication. Journal of Theoretical Biology 175:405–421.

Alcock, J. 1996. The relation between male body size, fighting, and mating success in Dawson's burrowing bee, *Amegilla dawsoni* (Apidae, Apinae, Anthophorini). Journal of Zoology 239:663–674.

Allen, J. J., L. M. Mäthger, A. Barbosa, K. C. Buresch, E. Sogin, J. Schwartz, C. Chubb, and R. T. Hanlon. 2010. Cuttlefish dynamic camouflage: responses to substrate choice and integration of multiple visual cues. Proceedings of the Royal Society B 277:1031–1039.

Anderson, R. A., L. D. Mcbrayer, and A. Herrel. 2008. Bite force in vertebrates: Opportunities and caveats for use of a nonpareil whole-animal performance measure. Biological Journal of the Linnean Society 93:709–720.

Aragón, P., P. López, and J. Martín. 2005. Roles of male residence and relative size in the social behavior of Iberian rock lizards, *Lacerta monticola*. Behavioral Ecology and Sociobiology 59:762–769.

Archer, J. 1988. *The behavioural biology of aggression*. Cambridge University Press, New York.

Austad, S. N. 1983. A game theoretical interpretation of male combat in the bowl and doily spider (*Frontinella pyramitela*). Animal behaviour 31:59–73.

Baerends, G. P., and J. M. Baerends-Van Roon. 1950. *An Introduction to the Study of the Ethology of the Cichlid Fishes*. Brill, Leiden.

Barton, K. 2013. MuMIn: Multi-model inference. R package version 1.9.13.:http://CRAN.R–project.org/package=MuMIn.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7:http://cran.r–project.org/package=lme4.

Bergman, T. J., and J. C. Beehner. 2008. A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (*Theropithecus gelada*). Biological Journal of the Linnean Society 94:231–240.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in ecology & evolution 24:127–35.

Bowmaker, J. K., E. R. Loew, and M. Ott. 2005. The cone photoreceptors and visual pigments of chameleons. Journal of Comparative Physiology A 191:925–32.

Bradbury, J., and S. Vehrencamp. 1998. *Principles of Animal Communication*. Sinauer, Massachusetts.

Brain, C. K. 1961. *Chamaeleo dilepis*—A Study on its Biology and Behaviour. Journal of the Herpetological Association of Rhodesia 15:15–20.

Brandt, Y. 2003. Lizard threat display handicaps endurance. Proceedings of the Royal Society B 270:1061–1068.

Brown, F. A., and M. I. Sandeen. 1948. Responses of the Chromatophores of the Fiddler Crab, *Uca*, to Light and Temperature. Physiological Zoology 21:361–371.

Buchanan, K. L., K. A. Spencer, A. R. Goldsmith, and C. K. Catchpole. 2003. Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). Proceedings of the Royal Society B 270:1149–1156.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York.

Burnham, K. P., D. R. Anderson, and K. P. Huyvaert. 2010. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65:23–35.

Burrage, B. R. 1973. Comparative ecology and behavior of *Chamaeleo pumilus pumilus* (Gmelin) and *C. namaquensis* A. Smith (Sauria: Chamaeleonidae). Annals of the South African Museum. 61: 1-158.

Bustard, H. R. 1965. Observations on the Life History and Behavior of *Chamaeleo hohnelii* (Steindachner). Copeia 4:401–410.

Bustard, H. R. 1967. The Comparative Behavior of Chameleons: Fight Behavior in *Chameleo gracilis* Hallowell. Herpetologica 23:44–50.

Bywater, C. L., M. J. Angilletta Jr., and R. S. Wilson. 2008. Weapon size is a reliable indicator of strength and social dominance in female slender crayfish (*Cherax dispar*). Functional Ecology 22:311–316.

Bywater, C. L., and R. S. Wilson. 2012. Is honesty the best policy? Testing signal reliability in fiddler crabs when receiver-dependent costs are high. Functional Ecology 26:804–811.

Carlson, B. A., S. M. Hasan, M. Hollmann, D. B. Miller, L. J. Harmon, and M. E. Arnegard. 2011. Brain evolution triggers increased diversification of electric fishes. Science 332:583–586.

Caryl, P. G. 1982. Telling the truth about intentions. Journal of Theoretical Biology 97:679–689.

Chaine, A. S., and B. E. Lyon. 2008. Intrasexual selection on multiple plumage ornaments in the lark bunting. Animal Behaviour 76:657–667.

Cook, E. G., T. G. Murphy, and M. A. Johnson. 2013. Colorful displays signal male quality in a tropical anole lizard. Die Naturwissenschaften 100:993–6.

Cooper, W. E., and N. Greenberg. 1992. Reptilian Coloration and Behavior. Pages 298–422 in *Biology of the Reptilia, Vol. 18: Hormones, Brain, and Behavior* (C. Gans, Ed.). University of Chicago Press, Chicago.

Cox, R. M., V. Zilberman, and H. B. John-Alder. 2008. Testosterone stimulates the expression of a social color signal in Yarrow’s spiny lizard, *Sceloporus jarrovii*. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology 309:505–514.

Crane, J. 1966. Combat, display and ritualization in Fiddler Crabs (Ocypodidae, genus *Uca*). Philosophical Transactions of the Royal Society B 251:459–472.

Crothers, L., E. Gering, and M. Cummings. 2011. Aposematic signal variation predicts male-male interactions in a polymorphic poison frog. Evolution 65:599–605.

Cuadrado, M. 1998. Models Painted with Female-like Colors Elicited Courtship by Male Common Chameleons: Evidence for a Courtship Releaser of common chameleon *Chamaeleo chamaeleon*. Journal of Ethology 16:73–79.

Cuadrado, M. 2000. Body Colors Indicate the Reproductive Status of Female Common Chameleons: Experimental Evidence for the Intersex Communication Function. Ethology 106:79–91.

Cuadrado, M. 2001. Mate guarding and social mating system in male common chameleons (*Chamaeleo chamaeleon*). Journal of Zoology 255:425–435.

Daanje, A. 1950. On Locomotory Movements in Birds and the Intention Movements Derived From Them. Behaviour 3:48–98.

Darwin, C. 1872. The expression of the emotions in man and animals. Murray, London.

Davies, N. B. 1978. Territorial defence in the speckled wood butterfly (*Pararge aegeria*): The resident always wins. Animal Behaviour 26:138–147.

DeNardo, D. F., and P. Licht. 1993. Effects of corticosterone on social behavior of male lizards. Hormones and Behavior.

Dey, C. J., J. Dale, and J. S. Quinn. 2014. Manipulating the appearance of a badge of status causes changes in true badge expression. Proceedings of the Royal Society B 281:20132680.

Dunham, L. A., and W. Wilczynski. 2014. Arginine vasotocin, steroid hormones, and social behavior in the green anole lizard, *Anolis carolinensis*. Journal of Experimental Biology 217:3670–3676.

Van Dyk, D. A., and C. S. Evans. 2008. Opponent assessment in lizards: examining the effect of aggressive and submissive signals. Behavioral Ecology 19:895–901.

Eaton, L., and K. A. Sloman. 2011. Subordinate brown trout exaggerate social signalling in turbid conditions. Animal Behaviour 81:603–608. Elsevier Ltd.

Endler, J. a., and P. W. Mielke. 2005. Comparing entire colour patterns as birds see them. Biological Journal of the Linnean Society 86:405–431.

Enquist, M. 1985. Communication during aggressive interactions with particular reference to variation in choice of behaviour. Animal Behaviour 33:1152–1161.

Enquist, M., E. Plane, and J. Roed. 1985. Aggressive communication in fulmars (*Fulmarus glacialis*) competing for food. Animal Behaviour 33:1007–1020.

Evans, M. R., A. R. Goldsmith, and S. R. A. Norris. 2000. The effects of testosterone on antibody production and plumage coloration in male house sparrows (*Passer domesticus*). Behavioral Ecology and Sociobiology 47:156–163.

Edwards, S. V, S. B. Kingan, J. D. Calkins, C. N. Balakrishnan, W. B. Jennings, W. J. Swanson, and M. D. Sorenson. 2005. Speciation in birds: genes, geography, and sexual selection. Proceedings of the National Academy of Sciences of the United States of America 102 (S1):6550–6557.

Feddersen-Petersen, D. 1991. The ontogey of social play and agonistic behaviour in selected canid species. Bonner zoologische Beiträge 42:97–114.

Filadelfi, A. M. C., A. Vieira, and F. M. Louzada. 2005. Circadian rhythm of physiological color change in the amphibian *Bufo ictericus* under different photoperiods. Comparative Biochemistry and Physiology A 142:370–375.

Freckleton, R. P. 2010. Dealing with collinearity in behavioural and ecological data: model averaging and the problems of measurement error. Behavioral Ecology and Sociobiology 65:91–101.

Fujii, R. 2000. The Regulation of Motile Activity in Fish Chromatophores. Pigment Cell Research 13:300–319.

Fujii, R., and R. R. Novales. 1969. Cellular aspects of the control of physiological color changes in fishes. American zoologist.

Goldman, J. M. J. M., and M. E. M. E. Hadley. 1969. In vitro demonstration of adrenergic receptors controlling melanophore responses of the lizard, *Anolis carolinensis*. Journal of Pharmacology and Experimental Therapeutics 166:1–7.

Gordon, M. 2014. Gmisc: A few handy misc functions for plots, tables, and more. R package version 0.6.4.:http://CRAN.R–project.org/package=Gmisc.

Grafen, A. 1990. Biological Signals as Handicaps. Journal of Theoretical Biology 144:517–546.

Grether, G. F., G. R. Kolluru, and K. Nersissian. 2004. Individual colour patches as multicomponent signals. Biological Reviews 79:583–610.

Guilford, T., and M. S. Dawkins. 1995. What are conventional signals? Animal Behaviour 49:1689–1695.

Hack, M. A., D. J. Thompson, and D. M. Fernandes. 1997. Fighting in Males of the Autumn Spider, *Metellina segmentata*: Effects of Relative Body Size, Prior Residency and Female Value on Contest Outcome and Duration. Ethology 103:488–498.

Hall, M. D., L. McLaren, R. C. Brooks, and S. P. Lailvaux. 2010. Interactions among performance capacities predict male combat outcomes in the field cricket. Functional Ecology 24:159–164.

Hänninen, L., and M. Pastell. 2009. CowLog: Open source software for coding behaviors from digital video. Behavior Research Methods 41:472–476.

Hardy, I. C. W., and M. Briffa. 2013. *Animal Contests*. Cambridge University Press, New York.

Harrell Jr., F. E. 2001. *Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis*. Springer, New York, NY.

Hart, N. S., and M. Vorobyev. 2005. Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors. Journal of Comparative Physiology A 191:381–92.

Hews, D. K., E. Hara, and M. C. Anderson. 2012. Sex and species differences in plasma testosterone and in counts of androgen receptor-positive cells in key brain regions of *Sceloporus* lizard species that differ in aggression. General and Comparative Endocrinology 176:493–499.

Hill, G. E., and K. J. McGraw. 2006. *Bird Coloration. Volume I. Mechanisms And Measurements*. Harvard University Press, Cambridge, MA, MA.

Himes, P. J., and M. E. Hadley. 1971. In vitro effects of steroid hormones on frog melanophores. The Journal of investigative dermatology.

Hinton, H., and G. Jarman. 1972. Physiological Colour Change in the Hercules Beetle. Nature 238:160–161.

Höglund, E., P. H. Balm, and S. Winberg. 2000. Skin darkening, a potential social signal in subordinate arctic charr (*Salvelinus alpinus*): the regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides. The Journal of Experimental Biology 203:1711–1721.

Hurd, P. L. 1997. Is Signalling of Fighting Ability Costlier for Weaker Individuals? Journal of Theoretical Biology 184:83–88.

Hurd, P. L., and M. Enquist. 2001. Threat display in birds. Canadian Journal of Zoology 79:931–942.

Hurd, P. L., and M. Enquist. 2005. A strategic taxonomy of biological communication. Animal Behaviour 70:1155–1170.

Hurd, P. L., C. A. Wachtmeister, and M. Enquist. 1995. Darwin's principle of antithesis revisited: A role for perceptual biases in the evolution of intraspecific signals. Proceedings of the Royal Society B 259:201–205.

Husak, J. F., A. K. Lappin, S. F. Fox, and J. A. Lemos-Espinal. 2006. Bite-Force Performance Predicts Dominance in Male Venerable Collared Lizards (*Crotaphytus antiquus*). Copeia 2006:301–306.

Huyghe, K., B. Vanhooydonck, H. Scheers, M. Molina-Borja, and R. Van Damme. 2005. Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. Functional Ecology 19:800–807.

Johnstone, R. A. 1997. The evolution of animal signals. Pages 155–178 in *Behavioural Ecology* (J. R. Krebs and N. B. Davies, Eds.). Blackwell Science, Oxford.

Johnstone, R. A., and A. Grafen. 1993. Dishonesty and the handicap principle. Animal Behaviour. .

Jonart, L. M., G. E. Hill, and A. V. Badyaev. 2007. Fighting ability and motivation: determinants of dominance and contest strategies in females of a passerine bird. Animal Behaviour 74:1675–1681.

Karsten, K. B., L. N. Andriamandimbiarisoa, S. F. Fox, and C. J. Raxworthy. 2009. Sexual selection on body size and secondary sexual characters in 2 closely related, sympatric chameleons in Madagascar. Behavioral Ecology 20:1079–1088.

Kästle, V. W. 1967. Soziale Verhaltensweisen von Chamäleonen aus der pumilus- und bitaeniatus-Gruppe. Zeitschrift für Tierpsychologie 24:313–341.

Kelber, A., M. Vorobyev, and D. Osorio. 2003. Animal colour vision – behavioural tests and physiological concepts. Biological Reviews of the Cambridge Philosophical Society 78:81–118.

Kelly, C. D. 2006. Fighting for harems: assessment strategies during male–male contests in the sexually dimorphic Wellington tree weta. Animal Behaviour 72:727–736.

Kelso, E. C., and P. A. Verrell. 2002. Do Male Veiled Chameleons, *Chamaeleo calyptratus*, Adjust their Courtship Displays in Response to Female Reproductive Status? Ethology 512:495–512.

Kemp, D. J. 2008. Resource-mediated condition dependence in sexually dichromatic butterfly wing coloration. Evolution 62:2346–2358.

Klukowski, M., B. Ackerson, and C. E. Nelson. 2004. Testosterone and daily activity period in laboratory-housed mountain spiny lizards, *Sceloporus jarrovi*. Journal of Herpetology 38:120–124.

Klukowski, M., and C. E. Nelson. 1998. The challenge hypothesis and seasonal changes in aggression and steroids in male northern fence lizards (*Sceloporus undulatus hyacinthinus*). Hormones and behavior 33:197–204.

Klukowski, M., and C. E. Nelson. 2001. Ectoparasite loads in free-ranging northern fence lizards, *Sceloporus undulatus hyacinthinus*: effects of testosterone and sex. Behavioral Ecology and Sociobiology 49:289–295.

Knapp, R., and M. C. Moore. 1995. Hormonal responses to aggression vary in different types of agonistic encounters in male tree lizards, *Urosaurus ornatus*. Hormones and Behavior 29:85–105.

Kodric-Brown, A., and J. H. Brown. 1984. Truth in advertising: The kinds of traits favored by sexual selection. American Naturalist 124:309–323.

Koga, T., and S. Ikeda. 2010. Perceived predation risk and mate defense jointly alter the outcome of territorial fights. Behavioral Ecology and Sociobiology 64:827–833.

Korzan, W. J., Ø. Øverli, and C. H. Summers. 2006. Future social rank: forecasting status in the green anole (*Anolis carolinensis*). Acta Ethologica 9:48–57.

Korzan, W. J., T. R. Summers, P. J. Ronan, and C. H. Summers. 2000. Visible Sympathetic Activity as a Social Signal in *Anolis carolinensis*: Changes in Aggression and Plasma Catecholamines. Hormones and Behavior 199:193–199.

Kotiaho, J. S., R. V Alatalo, J. Mappes, M. G. Nielsen, S. Parri, and A. Rivero. 1998. Energetic costs of size and sexual signalling in a wolf spider. Proceedings of the Royal Society B 265:2203.

Krebs, J. R., and R. Dawkins. 1984. Animal signals: mind-reading and manipulation. Pages 380–402 in *Behavioural Ecology: An Evolutionary Approach*. 2nd Ed. (J. R. Krebs and N. B. Davies, Eds.). Blackwell Scientific Publications, Oxford.

Lailvaux, S. P., A. Herrel, B. Vanhooydonck, J. J. Meyers, and D. J. Irschick. 2004. Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). Proceedings of the Royal Society B 271:2501–2508.

Lailvaux, S. P., and D. J. Irschick. 2007. The Evolution of Performance-Based Male Fighting Ability in Caribbean *Anolis* Lizards. Evolution 170:573–586.

Lappin, A. K., Y. Brandt, J. F. Husak, J. M. Macedonia, and D. J. Kemp. 2006a. Gaping Displays Reveal and Amplify a Mechanically Based Index of Weapon Performance. The American Naturalist 168:100–113.

Lappin, A. K., P. S. Hamilton, and B. K. Sullivan. 2006b. Bite-force performance and head shape in a sexually dimorphic crevice-dwelling lizard, the common chuckwalla [*Sauromalus ater* (= *obesus*)]. Biological Journal of the Linnean Society 88:215–222.

Lappin, A. K., and J. F. Husak. 2005. Weapon Performance, Not Size, Determines Mating Success and Potential Reproductive Output in the Collared Lizard (*Crotaphytus collaris*). American Naturalist 166:426–436.

Laucht, S., and J. Dale. 2012. Correlations of condition, testosterone, and age with multiple ornaments in male house sparrows: patterns and implications. The Condor 114:865–873.

Laucht, S., B. Kempenaers, and J. Dale. 2010. Bill color, not badge size, indicates testosterone-related information in house sparrows. Behavioral ecology and sociobiology 64:1461–1471.

Lessells, C. M., and P. T. Boag. 1987. Unrepeatable Repeatabilities: A Common Mistake. The Auk 104:116–121.

Ligon, R. A. 2014. Defeated chameleons darken dynamically during dyadic disputes to decrease danger from dominants. Behavioral Ecology and Sociobiology 68:1007–1017.

Ligon, R. A., and K. J. McGraw. 2013. Chameleons communicate with complex colour changes during contests: different body regions convey different information. Biology Letters 9:20130892.

Ligon, R.A., and K. L. McCartney. 2016. Biochemical Regulation of Pigment Motility in Vertebrate Chromatophores: A Review of Physiological Color Change Mechanisms. Current Zoology. 62: 1-29.

Losos, J. B., D. a Creer, and J. A. Schulte. 2002. Cautionary comments on the measurement of maximum locomotor capabilities. Journal of Zoology 258:57–61.

Marchetti, K. 1993. Dark habitats and bright birds illustrate the role of the environment in species divergence. Nature 362:149–152.

Martín, J., and P. López. 2009. Multiple color signals may reveal multiple messages in male Schreiber's green lizards, *Lacerta schreiberi*. Behavioral Ecology and Sociobiology 63:1743–1755.

Martinez, M., A. Salvador, and V. M. Simon. 20AD. Behavioral Changes Over Several Successful Agonistic Encounters Between Male Mice : Effects of Type of "Standard Opponent." Aggressive Behavior 20:441–51.

Matsumasa, M., and M. Murai. 2005. Changes in blood glucose and lactate levels of male fiddler crabs: Effects of aggression and claw waving. Animal Behaviour 69:569–577.

Matsumasa, M., M. Murai, and J. H. Christy. 2013. A low-cost sexual ornament reliably signals male condition in the fiddler crab *Uca beebei*. Animal Behaviour 85:1335–1341. Elsevier Ltd.

Maynard Smith, J., and D. Harper. 2003a. *Animal Signals*. Oxford University Press, New York.

Maynard Smith, J., and D. Harper. 2003b. Signals during contests. Pages 90–111 in *Animal Signals*. Oxford University Press, New York.

Maynard Smith, J., and D. G. Harper. 1988. The evolution of aggression: can selection generate variability? Philosophical Transactions of the Royal Society B 319:557–570.

McCartney, K. L., R. A. Ligon, M. W. Butler, D. F. Denardo, and K. J. McGraw. 2014. The effect of carotenoid supplementation on immune system development in juvenile male veiled chameleons (*Chamaeleo calyptratus*). Frontiers in zoology 11:26. Frontiers in Zoology.

McEvoy, J., G. M. While, S. M. Jones, and E. Wapstra. 2015. Examining the Role of Testosterone in Mediating Short-Term Aggressive Responses to Social Stimuli in a Lizard. Plos One 10:e0125015.

McGlothlin, J. W., J. M. Jawor, T. J. Greives, J. M. Casto, J. L. Phillips, and E. D. Ketterson. 2008. Hormones and honest signals: Males with larger ornaments elevate testosterone more when challenged. Journal of Evolutionary Biology 21:39–48.

Maia, R., D. R. Rubenstein, and M. D. Shawkey. 2013. Key ornamental innovations facilitate diversification in an avian radiation. Proceedings of the National Academy of Sciences 110: 10687-10692.

McGraw, K. 2006. Mechanics of carotenoid-based coloration. Pages 177–242 in *Bird Coloration: Volume I, Mechanisms and Measurements*. (G. Hill and K. McGraw, Eds.). Harvard University Press, Cambridge, Mass.

McGraw, K. J., S. M. Correa, and E. Adkins-Regan. 2006. Testosterone upregulates lipoprotein status to control sexual attractiveness in a colorful songbird. Behavioral Ecology and Sociobiology 60:117–122.

McGraw, K. J., and R. S. Parker. 2006. A novel lipoprotein-mediated mechanism controlling sexual attractiveness in a colorful songbird. Physiology & behavior 87:103–8.

Measey, G. J., K. Hopkins, and K. A. Tolley. 2009. Morphology, ornaments and performance in two chameleon ecomorphs: is the casque bigger than the bite? Zoology 112:217–226.

Meyers, J. J., D. J. Irschick, B. Vanhooydonck, and a. Herrel. 2006. Divergent roles for multiple sexual signals in a polygynous lizard. Functional Ecology 20:709–716.

Molnár, O., K. Bajer, B. Mészáros, J. Török, and G. Herczeg. 2013. Negative correlation between nuptial throat colour and blood parasite load in male European green lizards supports the Hamilton-Zuk hypothesis. Naturwissenschaften 100:551–558.

Molles, L. E., and S. L. Vehrencamp. 2001. Songbird cheaters pay a retaliation cost: evidence for auditory conventional signals. Proceedings of the Royal Society B 268:2013–2019.

Moore, M. C. 1987. Circulating steroid hormones during rapid aggressive responses of territorial male mountain spiny lizards, *Sceloporus jarrovi*. Hormones and behavior 21:511–521.

Moore, M. C., and J. Lindzey. 1992. The physiological basis of sexual behavior in male reptiles. Pages 70–113 in *Biology of the Reptilia. Physiology E: Hormones, Brain and Behavior, vol. 18* (C. Gans and D. Crews, Eds.). University of Chicago Press, Chicago.

Mowles, S. L., P. A. Cotton, and M. Briffa. 2011. Flexing the abdominals: do bigger muscles make better fighters? Biology Letters 7:358–60.

Moynihan, M. 1982. Why is lying about intentions rare during some kinds of contests? Journal of Theoretical Biology 97:7–12.

Muske, L. E., and R. D. Fernald. 1987. Control of a teleost social signal. I. Neural basis for differential expression of a color pattern. Journal of Comparative Physiology A 160:89–97.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4:133–142.

Nečas, P. 1999. *Chameleons: Nature's hidden jewels*. Chimaira, Frankfurt.

Nery, L. E. M., and A. M. de L. Castrucci. 1997. Pigment Cell Signalling for Physiological Color Change. Comparative Biochemistry and Physiology A 29:1135–1144.

Norris, D. O. 2007. *Vertebrate Endocrinology*. 4th edition. Elsevier, Burlington, MA.

O'Connor, K. I., N. B. Metcalfe, and A. C. Taylor. 1999. Does darkening signal submission in territorial contests between juvenile Atlantic salmon, *Salmo salar*? Animal behaviour 58:1269–1276.

Oberweger, K., and F. Goller. 2001. The metabolic cost of birdsong production. The Journal of experimental biology 204:3379–3388.

Olsson, M., E. Wapstra, T. Madsen, and B. Silverin. 2000. Testosterone, ticks and travels: a test of the immunocompetence-handicap hypothesis in free-ranging male sand lizards. Proceedings of the Royal Society B 267:2339–2243.

Osorio, D., and M. Vorobyev. 2005. Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. Proceedings of the Royal Society B 272:1745–1752.

Owens, I. P. F., and I. R. Hartley. 1991. "Trojan Sparrows": Evolutionary consequences of dishonest invasion for the badges-of-status model. American Naturalist 138:1187–1205.

Oyegbile, T. O., and C. a. Marler. 2005. Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. Hormones and Behavior 48:259–267.

Parcher, S. R. 1974. Observations on the natural histories of six Malagasy Chamaeleontidae. Zeitschrift fur Tierpsychologie 34:500–523.

Parker, G. A. 1974. Assessment strategy and the evolution of fighting behaviour. Journal of Theoretical Biology 47:223–243.

Penteriani, V., M. del Mar Delgado, C. Alonso-Alvarez, and F. Sergio. 2007. The importance of visual cues for nocturnal species: eagle owls signal by badge brightness. Behavioral Ecology 18:143–147.

Persons, M. H., L. J. Fleishman, M. A. Frye, and M. E. Stimphil. 1999. Sensory response patterns and the evolution of visual signal design in anoline lizards. Journal of Comparative Physiology A 184:585–607.

