Figueiredo Passos, L, Young, R and Garcia, G

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Flashy male Jamaican anoles (*Anolis grahami*) show accelerated telomere attrition

Abstract

Secondary sexual traits have evolved through sexual selection, many species have developed signals that can indicate their level of other fitness-relevant traits such as fight ability. Previous studies have shown that male sexual signals are honest signals about quality in an intrasexual context, demonstrating a direct relationship between the signal's design and the fighting ability of its possessor. However, signals can be costly since conspicuous signals are more likely to attract predators or be energetically expensive. Here we have analysed if dewlap size and colouration were reliable signs of a male's bite force, and the physiological costs associated with larger dewlaps and intense colouration in Jamaican anoles (*Anolis grahami*). We analysed dewlap size and colouration against bite force, and telomere attrition. Our results supported the hypothesis that dewlap size and colour intensity were honest predictors of an individual's fighting potential as indicated by bite force. However, we have also found a relationship between colour intensity with higher telomere attrition rates, thereby indicating a possible cost of this trait for the individual.

Keywords: *Bite-force, Dewlap, Telomere, Sexual selection*

Introduction

Secondary sexual traits have evolved through sexual selection, by female preference, inter-male competition or, in some cases, both (Zahavi, 1975; Berglund et al., 1996; Lailvaux & Irschick, 2007). In this context, many species have developed signals that can also indicate the level of other fitness-relevant traits such as fight ability (Emlen, 2008; Putman et al., 2018). Males can express this information in the form of colouration or ornamentation. Conspecific males interpret such signals to evaluate possible competitors, and females use this information to evaluate potential mates (Berglund et al., 1996). Traits that honestly signal fighting ability are advantageous as they can predict contest outcomes and, thus, males can avoid unwinnable physical combats and the costs associated with them (Andersson, 1994).

To establish whether a signal is reliable, the trait should be evaluated as to whether the size/shape or colouration of a secondary sexual character is predictive of ecologically relevant performance abilities (Perry et al.,2004). Different studies demonstrate that male secondary sexual signals express reliable information, demonstrating a direct relationship between the signal's design (mainly size and colour) and the fighting ability of its possessor expressed as bite force (e.g.Jennions & Backwell, 1996; Lailvaux & Irschick. 2007).

Signals inherent involve costs, more conspicuous signals (or more time devoted to signalling) are more likely to attract predators or be energetically costly to develop (Engqvist et al., 2015). One long-standing hypothesis about secondary sexual signals suggests that their honesty or reliability is related to how costly they are to produce and maintain (Lailvaux et al., 2012). An example of this is carotenoid-based colours (i.e. yellow/red spectrum), which are appropriate for honest signalling due to the costs related to pigment acquisition and the trade-offs between energetic allocation in ornaments against other metabolic processes such protection against oxidative DNA damage (de Lanuza et al., 2014).

The genus *Anolis* is characterised by having an extendible throat fan called a dewlap. The dewlap is a versatile signal structure being used in different contexts as a fundamental part of sexual/territorial display behaviours (Vanhooydonck et al., 2005). The dewlap extension is used as a threat or challenge to other males and predators (Jenssen et al., 2001) and to attract potential mates. Females show preference for males with certain dewlap characteristics and are more receptive to these males performing dewlap extensions (Greenberg & Noble, 1944; Crews, 1975). Studies have evaluated the relationship between dewlap size and fighting capacity (i.e. bite force) in anoles species with mixed results depending on the species (Vanhooydonck et al., 2005), level of sexual dimorphism (Lailvaux & Irschick, 2007), territoriality (Vanhooydonck et al., 2005) and level of within-population competition (Baeckens et al., 2018).

Here we aimed to analyse, if dewlap size and dewlap colouration were reliable signs of a male's bite force and if there is any physiological cost associated with these signals (using telomere attrition) in captive Jamaican anoles (*Anolis grahami,* Gray 1845). Consistent with previous studies, we expected dewlap size to be a good predictor of bite force, more importantly we predicted that there would be a biological cost associated with more intense coloured dewlaps.

Methods

Subjects

During this experiment 10 adult males, of unknown age, were used for data collection. All animals were hand caught in Nonsuch Island, Bermuda and transported by air to Chester Zoo, under licence 16-07-05-46, after clearance from a veterinary surgeon. The Jamaican anole (*Anolis grahami)*, despite being an invasive species in Bermuda is the most observed lizard on the island (Bacon et al. 2011). Subject animals weighted on average 10.20± 2.12g and had a snout-ventral length of 6.65±0.30cm and all individuals experiment exhibited breeding behaviour over the course of this study.

