

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

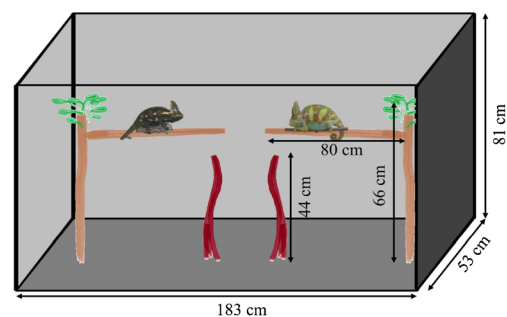


Fig. 1 Schematic of trial arena used during aggressive interactions. Chameleons were visually isolated from one another by an opaque divider in the middle of the arena (not shown) during a 5-min acclimation period before the divider was removed and the trial commenced. Chameleons are not shown to scale

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 away 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

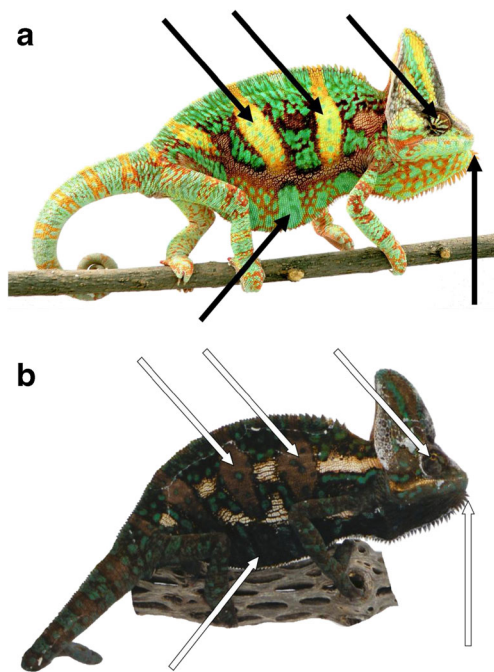


Fig. 2 Photographs illustrating the five body regions measured for brightness at the beginning and end of aggression trials. **a** A bright individual; **b** a darkened individual

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 ($t_{14}=−4.16$, $p<0.001$).

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

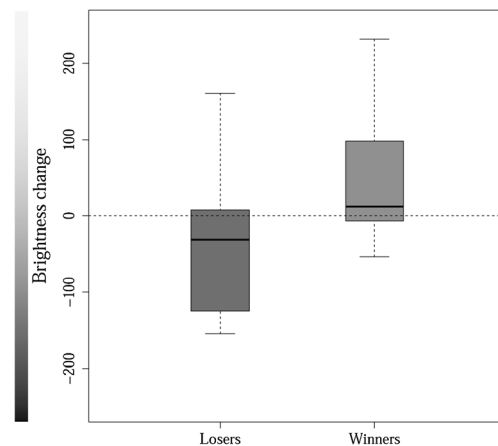


Fig. 3 Box and whiskers plot showing a statistically significant difference in brightness changes between winning and losing veiled chameleons after agonistic interactions. Positive values indicate brightening, and negative values indicate darkening. **Bold horizontal lines** represent median values,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

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	<i>p</i>
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