Pike, T. W. 2011. Using digital cameras to investigate animal colouration: estimating sensor sensitivity functions. Behavioral Ecology and Sociobiology 65:849–858.

Pike, T. W. 2012. Preserving perceptual distances in chromaticity diagrams. Behavioral Ecology 23:723–728.

Plasman, M., V. H. Reynoso, L. Nicolás, and R. Torres. 2015. Multiple colour traits signal performance and immune response in the Dickerson's collared lizard *Crotaphytus dickersonae*. Behavioral Ecology and Sociobiology 69:765–775.

Pryke, S. R., and S. Andersson. 2003. Carotenoid-based epaulettes reveal male competitive ability: experiments with resident and floater red-shouldered widowbirds. Animal Behaviour 66:217–224.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria:http://www.R–project.org/.

Van Rhijn, J. G., and R. Vodegel. 1980. Being honest about one’s intentions: an evolutionary stable strategy for animal conflicts. Journal of theoretical biology 85:623–41.

Rieppel, O. 1981. The skull and jaw adductor musculature in chameleons. Revue Suisse de Zoologie 88:433–445.

Rodgers, G. M., N. W. Gladman, H. F. Corless, and L. J. Morrell. 2013. Costs of colour change in fish: food intake and behavioural decisions. The Journal of experimental biology 216:2760–2767.

Rohwer, S. 1975. The social significance of avian winter plumage variability. Evolution 29:593–610.

Rohwer, S. 1977. Status signaling in Harris sparrows: Some experiments in deception. Behaviour 61:107–129.

Rohwer, S. 1982. The Evolution of Reliable and Unreliable Badges of Fighting Ability. American Zoologist 22:531–546.

Rohwer, S., and F. C. Rohwer. 1978. Status signalling in harris sparrows: Experimental deceptions achieved. Animal Behaviour 26:1012–1022.

Ryan, M. J. 1988. Energy, Calling, and Selection. American Zoologist 28:885–898.

Sacchi, R., F. Pupin, A. Gentilli, D. Rubolini, S. Scali, M. Fasola, and P. Galeotti. 2009. Male-male combats in a polymorphic lizard: residency and size, but not color, affect fighting rules and contest outcome. Aggressive Behavior 35:274–283.

Santos, E. S. A, D. Scheck, and S. Nakagawa. 2011. Dominance and plumage traits: Meta-analysis and metaregression analysis. Animal Behaviour 82:3–19.

Sartori, C., and R. Mantovani. 2010. Genetics of fighting ability in cattle using data from the traditional battle contest of the Valdostana breed. Journal of Animal Science 88:3206–3213.

Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. Methods in Ecology and Evolution 1:103–113.

Schneider, C. a, W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675. Nature Publishing Group.

Schuett, G. W., H. J. Harlow, J. D. Rose, E. A. Van Kirk, and W. J. Murdoch. 1996. Levels of Plasma Corticosterone and Testosterone in Male Copperheads (*Agkistrodon contortrix*) Following Staged Fights. Hormones and Behavior 30:60–68.

Searcy, W. A., and S. Nowicki. 2005a. *The Evolution of Animal Communication*. Princeton University Press, New Jersey.

Searcy, W. A., and S. Nowicki. 2005b. Signaling when interest overlap. Pages 24–77 in *The Evolution of Animal Communication*. Princeton University Press, New Jersey.

Searcy, W. A., and S. Nowicki. 2005c. Signaling when interests oppose. Pages 134–180 in *The Evolution of Animal Communication*. Princeton University Press, New Jersey.

Seehausen, O., Y. Terai, I. S. Magalhaes, K. L. Carleton, H. D. J. Mrosso, R. Miyagi, I. van der Sluijs, M. V Schneider, M. E. Maan, H. Tachida, et al. 2008. Speciation through sensory drive in cichlid fish. Nature 455:620–6.

Senar, J. C. 1999. Plumage coloration as a signal of social status. Proceedings of the International Ornithological Congress 22:1669–1686.

Senar, J. C. 2006. Color displays as intrasexual signals of aggression and dominance. Pages 87–136 in *Bird Coloration. Volume II. Function and Evolution* (G. Hill and K. McGraw, Eds.). Harvard University Press, Cambridge, MA.

Senar, J. C., and D. Escobar. 2002. Carotenoid derived plumage coloration in the siskin *Carduelis spinus* is related to foraging ability. Avian Science 2:19–24.

Siddiqi, A., T. W. Cronin, E. R. Loew, M. Vorobyev, and K. Summers. 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. The Journal of Experimental Biology 207:2471–2485.

Siefferman, L., and G. E. Hill. 2005. UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. Animal Behaviour 69:67–72.

Silverin, B., M. Baillien, and J. Balthazart. 2004. Territorial aggression, circulating levels of testosterone, and brain aromatase activity in free-living pied flycatchers. Hormones and Behavior 45:225–234.

Singh, L. A. K., L. N. Acharjyo, and H. R. Bustard. 1984. Observation of the Reproductive Biology of the Indian Chameleon *Chamaeleo zeylanicus* (Laurenti). Journal of the Bombay Natural History Society 81:86–92.

Singmann, H., and B. Bolker. 2014. afex: Analysis of Factorial Experiments. R package version 0.11-131:http://CRAN.R–project.org/package=afex.

Slotow, R., J. Alcock, and S. I. Rothstein. 1993. Social status signalling in white-crowned sparrows: an experimental test of the social control hypothesis. Animal Behaviour 46:977–989.

Smith, L. C., and H. B. John-Alder. 1999. Seasonal specificity of hormonal, behavioral, and coloration responses to within- and between-sex encounters in male lizards (*Sceloporus undulatus*). Hormones and Behaviorehavior 36:39–52.

Steffen, J. E., and C. C. Guyer. 2014. Display behaviour and dewlap colour as predictors of contest success in brown anoles. Biological Journal of the Linnean Society 111: 646-655.

Stevens, M., C. A. Párraga, I. C. Cuthill, J. C. Partridge, and T. S. Troscianko. 2007. Using digital photography to study animal coloration. Biological Journal of the Linnean Society 90:211–237.

Stoddard, M. C., and R. O. Prum. 2008. Evolution of Avian Plumage Color in a Tetrahedral Color Space: A Phylogenetic Analysis of New World Buntings. The American Naturalist 171:755–776.

Stuart-Fox, D. M., D. Firth, A. Moussalli, and M. J. Whiting. 2006. Multiple signals in chameleon contests: designing and analysing animal contests as a tournament. Animal Behaviour 71:1263–1271.

Stuart-Fox, D., and A. Moussalli. 2008. Selection for social signalling drives the evolution of chameleon colour change. PLoS biology 6:e25. doi:10.1371/journal.pbio.0060025.

Stuart-Fox, D., and A. Moussalli. 2009. Camouflage, communication and thermoregulation: lessons from colour changing organisms. Philosophical Transactions of the Royal Society B 364:463–470.

Stuart-Fox, D., A. Moussalli, and M. J. Whiting. 2007. Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. The American Naturalist 170:916–30.

Stuart-fox, D., A. Moussalli, and M. J. Whiting. 2008. Predator-specific camouflage in chameleons. Biology letters 4:326–329.

Stuart-fox, D., M. J. Whiting, and A. Moussalli. 2006. Camouflage and colour change: antipredator responses to bird and snake predators across multiple populations in a dwarf chameleon. Biological Journal of the Linnean Society 88:437–446.

Summers, C. H., and N. Greenberg. 1994. Somatic Correlates of Adrenergic Activity during Aggression in the Lizard, *Anolis carolinensis*. Hormones and Behavior 28:29–40.

Tedore, C., and S. Johnsen. 2012. Weaponry, color, and contest success in the jumping spider *Lyssomanes viridis*. Behavioural processes 89:203–11. Elsevier B.V.

Teyssier, J., S. V. Saenko, D. van der Marel, and M. C. Milinkovitch. 2015. Photonic crystals cause active colour change in chameleons. Nature Communications 6:6368.

Tibbetts, E. A. 2010. The condition dependence and heritability of signaling and nonsignaling color traits in paper wasps. The American naturalist 175:495–503.

Tibbetts, E. A. 2008. Resource value and the context dependence of receiver behaviour. Proceedings of the Royal Society B 275:2201–2206.

Tibbetts, E. A. 2013. The function, development, and evolutionary stability of conventional signals of fighting ability. Advances in the Study of Behavior. 45: 49-80.

Tibbetts, E. A. 2008. Resource value and the context dependence of receiver behaviour. Proceedings of the Royal Society B 275:2201–2206.

Tibbetts, E. A., and T. R. Curtis. 2007. Rearing conditions influence quality signals but not individual identity signals in *Polistes* wasps. Behavioral Ecology 18:602–607.

Tibbetts, E. A., and J. Dale. 2004. A socially enforced signal of quality in a paper wasp. Nature 432:218–222.

Tibbetts, E. A., and A. Izzo. 2010. Social Punishment of Dishonest Signalers Caused by Mismatch between Signal and Behavior. Current Biology 20:1637–1640. Elsevier Ltd.

Tibbetts, E. A., and R. Lindsay. 2008. Visual signals of status and rival assessment in *Polistes dominulus* paper wasps. Biology letters 4:237–239.

Tokarz, R. R. 1987. Effects of corticosterone treatment on male aggressive behavior in a lizard (*Anolis sagrei*). Hormones and behavior 21:358–370.

Trench, C. C. 1912. Notes on the Indian Chameleon (*Chamaeleon calcaratus*). Journal of the Bombay Natural History Society 21:687–689.

Umbers, K. 2011. Turn the temperature to turquoise: Cues for colour change in the male chameleon grasshopper (*Kosciuscola tristis*) (Orthoptera: Acrididae). Journal of Insect Physiology 57:1198–1204. Elsevier Ltd.

Umbers, K. D. L., L. Osborne, and J. S. Keogh. 2012. The effects of residency and body size on contest initiation and outcome in the territorial dragon, *Ctenophorus decresii*. PloS ONE 7:e47143.

Vanhooydonck, B., A. Y. Herrel, R. Van Dammes, and D. J. Irschick. 2005. Does dewlap size predict male bite performance in Jamaican *Anolis* lizards? Functional Ecology. 19: 38-42.

Vanhooydonck, B., F. B. Cruz, C. S. Abdala, D. L. M. Azócar, M. F. Bonino, and A. Herrel. 2010. Sex-specific evolution of bite performance in *Liolaemus* lizards (Iguania: Liolaemidae): The battle of the sexes. Biological Journal of the Linnean Society 101:461–475.

Veron, J. E. N. 1974. The role of physiological colour change in the thermoregulation of *Austrolestes annulosus* (Selys) (Odonata). Australian Journal of Zoology 22:457–469.

Vorobyev, M., and D. Osorio. 1998. Receptor noise as a determinant of colour thresholds. Proceedings of the Royal Society B 265:351–8.

Walker, L. K., M. Stevens, F. Karadas, R. M. Kilner, and J. G. Ewen. 2013. A window on the past: male ornamental plumage reveals the quality of their early-life environment. Proceedings of the Royal Society B 280:20122852.

Walton, B. M., and A. F. Bennett. 1993. Temperature-Dependent Color Change in Kenyan Chameleons. Physiological Zoology 66:270–287.

Ward, S., H. M. Lampe, and P. J. Slater. 2004. Singing is not energetically demanding for pied flycatchers, *Ficedula hypoleuca*. Behavioral Ecology 15:477–484.

Weiner, S. A., W. A. Woods, and P. T. Starks. 2009. The energetic costs of stereotyped behavior in the paper wasp, *Polistes dominulus*. Naturwissenschaften 96:297–302.

Whiting, M. J., D. M. Stuart-Fox, D. O'Connor, D. Firth, N. C. Bennett, and S. P. Blomberg. 2006. Ultraviolet signals ultra-aggression in a lizard. Animal Behaviour 72:353–363.

Wilson, R. S., and M. J. Angilletta Jr. 2015. Dishonest signaling during aggressive interactions: Theory and empirical evidence. Pages 205–228 in *Animal Signaling and Function: An Integrative Approach* (D. J. Irschick, M. Briffa and J. Podos, Eds.). Wiley-Blackwell, Hoboken, New Jersey.

Wilson, R. S., M. J. A. Jr, R. S. James, C. Navas, and F. Seebacher. 2007. Dishonest Signals of Strength in Male Slender Crayfish (*Cherax dispar*) during Agonistic Encounters. The American Naturalist 170:284–291.

Wunderlin, J., and C. Kropf. 2013. Rapid Colour Change in Spiders. Pages 361–370 in *Spider Ecophysiology* (W. Nentwig, Ed.). Springer, Berlin.

Wyman, M., M. Mooring, B. McCowan, M. Penedos, and L. Hart. 2008. Amplitude of bison bellows reflects male quality, physical condition and motivation. Animal Behaviour 76:1625–1639.

Wyszecki, G., and W. S. Stiles. 1982. Color Science: Concepts and Methods, Quantitative Data and Formulae. 2nd Ed. Wiley, New York.

Zahavi, A. 1975. Mate selection-a selection for a handicap. Journal of Theoretical Biology 53:205–214.

Zuur, A. F., E. N. Ieno, and C. S. Elphick. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3–14.

APPENDIX A

CHAPTER 1 TABLES AND FIGURES

Figure 1. a) Experimental trial arena with live chameleon displaying towards robochameleon. b) Custom-painted plastic veiled chameleon models used during behavioral trials. Custom paints were designed to mimic actual veiled chameleon display coloration, as seen by veiled chameleons (i.e., using visual models). The three models were systematically rotated throughout trials to avoid pseudo-replication. c) Morphological measurements taken from the head of each male veiled chameleon after bite-force measurements had been collected. Lower-jaw length (LJL) was taken from the tip of the snout to the back of the lower jaw. Head height (HH) was taken from the back of the lower jaw to the top of the casque. Head length (HL) was taken from the tip of the casque to the tip of the snout. Casque height (CH) was taken from the tip of the casque to the mid-point between the posterior portion of the supraorbital process and the posterior of the casque. Casque width, taken at the halfway point of the casque height (CH) measurement is not shown. d) Bite plates and load cell force transducer (arrow) set up to measure the force with which chameleons bite. Yellow rubber on bite plates provides a compressible surface which prevents damage to the chameleons' teeth.

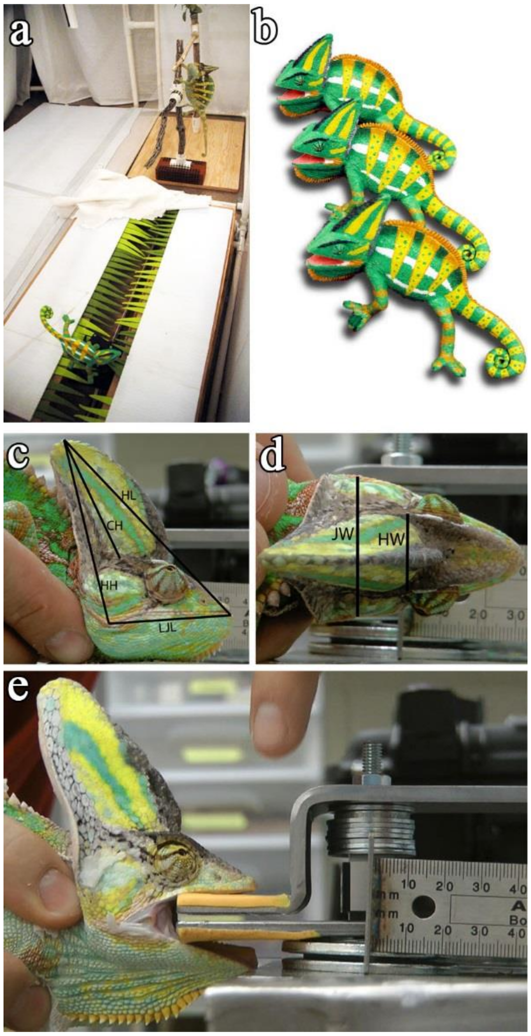


Figure 2. Relationships between morphology, testosterone, and bite force. a) Relative importance values and parameter estimates of morphological variables predicting testosterone. b) Linear relationship between testosterone and jaw width, the variable with the highest relative importance based on multimodel averaging. c) Relative importance values and parameter estimates of morphological variables predicting bite force. d) Linear relationship between bite force and casque width, the variable with the highest relative importance based on multimodel averaging. Raw measurements are shown in (b) and (d), but parameter estimates were obtained using standardized values.

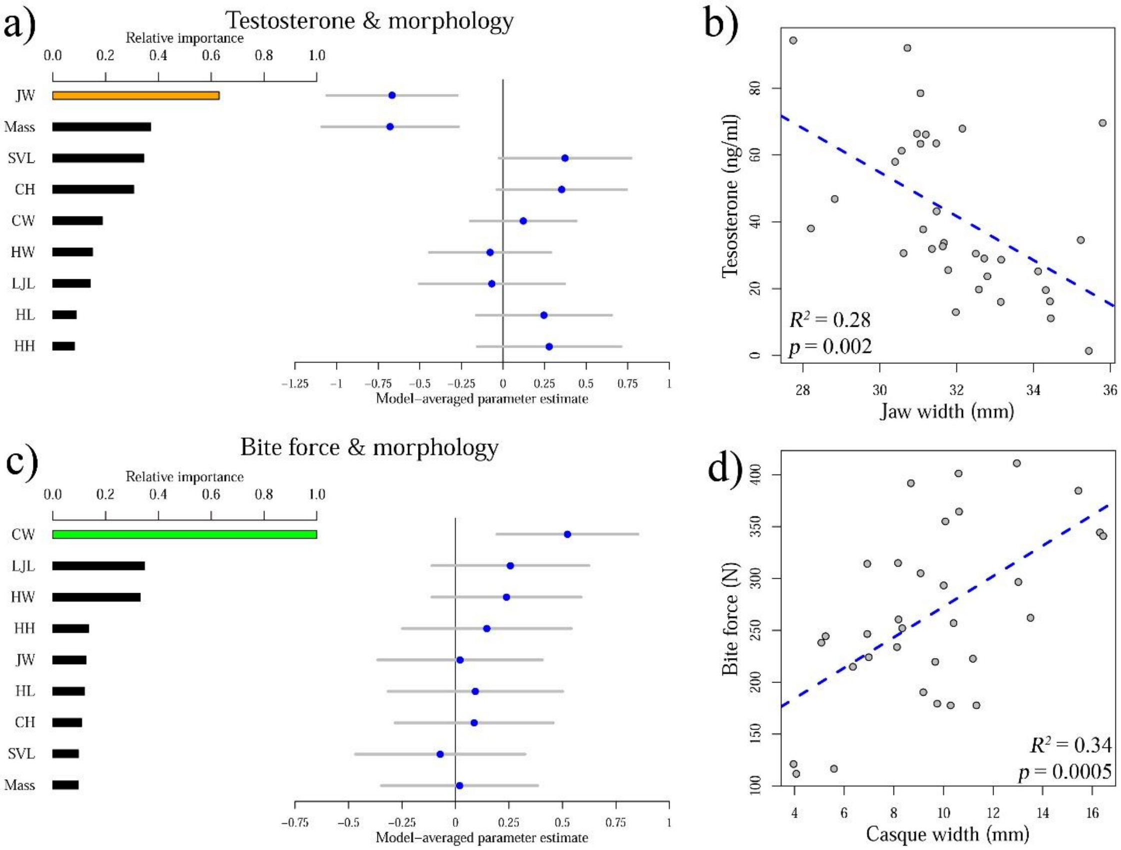


Figure 3. Relationships between phenotypic characters (testosterone, bite force, morphology), likelihood of approaching the robotic chameleon, and peak aggression. a) Relationship between robochameleon approaches and SVL, the only variable in the single model that performed better than the null model. b) Relative importance values and parameter estimates of phenotypic characters predicting peak aggression displayed by chameleons toward robotic chameleons. c) Linear relationship between peak aggression score and SVL. Raw values are shown in (a) and (c), but parameter estimates were obtained using standardized values.

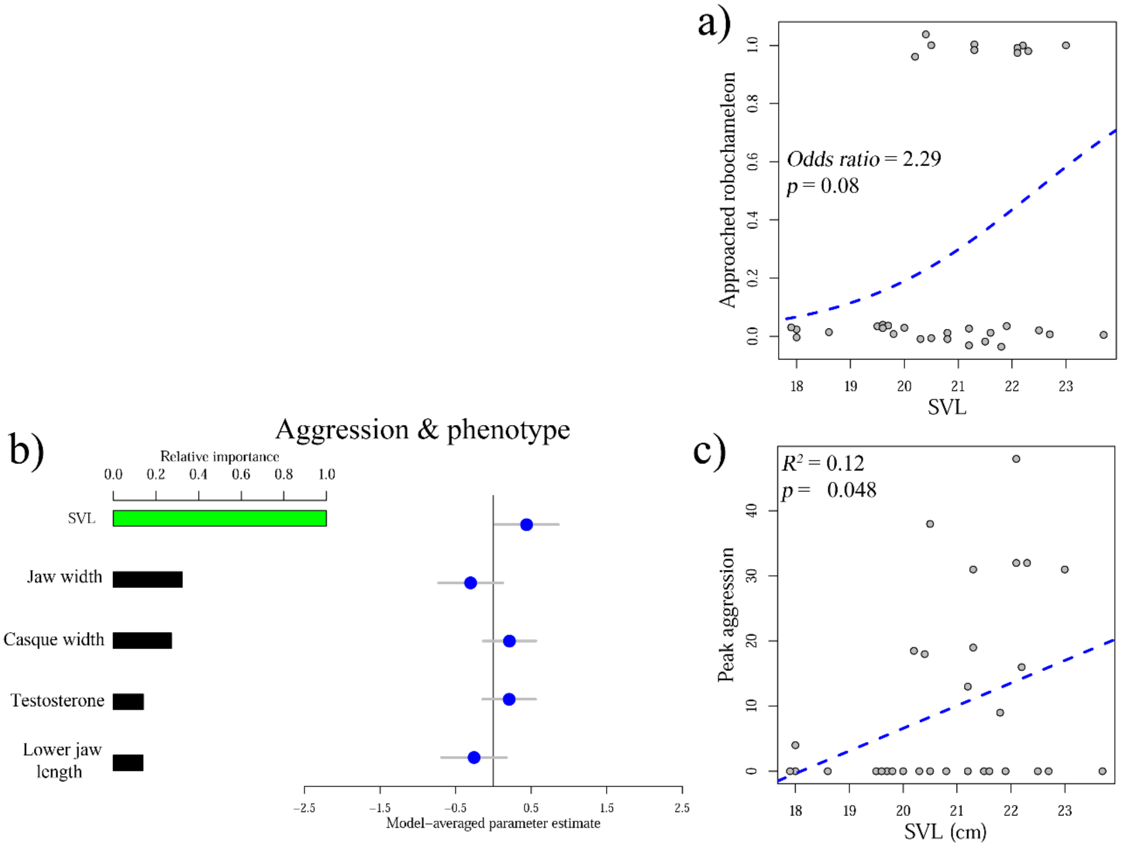


Figure 4. Relationships between color change metrics, testosterone, and bite force. a) Relative importance values and parameter estimates of the color variables predicting testosterone. b) Linear relationship between testosterone and the maximum speed of chameleon head brightening c) Relative importance values and parameter estimates of color change metrics predicting bite force (variables with RI values below 0.10 excluded for clarity). d) Linear relationship between bite force and the time it took for a chameleon to reach maximum stripe brightness, the variable with the highest relative importance for predicting bite force, based on multimodel averaging. Raw numeric values shown (b,d), but parameter estimates were obtained using standardized values.