All the experimental methods described here were approved by the Chester Zoo's Ethics Committee, UK and conform to all regulations and laws in all relevant countries in relation to care of experimental animal subjects. Additionally, we can confirm, from our post-experimental monitoring that no animals suffered any injuries, became ill or had their survivorship negatively affected as a result of this study.

Lizard housing

Lizards were kept as a group of one male and two females in ExoTerra 60 cm x 45 cmx90 cm screen terrariums inside an isolated and temperature-controlled room at Chester Zoo, UK. A 12 hour photoperiod was maintained with an average temperature of 30 °C during the day and 24° C at night. Temperature and humidity (around 60%) were monitored with a thermometer/hygrometer. Each terrarium was supplied with a basking lamp, soil substrate, and a potted plant. The terraria were sprayed daily with water, and lizards were fed live crickets 3 times a week. The side of the terrariums were covered with black plastic between adjacent terrariums to avoid visual contact between different lizards groups.

Dewlap area

To obtain a reliable measure of dewlap size, lizards were positioned sideways side against a 1-cm^2 gride paper and the base of the second ceratobranchial was carefully pulled forward with a pair of tweezers until completely extended (Figure 1). Before taking a digital picture, animals were placed in such a manner that the extended dewlap was parallel to the lens of the camera (Canon PowerShot SX520HS digital camera). All measurements were made in an identical manner using the same settings on the camera. We calculated the total dewlap area for each individual using ImageJ software (Schneider et al., 2012).

Dewlap colouration

We used a USB-2000 portable diode-array spectrometer and a PX-2 xenon strobe light source (both from Ocean Optics, Dunedin, USA) to perform spectrophotometric measurements. Spectral analyses were conducted in the 300 and 700 nm range. Spectral reflectance measurements were always taken of each male from the centre of the dewlap, three measurements per lizard. Spectralon white standard measurements were taken between each individual to account for lamp drift. We calculated the colorimetric parameters using the Pavo package (Maia et al, 2013) for R studio (R Studio Team, 2015): brightness (mean reflectance across 320–700nm), hue (wavelength corresponding to [{max reflectance−min reflectance}/2]), and red chroma (sum of reflectance from 605–700 divided by brightness).

Bite force

We induced the lizards to bite a force transducer by tapping them gently on the side of the mouth, this method resulted in a characteristic threat response where the jaws are opened maximally. Lizards were held by the researcher for immobilisation and then encouraged to bite a force sensitive resistor connected to force a transducer and charge amplifier (Kistler Inc., Winterthur, Switzerland). When the free end of the holder (i.e. bite plates) was placed between the jaws of the animal, prolonged and repeated biting resulted. The force resistor placement on the lizards' mouth was standardised for all animals. For more detailed descriptions of setup and biting procedure, see Herrel et al. (1999). The bite force of all lizards were measured five times and only the maximum value was used for analysis.

Telomere erosion

To analyse telomere erosion, animals were swabbed using a buccal swab twice over a six months interval. Swabs were placed in tubes with dry capsules for stabilisation until laboratory analysis. After a DNA extraction using a DNeasy Blood and Tissue Kit (Qiagen, Australia), a real-time quantitative PCR (qPCR) allowed the measurement of telomere length. Telomere primers to used were: Telb1 (5’-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3’) and Telb2 (5’-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3’). The 18S ribosomal RNA (18S) gene (92 bp in *Anolis*) was selected as the reference gene as it had previously been successfully used in a reptile (Plot et al., 2012). The primer sequences used were 18S-F (5’-GAGGTGAAATTCTTGGACCGG-3’) and 18S-R (5’-CGAACCTCCGACTTTCGTTCT-3’). The qPCR was performed in a final volume of 20 µl for both telomeres and 18S. 10 ng of DNA was used per reaction, and the primers at a concentration of 250 nM. Reactions were run in triplicate for each sample. The measurement of the relative telomere length was calculated by using an adaptation of the qPCR method described by performed following protocol described by Giraudeau et al. (2016) and Dutra et al. (2020). The relative telomere length calculation consisted of the ratio of telomere repeat copy (T) to the reference gene (S; 18S) copy number (S); T/S.

Statistical analysis

Prior to statistical analyses, data on bite force was $\log_{10}$ transformed to meet the assumptions of normality and homoscedascity (Shapiro-Wilk’s test with $W \geq 0.95$). To remove the influence of body size on the variables, we used analyses of covariance (ANCOVAs) with SVL as covariate. To test if dewlap size and colour were a good predictor of a male’s bite-force and telomere attrition we used ANCOVA with size as a covariate. We used RStudio version 1.2.5033 (R Studio Team, 2015) for all statistical analyses.