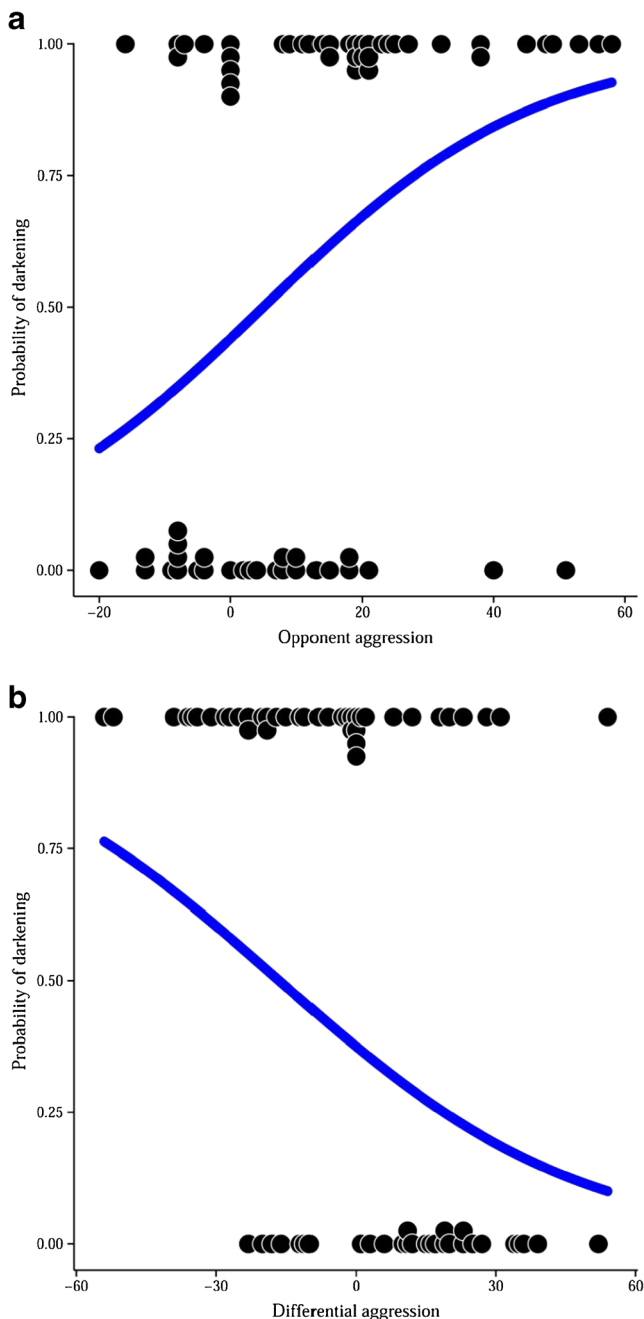


Fig. 4 Relationship between the probability of skin darkening for male veiled chameleons and **a** the aggression received over the course of agonistic interactions and **b** differential aggression scores (aggression exhibited minus opponent aggression). Solid circles indicate aggression score and darkening 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