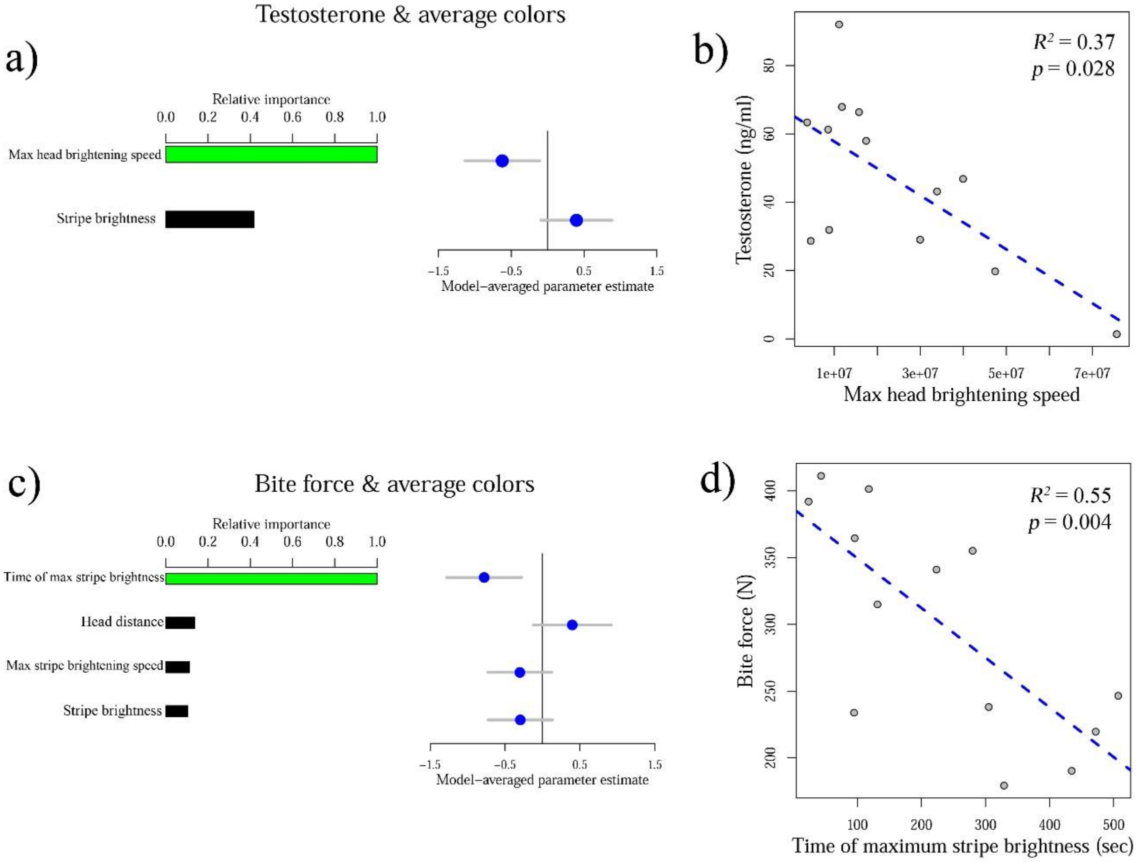
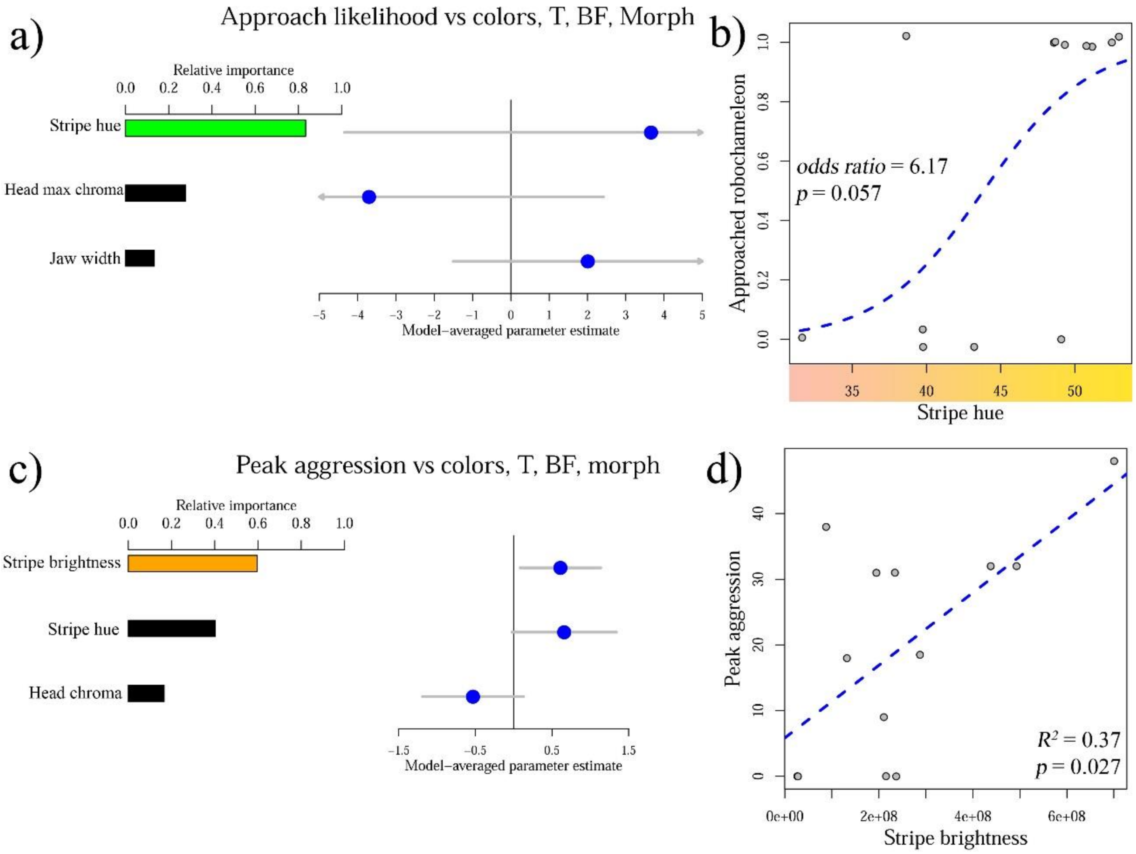


Figure 5. Relationships between color change metrics, phenotype (morphology, testosterone, bite force), and the likelihood of a chameleon approaching a robotic stimulus chameleon (a,b) or peak aggression exhibited by chameleons during aggressive trials with robochameleons (c,d). a) Relative importance values and parameter estimates of color change metrics and phenotype in predicting the likelihood of a chameleon approaching the robotic chameleon. Variables with RI values below 0.10 excluded for clarity. b) Relationship between approach likelihood and stripe hue, the variable with the highest relative importance based on multiple model averaging. c) Relative importance values and parameter estimates of variables influencing peak aggression score. d) Linear relationship between peak aggression and maximum stripe brightness, the variable with the highest relative importance for predicting bite force, based on multimodel averaging. Raw numeric values shown in (b) and (d), but parameter estimates were obtained using standardized values.



SUPPLEMENTARY MATERIAL

Supplementary Table 1. Descriptions of quantified aggressive behaviors displayed by adult male veiled chameleons during encounters with standardized, robotic chameleon stimulus.

Behavior	Description	Aggression score
Knock opponent off perch	Chameleon aggressively dislodges opponent from perch	5
Bite-release	Biting followed by immediate release of opponent	5
Bite-clamp	Sustained biting (locked on to opponent with mouth)	5
Attack	Initiation of physical contact	5
Fighting	Physical contact and intent to bite or displace opponent	5
Lunge	Fast, directed head or body thrust towards opponent	4
Approach	Directed movement towards opponent	4
Lateral display	Lateral compression, dorso-ventral expansion, physical orienting of body perpendicularly to opponent	3
Swaying	Lateral, side-to-side movement of entire body	2
Head bob	Rhythmic movement of head up and down	1
Tail curl	Tail curled and uncurled	1

Numeric values for each behavior exhibited were summed for each individual to compute overall peak aggression scores.

Supplementary Table 2. Correlations between morphological characters of adult male veiled chameleons.

	Mass	SVL	HW	CW	JW	HL	LJL	HH
Mass								
SVL	0.61***							
HW	0.27	0.52**						
CW	-0.18	-0.11	0.2					
JW	.87***	0.59***	0.21	-0.19				
HL	.65***	0.78***	0.43*	-0.3	.57***			
LJL	0.51**	.60***	0.41*	-0.16	.59***	.64***		
HH	.73***	.74***	0.35*	-0.28	.69***	.87***	.68***	
CH	.59***	.72***	0.40*	-0.14	0.52**	.90***	.59***	.92***

SVL = Snout-vent length, HW = Head width, CW = Casque width, JW = Jaw width, HL = Head length, LJL = Lower jaw length, HH = Head height.

* p < 0.05, ** p < 0.01, *** p < 0.001.

Supplementary Table 3. 95% confidence set of best-ranked models (the models whose cumulative Akaike weight, cum w_i, ≈ 0.95) used to determine the morphological variables that best explain male veiled chameleon testosterone.

Model	**df**	***Log L***	**AICc**	**ΔAICc**	**w_i**
Jaw width + SVL	4	-39.01	87.4	0.00	0.112
Casque height + Jaw width	4	-39.33	88.1	0.65	0.081
Casque height + Mass	4	-39.39	88.2	0.77	0.076
Jaw width	3	-40.89	88.6	1.16	0.063
Mass + SVL	4	-39.61	88.6	1.20	0.062
Head width + Jaw width + SVL	5	-38.57	89.4	1.91	0.043
Head height + Jaw width	4	-39.99	89.4	1.95	0.042
Casque width + Jaw width + SVL	5	-38.72	89.7	2.23	0.037
Head length + Jaw width	4	-40.24	89.9	2.46	0.033
Casque height + Mass + Lower jaw length	5	-38.91	90.0	2.59	0.031
Mass	3	-41.61	90.1	2.61	0.030
Casque height + Casque width + Jaw width	5	-38.99	90.2	2.75	0.028
Jaw width + Lower jaw length + SVL	5	-38.99	90.2	2.75	0.028
Casque height + Casque width + Mass	5	-39.03	90.3	2.83	0.027
Mass + Lower jaw length + SVL	5	-39.18	90.6	3.13	0.023
Mass + Head length	4	-40.62	90.7	3.21	0.022
Casque height + Head width + Jaw width	5	-39.22	90.7	3.21	0.022
Casque width + Jaw width	4	-40.66	90.7	3.30	0.022
Casque height + Jaw width + Lower jaw length	5	-39.31	90.8	3.41	0.020
Casque width + Mass + SVL	5	-39.32	90.9	3.41	0.020
Casque height + Mass + Head width	5	-39.33	90.9	3.45	0.020
Jaw width + Lower Jaw length	4	-40.75	90.9	3.49	0.020
Mass + Head width + SVL	5	-39.36	90.9	3.50	0.019
Casque width + Head height + Jaw width	5	-39.45	91.1	3.67	0.018
Head width + Jaw width	4	-40.88	91.2	3.74	0.017
Casque width + Head length + Jaw width	5	-39.71	91.6	4.21	0.014
Head height + Head width + Jaw width	5	-39.96	92.1	4.69	0.011
Casque width + Mass	4	-41.37	92.2	4.72	0.011
Casque width + Mass + Head length	5	-39.98	92.2	4.73	0.011
Head height + Jaw width + Lower jaw length	5	-39.98	92.2	4.74	0.010
Mass + Head width	4	-41.57	92.6	5.12	0.009
Head length + Head width + Jaw width	5	-40.19	92.6	5.16	0.009
Mass + Lower jaw length	4	-41.61	92.7	5.21	0.008

Supplementary Table 4. 95% confidence set of best-ranked models (the models whose cumulative Akaike weight, cum w_i, ≈ 0.95) used to determine the morphological variables that best explain male veiled chameleon bite force.

Model	**df**	***Log L***	**AICc**	**ΔAICc**	**w_i**
Casque width + Lower jaw length	4	-39.70	88.8	0.00	0.114
Casque width + Head width	4	-39.77	89.0	0.13	0.107
Casque width	3	-41.22	89.3	0.44	0.092
Casque width + Head height	4	-40.38	90.2	1.35	0.058
Casque width + Head width + Lower jaw length	5	-39.14	90.5	1.67	0.050
Casque width + Head length	4	-40.68	90.8	1.95	0.043
Casque width + Casque height	4	-40.70	90.8	2.00	0.042
Casque width + Lower jaw length + SVL	5	-39.42	91.1	2.23	0.037
Casque width + Head height + Head width	5	-39.55	91.3	2.49	0.033
Casque width + Head width + SVL	5	-39.56	91.3	2.50	0.033
Casque width + Jaw width	4	-40.98	91.4	2.55	0.032
Casque width + Jaw width + Lower jaw length	5	-39.64	91.5	2.66	0.030
Casque width + Mass	4	-41.03	91.5	2.66	0.030
Casque width + Mass + Lower jaw length	5	-39.67	91.6	2.73	0.029
Casque width + Head height + Lower jaw length	5	-39.68	91.6	2.75	0.029
Casque width + Casque height + Lower jaw length	5	-39.70	91.6	2.79	0.028
Casque width + Head length + Lower jaw length	5	-39.70	91.6	2.79	0.028
Casque width + Casque height + Head width	5	-39.72	91.7	2.82	0.028
Casque width + Head width + Jaw width	5	-39.73	91.7	2.84	0.028
Casque width + SVL	4	-41.14	91.7	2.88	0.027
Casque width + Head length + Head width	5	-39.75	91.7	2.89	0.027
Casque width + Mass + Head width	5	-39.76	91.8	2.92	0.027
Casque width + Head height + Jaw width	5	-40.36	92.9	4.10	0.015
Casque width + Head length + Jaw width	5	-40.67	93.6	4.72	0.011
Casque width + Mass + Head length	5	-40.68	93.6	4.74	0.011
Casque width + Casque height + Jaw width	5	-40.68	93.6	4.74	0.011

Supplementary Table 5. The single model that performed better than the null model using phenotypic characters (testosterone, bite force, mass, SVL, casque width, and jaw width) explaining the likelihood that a chameleon would approach the robotic chameleon.

Model	**df**	***Log L***	**AICc**	**ΔAICc**	**w_i**
SVL	2	-18.03	40.5	0.00	1.0

Supplementary Table 6. Models with higher likelihood (greater Akaike weight) than the null model used to determine the phenotypic characters that best explain peak aggression scores for male veiled chameleons.

Model	**df**	***Log L***	**AICc**	**ΔAICc**	**w_i**
SVL	3	-43.06	93.0	0.00	0.233
SVL + Jaw width	4	-41.83	93.1	0.16	0.215
SVL + Casque width	4	-42.10	93.7	0.69	0.165
SVL + Testosterone	4	-42.26	94.0	1.01	0.141
SVL + Lower jaw length	4	-42.26	94.0	1.02	0.140
SVL + Jaw width + Casque width	5	-41.11	94.5	1.55	0.107

Supplementary Table 7. Correlations between colorimetric variables from stripe and head patches of adult male veiled chameleons during color changes directed at a standardized stimulus (robotic chameleon). See *Supplementary Figure 1* for locations of color patches.

	Stripe brightness	Head brightness	Time of stripe brightness	Time of head brightness	Stripe brightness speed	Head brightness speed	Maximum stripe chroma	Maximum head chroma	Stripe speed	Head speed	Stripe distance	Head distance	Stripe hue
Stripe brightness													
Head brightness	0.27												
Time of stripe brightness	0.16	0.31											
Time of head brightness	0.31	0.66*	0.31										
Stripe brightness speed	0.72**	0.59*	0.14	0.66*									
Head brightness speed	0.10	0.69**	0.16	0.78**	0.59*								
Maximum stripe chroma	0.51	0.61*	0.13	0.39	0.46	0.34							
Maximum head chroma	0.27	0.39	0.40	0.21	0.13	0.27	0.65*						
Stripe speed	-0.15	0.39	0.43	0.19	0.29	0.39	-0.19	0.01					
Head speed	-0.14	0.38	0.40	0.53	0.31	0.4	0.24	0.38	0.49				
Stripe distance	0.03	0.45	0.17	0.37	0.23	0.12	0.28	-0.05	0.33	0.52			
Head distance	-0.17	0.14	0.60*	-0.02	-0.21	-0.15	0.02	0.36	0.54	0.49	0.38		
Stripe hue	0.55	0.58*	0.20	0.40	0.49	0.44	0.95***	0.61*	-0.16	0.18	0.21	-0.08	
Head hue	0.11	0.05	-0.16	-0.43	0	-0.25	-0.11	-0.09	0.21	-0.38	-0.22	0.10	-0.11

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary Table 8. The 2 models with higher likelihood (greater Akaike weight) than the null model used to determine the relevant importance of color signals in predicting circulating testosterone levels.

Model	**df**	***Log L***	**AICc**	**ΔAICc**	**w_i**
Head max brightening speed	3	-14.94	38.5	0.00	0.583
Head max brightening speed + Stripe brightness	4	-13.11	39.2	0.67	0.417

Supplementary Table 9. The 12 models with higher likelihood (greater Akaike weight) than the null model used to determine the color metrics that best explain male veiled chameleon bite force (among individuals that underwent color changing bouts in response to the robotic chameleon stimulus).

Model	**df**	***Log L***	**AICc**	**ΔAICc**	**w_i**
Stripe time	3	-12.712	34.1	0.00	0.229
Stripe time + Head distance	4	-11.063	35.1	1.04	0.136
Stripe time + Max stripe brightening speed	4	-11.256	35.5	1.42	0.113
Stripe time + Stripe brightness	4	-11.339	35.7	1.59	0.104
Stripe time + Head max chroma	4	-11.665	36.3	2.24	0.075
Stripe time + Head speed	4	-11.695	36.4	2.30	0.073
Stripe time + Head hue	4	-12.206	37.4	3.32	0.044
Stripe time + Head brightness	4	-12.225	37.4	3.36	0.043
Stripe time + Stripe hue	4	-12.350	37.7	3.61	0.038
Stripe time + Max head brightening speed	4	-12.475	37.9	3.86	0.033
Stripe time + Head time	4	-12.564	38.1	4.04	0.030
Stripe time + Stripe distance	4	-12.633	38.3	4.18	0.028
Stripe time + Stripe chroma	4	-12.650	38.3	4.21	0.028
Stripe time + Stripe speed	4	-12.692	38.4	4.29	0.027

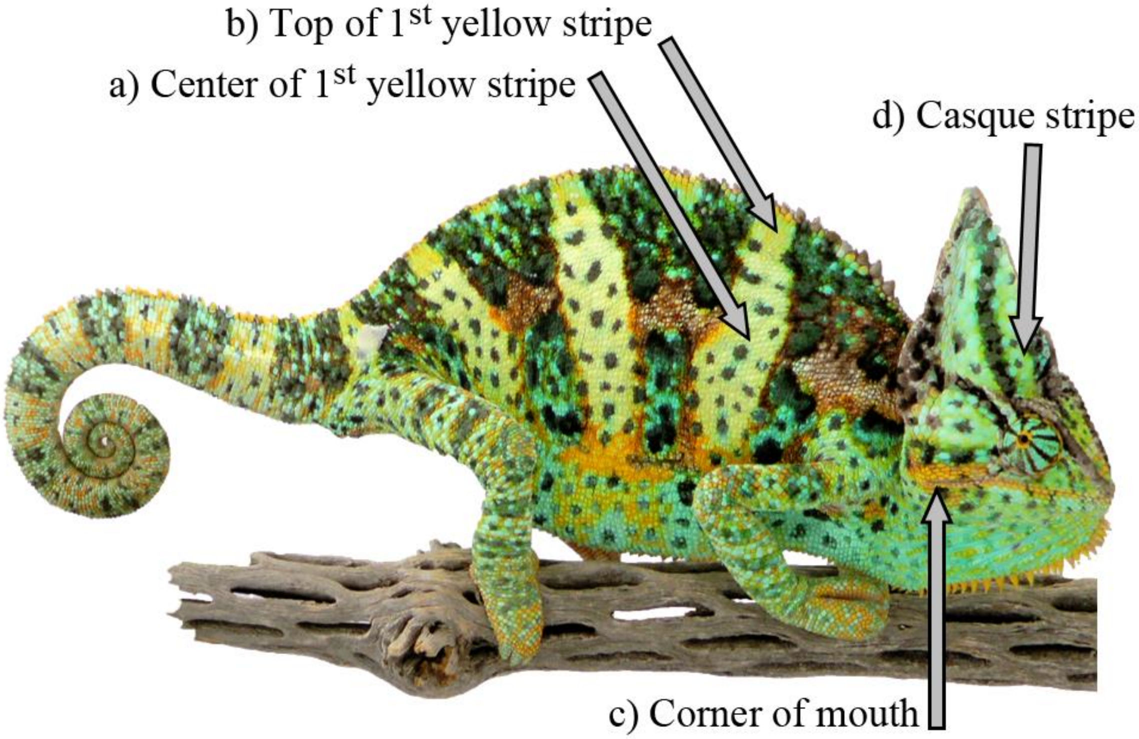
Supplementary Table 10. The 25 models with higher likelihood (greater Akaike weight) than the null model used to determine the relevant importance of color signals, morphology, testosterone, and bite force in predicting the likelihood that male veiled chameleons would approach the robochameleon.

Model	**df**	***Log L***	**AICc**	**ΔAICc**	**w_i**
Stripe hue + Head chroma	3	-2.95	14.6	0.00	0.258
Stripe hue	2	-5.55	16.3	1.73	0.109
Stripe hue + Jaw width	3	-4.32	17.3	2.73	0.066
Stripe hue + Head hue	3	-4.69	18.0	3.48	0.045
Stripe hue + Head width	3	-4.84	18.3	3.78	0.039
Stripe hue + Head brightness	3	-4.88	18.4	3.87	0.037
Stripe chroma	2	-6.83	18.9	4.30	0.030
Stripe hue + Head length	3	-5.12	18.9	4.34	0.029
Stripe hue + Head speed	3	-5.17	19.0	4.44	0.028
Stripe brightness	2	-6.98	19.2	4.60	0.026
Stripe hue + Mass	3	-5.29	19.3	4.68	0.025
Stripe hue + Stripe speed	3	-5.31	19.3	4.73	0.024
Stripe hue + Head distance	3	-5.33	19.3	4.76	0.024
Stripe hue + Max stripe brightness speed	3	-5.36	19.4	4.81	0.023
Stripe chroma + Jaw width	3	-5.36	19.4	4.83	0.023
Stripe hue + Lower jaw length	3	-5.36	19.4	4.83	0.023
Max stripe brightness speed	2	-7.11	19.4	4.84	0.023
Jaw width	2	-7.12	19.4	4.88	0.022
Head height + Jaw width	3	-5.41	19.5	4.92	0.022
Stripe hue + Bite force	3	-5.44	19.5	4.98	0.021
Stripe hue + Head time	3	-5.44	19.5	4.98	0.021
Stripe hue + Stripe brightness	3	-5.45	19.6	5.00	0.021
Stripe chroma + Head chroma	3	-5.48	19.6	5.07	0.020
Stripe hue + Testosterone	3	-5.50	19.7	5.11	0.020
Stripe hue + Max head brightness speed	3	-5.51	19.7	5.11	0.020

Supplementary Table 11. The 3 models with higher likelihood (greater Akaike weight) than the null model used to determine the relevant importance of color signals, testosterone, and bite force in predicting total aggression displayed by male veiled chameleons towards robochameleon models. Neither testosterone nor bite force was present in any of the top models (i.e. those with lower AICc values than the null model).

Model	**df**	***Log L***	**AICc**	**ΔAICc**	**w_i**
Stripe brightness	3	-14.89	38.5	0.00	0.598
Stripe hue	3	-15.82	40.3	1.86	0.236
Stripe hue + Head chroma	4	-14.00	41.0	2.56	0.166

Supplementary Figure 1. Body regions from which color and color-change measurements were collected. The brightness of the two stripe patches (a,b) were highly correlated (both $r > 0.88$, both $p < 0.0001$) with a previously calculated (Ligon and McGraw 2013) brightness principal component score calculated from six stripe color patches. The brightness (both r > 0.92, both $p < 0.001$) and speed (both $r > 0.66$, both $p < 0.001$) of the two head color patches (c, d) were highly correlated with composite principal component scores previously calculated from nine color patches (Ligon and McGraw 2013).



APPENDIX B

CHAPTER 2 TABLES AND FIGURES

Table 12. Comparison of generalized linear mixed models with different combinations of explanatory variables explaining display, brightening, approach, and attack behavior of unpainted chameleons during aggressive interactions. For each set of models, the response variable was a binary variable describing whether a painted chameleon's opponent performed the behavior (listed in the "Response" column) or not. Fixed effects include paint treatment (painted bright or painted dark) and approach behavior of the focal, painted chameleon (approached or not). The significance of differences in model fit is based on log-likelihood tests comparing each model to the null model containing only chameleon identity as a random effect (significant differences are in bold).

Response	Fixed effects	Random effect	*Df*	AIC	*logLik*	*p*
Opponent lateral display						
		ID	2	28.321	-12.16	
	Treatment	ID	3	26.258	-10.13	**0.044**
	Approach behavior	ID	3	20.177	-7.09	< **0.001**
	Treatment + Approach + Treatment*Approach	ID	5	20.509	-5.25	0.160
Opponent brightening						
		ID	2	19.417	-7.71	
	Treatment	ID	3	21.408	-7.70	0.923
	Approach behavior	ID	3	21.191	-7.60	< **0.001**
	Treatment + Approach + Treatment*Approach	ID	5	25.156	-7.58	0.983
Opponent approach						
		ID	2	41.074	-18.54	
	Treatment	ID	3	43.068	-18.53	0.939
	Approach behavior	ID	3	36.843	-15.42	< **0.001**
	Treatment + Approach + Treatment*Approach	ID	5	28.925	-9.46	**0.003**
Opponent attack						
		ID	2	29.929	-12.96	
	Treatment	ID	3	31.913	-12.96	0.897
	Approach behavior	ID	3	32.309	-13.15	1.000
	Treatment + Approach + Treatment*Approach	ID	5	27.370	-8.68	**0.011**

Table 13. The aggression painted chameleons received during an agonistic encounter depended on the interaction between his paint treatment and whether or not he approached his opponent.

Model	Parameter	Test statistic	*p*
Aggression received			
	Treatment	$F_{1,\,7.62} = 0.01$	0.94
	Approach	$F_{1,\,18.14} = 1.93$	0.18
	Treatment*Approach	$F_{1,\,7.62} = 6.18$	**0.04**

Table 14. Models explaining the relationship between steroid hormone levels in unpainted chameleons and painted chameleon treatment and approach behavior. Testosterone was linked to opponent paint treatment, being slightly higher when facing dark painted chameleons. Corticosterone levels of unpainted chameleons were influenced by the interaction of opponent treatment and approach behavior.

Model	Parameter	Test statistic	*p*
Testosterone			
	Opponent paint treatment	$F_{1,6.29} = 12.09$	**0.01**
	Opponent approach behavior	$F_{1,6.75} = 0.22$	0.07
	Opponent paint treatment * Opponent approach behavior	$F_{1,6.06} = 4.73$	0.15
Corticosterone			
	Opponent paint treatment	$F_{1,12.79} = 1.25$	0.28
	Opponent approach behavior	$F_{1,16.51} = 0.22$	0.64
	Opponent paint treatment * Opponent approach behavior	$F_{1,16.51} = 4.73$	**0.04**

Figure 7. Artificial paints (dashed lines) closely matched exemplar chameleon colors (solid lines) for six different colors (a – yellow, b – orange, c – green, d – blue-green, e – white, f - brown). Using chameleon visual models, all paints (with the exception of (c) green paint, chromatic *dS* = 1.11 JNDs, achromatic *dS* = 0.21 JNDs) are theoretically indistinguishable (*dS* < 1 JNDs) from the exemplar chameleon skin colors to chameleons.