Results

The ANCOVA analysis showed that dewlap size was positively significant for bite force ($F_{1,8}$=5.6, p=0.05). The dewlap colouration analysis was positively significant for brightness against bite force ($F_{1,8}$=10.6, p= 0.01), and telomere attrition ($F_{1,8}$=7.17, p=0.03). Yellow chroma ($F_{1,8}$=8.09, p=0.02), red chroma ($F_{1,8}$=6.26, p=0.03), and hue ($F_{1,8}$=5.67, p=0.04) were positively significant for telomere attrition. All other analyses did not result in statistically significant relationships.

Discussion

Our results show that dewlap size and colour intensity were honest predictors of an individual's bite force. Our analysis also demonstrated that intense dewlap colouration is associated with higher telomere attrition rates, thereby indicating a possible cost of this trait for the individual.

Anolis grahami are highly territorial lizards, with males defending their territories with visual displays, including dewlap extensions (Vanhooydonck et al., 2005). The reliability of such display could avoid the costly interactions physical combats may impose through bites during male-male competition (Andersson, 1994). Our results agree with previous studies showing that dewlap size is a reliable sign of combat performance, as measured by bite force. Previous studies have shown a relationship between bite-force and combat success, especially in *Anolis* species with high sexual dimorphism (Lailvaux & Irschick, 2007), such as *A. grahami.* This study reinforces the idea that dewlaps, among other signals, can act as a reliable indicator of combat ability to rival individuals; thereby, avoiding potentially physically damaging fights (Lailvaux & Irschick, 2007) .

The red colouration observed on dewlap of adult male *A. grahami* is associated with carotenoids (Macedonia et al., 2000) which are commonly obtained thought diet and they are an important antioxidant defence against reactive oxygen radicals (McGraw, 2005; Freeman-

Gallant et al.,2011). Pigment allocation to increase brightness of colourful ornaments could reduce the availability of carotenoids for use as antioxidants and, thereby, could increase DNA damage (McGraw & Ardia, 2003), leading to telomere attrition. A pioneering study on Australian painted dragons (*Ctenophorus pictus*) demonstrated this trade-off between telomere attrition and colour maintenance for signalling in a lizard species (Giraudeau et al., 2016), similar evidence has also been found in birds (Taff & Freeman-Gallant 2017). Our results support these findings suggesting that the maintenance of carotenoid-based colouration could lead to telomere shortening.

A central idea of life-history theory is that investment in present reproduction reduces survivorship and, consequently, future reproduction (Cox & Calsbeek 2009; Cox et al., 2010). The foundation for this hypothesis is that organisms that prioritise breeding over cellular maintenance (such as antioxidant production) often age quicker, having shorter life spans and higher rates of telomere attrition (Ricklefs & Wikelski, 2002). Physiological explanations for the trade-off are grounded in the notion that these two components compete for limited energy and nutrients (Zera & Harshman, 2001). Laboratory and field manipulations have demonstrated this prediction with respect to different physiological processes including DNA repair and resistance to oxidative stress using different species, including *Anolis* lizards (Cox et al., 2010). Our results support this hypothesis with male anoles investing in reproduction now at the cost of accelerated telomere attrition. Male anoles, by investing in an intense dewlap colouration, increase their chances of reproduction, as it has been shown that females have a preference for intense coloured dewlaps (Sigmund, 1983). If telomere attrition is, in fact, associated with increased senescence in anole lizards, then maintaining dewlap coloration with the associated benefits in terms of reproductive success but at the expense of longevity may be favoured by increasing reproductive output. Within a polygynous mating system, males can maximise reproductive success by monopolising females through male-male contests such as dewlap displays (Orrell & Jenssen, 2001*).* The breeding strategy Anolis consists of females laying multiple clutches of single eggs during a prolonged season (Cox & Calsbeek, 2010), giving males the opportunity to sire multiple batches of offspring in one breeding season.

In summary our results support previous studies showing that dewlap size and colouration are honest signals of bite force, but that these come with physiological costs. More studies using free-ranging animals and larger sample sizes are necessary to better understand this trade-off, including additional measurements such as circulating carotenoids and reactive oxygen radicals to fully understand the link between carotenoid-based colouration and telomere attrition (i.e., longevity).

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Availability of data and material

Data is available as Passos, Luiza (2022), “Jamaican Anoles Dewlap data”, Mendeley Data, V1, doi: 10.17632/2vphhkcrjj.1

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Figure 1. Jamaican anole (*Anolis grahami*) having its dewlap fully extended with the use of tweezers for total area measurement.