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

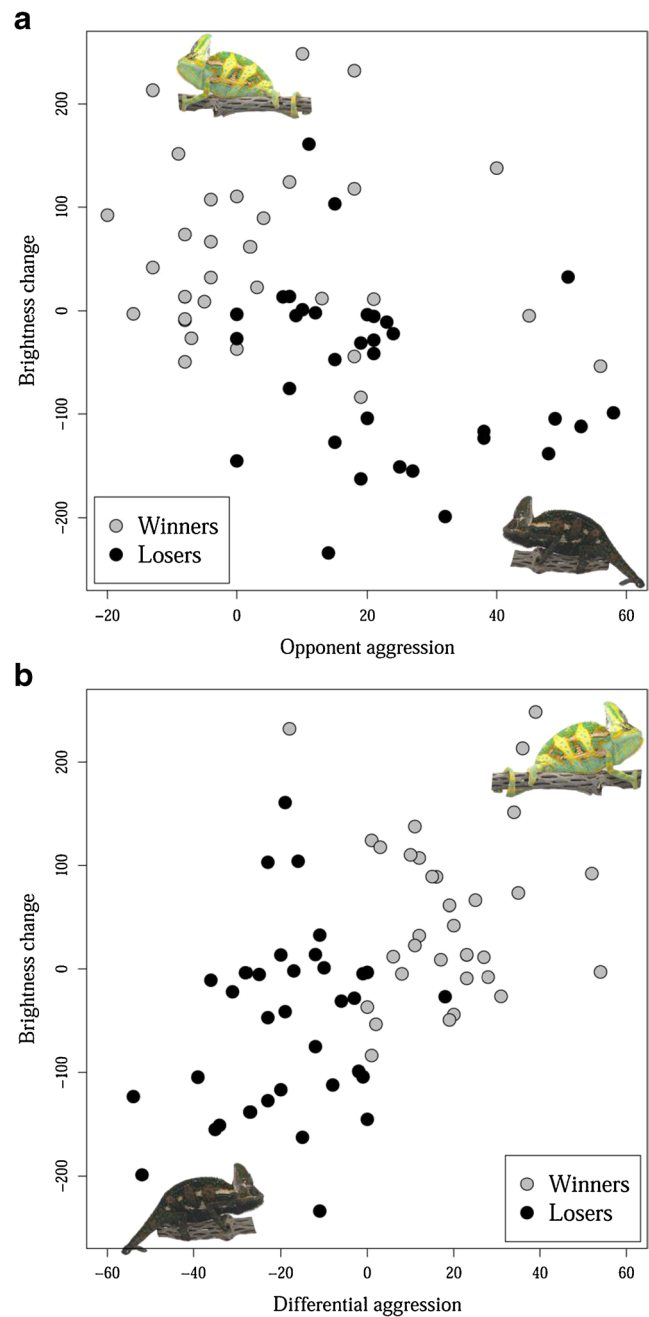


Fig. 5 Relationship between brightness change and **a** opponent aggression and **b** differential aggression (directed minus received) and during agonistic encounters between adult male veiled chameleons. Using generalized linear mixed models, these relationships were significant when considering winners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

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 $\bar{x} = 6.45 \pm 1.07$; $F_{1, 216} = 8.94$, $p = 0.004$) 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; $\bar{x} = 3.86 \pm 1.51$) to period 2 (immediately prior to darkening; $\bar{x} = 9.66 \pm 1.51$; $F_{1, 216} = 7.34$, $p = 0.008$) and then decreased significantly in period 3, once darkening began ($\bar{x} = 1.71 \pm 1.51$; $F_{1, 216} = 13.76$, $p < 0.001$). However, aggression levels did not change between period 3 (darkening) and period 4 (post-darkening; $\bar{x} = 1.50 \pm 1.51$; $F_{1, 216} = 0.01$, $p = 0.92$). All aggression values reported are least squares means values.

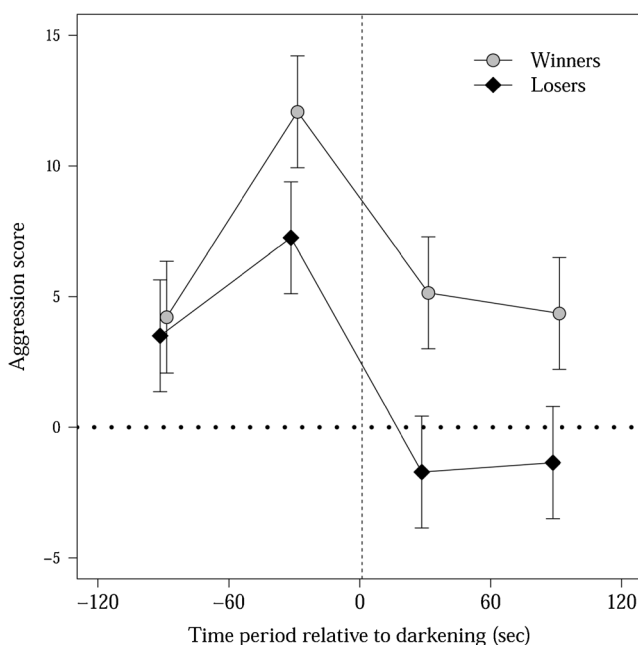


Fig. 6 Average aggression scores (\pm SE) for winners and losers of encounters between adult male veiled chameleons during four successive 60-s time periods, relative to the onset of darkening by the loser (time=0). Aggression was significantly different between winners and losers, and among the different 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 dorso-ventral 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 *Chamaeleo 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 arctic 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 *Chamaeleo gracilis*. Gen Comp Endocrinol 64:305–311
- Ott M, Schaeffl 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