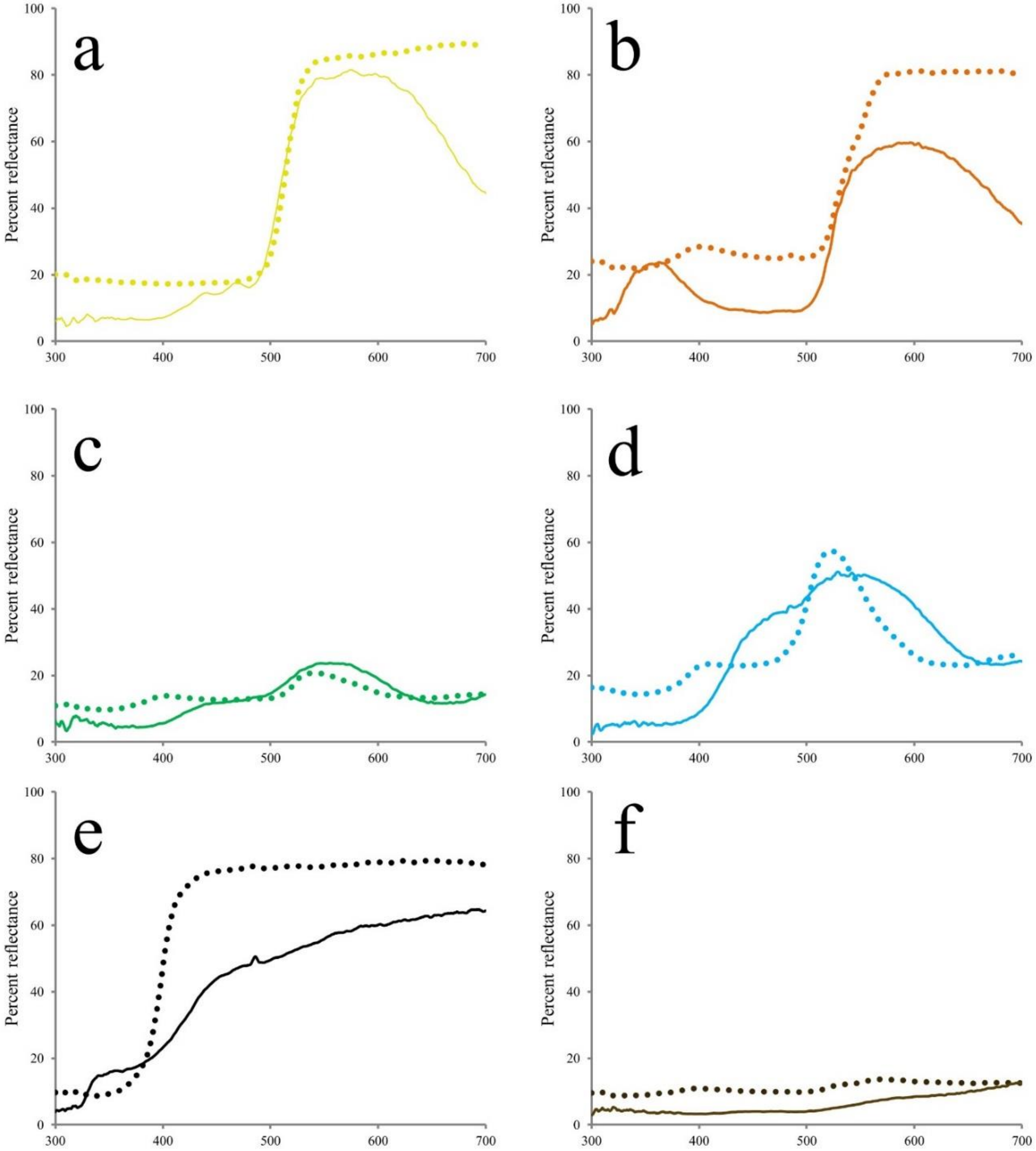


Figure 8. Chameleons displaying naturally bright, aggressive coloration (a) or dark, submissive coloration (b) look similar to chameleons experimentally painted bright (c) or dark (d). Non-toxic paint was applied based on individual-specific patterns, as can be seen when comparing a-c and b-d. Note, the white near the dorsal posterior region in (b) is due to shedding skin and was not recreated in (d).

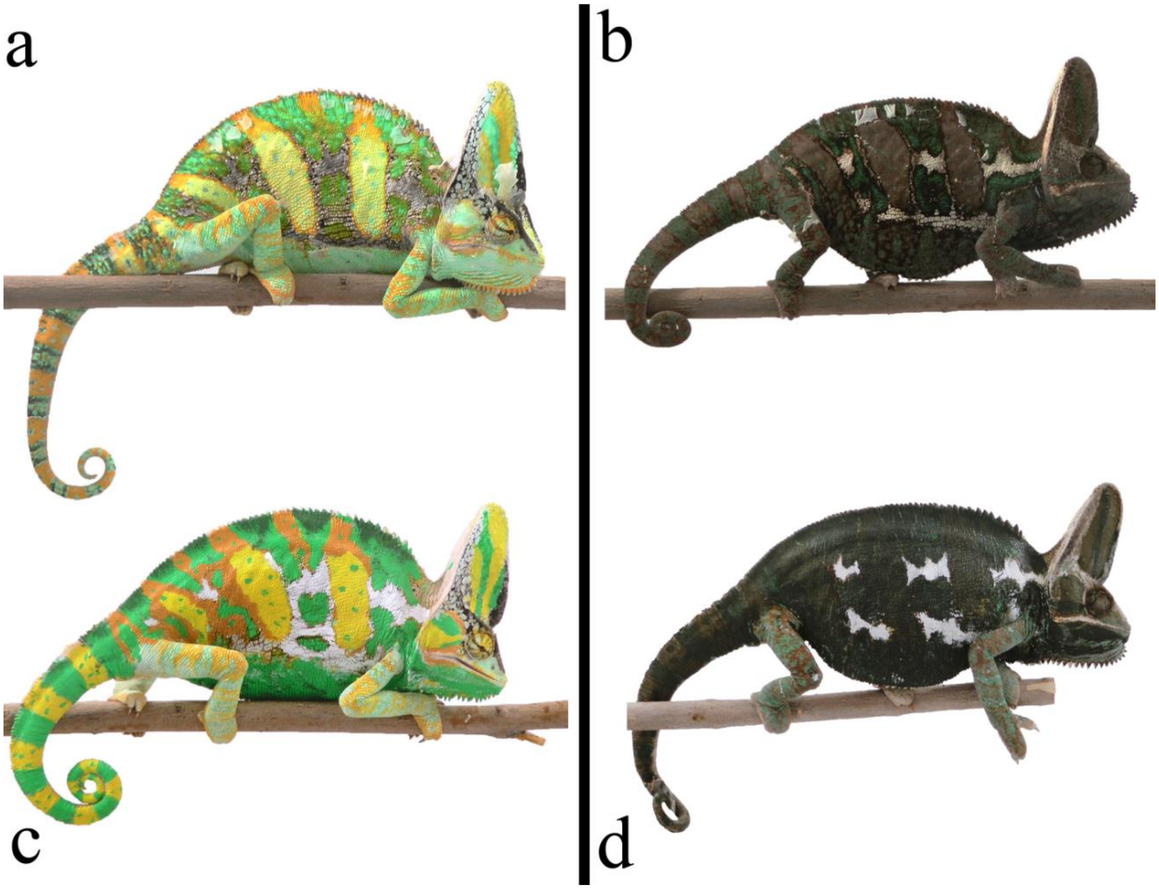


Figure 9. Mismatched chameleons, those whose experimentally manipulated color did not match their behavior, were more likely to be approached (a) and attacked (b) by their opponents. Sample sizes are located above each bar and the relevant model statistics are located in Table 1.

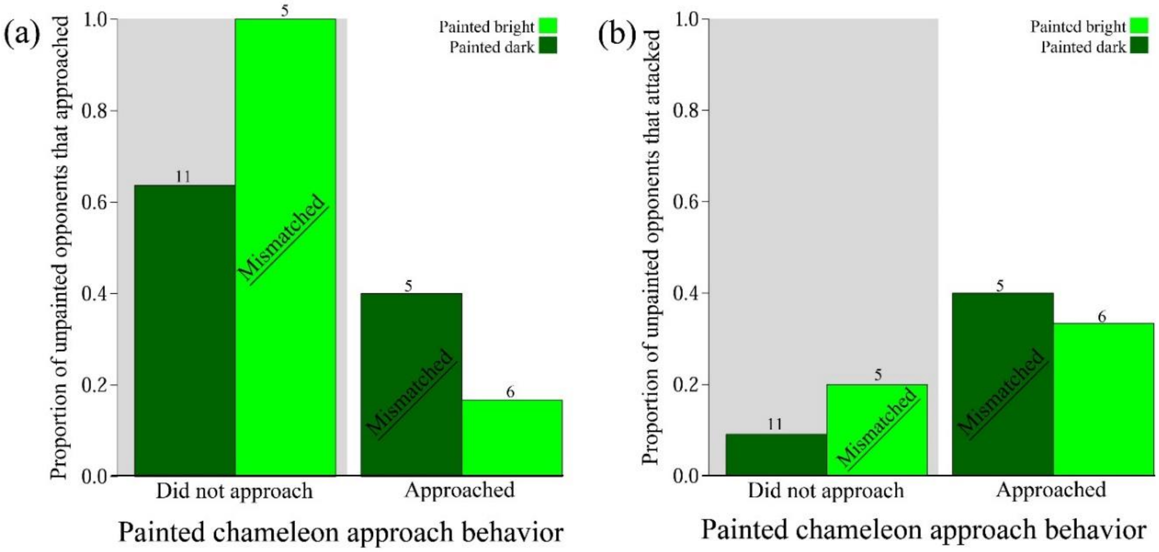


Figure 10. Mismatched chameleons, those whose approach behavior did not match their paint treatment, received higher levels of aggression than chameleons whose painted display color matched their behavior suggesting that social costs of dishonest signaling may play a role in maintaining honesty in chameleon color signals (Table 2).

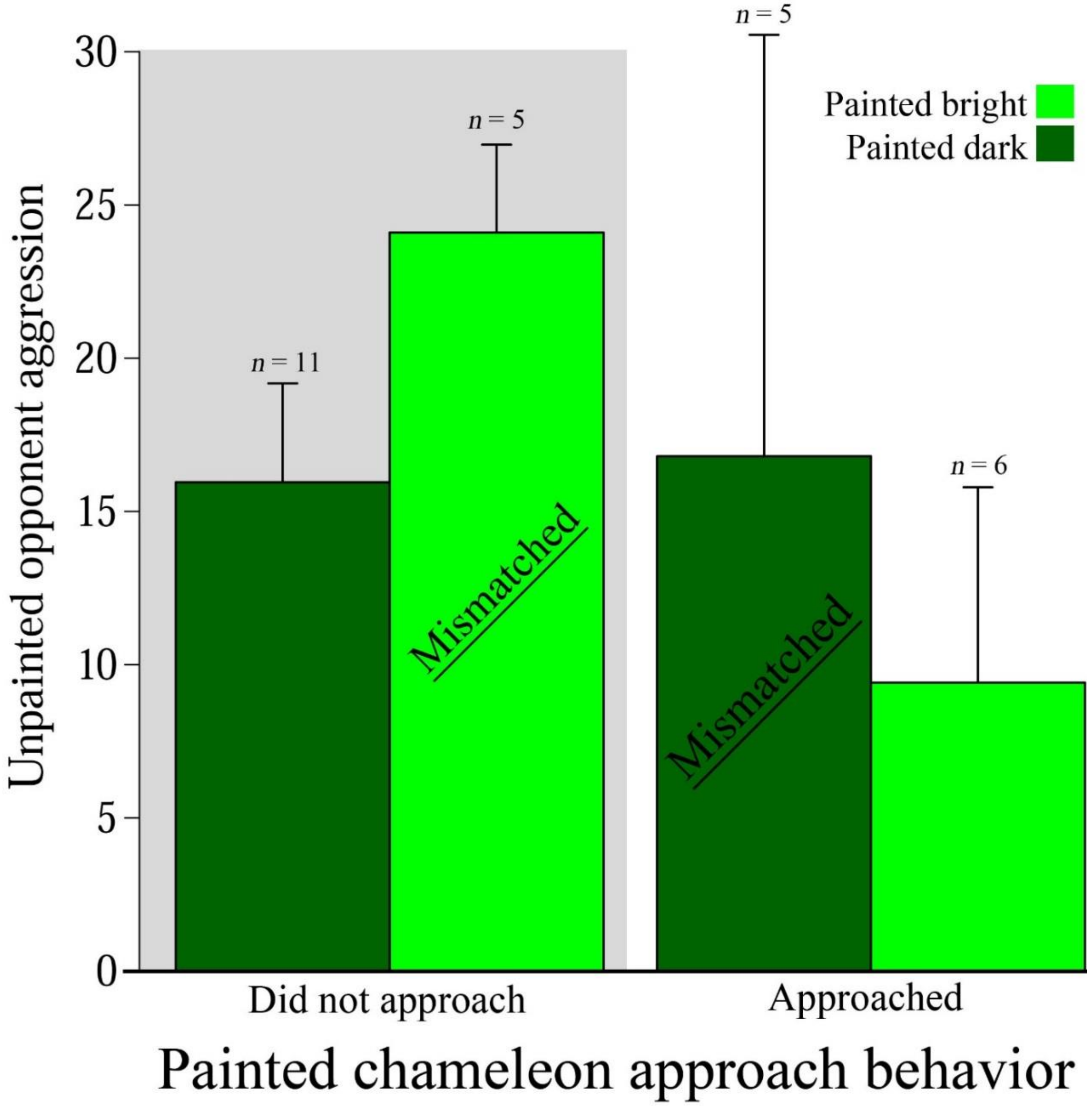
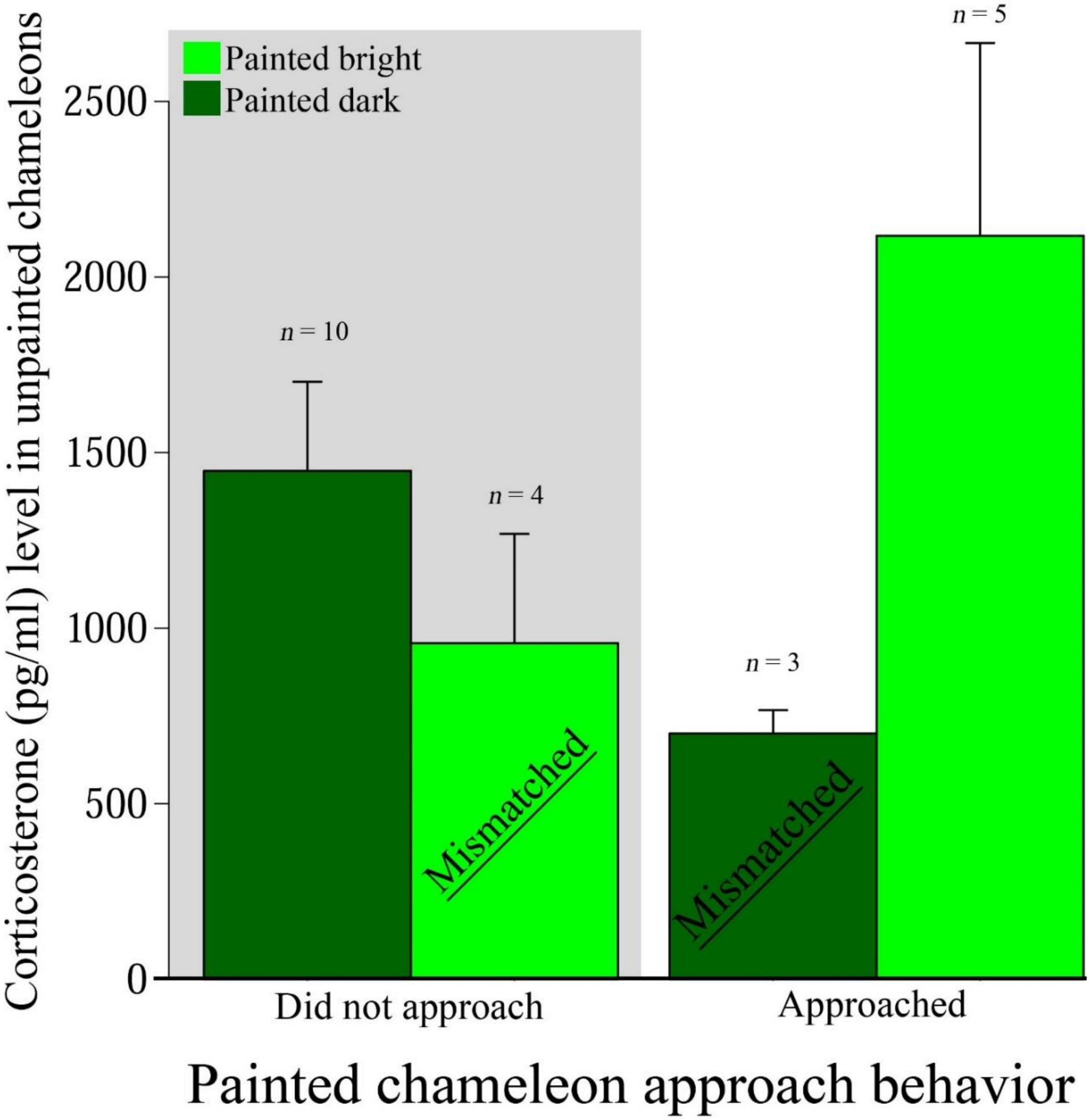


Figure 11. Corticosterone levels of unpainted chameleons were lower when facing mismatched opponents, those whose behavior did not match their experimentally manipulated display colors.



SUPPLEMENTARY MATERIALS

Supplementary Table 15. Behavioral metrics quantified to evaluate inter-observer reliability. Weighted behavioral metrics were used in the calculation of overall aggressive scores, calculated as the sum of all weighted behaviors multiplied by their respective weights.

Behavioral metric		Description	
Number of			Weight
Aggressive behaviors	Fights	Physical contact and intent to bite or displace opponent	5
	Knock-offs	Chameleon aggressively dislodges opponent from perch	5
	Attacks	Initiation of physical contact	5
	Bite-releases	Biting followed by immediate release of opponent	5
	Bite-clamps	Sustained biting (locked on to opponent with mouth)	5
	Approaches	Directed movement towards opponent	4
	Lunges	Fast, directed head or body thrust towards opponent	4
	Lateral displays	Lateral compression, dorso-ventral expansion, physical orienting of body perpendicularly to opponent	3
	Swaying bouts	Lateral, side-to-side movement of entire body	2
	Tail curls	Tail curled and uncurled	1
	Brightening bouts*	Rapid color change resulting in increasing brightness and chroma	
Submissive behaviors	Darkening bouts*	Rapid color change resulting decreasing brightness and chroma	
	Retreats†	Directed movement away from opponent	
	Times fleeing†	Rapid, directed movement way from opponent	
Composite scores			
	Aggression score	Summed total of weighted aggressive behaviors	
Did this behavior occur? (Yes/No)			
	Approach?	Did the chameleon approach?	

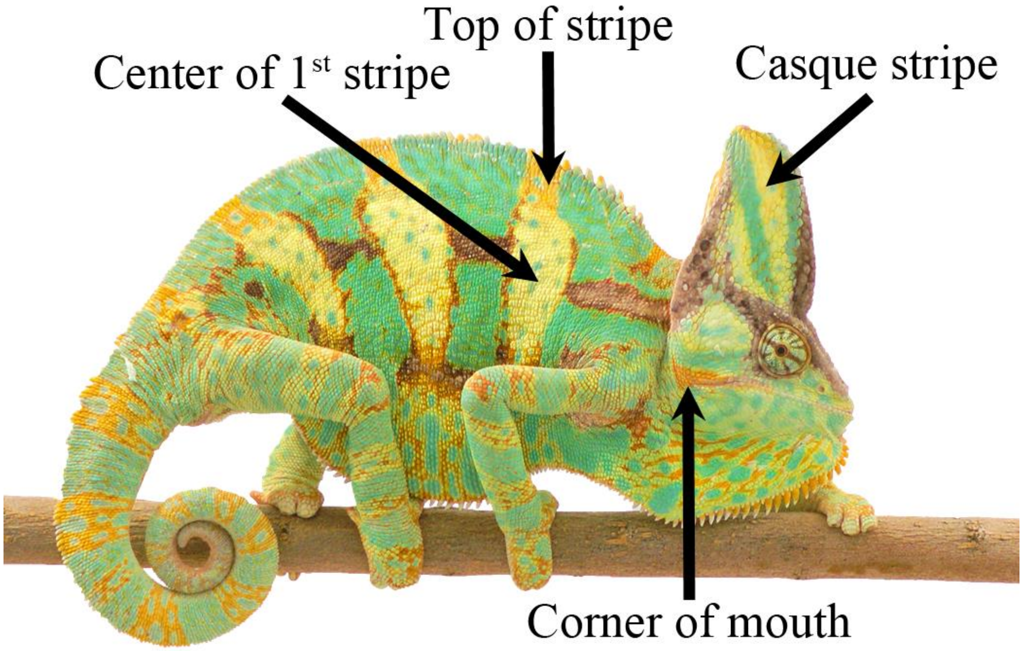
*Brightening and darkening were not assigned weights so that overall aggression scores would be comparable between painted and unpainted chameleons.

†Submissive behaviors were not assigned weights in this study.

Supplementary Table 16. Re-analysis of the aggression painted chameleons received during agonistic encounters with an outlier removed. Without this data point, aggression received depended only on whether or not a painted chameleon approached his opponent.

Model	Parameter	Test statistic	p
Aggression received			
	Treatment	$F_{1,\ 14.87} = 2.22$	0.16
	Approach	$F_{1,\ 14.87} = 8.04$	**0.01**
	Treatment*Approach	$F_{1,\ 14.87} = 0.04$	0.85

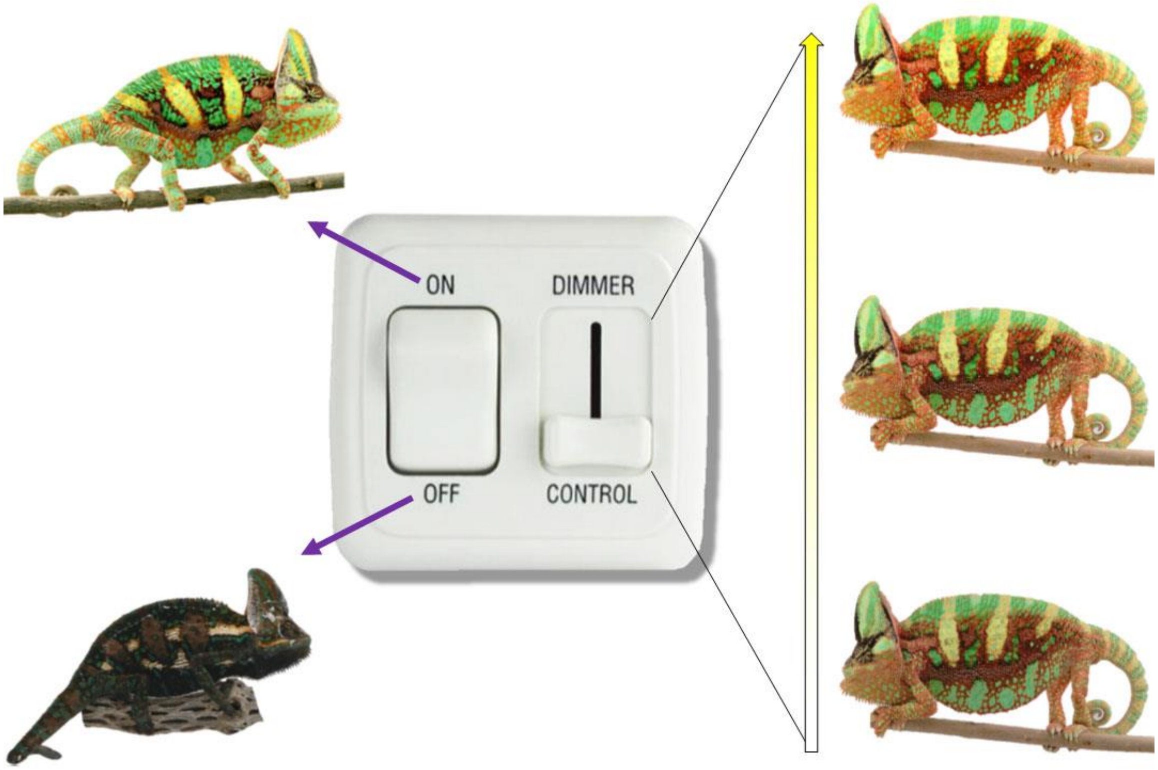
Supplementary Figure 12. Body regions from which color and color-change measurements were collected.



APPENDIX C

CHAPTER 3 FIGURE

Figure 13. Separate components of complex color change signals used by male veiled chameleons during agonistic interactions can be likened to the use of a light switch with dimming functionality. A chameleon's choice to brighten (signaling aggression) or darken (signaling submission) is analogous to the choice of whether to turn the light on or off. An individual chameleon's performance capability is also linked to the timing of this 'switch', where individuals with more powerful bites turn the switch 'on' earlier during aggressive interactions. In addition to the strategic signals of brightening and darkening (turning the light on or off), there is significant variation among chameleons with respect to the maximum brightness they reach and their speed of color change. This variation in dynamic color signals among chameleons can be likened to variation among different light switches, where the maximum intensity and speed with which the dimmer can be moved vary from switch to switch. Just as the variability in brightness and color change speed is likely a consequence of a chameleon's development and current health, the variability among the light switches is a consequence of the way the switches were built and how much power they can currently draw.



APPENDIX D

CHAMELEONS COMMUNICATE WITH COMPLEX COLOUR CHANGES DURING CONTESTS: DIFFERENT BODY REGIONS CONVEY DIFFERENT INFORMATION

Downloaded from rsbl.royalsocietypublishing.org on December 11, 2013

biology
letters

rsbl.royalsocietypublishing.org

Animal behaviour

Chameleons communicate with complex colour changes during contests: different body regions convey different information

Russell A. Ligon and Kevin J. McGraw

School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

Many animals display static coloration (e.g. of feathers or fur) that can serve as a reliable sexual or social signal, but the communication function of rapidly changing colours (as in chameleons and cephalopods) is poorly understood. We used recently developed photographic and mathematical modelling tools to examine how rapid colour changes of veiled chameleons *Chamaeleo calyptratus* predict aggressive behaviour during male–male competitions. Males that achieved brighter stripe coloration were more likely to approach their opponent, and those that attained brighter head coloration were more likely to win fights; speed of head colour change was also an important predictor of contest outcome. This correlative study represents the first quantification of rapid colour change using organism-specific visual models and provides evidence that the rate of colour change, in addition to maximum display coloration, can be an important component of communication. Interestingly, the body and head locations of the relevant colour signals map onto the behavioural displays given during specific contest stages, with lateral displays from a distance followed by directed, head-on approaches prior to combat, suggesting that different colour change signals may evolve to communicate different information (motivation and fighting ability, respectively).

Research

Cite this article: Ligon RA, McGraw KJ. 2013 Chameleons communicate with complex colour changes during contests: different body regions convey different information. Biol Lett 9: 20130892.
http://dx.doi.org/10.1098/rsbl.2013.0892

Received: 16 October 2013
Accepted: 14 November 2013

Subject Areas:
behaviour

Keywords:
agonistic signalling, combat, communication, *Chamaeleo calyptratus*, colour signals, physiological colour change

Author for correspondence:
Russell A. Ligon
e-mail: russell.ligon@gmail.com

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2013.0892 or via http://rsbl.royalsocietypublishing.org.

1. Introduction

The colour of most animals is relatively fixed (e.g. in dead tissues like exoskeleton, scales, feathers and hair), but some animals are capable of undergoing rapid, physiological colour change which allows them to display different colours and patterns in response to changing environmental contexts (e.g. predators [1], temperature [2] and humidity [3]). A few taxa (predominantly cephalopods, fish and reptiles) also are capable of physiological colour change during intraspecific interactions. Chameleons (Squamata: Chameleonidae) represent an intriguing subject for research on dynamic coloration because, unlike organisms that undergo localized colour change [4] or rely on achromatic pattern alterations [5], they exhibit complex colour changes during social interactions [6]. In fact, selection for conspicuous signals is likely to have driven the evolution of display colours for some chameleons [7]. The complexity of chameleon colour change may permit dynamic signalling opportunities for chameleons, whereby they use diverse chromatic elements on the body to reveal distinct information, either over the course of social interactions or across different behavioural contexts.

To date, most research on physiological colour-change signals has focused on simplified on/off signals [4,5] or mechanistic (e.g. cellular and endocrine) controls [8], with less emphasis on adaptive significance and information content [9]. The relative paucity of investigations undertaken on the signalling role of physiological colour change may be due, in part, to the technological and methodological challenges associated with quantifying such a dynamic trait. However, recent advances in the photographic quantification of colour [10,11] and the visual systems of animals [12] now enable rigorous, non-invasive analyses of colour change,

© 2013 The Author(s) Published by the Royal Society. All rights reserved.

Royal Society Publishing
Informing the science of the future

Downloaded from rsbl.royalsocietypublishing.org on December 11, 2013

as it occurs and is perceived by conspecifics during social interactions. Here, we present the first study using these photographic [10,11] and analytical methods [11,13], as well as organism-specific visual models [12,14], to examine how dynamic colour changes of veiled chameleons *Chamaeleo calyptratus* predict behaviours in male–male contests. Though animals engaged in contests have conflicting aims, there is a shared benefit in avoiding unnecessary escalation of such contests. Dynamic signals, for example those provided by multi-component colour changes, may allow contestants to flexibly communicate motivation or ability during contests and could therefore contribute to evolutionarily stable strategies [15].

Male veiled chameleons are well known for intense intrasexual aggression (see electronic supplementary material, S1), yet agonistic encounters are frequently settled prior to physical contact, putatively through visual signals including rapid, body-wide colour change [6]. Here, we attempt to uncover the components of colour change linked to escalation behaviour (likelihood of approaching an opponent) and contest outcome (likelihood of winning a fight) by evaluating 28 different colour patches (figure 1) from displaying veiled chameleons during staged agonistic encounters. Specifically, we focused on the rate and degree of colour change (calculated using known sensitivities of chameleon photoreceptors [14]), as well as the maximum brightness of each colour patch, during aggressive interactions.

2. Material and methods

In spring 2011, we staged a series of aggressive, dyadic encounters between 10 adult male veiled chameleons in a round-robin tournament format that matched each chameleon against every other chameleon in our study population (see electronic supplementary material, S2). Trials were recorded with two high-definition video cameras that also enabled us to take still photographs of each chameleon. Though chameleons can exhibit a complex suite of behaviours during agonistic encounters (e.g. hissing, tail-curling and rocking), the most salient signals relate to the probability of escalating and winning aggressive encounters [15]. Thus, we monitored both escalation likelihood (whether or not a chameleon moved in a directed fashion toward its opponent) and win/loss outcome (losing chameleons retreated from their opponent at some point during the trial). We then used matrices of approach behaviour and contest outcomes to determine ‘ability’ scores using Bradley–Terry models (see electronic supplementary material, S6). Both chameleons displayed aggressive behaviour and rapid colour change in 17 of our 45 contests.

We collected data on brightness and colour change from 28 different colour patches on each chameleon (encompassing a variety of chameleon body regions and colour types; figure 1) that were photographed every 4 s during display, approach and combat phases of the trials. We first equalized and linearized photographs [11], then used specialized mapping functions to convert RGB (red, green and blue) values from these photographs to relative stimulation values of the different chameleon photoreceptor types (see electronic supplementary material, S3). Photographically derived estimates of chameleon photoreceptor stimulation values obtained from an independent dataset did not differ from spectrophotometrically determined values (see electronic supplementary material, table S2 and figure S2).

Because chameleon display coloration and change has never been quantitatively analysed with respect to intraspecific variation in behaviour, we measured the amount and speed of colour change, as well as maximum display brightness (previously suggested to be an informative component of squamate colour signals [16]). We measured colour changes as: (i) the perceptual distance travelled (sum of distances between colour measurements taken at different times) and (ii) rate of colour change (perceptual distance/time), both measured using units of just noticeable differences, which capture perceivable changes in colour taking into account species-specific photoreceptor sensitivities [13,17] (see electronic supplementary material, S4). Brightness was quantified by calculating how each colour patch would stimulate chameleon double cones, though only maximum brightness was evaluated in model-averaging and regression analyses.

We used principal components analyses to reduce the number of variables within three body regions (head, stripes and main body; electronic supplementary material, S5 and table S4) and an information theoretic model-averaging approach to evaluate the relative importance (RI; the sum of Akaike weights for all models in which that variable appeared) of colour intensity and change (averaged for each chameleon across trials) in predicting the likelihood of approaching an opponent or winning aggressive interactions (see electronic supplementary material, S6). Lastly, we included predictor variables from the top models in regression analyses to determine the degree to which colour traits explained variation in approach and winning abilities.

3. Results

Approach and fighting abilities were highly correlated ($r = 0.84$, $p = 0.003$), though the colour metrics that best predicted these outcomes were different. Specifically, maximum head (RI = 0.32) and stripe brightness (RI = 0.60) achieved were the best predictors of approach ability during agonistic encounters (figure 2*a*; electronic supplementary material, table S5); in regression analysis, maximum stripe brightness explained 71% of the variation in approach likelihood (figure 2*c*). Chameleons who displayed brighter heads (RI = 0.80) that changed colour faster (RI = 0.31) were more likely to win physical encounters (figure 2*b*; electronic supplementary material, table S6). A regression containing only maximum head brightness explained 83% of the variation in fighting ability (figure 2*d*).

4. Discussion

We found that different aspects of chameleon competition (approach likelihood and contest outcome) were best predicted by separate components of display coloration—maximum stripe brightness and maximum head brightness, respectively. We also found that rate of colour change was an informative component of aggressive displays; chameleons whose head coloration changed faster were more likely to win agonistic encounters. Taken together, these correlational findings represent the first demonstration that multiple components of rapid colour change can be used to signal different aspects of competitive behaviour (e.g. motivation and fighting ability).

Selection may favour separate signal components for motivation and fighting ability [15] because these two aspects of competition can differentially affect contest outcomes; for example, regardless of true ability, animals who fight harder to defend mates or territories can often overcome less-motivated competitors. Separate signals may also be favoured when rival assessment is prolonged and animals progress through a series of escalation and threat behaviours, as occurs for slow-moving chameleons that have the opportunity to sequentially evaluate competitor intent and quality. Interestingly, the physical locations of the key colour-signalling elements of veiled

Downloaded from rsbl.royalsocietypublishing.org on December 11, 2013

Figure 1. Colour patches measured during male–male competition between veiled chameleons. (*a*,*b*) Interindividual variation in location of colour patches can be seen by comparing the location of colour patches in (*a*) to those in (*b*). Because exact locations of colour patches varied among individuals, we focused on similarly located colour patches of equivalent pigmentary and structural makeup. Descriptions of colour patches and relevant principal component loadings are located in the electronic supplementary material, table S4.

chameleons align closely with the behaviours they exhibit during agonistic encounters. Aggressive chameleons display laterally to one another from a distance before approaching, providing their opponents the opportunity to assess body stripe coloration (which best predicted escalation likelihood in our study). Next, as they approach and prepare to engage in head-to-head combat (see electronic supplementary material, S1 and video S1), they have close visual access to head coloration (which best predicted win/loss outcome). Separate plumage patches in lark buntings *Calamospiza melanocorys* also convey different information about approach intensity and physical aggression [18], but our study is the first to document the use of multiple, behaviourally accentuated colour-change signals to communicate different information.

Costly intraspecific conflict over resources is common throughout the animal kingdom, yet rapid colour change as an agonistic signal is incredibly rare and warrants special examination. One possible explanation for the link between rate of colour change and fighting ability is that expression of bright, rapidly changing colours is causally related to the physiological processes (e.g. hormone status and energetic reserves) associated with fighting ability [4]. An alternative,

Downloaded from rsbl.royalsocietypublishing.org on December 11, 2013

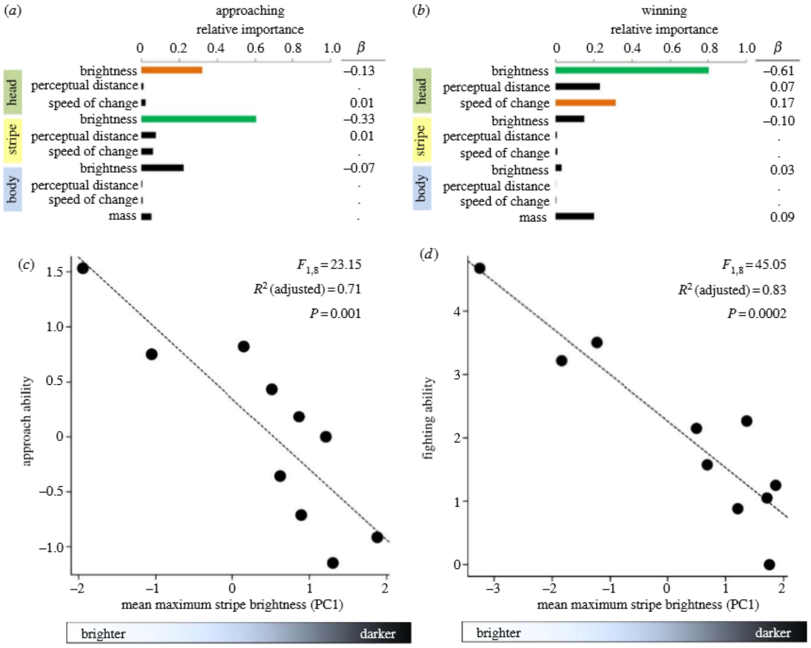


Figure 2. Explanatory variables associated with escalating and winning contests. Multi-model averaging indicates that average maximum stripe brightness (principal component, PC) best explains the likelihood of (*a*,*c*) a chameleon approaching his opponent and (*b*,*d*) that average maximum head brightness (PC) best explains the likelihood of a chameleon winning a fight. (*a*,*b*) Bar length is proportional to RI values of variables predicting (*a*) likelihood of approaching an opponent or (*b*) likelihood of winning an aggressive interaction, with green bars indicating RI values greater than 0.60 and orange bars indicating RI values greater than 0.30. Average parameter estimates of regression coefficients (*β*) were obtained using multi-model-averaging approaches and dots indicate parameter estimates with absolute values less than 0.005. (*c*) Regression of chameleon stripe brightness and approach ability calculated across trials. Average stripe brightness values represent PC scores, all of which had negative loadings of maximum brightness values (chameleons with brighter bodies had negative PC scores). (*d*) Regression of head brightness on fighting ability. Colour-metric predictor variables in (*a*–*d*) represent principal component scores from multiple colour patches within a region (see electronic supplementary material, table S4). Abilities plotted in (*c*,*d*) represent Bradley–Terry 'ability' scores.

though not exclusive, explanation suggests that it is more costly for strong individuals facing strong opponents to signal weakness before signalling strength (analogous to brightening slowly) than it is to initially signal strength [15], providing the evolutionary pressure to rapidly signal fighting ability when strong. Regardless of the explanation, documenting the behavioural contexts in which colour change signals occur is an important first step in understanding the function and evolution of this relatively rare signal type and should markedly inform our views on competitive signalling theory. Moreover, future detailed studies of the physiological mechanisms underlying display coloration will be key for revealing the information communicated by rapidly changing colours.

Acknowledgements. We thank Ellis Loew, Jim Bowmaker, Matthew Toomey, Martin Stevens, Thomas Pike, Kristen McCartney, Sarah Bruemmer, Megan Best and Brianna Bero-Buell for invaluable assistance, Dale DeNardo, the McGraw lab and two anonymous reviewers for helpful manuscript suggestions, and David, Sandy and Veronica Ligon for support. See full acknowledgements in the electronic supplementary material, S8.

Funding statement. Additional financial support was provided by an ASU GPSA grant.

References

1. Allen JJ, Mäthger LM, Barbosa A, Buresch KC, Sogin E, Schwartz J, Chubb C, Hanlon RT. 2010 Cuttlefish dynamic camouflage: responses to substrate choice and integration of multiple visual cues. *Proc. R. Soc. B* **277**, 1031–1039. (doi:10.1098/rspb.2009.1694)

2. Veron JEN. 1974 The role of physiological colour change in the thermoregulation of *Austrolestes annulosus* (Selys) (Odonata).

Downloaded from rsbl.royalsocietypublishing.org on December 11, 2013

Aust. J. Zool. **22**, 457–469. (doi:10.1071/Z09740457)

3. Hinton HE, Jarman GM. 1973 Physiological colour change in the elytra of the hercules beetle, *Dynastes hercules*. *J. Insect Physiol.* **19**, 533–549. (doi:10.1016/0022-1910(73)90064-4)
4. Summers CH, Greenberg N. 1994 Somatic correlates of adrenergic activity during aggression in the lizard, *Anolis carolinensis*. *Horm. Behav.* **28**, 29–40. (doi:10.1006/hbeh.1994.1003)
5. Adamo SA, Hanlon RT. 1996 Do cuttlefish (Cephalopoda) signal their intentions to conspecifics during agonistic encounters? *Anim. Behav.* **52**, 73–81. (doi:10.1006/anbe.1996.0153)
6. Nečas P. 1999 *Chameleons: nature's hidden jewels*. Frankfurt, Germany: Chimaira.
7. Stuart-Fox D, Moussalli A. 2008 Selection for social signalling drives the evolution of chameleon colour change. *PLoS Biol.* **6**, e25. (doi:10.1371/journal.pbio.0060025)
8. Nery LEM, Castrucci AML. 1997 Pigment cell signalling for physiological color change. *Comp. Biochem. Physiol. A* **29**, 1135–1144. (doi:10.1016/S0300-9629(97)00045-5)
9. Stuart-Fox D, Moussalli A. 2009 Camouflage, communication and thermoregulation: lessons from colour changing organisms. *Phil. Trans. R. Soc. B* **364**, 463–470. (doi:10.1098/rstb.2008.0254)
10. Stevens M, Párraga CA, Cuthill IC, Partridge JC, Troscianko TS. 2007 Using digital photography to study animal coloration. *Biol. J. Linn. Soc.* **90**, 211–237. (doi:10.1111/j.1095-8312.2007.00725.x)
11. Pike TW. 2011 Using digital cameras to investigate animal colouration: estimating sensor sensitivity functions. *Behav. Ecol. Sociobiol.* **65**, 849–858. (doi:10.1007/s00265-010-1097-7)
12. Vorobyev M, Osorio D, Bennett ATD, Marshall NJ, Cuthill IC. 1998 Tetrachromacy, oil droplets and bird plumage colours. *J. Comp. Physiol. A* **183**, 621–633. (doi:10.1007/s003590050286)
13. Pike TW. 2012 Preserving perceptual distances in chromaticity diagrams. *Behav. Ecol.* **23**, 723–728. (doi:10.1093/beheco/ars018)
14. Bowmaker JK, Loew ER, Ott M. 2005 The cone photoreceptors and visual pigments of chameleons. *J. Comp. Physiol. A* **191**, 925–932. (doi:10.1007/s00359-005-0014-4)
15. Enquist M. 1985 Communication during aggressive interactions with particular reference to variation in choice of behaviour. *Anim. Behav.* **33**, 1152–1161. (doi:10.1016/S0003-3472(85)80175-5)
16. Molnár O, Bajer K, Török J, Herczeg G. 2012 Individual quality and nuptial throat colour in male European green lizards. *J. Zool.* **287**, 233–239. (doi:10.1111/j.1469-7998.2012.00916.x)
17. Vorobyev M, Osorio D. 1998 Receptor noise as a determinant of colour thresholds. *Proc. R. Soc. B* **265**, 351–358. (doi:10.1098/rspb.1998.0302)
18. Chaine AS, Lyon BE. 2008 Intrasexual selection on multiple plumage ornaments in the lark bunting. *Anim. Behav.* **76**, 657–667. (doi:10.1016/j.anbehav.2008.03.014)

Chameleons communicate with complex colour changes during contests: different body regions convey different information

Russell A. Ligon*, Kevin J. McGraw

SUPPLEMENTARY INFORMATION

1. Study system

We obtained ten, wild-caught male veiled chameleons *Chamaeleo calyptratus* from a feral population in Florida [1]. Veiled chameleons are large, omnivorous, territorial lizards native to the mountainous regions of southwestern Arabia [2,3]. Like many chameleons, male veiled chameleons display intense antagonistic behaviour towards conspecific males [3] and exhibit rapid, body-wide colour changes during intraspecific interactions [4] (*pers. obs*). Upon seeing each other, adult male veiled chameleons typically begin an elaborate display that encompasses both morphological and chromatic transformations. The two males rapidly compress their bodies laterally, dorsoventrally expand their bodies, orient their bodies perpendicular to the direction of their opponents, and curl their tails into a disk-like shape (*cf.* [5]), all to enhance their apparent body size. Additionally, displaying males become noticeably brighter and more colourful to the human eye. At any point during the interaction, either chameleon can cease aggressive behaviours and begin to retreat. If neither chameleon retreats, however, these interactions will escalate to physical combat, including head-butting, lunging, and biting.

2. Aggression trials

2.1 Trial setup

Over a period of six weeks (15 March - 6 May 2011), we staged dyadic encounters between 10 adult male veiled chameleons in a tournament format that matched each chameleon against every other. Each chameleon faced no more than one other chameleon per day and never faced a chameleon with which they had any previous experience. Prior to each trial, chameleons were placed separately into cloth bags and set in a heated, darkened box, during which time we obtained the body mass of each individual using a digital balance. After a 10-minute thermal equilibration period, chameleons were sequentially placed on opposite, visually isolated ends of the trial arena (183 x 53 x 81 cm). We then allowed the chameleons to acclimate for five minutes before the central divider was removed and the trial began. Trials were recorded from behind a blind with two Panasonic HDC-TM 700 video cameras (Osaka, Japan), which enabled us to take still photographs while recording video, with one camera focused on each chameleon.

Each half of the trial arena contained a vertical perch (66 cm tall) with plastic foliage to provide cover for chameleons during the acclimation period, a horizontal perch (80 cm long) to allow the chameleons to approach one another, and a second vertical perch (44 cm tall) to allow chameleons to climb up toward or down and away from opponents (Supplementary Figure 1). The order in which chameleons of each dyad were placed into the arena was randomized.

Supplementary Figure 1. Trial arena used to stage agonistic encounters between adult male veiled chameleons *Chamaeleo calyptratus*.

2.2 Behaviours monitored

We monitored chameleon behaviour by watching the LCD screens of the video cameras from behind a blind, and trials were terminated once a clear winner was established (see below) or if no interaction occurred within 30 minutes. If, during the trial, there appeared to be a risk of serious injury (biting and twisting, biting at an awkward angle), the trial was terminated and the animals separated to avoid serious physical injury. However, most conflicts were decided prior to physical contact or very quickly thereafter, and, throughout the 45 aggressive trials, such interruption was necessary only once. This single trial did result in broken skin for one individual, but in no other trial did chameleons sustain any other body injury.

3. Using colour photography to measure chameleon colour change

Although most recent studies of animal colouration have relied on spectrophotometry to objectively quantify colour, there are distinct limitations of this methodology for some applications. Specifically, spectrophotometers can only capture point samples of colour in space and time and, with the exception of some extremely expensive equipment, require the measured sample to come into direct contact with the measurement probe. Fortunately, recent advances in the standardization and quantification of colour obtained from photographs have enabled researchers to undertake detailed studies of animal colouration using digital cameras [6,7]. Using recently developed methods, we: 1) identified a suite of colour patches to measure from each chameleon, 2) standardized photographs [6,7] using a specialized colour standard [8], 3) calculated the receptor sensitivity functions of our digital cameras [7], and 4) used a large set of reflectance data and the known properties of chameleon photoreceptors to create mapping functions to convert RGB camera output into the relative quantum catch of three chameleon cones [7].

Although veiled chameleons possess four classes of cones, we focused on the relative stimulation of the short-, medium-, and long-wavelength-sensitive cone classes. Despite the fact that logistical constraints prevented us from measuring the UV reflectance of colour patches using colour photography while simultaneously measuring reflectance in the human-visual portion of the light spectrum [7], we feel that this approach was unlikely to generate incomplete or inaccurate data for two reasons. First, full-spectrum spectrophotometry and ultraviolet-filtered photography of veiled chameleon display colouration revealed few regions with significant ultraviolet reflectance (RAL, *unpublished data*). Second, our trials were conducted indoors, under artificial lighting that lacked UV transmittance.

3.1 Colour identification

We analyzed the video recordings of each trial to determine the onset and duration of each colour-changing bout during the course of an interaction. We evaluated pictures taken at approximately 4 s intervals (a rate determined by the processing ability of the cameras) starting from 30 s prior to the onset of colour change (determined by a human observer), through 30 s after the colour-changing bout was determined to be complete. Because it is currently unknown what, if any, aspects of physiological colour change are most informative for chameleons when evaluating one another during agonistic encounters, we measured the colour of 28 different patches (Fig. 1) on each chameleon in each photo throughout the course of each brightening event. We chose these patches because they represented all of the major colour classes exhibited by veiled chameleons (e.g., greens, blues, yellows, oranges, etc.) during colourful aggressive displays and occurred in body areas accentuated by chameleons during behavioural displays (RAL, *pers obs*). However, the colour and pattern of veiled chameleons varies a great deal, both within and among chameleons. Although the exact location of a particular colour patch varied slightly within a region among individuals (*cf.* Fig. 1a and 1b), we felt that it was more informative from a chromatic-signaling perspective to make comparisons between similarly located colour patches of equivalent pigmentary and structural makeup than to inflexibly focus on a narrowly-defined body location that might contain different colour types among individuals. Within individuals, we focused our analyses on patches defined by the general descriptions outlined in Supplementary Table 4 and measured perceptual changes in these same patches throughout behavioural interactions.

3.2 Photograph standardization (linearization and channel equalization)

A first step in the process of using digital photography to measure colouration is to ensure that the R (red), G (green), and B (blue) channels of the camera response are linearized and equalized. Although there are costly (in both time and money) technical methods to perform these functions [8], we used a simple and highly effective approach to achieve a similar result. Specifically, we used a specialized colour standard (ColourChecker Passport, X-Rite Photo) commonly used in photographic and videographic calibration in conjunction with a software plug-in (PictoColour® inCamera™, PictoColour Software, Burnsville, MN) for Adobe Photoshop. This plug-in creates custom International Colour Consortium (ICC) digital profiles by "evaluating the colour data captured by the camera…and comparing the data to the known colour values of the [standard colour] charts." As described by Bergman and Beehner [8], we manually aligned the grid from the inCamera tool to the colour grid of the ColourChecker Passport in a given reference photograph, allowing inCamera to create a custom profile that adjusted pixel values to those of the known colours of the standard. For each reference photograph (containing the ColourChecker Passport, taken at the beginning of each trial day), we created a unique profile that could then be assigned within Photoshop to all other pictures taken under identical conditions (alleviating the need to have a colour standard in every single picture or frame). This method is defined as the "sequential method" by Bergman and Beehner [8] and was found by these authors to provide results that are more accurate than the "adjacent method" (colour standard in the same photograph as the colourful object of interest).

We assessed linearity of our colour responses by using linear regression of the relationship between the different channels (R, G, and B) and the known reflectance values of the six gray standards on the colour standard. This relationship was examined for linearity under varying light conditions, after the inCamera colour corrections were applied. Analyses on two independent data sets using these methods uncovered extremely high R^2 values ($R^2 >$ 0.98) for all three channels (M. Amarello unpublished data; R. Ligon unpublished data). RGB equalization (R = G = B) was also tested and found to be within the limits described by Bergman and Beehner [8] (> 95% RGB values within 5% of one another).

3.3 Calculating camera sensitivity functions

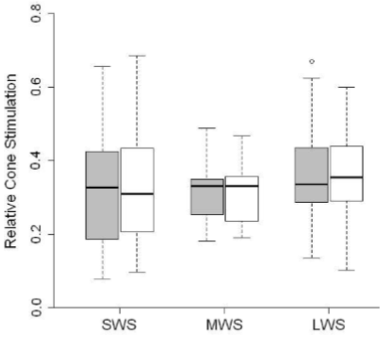
Direct measurement of camera sensor sensitivity functions is technically demanding, time consuming, and expensive [6,7]. However, Pike [7] recently described a method that enables individuals to estimate these sensitivities using a custom Matlab (MathWorks, Natick, MA) function. Users define several key components of sensor sensitivity functions (likely peak and trough locations, for example) before the quadratic programming function identifies the camera sensor sensitivities by comparing the RGB output of known colour standards to the radiance values (reflectance x irradiance) of the same standards. For additional information on the technical aspects of this procedure, see reference 7.

3.4 Mapping functions for converting RGB values to chameleon quantum catch

Published data on ocular media and cone sensitivities from several species of chameleons provided the basis of our chameleon-specific visual models [9]. Because the visual pigments found in chameleon photoreceptors possess absorbance characteristic of both rhodopsin and porphyropsin, we constructed cone sensitivity functions using a combination of both pigment types. We originally designed this model to calculate the quantum catch (the number of photons absorbed) of the different cone classes within chameleon eyes using spectral data. To calculate the quantum catch of the different chameleon photoreceptors using the linearized and equalized RGB output of a camera, however, we had to create a mapping function to convert between the two (spectrophotometrically and photographically determined) colour spaces.

The mapping function to obtain the quantum catch of the different chameleon cone types consists of a series of coefficients by which RGB values are multiplied (Supplementary Table 1). To obtain the coefficients necessary to convert between the two colour spaces, we simulated what our camera would see when viewing 1250 Munsell colour chips and what a chameleon would see (one cone type at a time) when viewing the same 1250 colours. Using matrix algebra within Matlab, we were able to explore the effectiveness of several different equations of the type described by Westland & Ripamonti [10]. The model, included below, that gave us the best fit contained several higher order polynomials. Following the notation of Stevens et al. [6], R, G, and B describe calibrated and linearized pixel values for a given colour, b_i represents the coefficients specific to receptor class i (Supplementary Table 1), and Q_i represents the quantum catch for receptor class i.

$$Q_i = b_{i1}R+b_{i2}G+b_{i3}B+b_{i4}R^2+b_{i5}G^2+b_{i6}B^2 +b_{i7}RGB+b_{i8}GB+b_{i9}B^2G^2+b_{i10}R^3+b_{i11}G^3 +b_{i12}B^3 \tag{1}$$



Supplementary Figure 2. Comparison of spectrophotometrically (grey boxes) and photographically (white boxes) determined estimates of the relative stimulation of short- (SWS), medium- (MWS), and long-wavelength-sensitive (LWS) cones of a chameleon when viewing an X-Rite™ colour standard. Paired t-tests comparing these methods (Supplementary Table 2) revealed no significant differences between photographically and spectrophotometrically derived estimates.

After obtaining the mapping coefficients (b_i, Supplementary Table 1) required to convert between photographically determined quantum catch and spectrophotometrically determined quantum catch, we converted RGB values to the relative stimulation of the different photoreceptor types and validated our conversion equation by testing it on an independent data set (Supplementary Figure 2).

Supplementary Table 1. Coefficients for polynomials used to convert between RGB and quantum catch of chameleon photoreceptors.

	b1	b2	b3	b4	b5	b6	b7	b8	b9	b10	b11	b12
LWS	0.427	1.090	-0.500	0.473	-1.514	0.240	-0.766	1.117	0.532	-0.051	0.709	-0.567
MWS	-0.069	1.310	-0.254	0.093	-0.510	0.210	-0.394	0.351	0.296	0.060	0.286	-0.342
SWS	0.027	-0.010	1.004	-0.114	0.146	-0.407	-0.542	-0.276	-0.578	0.148	0.125	1.440

Supplementary Table 2. Outcome of paired t-tests comparing spectrophotometric and photographic estimates of the relative quantum catch of the long- (LWS), medium- (MWS), and short-wavelength-sensitive (SWS) cones of a chameleon, in terms of the mean difference between estimates, the confidence interval for this difference, and whether this difference was significantly different from zero.

Cone Class	**Mean Difference**	**95% CI**	***t*-value**	**df**	***p*-value**
LWS	0.0099	(-0.0053 – 0.0252)	1.346	23	0.192
MWS	-0.0002	(-0.0140 – 0.0137)	-0.023	23	0.982
SWS	-0.0098	(-0.0310 – 0.0115)	-0.952	23	0.351

3.5 Pattern analyses

We used the pattern analysis techniques described by Stoddard and Stevens [11] to quantify chameleon body patterns at different time points during aggressive trials. Briefly, we used a custom Matlab function written by Martin Stevens to analyze the 'granularity' of images, which allowed us to determine the relative importance of different marking sizes to the overall chameleon pattern. We chose five body regions from which to extract images for pattern analyses (Supplementary Figure 3) and, in an attempt to standardize our pattern analyses, limited our analyses to photographs taken when chameleons were perpendicular to the camera.

From each of the five body regions analyzed for pattern, we extracted seven images (by fast Fourier transforming the original image and applying seven isotropic band pass filters [12]) containing information based on different spatial scales. These filters capture pattern element information at different scales, with small filter sizes corresponding to large markings and large filters corresponding to small markings [11]. Evaluating these seven

Supplementary Figure 3. Body regions of veiled chameleons analyzed for pattern elements. We analyzed pattern elements from five square images from the casque (1), first lateral stripe (2), center horizontal stripe between the first and second lateral stripes (3), the first tail band (4), and central body region (5). Figure is in grayscale to illustrate appearance of pictures analyzed for pattern using methods described in main body of the text.

images ('granularity bands' [12]) allowed us to determine the contribution of different pattern element sizes to overall chameleon body pattern.

After filtering images, we measured the overall pattern 'energy' (E) for each granularity band by dividing the number of pixels in the image by the sum of the squared pixel values [13]. The values of E across all granularity bands constitute the 'granularity spectrum' of an image [13], and a number of informative values can be calculated from this spectrum. As in Stoddard and Stevens [11], we focused our subsequent analyses on three pattern properties that capture different components of pattern information. First, we calculated the bandwidth of maximum energy (E_{max}), which corresponds to the most prevalent pattern element size. Second, as a measure of the relative importance of the highest marking size, we calculated the proportion of energy contained within the predominant marking size relative to the energy across all bandwidths (E_{prop}). Third, we calculated the total energy (E_{tot}) across all bandwidths as a measure of overall pattern contrast [11,13].

In an effort to quantify the extent of pattern-element change over time, we evaluated pattern elements from standardized photographs taken of chameleons taken at the beginning of a given trial, and compared these measurements to those obtained from the same chameleons later in the trial, at the 'moment of determination' (i.e. the time point we identified at which the losing chameleon first began to display submissive behaviours, which typically falls within the 30 s buffer period of the brightening events we evaluated for chromatic changes). In the case of physical interaction, this was when the loser began to retreat or scramble away from the winner. In the case of non-physical interactions, the moment of determination was identified as the time point at which the loser began to darken or retreat (whichever came first).

To evaluate intra-individual variation in pattern within a given trial, we included images taken from trials conducted at different times and containing different individuals (i.e. trials separate from those conducted and analyzed to uncover the importance of colour change as a signal). However, we restricted our analyses to instances in which we could analyze the pattern of a given chameleon at the beginning of a trial and at the moment of determination. Within-individual comparisons allowed us to use paired t-tests to evaluate differences within individuals over time.

We found that dominant marking filter size (E_{max}) did not differ within chameleons at different time points (Supplementary Table 3). Additionally there were no differences within chameleons for the proportion of energy contained within the dominant granularity band (E_{prop}) for four out of the five body regions measured (Supplementary Table 3). Lastly, the total energy of the granularity spectrum (E_{tot}), an overall measure of pattern contrast, did not differ statistically over time for three out of the five body regions analyzed (Supplementary Table 3). Because the pattern elements of colour display appear to be largely invariant within individual chameleons, and because our interest was in the changing nature of colour signals, we restricted our analyses to dynamic chromatic variables.

where e_i refers to the noise in cone type i (L = long wavelength, M = medium wavelength, S = short wavelength sensitive cones), and Δf_i corresponds to the difference in the response of cone class i between the two colours being compared. Receptor noise levels (e_i) vary as a function of the relative densities of the different cone types [15]; however, this information has not been determined for chameleons. Therefore, we used a relatively conservative estimate of equal receptor noise levels ($e = 0.05$) across long- (LWS), medium- (MWS), and short- (SWS) wavelength-sensitive cones for our analyses. Because perceptual distances are influenced by error estimates and we wanted to ensure that the inferences we drew regarding the importance of perceivable colour changes were robust to different noise estimates, we also evaluated all models using biologically informed [9], yet conservative, estimates of relative cone densities of LWS:MWS:SWS set to 6:3:1. Results from these analyses were not qualitatively different than those obtained using equal receptor noise levels of 0.05.

After determining relative discriminabilities, we used the custom R (R Core Development Team) scripts provided by Pike [16] to calculate the Cartesian coordinates of each colour patch across time within the chromaticity space he describes. This chromaticity space preserves the perceptual distance between colours, such that the Euclidean distance between any two colours is exactly equal to their perceptual distances, in JNDs. Because each colour has x and y coordinates within this two-dimensional space, we were then able to calculate changes in Euclidean distances (ΔJND) between colour measurements taken at different times. By dividing ΔJND measurements by the elapsed time between the pictures from which colour values were taken, we were also able to calculate a rate of colour change within chameleon-specific perceptual space.

5. Variable selection and data reduction

We focused our analyses on three metrics of chameleon colouration: i) the maximum brightness of each body region achieved by a chameleon during a colour changing event, ii) the maximum speed of colour change (JND/sec) of each body region during a colour changing event, and iii) the total perceptual distance (sum of all ΔJNDs) travelled by each body region during a colour changing event. In total, we attempted to collect colour measurements from each of 28 different colour patches for a total of 3,190 photographs (i.e. each picture during colour changing bouts per chameleon per trial). Occasionally, however, some colour patches were not visible. In these cases, we used linear interpolation to determine likely values of missing colours [17,18]. This method represents a conservative approach to obtain measurements of colour movement within chameleon perceptual space because interpolated, straight-line values result in the minimum distance (in terms of JNDs) that a colour can travel through chromaticity space while still changing from one (measured) colour to another (measured) colour. Additionally, because interpolated values minimize distances travelled in chromaticity space, measures of the speed of colour change (ΔJND) will also be minimized using interpolated values.

To reduce the number of variables evaluated in our analyses, we performed separate principal components analyses (PCA) on the three chromatic variables of interest (maximum brightness, maximum JND/sec, total JND distance) for each of three body regions (Supplementary Table 4). Specifically, we analyzed all colour measurements collected from the head region (9 patches), from striped regions of the body (7 patches), and from non-striped body regions separately (12 patches). This resulted in nine distinct chromatic variables (max brightness, max speed of colour change, and total discriminable colour change from each of the three body regions), which we analyzed using an information theoretic approach [19] (see Supplementary Information 6). Additionally, we included the body mass of each chameleon prior to a given contest in our analyses, though we scaled these values to a mean of 0 and a standard deviation of 1 to facilitate direct comparisons between mass and scaled PC colour scores.

Supplementary Table 4. Colour regions measured during agonistic encounters between adult male veiled chameleons and corresponding factor loadings onto the principal components used in analyses evaluating characters predicting behaviour and contest outcome. Colour identification codes (ID) are used in Figures 1a and 1b.

ID	Colour Patch	Brightness PCs			Maximum Speed PCs			Maximum Distance PCs		
		Head	Body	Stripes	Head	Body	Stripes	Head	Body	Stripes
	Eigenvalues	4.325	5.095	3.524	3.247	4.799	3.517	5.565	7.388	4.875
	Proportion of Variance	0.481	0.425	0.503	0.361	0.400	0.502	0.618	0.616	0.696
						PC loadings				
a	Corner of mouth	-0.078			0.376			0.357		
b	Cheek, below corner patch (not orange spot)	-0.389			0.233			0.356		
c	Central casque stripe, top (yellow)	-0.398			0.371			0.335		
d	Central casque stripe, bottom (yellow)	-0.332			0.368			0.328		
e	Posterior casque stripe, top (green)	-0.393			0.146			0.320		
f	Posterior casque stripe, bottom (green)	-0.374			0.354			0.353		
g	Eye, background colour	-0.392			0.196			0.314		
h	Eye, stripe colour	-0.211			0.442			0.351		
i	Front-most throat cone	-0.285			0.387			0.279		
j	Behind 1st stripe, dorsal, light		-0.346			0.269			0.306	
k	Behind 2nd stripe, dorsal, light		-0.338			0.263			0.305	
l	Behind 1st stripe, dorsal, dark colour		-0.153			0.345			0.279	
m	Behind 2nd stripe, dorsal, dark colour		-0.168			0.375			0.322	
n	Center of top lateral stripe in front of 1st yellow stripe		-0.345			0.278			0.287	
o	Center of top lateral stripe in front of 2nd yellow stripe		-0.303			0.179			0.292	
p	Below top lateral stripe, between yellow stripes, light		-0.331			0.305			0.318	
q	Below top lateral stripe, between yellow stripes, dark		-0.158			0.154			0.242	
r	Belly below 2nd yellow stripe, light		-0.325			0.260			0.300	
s	Belly below 2nd yellow stripe, dark		-0.112			0.276			0.222	
t	Non-yellow tail band, posterior to closest yellow band		-0.372			0.318			0.281	
u	Rear belly (blue equivalent)		-0.335			0.357			0.291	
v	Front full stripe, yellow, center			-0.402			0.348			0.400
w	Mid full stripe, yellow, center			-0.317			0.266			0.363
x	Dots in front stripe, central			-0.443			0.265			0.389
y	Dots in mid stripe, central			-0.428			0.45			0.390
z	Orange surrounding 1st yellow stripe, dorsal			-0.417			0.447			0.377
§	Orange surrounding middle yellow stripe, dorsal			-0.315			0.47			0.361
Ø	Yellow tail band closest to body			-0.292			0.339			0.364

6. Model averaging

Our primary interest was to determine the colourimetric variable(s) that best explained the approach likelihoods and contest outcomes of aggressive interactions between adult male veiled chameleons. Colour variables related to the likelihood of approaching an opponent may be related to the underlying motivation of a given chameleon to engage in a fight, while colour traits linked to contest outcome may reveal a male's competitive ability and resource holding potential. Alternatively, colour signals that predict contest outcome could also be related to motivation, given that motivation has been shown to influence success in competitive interactions [20].

For both approaching and winning, we first identified individual ‘ability’ scores using Bradley-Terry (BT) analysis [21,22] which can be used to identify underlying 'ability' parameters (representing the likelihood of one individual beating another) from a matrix of contest outcomes. We restricted our BT analyses to a subset of trials (n = 17 out of the original 45 trials) in which both chameleons exhibited some degree of brightening colour display and for which there were no missing colour values. On average, each chameleon was represented in over three of these trials ($\bar{x}$ = 3.4, range 1-5). After identifying approach ability and fighting ability using BT models, we used Akaike's information criterion (AIC) within an information-theoretic (IT) framework [19] to evaluate a set of generalized linear models to determine the colourimetric and body mass variables (averaged across trials) that best explained the likelihood of approaching and the likelihood of winning aggressive encounters.

Although an all-subset approach, wherein researchers evaluate every possible combination of explanatory variables, is commonly used in behavioural ecology when explicit predictions cannot be made regarding the relative strengths of models containing different combinations of explanatory variables [23], we followed the recommendations of Grueber et al. [24] and evaluated only a specific, biologically plausible, subset of the total number of possible models when analysing 10 predictor variables (i.e. 9 colour variables + body mass). Specifically, we began by evaluating the predictive ability of models that included each of our 10 explanatory variables individually (individual predictor models). Next, we considered models that included all colour variables for a given body region (body region models) and models that included a given colour variable for all body regions (colorimetric models). Next, we evaluated the effect of adding body mass to the body region and colorimetric models. At this point, we assessed models that contained the strongest single predictor variable (assessed by AICc score) and one additional relevant (i.e. same colour variable/different region, same body region/different colour variable) parameter. Lastly, we evaluated the effect of including mass in the top model as well as coupling body mass with the individual variables in the top model (if it contained more than one colour variable).

Multiple models may be well-supported within the framework of IT, but it is still possible to evaluate the relative importance of specific variables in explaining the dependent variable [19,25]. In instances when IT-AIC analyses do not clearly identify a single best model (Akaike weight < 0.9), evaluation of multiple well-supported hypotheses is typically required [19]. Model averaging is a technique that allows researchers to incorporate parameter estimates from multiple models, which provides weighted estimates according to the support of the model. Specifically, the regression coefficient estimate is obtained for each model in which a given variable is present and multiplied by the Akaike weight of that model (w_i), then summed. This formula can be represented as:

$$\sum_{i=1}^{R} w_i \hat{\beta}_i \tag{3}$$

where $\hat{\beta}_i$is the estimate for the predictor in a given model i, and w_i is the Akaike weight of that model. Variables that are represented infrequently or only show up in poorly supported models will exhibit negligible parameter estimates, while variables that turn up repeatedly in strongly supported models will have appreciably larger parameter estimates and contribute more to predictive models. In addition to model-averaged parameter estimates, we also calculated the relative importance (RI) for each predictor variable as the sum of Akaike weights for all models in which that variable appeared. Because Akaike weights from a given model set sum to 1, RI values can range from 0 to 1. An RI value near 0 indicates that the variable is infrequently represented or present only in poorly-supported (low w_i) models, while a high RI value indicates that the variable is present in many, highly-supported (large w_i) models.

7. Full acknowledgements

We thank Ellis Loew and Jim Bowmaker for providing the raw data necessary to complete our visual models. Matthew Toomey provided assistance in designing and implementing the chameleon visual models. We thank Martin Stevens and Thomas Pike for providing explanations of the processes required to calculate quantum catch using photographic methods. Thomas Pike also provided Matlab code, as well as invaluable assistance explaining the processes required, for estimating camera sensor sensitivities, determining photograph-to-cone catch conversions, and converting relative cone stimulation to Cartesian coordinates in perceptual distance-chromaticity space. Martin Stevens also provided the Matlab code used in the granularity analyses. We thank Kristen McCartney, Sarah Bruemmer, Megan Best, and Brianna Bero-Buell for assistance with chameleon husbandry, data collection, and photographic analyses. We thank Dale DeNardo, the McGraw lab, and two anonymous reviewers for comments and suggestions on an earlier version this manuscript. We also thank David and Sandy Ligon for financial support, and Veronica Ligon for emotional support. Additional funding was provided by the Arizona State University Graduate and Professional Students Association JumpStart research grant.

8. References

1 Krysko, K. L., Enge, K. M. & King, F. W. 2004 The Veiled Chameleon, *Chamaeleo calyptratus*: A New Exotic Lizard Species from Florida. *Florida Sci.* **67**, 249–253.

2 Hillenius, D. 1966 Notes on chameleons III. The chameleons of southern Arabia. *Beaufortia* **13**, 91–108.

3 Nečas, P. 1999 *Chameleons: Nature's hidden jewels.* Frankfurt: Chimaira.

4 Nečas P 1991 Chamaeleo calyptratus calyptratus. *Herpetofauna* **13**, 6–10.

5 Bustard, H. R. 1967 The Comparative Behavior of Chameleons: Fight Behavior in *Chameleo gracilis* Hallowell. *Herpetologica* **23**, 44–50.

6 Stevens, M., Párraga, C. A., Cuthill, I. C., Partridge, J. C. & Troscianko, T. S. 2007 Using digital photography to study animal coloration. *Biol. J. Linn. Soc.* **90**, 211–237.

7 Pike, T. W. 2011 Using digital cameras to investigate animal colouration: estimating sensor sensitivity functions. *Behav. Ecol. Sociobiol.* **65**, 849–858.

8 Bergman, T. J. & Beehner, J. C. 2008 A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (*Theropithecus gelada*). *Biol. J. Linn. Soc.* **94**, 231–240.

9 Bowmaker, J. K., Loew, E. R. & Ott, M. 2005 The cone photoreceptors and visual pigments of chameleons. *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.* **191**, 925–32.

10 Westland, S. & Ripamonti, C. 2004 *Computational colour science using MATLAB*. Chichester: John Wiley & Sons Ltd.

11 Stoddard, M. C. & Stevens, M. 2010 Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proc. R. Soc. B-Biological Sci.* **277**, 1387–93.

12 Barbosa, A., Mäthger, L. M., Buresch, K. C., Kelly, J., Chubb, C., Chiao, C.-C. & Hanlon, R. T. 2008 Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. *Vision Res.* **48**, 1242–53.

13 Chiao, C.-C., Chubb, C., Buresch, K., Siemann, L. & Hanlon, R. T. 2009 The scaling effects of substrate texture on camouflage patterning in cuttlefish. *Vision Res.* **49**, 1647–56.

14 Osorio, D. & Vorobyev, M. 2005 Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc. R. Soc. B-Biological Sci.* **272**, 1745–52.

15 Vorobyev, M., Osorio, D., Bennett, a T., Marshall, N. J. & Cuthill, I. C. 1998 Tetrachromacy, oil droplets and bird plumage colours. *J. Comp. Physiol. A.* **183**, 621–33.

16 Pike, T. W. 2012 Preserving perceptual distances in chromaticity diagrams. *Behav. Ecol.* **23**, 723–728.

17 Vasseur, D. a. & Yodzis, P. 2004 the Color of Environmental Noise. *Ecology* **85**, 1146–1152.

18 Yu, J., Smith, V. A., Wang, P. P., Hartemink, A. J. & Jarvis, E. D. 2004 Advances to Bayesian network inference for generating causal networks from observational biological data. *Bioinformatics* **20**, 3594–603.

19 Burnham, K. & & Anderson, D. 2002 *Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn.* New York: Springer.

20 Jonart, L. M., Hill, G. E. & Badyaev, A. V. 2007 Fighting ability and motivation: determinants of dominance and contest strategies in females of a passerine bird. *Anim. Behav.* **74**, 1675–1681.

21 Bradley, R. & Terry, M. 1952 Rank analysis of incomplete block designs I: The method of paired comparisons. *Biometrika* **39**, 324–345.

22 Firth, D. 2005 Bradley-Terry Models in R. *J. Stat. Softw.* **12**, 1–8.

23 Symonds, M. R. E. & Moussalli, A. 2010 A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behav. Ecol. Sociobiol.* **65**, 13–21.

24 Grueber, C. E., Nakagawa, S., Laws, R. J. & Jamieson, I. G. 2011 Multimodel inference in ecology and evolution: challenges and solutions. *J. Evol. Biol.* **24**, 699–711.

25 Burnham, K. P., Anderson, D. R. & Huyvaert, K. P. 2010 AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav. Ecol. Sociobiol.* **65**, 23–35.

Supplementary Table 5. 95% confidence set of best-ranked linear models (the 10 models whose cumulative Akaike weight, cum $w_{i,}$ $\approx$ 0.95) used to determine the variables that best explain whether a male veiled chameleon will *approach* another male chameleon during an agonistic encounter.

Model	**Log *L***	***k***	**AIC$_c$**	**ΔAIC$_c$**	**w_i**
STRIPESMaxB.PC1	-6.84	1	20.17	0.00	0.229
HEADMaxB.PC1	-6.87	1	20.24	0.07	0.222
BODYMaxB.PC1	-7.39	1	21.28	1.11	0.132
STRIPESMaxB.PC1 + HEADMaxB.PC1	-6.17	2	22.04	1.87	0.090
STRIPESMaxB.PC1 + BODYMaxB.PC1	-6.33	2	22.37	2.20	0.076
STRIPESMaxB.PC1 + STRIPESMaxD.PC1	-6.47	2	22.65	2.48	0.066
STRIPESMaxB.PC1 + STRIPESMaxSpeed.PC1	-6.77	2	23.25	3.08	0.049
STRIPESMaxB.PC1 + ScaledMass	-6.83	2	23.38	3.21	0.046
STRIPESMaxB.PC1 + HEADMaxB.PC1	-6.17	3	24.33	4.16	0.029
HEADMaxSpeed.PC1	-9.73	1	25.97	5.80	0.013

Model variables where '*MaxB*' represents maximum brightness, '*MaxD*' represents maximum perceptual distance travelled, and '*MaxSpeed*' represents maximum speed of chameleon-perceivable colour change. Body regions (Head, Stripes, Body) refer to those summarized in Table S4. Model statistics include Log-Likelihood function (Log *L*), number of estimated parameters (*k*), selection criterion (AIC$_c$), distances from best model (ΔAIC$_c$), and Akaike weights (w_i). Akaike weights (w_i) can be considered the probability of a given model *i*, given the variables evaluated and the data analyzed (Burnham et al. 2011).

Supplementary Table 6. 95% confidence set of best-ranked linear models (the 11 models whose cumulative Akaike weight, cum $w_{i,}$ $\approx$ 0.95) used to determine the variables that best explain whether a male veiled chameleon will *win* an agonistic encounter with another male chameleon.

Model	**Log *L***	***k***	**AIC$_c$**	**ΔAIC$_c$**	**w_i**
HEADMaxB.PC1	-9.20	1	24.89	0.00	0.215
HEADMaxB.PC1 + HEADMaxD.PC1	-7.78	2	25.26	0.37	0.179
HEADMaxB.PC1 + HEADMaxSpeed.PC1	-8.09	2	25.89	1.00	0.130
HEADMaxB.PC1 + STRIPESMaxB.PC1	-8.65	2	27.02	2.13	0.074
HEADMaxB.PC1 + BODYMaxB.PC1	-8.70	2	27.12	2.23	0.070
HEADMaxSpeed.PC1 + ScaledMass	-8.71	2	27.12	2.23	0.070
HEADMaxB.PC1 + ScaledMass	-8.90	2	27.51	2.62	0.058
HEADMaxB.PC1 + HEADMaxSpeed.PC1 + ScaledMass	-6.83	3	27.65	2.76	0.054
STRIPESMaxB.PC1	-10.78	1	28.05	3.16	0.044
HEADMaxB.PC1 + HEADMaxD.PC1 +	-7.10	3	28.19	3.30	0.041
HEADMaxB.PC1 + BODYMaxB.PC1	-7.65	3	29.30	4.41	0.023

Model variables and model statistics as described in Table S5.

APPENDIX E

DEFEATED CHAMELEONS DARKEN DYNAMICALLY DURING DYADIC DISPUTES TO DECREASE DANGER FROM DOMINANTS

Behav Ecol Sociobiol (2014) 68:1007–1017
DOI 10.1007/s00265-014-1713-z

ORIGINAL PAPER

Defeated chameleons darken dynamically during dyadic disputes to decrease danger from dominants

Russell A. Ligon

Received: 22 January 2014 / Revised: 15 March 2014 / Accepted: 17 March 2014 / Published online: 17 April 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Research on intraspecific aggression has typically focused on dominant individuals, but a better understanding of the consequences and mechanisms of agonistic encounters requires a balanced perspective that includes knowledge of subordinate animal behaviors. In contrast to signals of fighting ability, signals of submission are an understudied component of agonistic communication that could provide important insights into the dynamics, function, and evolution of intraspecific competition. Here, I use a series of staged agonistic trials between adult male veiled chameleons *Chamaeleo calyptratus* to test the hypothesis that rapid skin darkening serves as a submissive signal to resolve agonistic activity. Concordant with this hypothesis, I found that losing chameleons darkened over the course of aggressive trials while winners brightened, and the likelihood of darkening increased when individuals were attacked more aggressively. Additionally, I found that the degree of brightness change exhibited by individual chameleons was tied to both overall and net aggression experienced during a trial, with chameleons who received high levels of aggression relative to their own aggression levels darkening to a greater extent than individuals receiving relatively less aggression. Lastly, I found that aggression increased for losers and winners prior to the onset of darkening by the eventual loser but that both chameleons reduced aggression after the losing chameleon began to darken. Based on the theoretical prediction that signals of submission should be favored when retreat options are restricted, I suggest that limited escapability imposed by chameleon morphology, physiology, and ecology favored the evolution of a pigment-based signal of submission in this group.

Keywords Submissive signals · Physiological color change · Aggression · Communication · Reptiles · *Chamaeleo calyptratus* · Color signals

Introduction

Despite a long history of research into the function and evolution of aggressive interactions and signaling in animals (Bradbury and Vehrencamp 1998), the majority of such research has been heavily focused on characteristics associated with "winning" competitive encounters (e.g., 1,314 papers related to "winning" compared to 482 related to "losing" in a recent literature search, Supplementary Material 1). Because selection should favor the development, growth, and elaboration of traits that increase the likelihood of success during antagonistic events, the focus on "winning" traits makes intuitive sense, but this line of research typically ignores half of the aggressive equation—the losing half. Losing a single encounter does not preclude future success for an individual in many cases (e.g., females of numerous species do not exhibit preferences for dominant males; Qvarnstrom and Forsgren 1998), and many organisms have evolved traits and strategies to mitigate the short- and long-term costs associated with defeat. One such strategy is the adoption of submissive or appeasement behaviors (Lorenz 1966), which an animal performs to signal nonaggressive intentions and inhibit additional aggression from the winning individual. Submissive behaviors or signals vary widely across taxa (Koutnik 1980; East et al. 1993; Issa and Edwards 2006; Van Dyk and Evans 2008), but are typically given when an

---

Communicated by E. Fernandez-Juricic

Electronic supplementary material The online version of this article (doi:10.1007/s00265-014-1713-z) contains supplementary material, which is available to authorized users.

R. A. Ligon (✉)
School of Life Sciences, Arizona State University, Tempe, AZ 85281, USA
e-mail: russell.ligon@gmail.com

animal is unlikely to win an aggressive encounter and further interaction with the competitor is not beneficial. Because unnecessary expenditure of time and energy is costly, natural selection should favor dominant animals that recognize and respond to signals of submission as well as subordinate animals that give such signals when continued effort is unlikely to yield any reward (Matsumura and Hayden 2006).

Notwithstanding the apparent value of submissive signals in mitigating unnecessary costs during aggressive interactions, there are conceivably simpler ways, such as fleeing, for losers to end aggressive interactions. Therefore, signals of submission are likely to evolve only under particular circumstances, outlined by a game theory model developed by Matsumura and Hayden (2006). First, signals of submission are more likely to evolve when the costs of injury are similar to the value of the contested resource (e.g., food, territory, access to mates). If, however, the resource value-to-cost ratio of a fight is sufficiently high, combatants will escalate and prolong aggressive encounters simply because *any* chance of winning makes continued aggression worthwhile (signals of submission are unlikely to be given in these situations; Enquist and Leimar 1990). Second, signals of submission should be favored when winners do not gain additional benefits from winning prolonged or escalated fights (compared to winning abbreviated contests). If winners do not gain additional benefits from prevailing in lengthy contests, then they will be more likely to recognize and respond to honest signals of submission. Third, signals of submission should be favored when losers have limited ability to rapidly or safely retreat. It is assumed that there is a small risk in signaling submission, and the benefits of such signaling only outweigh the risk if there is no simple alternative (i.e., if fleeing is dangerous or difficult). Lastly, signals of submission should be favored when the ability of combatants to estimate the resource holding potential of opponents is good, but not perfect.

Few studies have explicitly tested these theoretical predictions regarding when submissive signals should be given and the social environments that favor the use of such signals: However, several recent studies have begun to explore signals of submission in their own right. As predicted, signals of submission can reduce costs associated with aggressive interactions (Issa and Edwards 2006), and subordinate individuals are more likely to signal submission when they receive higher levels of aggression or are of lower rank (O'Connor et al. 1999; Höglund et al. 2000; Batista et al. 2012). Additionally, it appears that signals of submission can, in some cases, be modulated to maximize detection (Eaton and Sloman 2011), perhaps because it is vitally important to have such signals recognized. In concert with theoretical predictions, such empirical findings provide a basic framework for the social and environmental contexts in which signals of submission are likely to be most valuable, though specific predictions concerning the dynamics of submissive signals will likely vary among species.

Although the relative importance of the factors predicted by Matsumura and Hayden (2006) to favor the evolution of submissive signals is unknown, the need for unambiguous signals of submission seems particularly important when losing animals have limited ability to rapidly or safely retreat. Morphological, physiological, or environmental constraints that limit the escapability of contestants should all favor the evolution of distinctive signals of submission, which would allow rapid de-escalation of intense physical aggression without requiring the immediate physical and spatial separation created by fleeing. Chameleons provide an ideal study system for testing this idea, given their conspicuous and dynamic color changes in social settings (Stuart-Fox and Moussalli 2008; Ligon and McGraw 2013) and their limited mobility (Peterson 1984; Abu-Ghalyun et al. 1988; Fischer et al. 2010). Here, I investigate the use of a rapid physiological color change as a social signal of submission by veiled chameleons *Chamaeleo calyptratus*. Individuals of this species, like chameleons in general (Peterson 1984; Nečas 1999; Fischer et al. 2010), possess a suite of adaptations that allow them to utilize habitats characterized by small branches in bushes or tree canopies. These adaptations, including lateral body compression (Nečas 1999), a modified shoulder girdle (Peterson 1984; Fischer et al. 2010), and increased density of tonic muscle fibers (Abu-Ghalyun et al. 1988), coupled with the heterogeneous, spatially dispersed, narrow perch options that characterize the habitats chameleons tend to inhabit, combine to limit a losing individual's ability to rapidly escape danger from aggressive conspecifics during antagonistic encounters. Additionally, chameleons are highly visual animals (Harkness 1977; Ott and Schaeffel 1995; Nečas 1999; Bowmaker et al. 2005) that rely on complex chromatic signals to modulate aggressive interactions (Ligon and McGraw 2013) and signal reproductive status (Cuadrado 2000). Despite abundant evidence that several species of chameleons assume darker coloration after losing aggressive encounters (Bustard 1965, 1967; Nečas 1999; Stuart-Fox 2006; Stuart-Fox et al. 2006; Karsten et al. 2009), no empirical study had yet been conducted to assess darkening as a social signal of submission in this group. I hypothesized that rapid darkening of individual chameleons serves as a signal of submission indicating cessation of aggression (on the part of the submissive animal) and reducing aggression from non-darkening (winning) individuals. Therefore, I predicted that (i) skin darkening would be more frequent among losers than winners, (ii) intensity of opponent aggression would increase the likelihood and degree of darkening, and (iii) skin darkening would reduce aggression received from dominant individuals (sensu O'Connor et al. 1999). To test these predictions, I staged a series of agonistic encounters between 40 captive, adult male veiled chameleons. From each of these trials, I recorded the timing of all aggressive (e.g., bites, lunges, lateral displays) and submissive (e.g., avoidance, retreats) behaviors, the initial and final coloration displayed by each chameleon, and the onset of skin darkening to determine whether rapid darkening is associated with submissive behavior and with a subsequent reduction in aggression by the opponent.

Methods

Study species

Veiled chameleons are large, omnivorous, territorial lizards native to the mountainous regions of southwest Arabia (Nečas 1999) and are an ideal species in which to examine complex color change signals because they exhibit rapid, body-wide chromatic changes during intraspecific interactions (Kelso and Verrell 2002; Ligon and McGraw 2013). Like many chameleon species, male veiled chameleons frequently display intense antagonistic behavior toward conspecific males, probably to defend territories or females (sensu Cuadrado 2001). Upon seeing another adult male, veiled chameleons typically begin an elaborate display that encompasses both morphological and colorimetric transformations (Nečas 1999; Ligon and McGraw 2013). Aggressive males rapidly brighten undergo lateral compression of the body, rapid expansion along the dorsoventral axis, and a curling of the tail into a disk-like shape. At any point during the interaction, either chameleon can cease aggressive behaviors and begin to retreat. Based on personal observations, retreat behavior frequently seems to be temporally linked with darkening by the retreating individual (Supplementary video). If neither chameleon retreats after both males have begun to display toward one another, these interactions can escalate to physical violence including head-butting, lunging, and biting.

Housing

I studied 40 adult male veiled chameleons that were obtained from a private breeder and a feral population in Florida, USA. During the course of this study, chameleons were housed individually in visually isolated cages (89×56×53 cm) with screen roofs and doors. Cages were located within a temperature-controlled (26±2 °C) vivarium at Arizona State University. All cages contained live, dead, and artificial plants for climbing and shelter, and were misted four times daily. Additionally, each cage was equipped with a heat lamp (Zoo Med Repti-Basking Spot Lamp, 50 watt, Zoo Med Laboratories Inc., San Luis Obispo, CA, USA) and a UV light source (Zoo Med Reptisun 5.0 UVB Fluorescent Bulbs, Zoo Med Laboratories Inc.). Room lights were set to a 14:10-h light/dark schedule and cage lights turned on 30 min after and turned off 30 min before room lights to mimic dawn and dusk.

Insect food items (including crickets, cockroaches, caterpillars, and mealworms) were dusted with supplements (Rep-Cal Phosphorus-free Calcium, 0 % D3, Rep-Cal Research Labs, Los Gatos, CA, USA and Zoo Med Reptivite Reptile Vitamins, Zoo Med Laboratories Inc., San Luis Obispo, CA, USA) and provided to chameleons on alternate days.

Trial setup

In summer 2012, I staged 79 aggression trials between pairs of chameleons over a 9-week period. After measuring body mass (to the nearest 1 g, with a digital scale), I placed chameleons on opposite, visually isolated ends of a trial arena (183×53×81 cm) and allowed them to acclimate for 5 min before the central divider was removed and the trial begun. Each side of the trial arena had a vertical perch (66 cm) located away from the center of the arena with plastic foliage at the top, a horizontal perch (80 cm) extending toward the center of the arena, and a second vertical perch (40 cm) near the center (Fig. 1). Plastic foliage gave the chameleons a place to partially hide, the horizontal perches (and the floor of the arena) allowed chameleons to approach one another, and the vertical perches provided avenues for approach or escape. Trials were recorded from behind a blind with two Panasonic HDC-TM 700 video cameras, with one camera focused on each chameleon. Chameleons were allowed to interact with one another for 15 min or until one chameleon retreated more than once during the trial. Chameleons involved in each contest were always unfamiliar with one another, and most (n=37) chameleons appeared in four trials. However, due to logistical constraints, one chameleon participated in only two trials, one chameleon participated in three trials, and one chameleon in five trials.

Behavior

Initial observations of veiled chameleons during agonistic encounters revealed that many of the behaviors previously

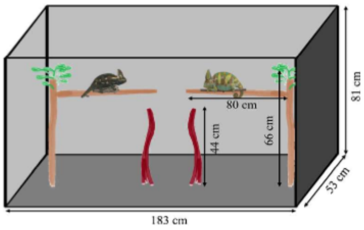


Fig. 1 Schematic of trial arena used during aggressive interactions. Chameleons were visually isolated from one another by an opaque divider in the middle of the arena (not shown) during a 5-min acclimation period before the divider was removed and the trial commenced. Chameleons are not shown to scale

Table 1 Descriptions and ranking of aggressive and submissive behaviors displayed by adult male veiled chameleons during agonistic encounters

Behavior	Description	Aggression score
Knock opponent off perch	Chameleon aggressively dislodges opponent from perch	5
Bite-release	Biting followed by immediate release of opponent	5
Bite-clamp	Sustained biting (locked on to opponent with mouth)	5
Attack	Initiation of physical contact	5
Fighting	Physical contact and intent to bite or displace opponent	5
Lunge	Fast, directed head or body thrust toward opponent	4
Approach	Directed movement toward opponent	4
Lateral display	Lateral compression, dorsoventral expansion, physical orienting of body perpendicularly to opponent	3
Swaying	Lateral, side-to-side movement of entire body	2
Head bob	Rhythmic movement of head up and down	1
Tail curl	Tail curled and uncurled	1
Retreat	Directed movement away from opponent	−4
Flee	Rapid, directed movement way from opponent	−5

Numeric values for each behavior displayed were summed for each individual for a given time period to compute an aggression score for that time period

described for the Madagascan chameleons *Furcifer labordi* and *Furcifer verrucosus* (Karsten et al. 2009) were frequently used in this study as well. Therefore, sensu Karsten et al. (2009), I recorded 13 aggressive and submissive behaviors of veiled chameleons during the course of each trial (Table 1). To determine overall aggression scores for individual chameleons, I ranked these behaviors according to their frequency, intensity, and apparent influence on contest outcome (sensu Karsten et al. 2009) and awarded chameleons the requisite number of points for each behavior. As in Karsten et al. 2009, aggressive displays and behaviors received positive values, with those that carried greater costs or risks receiving higher values (Table 1). For example, *head-bobbing* and *tail-curling* behaviors were exhibited only during aggressive displays but are presumed to represent lower escalation than *full body swaying*. Additionally, the behaviors that put chameleons at greater risk (such as approaching and attacking) received the highest values. Conversely, submissive behaviors that minimized risk and ended contests were given negative values (with *retreating* being approximately the opposite of *approaching*, and *fleeing* being the least aggressive behavior exhibited).

When examining aggressive behaviors statistically (see “Statistics”), I used the sum of the weighted aggressive behaviors (Table 1). In addition to scoring the behaviors exhibited by each chameleon during aggressive interactions, I classified “winners” and “losers” of each trial based on the behaviors displayed by the chameleons during the trial. Specifically, losing chameleons were those that retreated (exhibiting directed movement away from their opponent) at some point during the trial. In the 34 trials with a definitive outcome, only once did a chameleon approach and re-aggress his opponent following a retreat, giving me reasonable confidence in the use of this metric in differentiating “winners” and “losers.”

Photography and skin darkening

At the beginning and end of each trial, I scored the brightness of each chameleon using calibrated and linearized photographs (Stevens et al. 2007; Pike 2011). Following the methods of Bergman and Beehner (2008), photographs were equalized and linearized using a specialized color standard (ColorChecker Passport, X-Rite Photo) in conjunction with a software plug-in (PictoColor® inCamera™, PictoColor Software, Burnsville, MN, USA) for Adobe Photoshop (Adobe Systems Inc., San Jose, CA, USA). This plug-in allows users to create custom International Color Consortium (ICC) digital profiles from reference photographs containing the color standard and apply these profiles to all photographs taken under similar conditions.

I quantified the overall brightness of each chameleon by measuring the brightness of five body regions (Fig. 2) and averaging these values. Specifically, I summed the red, green, and blue (RGB) values obtained in Adobe Photoshop from a 5×5-pixel area within each body region. Summed RGB values explained 96 % of the variation in spectrophotometrically determined brightness values of the 24-color patches of the X-Rite color standard ($F_{1,\ 22}=590$, $R^2=0.96$, $p<0.001$), giving me a high degree of confidence that summed RGB values capture variation in overall brightness.

Changes in overall brightness were determined by subtracting the average brightness displayed by a given chameleon at the end of a trial from the average brightness of the same chameleon at the beginning of the trial. Thus, chameleons that had positive values for brightness change

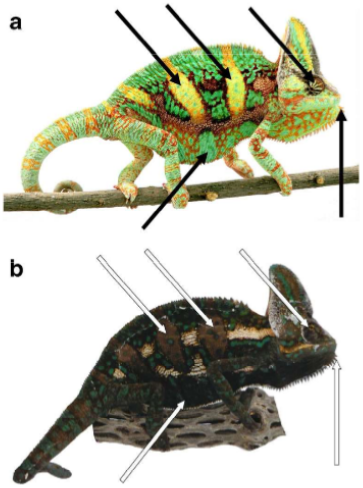


Fig. 2 Photographs illustrating the five body regions measured for brightness at the beginning and end of aggression trials. **a** A bright individual; **b** a darkened individual

brightened over the course of a trial and chameleons that exhibited negative color change values darkened. It should be noted that I only incorporated data from a given body region for each chameleon into the brightness calculations if that chameleon's opponent also had complete data for that body region. If, for example, individual "A" was partially obscured at the end of the trial and I could not measure his eye brightness at this time point, I would calculate his average beginning (and end) brightness without the eye brightness values. Additionally, when I calculated his opponent's brightness and brightness change, I would exclude data from the eye region so that simple differences in sampling regime could not induce any artificial differences between individuals or sampling time points.

Statistics

Darkening differences between winners and losers

To determine whether losing chameleons were more likely to darken than winning chameleons, I used a generalized linear mixed model with darkening as a binary response variable (i.e., did the chameleon darken or not), binomial error structure, and logit-link function (parameter estimates obtained via maximum likelihood). I included chameleon and trial identity as random effects in this model to avoid pseudoreplication and the problems associated with non-independence of the behavioral responses of two individuals engaged in a given dyadic encounter (Briffa et al. 2013). Additionally, I used paired *t* tests to analyze overall differences in brightness change between winners and losers (normality of color data assessed with Shapiro–Wilk tests).

Opponent aggression and darkening

To examine the relationship between opponent aggression and the likelihood of darkening, as well as the relationship between differential aggression (aggression directed at an opponent minus aggression received from an opponent) and likelihood of darkening, I used generalized linear mixed models with darkening as a binary response variable, binomial error structures, and logit-link functions (parameter estimates obtained via maximum likelihood). I included chameleon and trial identity as random effects in both models. I also evaluated the relationship between both (i) opponent aggression and (ii) differential aggression and the overall brightness change exhibited during each trial using linear mixed effects models fitted using restricted maximum likelihood and including chameleon and trial identity as random effects.

Temporal changes in aggression relative to darkening

Because I was also interested in how aggression changed over time relative to the onset of darkening, I watched behavioral trials and recorded the time when an individual began to visibly darken, using this time point as the "onset of darkening." The efficacy of this method for identifying the onset of darkening was validated in a subset of trials ($n=5$) where I quantitatively evaluated mean brightness values before and after the onset of darkening (Supplementary Material 2). Following identification of the onset of darkening, I investigated changes in aggression over time used a repeated-measures mixed model, with chameleon and trial identity as random effects, using restricted maximum likelihood and an autoregressive covariance structure (degrees of freedom calculated with the Kenward–Roger approximation). Specifically, I used this model to examine the total aggressive behaviors exhibited during four successive 60-s periods beginning 120 s before the onset of darkening by the loser and lasting until 120 s after darkening began. I chose these time periods because my observations suggested that most salient variation in aggressive behavior typically occurred just prior to and immediately after contest outcome was determined, typically the point at which one chameleon began to darken. This repeated-measures mixed model was conducted on a subset of 28 trials that had a definitive winner and loser and where the loser darkened at some point during the trial (i.e., darkening was required to examine changes in aggression relative to darkening).

Data analyzed and programs used

Mixed model analyses were conducted on the subset of the original trials with definitive outcomes ($n=34$), and the paired t tests evaluating differences between winners and losers with respect to color change were conducted on a subset of 15 trials wherein no individual was represented more than once as a winner or loser. The repeated-measures mixed model was run using PROC GLIMMIX in SAS 9.2 (SAS Institute Inc., Cary, NC, USA), and all other statistical analyses were conducted in the R computing environment (R Development Core Team 2011).

Results

Darkening differences between winners and losers

I found that losing chameleons darkened more often (28 of 34 trials) than winning chameleons (11 of 34 trials) and that whether a chameleon won or lost a fight had a significant influence on his likelihood of darkening (Table 2, odds ratio=14.95). This odds ratio means that losing chameleons were almost 15 times more likely to darken than winners. Additionally, when evaluating the subset of trials ($n=15$) with definitive outcomes and in which no individual was represented more than once as a winner or loser, I found that winners tended to brighten (average change in brightness=47.13) and losers tended to darken (average change in brightness=−38.88) over the course of a trial (Fig. 3). This difference in color change between winners and losers was significant ($t_{14}=-4.16$, $p<0.001$).

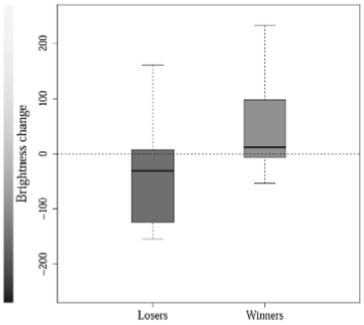


Fig. 3 Box and whiskers plot showing a statistically significant difference in brightness changes between winning and losing veiled chameleons after agonistic interactions. Positive values indicate brightening, and negative values indicate darkening. *Bold horizontal lines* represent median values, while *lower and upper boundaries of the boxes* represent the first and third quartiles, respectively. *Whiskers* indicate the entire span of the data

Opponent aggression and darkening

The probability of a chameleon darkening over the course of an aggressive interaction increased with the total aggression that chameleon received (Table 2; Fig. 4a), but only if two chameleons that were involved in an escalated interaction in which they both were highly aggressive and neither darkened were excluded (with these individuals, parameter estimate=0.01, SE=0.01 $p=0.22$, $z=1.24$). Additionally, the differential aggression experienced by a chameleon was negatively related to observed brightness changes, such that chameleons who exhibited numerous aggressive behaviors but had few directed toward them were less likely to darken than chameleons who received more aggression than they exhibited (Table 2; Fig. 4b).

I also found a significant, negative relationship between opponent aggression and the extent of brightness change over the course of the trial (Table 2; Fig. 5a), though this relationship held only when excluding the two chameleons involved in the previously mentioned, highly aggressive interaction (with outliers included, parameter estimate=−0.72, SE=0.49, $F_{1,34}=2.17$, $p=0.15$). This negative relationship suggests that chameleons darkened more when facing highly aggressive

Table 2 Summary output from generalized linear mixed models evaluating the factors influencing the likelihood of darkening and the degree of brightness change by male veiled chameleons during agonistic encounters

Response variable	Fixed effect (explanatory variable)	Random effects	Estimate	SE	Test statistic	p
Likelihood of darkening	Win/loss outcome	ID, trial	−2.70	0.62	$z=-4.34$	**<0.0001**
	Opponent aggression	ID, trial	0.48	0.02	$z=2.79$	**0.005**
	Differential aggression	ID, trial	−0.04	0.01	$z=-3.24$	**0.001**
Change in brightness	Opponent aggression	ID, trial	−1.98	0.54	$F_{1,32}=13.48$	**0.0009**
	Opponent aggression (winners only)	ID	−0.21	0.62	$F_{1,16}=0.11$	0.74
	Opponent aggression (losers only)	ID	0.49	0.61	$F_{1,10}=0.64$	0.44
	Differential aggression	ID, trial	2.21	0.43	$F_{1,33}=26.81$	**<0.0001**
	Differential aggression (winners only)	ID	0.64	0.89	$F_{1,16}=0.52$	0.48
	Differential aggression (losers only)	ID	1.20	0.87	$F_{1,10}=1.89$	0.20

Significant terms in bold

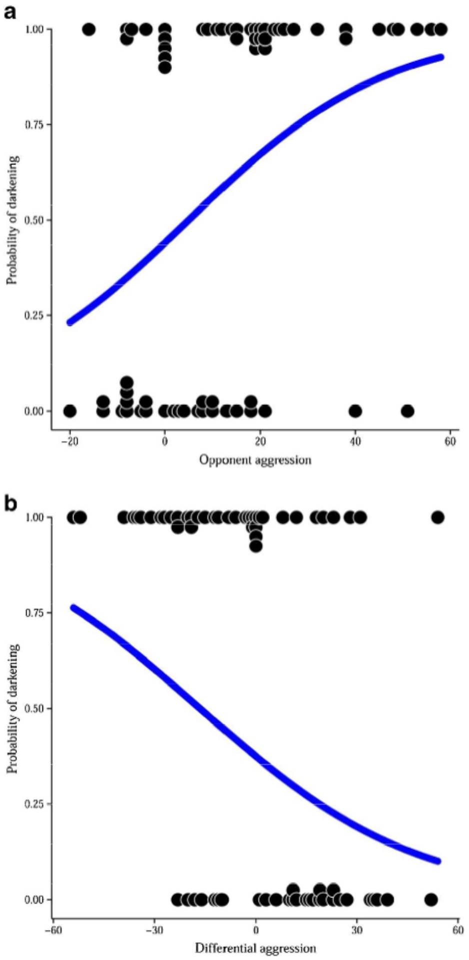


Fig. 4 Relationship between the probability of skin darkening for male veiled chameleons and **a** the aggression received over the course of agonistic interactions and **b** differential aggression scores (aggression exhibited minus opponent aggression). *Solid circles* indicate aggression score and darkening result (1=yes, 0=no) for each individual, while the *blue line* is the resulting probability estimate based on these data. Each chameleon either did or did not darken, but overlapping data points are offset for clarity

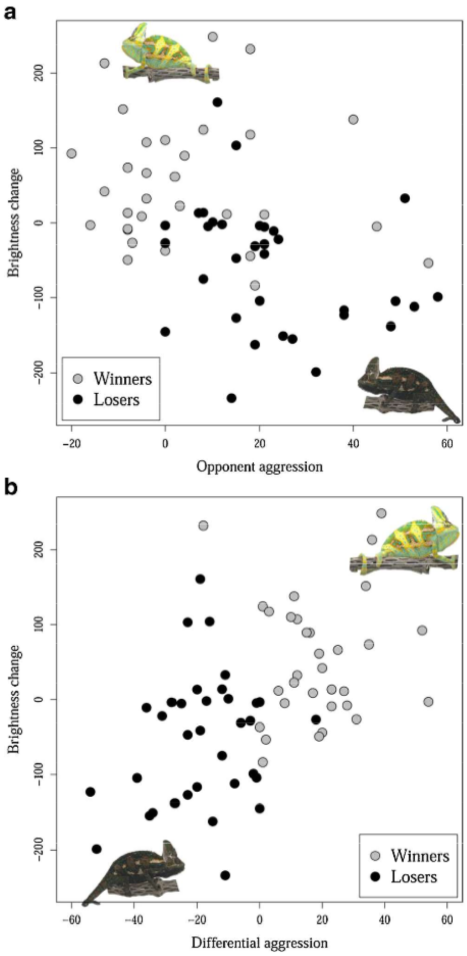


Fig. 5 Relationship between brightness change and **a** opponent aggression and **b** differential aggression (directed minus received) and during agonistic encounters between adult male veiled chameleons. Using generalized linear mixed models, these relationships were significant when considering winners and losers together, but not when evaluating winners or losers separately. See "Results" for details

opponents. However, opponent aggression did not have a significant influence on changes in brightness when evaluating winners or losers separately (Table 2). Additionally, there was a positive relationship between differential aggression (directed minus received) and the extent of brightness change over the course of the trial (Table 2). Specifically, those chameleons that were more aggressive than their opponents achieved higher differential aggression scores and became brighter over the course of a trial (Fig. 5b). Conversely, chameleons that received a great deal more aggression than they delivered (resulting in negative scores for differential aggression) became significantly darker over the course of a trial. However, the relationship between differential aggression and brightness change was not significant when evaluating losing or winning chameleons separately (Table 2).

Temporal changes in aggression relative to darkening

Using a repeated-measures mixed model, I found that aggression was significantly lower for losers (average least square means for aggression score=1.92±1.07) than for winners (average least square means $\bar{x}$= 6.45±1.07; $F_{1,\ 216}$=8.94, p= 0.004) and varied significantly over time ($F_{3,\ 216}$=6.31, p<0.001; Fig. 6). However, there was no significant effect of the interaction between time and winner/loser class on aggression ($F_{3,\ 216}$=0.78, p=0.51), with winners and losers showing similar changes in aggression over time. Post hoc analyses revealed that aggression scores increased from period 1 (lasting from 120 to 60 s before the onset of darkening; $\bar{x}$= 3.86±1.51) to period 2 (immediately prior to darkening; $\bar{x}$= 9.66±1.51; $F_{1,\ 216}$=7.34, p=0.008) and then decreased significantly in period 3, once darkening began ($\bar{x}$= 1.71±1.51; $F_{1,\ 216}$=13.76, p<0.001). However, aggression levels did not change between period 3 (darkening) and period 4 (post-darkening; $\bar{x}$= 1.50±1.51; $F_{1,\ 216}$=0.01, p=0.92). All aggression values reported are least squares means values.

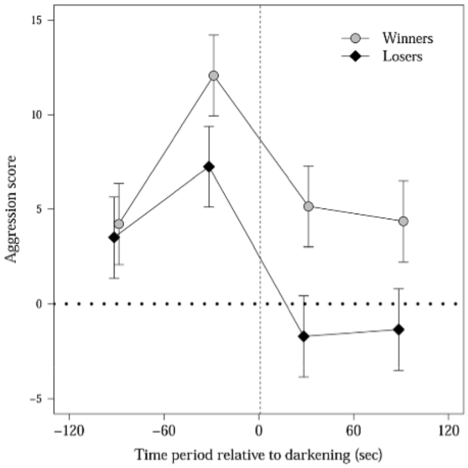


Fig. 6 Average aggression scores (± SE) for winners and losers of encounters between adult male veiled chameleons during four successive 60-s time periods, relative to the onset of darkening by the loser (time=0). Aggression was significantly different between winners and losers, and among the different time periods, but there was no significant interaction between time and winner/loser class. Plotted points represent least squares means for aggression during each 60 sec time period

Discussion

This study provides correlational evidence that rapid darkening during agonistic encounters between male veiled chameleons serves as a signal of submission, indicating a swift cessation of aggression from the darkening individual and leading to a marked reduction in aggression from the dominant competitor. Additionally, losing chameleons were more likely to darken than winning chameleons and the probability of darkening was linked to opponent aggression (both total and differential). Furthermore, there was a significant relationship between opponent aggression (again, both total and differential) and the degree of brightness change exhibited by chameleons. In conjunction with recent findings relating to the importance of chromatic changes for signaling motivation and fighting ability in this species (Ligon and McGraw 2013), these results support the idea that veiled chameleons rely on rapid changes in color and brightness to communicate with conspecifics and to minimize the costliness of aggressive interactions through rapid color change signals at both the beginning *and* end of agonistic encounters.

Rapid changes in skin brightness appear to represent an important signal in veiled chameleons, where chameleons less likely to win an aggressive encounter (based on opponent aggression and differential aggression) are more likely to darken, while those chameleons that are more likely to win are also more likely to brighten (this study) or achieve higher maximum brightness (Ligon and McGraw 2013). It is interesting to note, however, that while overall changes in brightness are associated with contest dynamics (i.e., differential aggression) and opponent behavior (i.e., opponent aggression), darkening does not appear to represent a graded signal of submission for losing chameleons. That is, among losing chameleons, there were no significant relationships between the degree of darkening and either opponent aggression or differential aggression. Because submissive chameleons should benefit from unambiguous signals (i.e., not graded) of submission that result in the immediate cessation of directed aggression from dominant individuals, this finding makes intuitive sense—though it does conflict with the findings of O'Connor et al. (1999) and my own predictions based on those findings. Regardless, it remains true that all submissive chameleons did not darken to the same extent, and future work exploring the physiological (e.g., energetic requirements of pigment translocation) and ecological (e.g., appearance to predators) costs associated with rapid darkening will shed important light on the causes of the variation that appears to exist within this putative signal of submission. One logical place to begin such an investigation would be interactions such as the one I excluded when analyzing the influence of opponent aggression on darkening (see "Results" for statistical conclusions with and without these outliers). In this trial, the chameleons engaged in a uniquely escalated fight, and interestingly, both were still brighter at the end of the trial than at the beginning. Exploring the causes and consequences of extreme cases that do not fit the general trend could lend insight into the mechanisms of darkening as a signal of submission.

Though the results of the present study suggest that rapid skin darkening may serve as a signal of submission for veiled chameleons engaged in aggressive contests with conspecifics, additional work must be done to determine if darkening is, in fact, a signal of submission or is, instead, merely a physiological by-product of submission (i.e., a cue). For example, the fact that several winning chameleons also darkened over the course of aggressive interactions suggests that darkening could be related to shared physiological processes that may tend to manifest more strongly in losers than in winners (e.g., stress). Despite such a possibility, in this study I found strong evidence that darkening is followed by a reduction in opponent aggression. However, in the absence of an experimental manipulation of chameleon coloration and brightness, we cannot be sure that changes in opponent behavior arose as a response to skin darkening as opposed to, say, submissive behaviors associated with the color change. Future studies aimed at elucidating the specific signaling content of rapid darkening could artificially manipulate color and brightness of combative chameleons to determine if (i) the dark display coloration associated with submission is sufficient to reduce aggression from opponents and (ii) if individuals who perform aggressive behaviors in spite of their artificially manipulated submissive coloration receive more aggression from opponents (i.e., punishment costs). Social costs associated with dishonest signals may play a role in enforcing signal honesty (e.g., Molles and Vehrencamp 2001; Van Dyk and Evans 2008), which is an important consideration when investigating how this putative signal evolved.

Though selection typically favors individuals possessing the attributes that increase the likelihood of winning an aggressive encounter, every definitive dyadic contest creates a "loser." For many vertebrates, losing such a contest can have substantial, immediate effects on behavior and physiology (Hannes et al. 1984; Huhman et al. 1992; Schuett et al. 1996; Höglund et al. 2000), and the prevalence of losing has likely favored the evolution of mechanisms to minimize the consequences of such losses. While short-term responses to losing can decrease the time needed for recovery (e.g., through increased respiration rates; Huntingford et al. 1995) or minimize additional risk (via signals of submission; O'Connor et al. 1999; Issa and Edwards 2006, this study), longer-term responses (such as the ability to recognize conspecifics with whom an individual has previously interacted; Forster et al. 2005; Stuart-Fox and Johnston 2005) can also reduce the costs of future interactions with that individual. In many cases, it appears that losing individuals that recognize previously dominant opponents save time and energy, while reducing risk, by simply submitting more quickly than when faced with unfamiliar opponents (e.g., O'Connor et al. 2000). Although it is currently unknown whether chameleons possess the ability to recognize previous opponents, it seems likely that the strength of the graded signals displayed by chameleons during an initial contest may inform subsequent interactions, though this possibility has not yet been tested in any taxon.

While the results of the current study suggest that rapid skin darkening serves as a signal of submission in veiled chameleons, the question of why a signal of submission may have evolved in veiled chameleons in the first place remains unanswered. To explore this question, I revisit the theoretical predictions of Matsumura and Hayden (2006) which state that signals of submission should evolve when, among other things, losing animals have limited ability to quickly and safely escape their attackers. I suggest that submissive color change signals evolved in veiled chameleons because their specialized arboreality (utilizing small branches in bushes and forest canopies) and slow-moving lifestyle severely restricts their ability to rapidly and safely flee from dominant individuals. Because most chameleons are predominantly arboreal (Nečas 1999), the majority of aggressive, territorial interactions between adult males are likely to occur well-above the ground. Despite the numerous morphological adaptations of chameleons to tree-living, including fused digits, laterally compressed bodies, prehensile tails, prevalence of tonic muscle fibers, and skeletal structures, small-branch arboreality imposes significant locomotor limitations (Peterson 1984; Abu-Ghalyun et al. 1988; Fischer et al. 2010), and rapid movement through the branches away from an opponent is not likely a viable option for most chameleons. Additional support for the idea that limited escape opportunities favor the evolution of submissive signals can be found in the life history of the weakly electric fish *Gymnotus omarorum*, which relies on electrical impulses to signal subordinate status (Batista et al. 2012). This species lives in a rapidly changing environment where optimal habitats are occasionally restricted to small patches within lakes, thereby increasing population density and reducing the opportunities for subordinate animals to escape dominant individuals (G. Batista and A. Silva, personal communication). In the case of both chameleons and electric fish, the inability to escape may have been a key factor favoring the evolution of signals of submission serving to limit aggression from dominant individuals.

Whenever selection pressures favor the evolution of submissive signals, there are undoubtedly a number of factors that influence signal design. So why did rapid skin darkening per se evolve as the signal of submission in chameleons? It may be that preexisting dermal structures (chromatophores) associated with darkening, which likely evolved for non-signaling functions (e.g., thermoregulation; Burrage 1973; Walton and Bennett 1993), were simply co-opted for use in signaling situations because body-wide darkening consistently provides maximum contrast, making this signal unambiguous. Alternatively, darkening may have evolved as a signal of submission because it is essentially the opposite of the changes exhibited by antagonistic chameleons, which frequently undergo dramatic skin brightening when displaying aggression toward opponents (Stuart-Fox and Moussalli 2008; Ligon and McGraw 2013). Darwin's (1872) principle of antithesis suggests that animals (including humans) frequently rely on behavioral displays occupying opposite ends of a spectrum to convey opposite meanings. Hurd et al. (1995) followed up on this concept, using neural network simulations to show that coevolution of two, simultaneously evolving signals in a system driven by perceptual drive "will act to draw evolving patterns away from each other and other stimuli." In fact, these authors found that two stimuli evolved "not just to be different but to be each other's opposite." The idea that losing chameleons should appear as different from aggressive chameleons as possible to create an unambiguous signal of submission is consistent with both Darwin's verbal argument and the mathematically based neural network model of Hurd et al. (1995). In addition to rapid skin darkening, losing chameleons de-emphasize body size by elongating their bodies and straightening their tails (Bustard 1967, personal observation), the effect of which is to appear thin and small. This appearance stands in sharp contrast to aggressively displaying chameleons, which expand along the dorsoventral axis and curl their tails, maximizing their apparent body size.

Though losing chameleons were significantly more likely to darken than winners in this study, winning chameleons also darkened with some regularity over the course of staged agonistic encounters (in 11 of 34 trials with a distinct winner and loser). While changes in stress hormone profiles often differ between winners and losers of aggressive interactions, with losers of one-time interactions frequently exhibiting higher plasma concentrations of the hormones associated with stress (e.g., glucocorticoids, corticotropins; Hannes et al. 1984; Huhman et al. 1992; Schuett et al. 1996; Höglund et al. 2000), winners also frequently experience short-term spikes in stress hormones (Hannes et al. 1984; Øverli et al. 1999; Summers et al. 2005). If skin darkening of chameleons is under hormonal control and is associated with the stress response (Höglund et al. 2000), then post-conflict skin darkening could arise in winners as a result of conflict-induced increases in stress. In fact, evidence suggests that physiological color change of chameleons is under both hormonal and nervous system control (Hogben and Mirvish 1928; Okelo 1986), so shared pathways involving the pituitary peptide alpha-melanocyte-stimulating hormone (α-MSH; the most potent naturally occurring melanin-dispersing agent known; Eberle 1988) and stress may indeed be involved in the darkening occasionally exhibited by winners. However, the time scale of darkening among winners, in that they typically darken only *after* contest outcome has been determined, suggests that the intraspecific signaling function of post-fight darkening of winners is of limited communication value. Undoubtedly, new insights into the function and evolution of skin darkening in both winning and losing veiled chameleons will be gained once experimental manipulations of color and empirical quantification of circulating hormones are performed to better understand the mechanistic bases of skin darkening during aggressive interactions and the role of color, separate from the confounding influences of behavior, in mediating these conflicts.

Ethical standards

All trials and procedures described in this manuscript were approved by the Arizona State University Institutional Animal Care and Use Committee (Protocol #10-1124R) and comply with the current laws of the USA. Although aggressive interactions sometimes included head-butting, lunging, and biting, most encounters were decided prior to physical contact or very shortly thereafter. However, as an added precaution, I employed a "chameleon safety rule" whereby any apparent risk of serious injury (biting and twisting, biting at an awkward angle) was cause to separate animals and terminate the trial. Throughout the 79 aggressive trials, no such interruption was required and no serious injuries occurred.

Acknowledgments I thank Megan Best, Sarah Bruemmer, Brianna Bero-Buell, and Andrea Carpenter for their invaluable assistance with experimental procedures, data collection, and dedicated chameleon care. I thank the Animal Behavior Society, the American Society for Ichthyologists and Herpetologists, the ASU chapter of Sigma Xi, and the ASU Graduate and Professional Students Association for financial support of this research. I also thank my parents, David and Sandy Ligon, as well as Richard and Janet Steele and two anonymous donors for their generous financial support to this project. I thank my advisor Kevin McGraw, Dale DeNardo, and members of the McGraw lab group for input that greatly improved this manuscript. I also thank my wife, Veronica Ligon, for her support throughout this project.

References

Abu-Ghalyun Y, Greenwald L, Hetherington TE, Gaunt AS (1988) The physiological basis of slow locomotion in chamaeleons. J Exp Zool 245:225–231

Batista G, Zubizarreta L, Perrone R, Silva A (2012) Non-sex-biased dominance in a sexually monomorphic electric fish: fight structure and submissive electric signalling. Ethology 118:398–410

Bergman TJ, Beehner JC (2008) A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (*Theropithecus gelada*). Biol J Linn Soc 94:231–240

Bowmaker JK, Lowe ER, Ott M (2005) The cone photoreceptors and visual pigments of chameleons. J Comp Physiol A 191:925–932

Bradbury J, Vehrencamp S (1998) Principles of animal communication. Sinauer, Massachusetts

Briffa M, Hardy ICW, Gammell MP, Jennings DJ, Clarke DD, Goubault M (2013) Analysis of animal contest data. In: Hardy ICW, Briffa M (eds) Animal contests. Cambridge University Press, New York, pp 47–85

Burrage BR (1973) Comparative ecology and behaviour of *Chamaeleo pumilus* (Gmelin) and *C. namaquensis* A. Smith (Sauria: Chamaeleonidae). Ann S Afr Mus 61:1–158

Bustard HR (1965) Observations on the life history and behavior of *Chamaeleo hohnelii* (Steindachner). Copeia 1965:401–410

Bustard HR (1967) The comparative behavior of chameleons: fight behavior in *Chameleo gracilis* Hallowell. Herpetologica 23:44–50

Cuadrado M (2000) Body colors indicate the reproductive status of female common chameleons: experimental evidence for the intersex communication function. Ethology 106:79–91

Cuadrado M (2001) Mate guarding and social mating system in male common chameleons (*Chamaeleo chamaeleon*). J Zool 255:425–435

Darwin C (1872) The expression of the emotions in man and the animals. Murray, London

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0, http://www.R-project.org/

East ML, Hofer H, Wickler W (1993) The erect 'penis' is a flag of submission in a female-dominated society: greetings in Serengeti spotted hyenas. Behav Ecol Sociobiol 33:355–370

Eaton L, Sloman KA (2011) Subordinate brown trout exaggerate social signaling in turbid conditions. Anim Behav 81:603–608

Eberle AN (1988) The melanotropins: chemistry, physiology and mechanisms of action. Karger, Switzerland

Enquist M, Leimar O (1990) The evolution of fatal fighting. Anim Behav 39:1–9

Fischer MS, Krause C, Lilje KE (2010) Evolution of chameleon locomotion, or how to become arboreal as a reptile. Zoology 113:67–74

Forster GL, Watt MJ, Korzan WJ, Renner KJ, Summers CH (2005) Opponent recognition in male green anoles, *Anolis carolinensis*. Anim Behav 69:733–740

Hannes RP, Franck D, Liemann F (1984) Effects of rank order fights on whole-body and blood concentrations of androgens and corticosteroids in the male swordtail (*Xiphophorus helleri*). Z Tierpsychol 65: 53–65

Harkness L (1977) Chameleons use accommodation cues to judge distance. Nature 267:346–349

Hogben LT, Mirvish L (1928) The pigmentary effector system. V. The nervous control of excitement pallor in reptiles. J Exp Biol 5:295–308

Höglund E, Balm PH, Winberg S (2000) Skin darkening, a potential social signal in subordinate artic charr (*Salvelinus alpinus*): the regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides. J Exp Biol 203:1711–1721

Huhman KL, Moore TO, Mougey EH, Meyerhoff JL (1992) Hormonal responses to fighting in hamsters: separation of physical and psychological causes. Physiol Behav 51:1083–1086

Huntingford FA, Taylor AC, Smith IP, Thorpe KE (1995) Behavioural and physiological studies of aggression in swimming crabs. J Exp Mar Biol Ecol 193:21–39

Hurd PL, Wachtmeister CA, Enquist M (1995) Darwin's principle of antithesis revisited: a role for perceptual biases in the evolution of intraspecific signals. Proc R Soc Lond B 259:201–205

Issa FA, Edwards DH (2006) Ritualized submission and the reduction of aggression in an invertebrate. Curr Biol 16:2217–2221

Karsten KB, Andriamandimbiarisoa LN, Fox SF, Raxworthy CJ (2009) Social behavior of two species of chameleons in Madagascar: insights into sexual selection. Herpetologica 65:54–69

Kelso EC, Verrell PA (2002) Do male veiled chameleons, *Chamaeleo calyptratus*, adjust their courtship displays in response to female reproductive status? Ethology 108:495–512

Koutnik DL (1980) Submissive signalling in mule deer. Anim Behav 28: 312–313

Ligon RA, McGraw KJ (2013) Chameleons communicate with complex colour changes during contests: different body regions convey different information. Biol Lett 9:20130892

Lorenz K (1966) On aggression. Methuen, London.

Matsumura S, Hayden TJ (2006) When should signals of submission be given?—a game theory model. J Theor Biol 240:425–433

Molles LE, Vehrencamp SL (2001) Songbird cheaters pay a retaliation cost: evidence for auditory conventional signals. Proc R Soc Lond B 268:2013–2019

Nečas P (1999) Chameleons: Nature's hidden jewels. Chimaira, Frankfurt

O'Connor KI, Metcalfe NB, Taylor AC (1999) Does darkening signal submission in territorial contests between juvenile Atlantic salmon, *Salmo salar*? Anim Behav 58:1269–1276

O'Connor KI, Metcalfe NM, Taylor AC (2000) Familiarity influences body darkening in territorial disputes between juvenile salmon. Anim Behav 59:1095–1101

Okelo O (1986) Neuroendocrine control of physiological color change in *Chameleo gracilis*. Gen Comp Endocrinol 64:305–311

Ott M, Schaeffel F (1995) A negatively powered lens in the chameleon. Nature 373:692–694

Øverli Ø, Harris CA, Winberg S (1999) Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. Brain Behav Evol 54:263–275

Peterson JA (1984) The locomotion of *Chamaeleo* (Reptilia: Sauria) with particular reference to the forelimb. J Zool (202: 1-42

Pike TW (2011) Using digital cameras to investigate animal colouration: estimating sensor sensitivity functions. Behav Ecol Sociobiol 65: 849–858

Qvarnstrom A, Forsgren E (1998) Should females prefer dominant males? Trends Ecol Evol 13:498–501

Schuett GW, Harlow HJ, Rose JD, Van Kirk EA, Murdoch WJ (1996) Levels of plasma corticosterone and testosterone in male copperheads (*Agkistrodon contortrix*) following staged fights. Horm Behav 30:60–68

Stevens M, Párraga CA, Cuthill IC, Partridge JC, Troscianko TS (2007) Using digital photography to study animal coloration. Biol J Linn Soc 90:211–237

Stuart-Fox D (2006) Testing game theory models: fighting ability and decision rules in chameleon contests. Proc R Soc Lond B 273:1555–1561

Stuart-Fox DM, Johnston GR (2005) Experience overrides colour in lizard contests. Behaviour 142:329–350

Stuart-Fox DM, Moussalli A (2008) Selection for social signalling drives the evolution of chameleon colour change. PLoS Biol 6:e25

Stuart-Fox DM, Firth D, Moussalli A, Whiting MJ (2006) Multiple signals in chameleon contests: designing and analysing animal contests as a tournament. Anim Behav 71:1263–1271

Summers CH, Korzan WJ, Lukkes JL, Watt MJ, Forster GL, Øverli O, Höglund E, Larson ET, Ronan PJ, Matter JM, Summers TR, Renner KJ, Greenberg N (2005) Does serotonin influence aggression? Comparing regional activity before and during social interaction. Physiol Biochem Zool 78:679–694

Van Dyk DA, Evans CS (2008) Opponent assessment in lizards: examining the effect of aggressive and submissive signals. Behav Ecol 19:895–901

Walton BM, Bennett AF (1993) Temperature-dependent color change in Kenyan chameleons. Physiol Zool 66:270–287

APPENDIX F

COAUTHOR PERMISSIONS FOR INCLUSION OF PUBLISHED WORKS

June 25, 2015

To whom it may concern,

I, Kevin McGraw, grant Russell Ligon permission to include, as part of his dissertation, our manuscript:

Ligon RA & KJ McGraw. 2013. Chameleons communicate with complex colour changes during contests: different body regions convey different information. Biology Letters. 9: 2013082.

Sincerely,

Kevin J. McGraw