

Messages conveyed by assorted facets of the dewlap, in both sexes of *Anolis sagrei*

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Abstract Dewlaps of *Anolis* lizards are complex multicomponent signaling devices that have been intensively studied. Yet, the functions and multiple messages conveyed by the dewlap remain largely unknown. Here, we assess some aspects of sexual identity, individual quality, and social status that may be signaled by the dewlap in both sexes of *Anolis sagrei*. In addition, we investigate whether diverse facets of dewlap signaling provide additive information (redundant message hypothesis) or highlight different characteristics of the sender (multiple-message hypothesis). To do so, components of dewlap design (area, color, and pattern ratio) and use (dewlap extension frequency during intersexual context) were quantified and investigated for relationships with sexual identity (sex), individual quality (performance and health state measurements), and social status (mirror-motivated aggression). First, we found that body size together with relative dewlap area and color act as redundant messages in the advertisement of sexual identity. Second, we found that dewlap coloration in the center and edge region signals aspects of individual quality, specifically health state, but only in males. The dewlap center and edge acts primarily as redundant signals, at least for body condition and immune response. However, different color components irrespective of dewlap region convey nonredundant information about aspects of health state in males, supporting the multiple-message hypothesis. Surprisingly, dewlap use in *A. sagrei* males conveyed no

information about the tested quality measurements nor about mirror-motivated aggression. Neither dewlap design nor use in females was related to any of these parameters. In contrast to males, correlations between components of dewlap design and use during intersexual interactions were found for females, suggesting important signaling functions of the female dewlap in a courtship context.

Keywords Sexual identity · Individual quality · Social status · Redundant messages · Multiple messages

Introduction

For centuries, biologists have been fascinated by signals involved in sexual selection including the massive horn of rhinoceros beetles or the brilliantly colored peacock's tail. These secondary sexual characters are not purely decorative, but often provide reliable information about a signaler's sexual identity, individual quality, and/or social status during intraspecific interactions (Andersson 1994; Bradbury and Vehrencamp 1998). Conveying this particular information may have important fitness implications in terms of survival and reproductive success and is therefore crucial for each signaler (Johnstone 1995). In conveying information, the integration of several morphological and behavioral components is often involved, rather than the use of either trait alone (Guilford and Dawkins 1991). In male guppies, for instance, the courtship signal includes both male coloration and displays (Kodric-Brown and Nicoletto 2001). Even traits that are traditionally considered single signals may actually consist of several components (Candolin 2003; Grether et al. 2004). In male peacock blennies (*Salarias pavo*), for example, the size of the yellow head crest signals developmental quality,

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whereas its color has been found to signal the current health state (Locatello et al. 2012).

Two main hypotheses have been proposed to explain the evolution of such multicomponent displays. First, the redundant message hypothesis (Møller and Pomiankowski 1993; Johnstone 1996) states that all components broadcast the same property of the sender (e.g., its species identity, sex, or quality). Repeating the same message in different ways has been shown to enhance the detection, recognition, discrimination, and memorability of signals by human receivers (reviewed in Rowe 1999). Besides, animals may have evolved multiple redundant signal components to convey their message under a wide range of circumstances, as the efficacy of a particular signal component may depend on several environmental conditions (e.g., light level, humidity, and ambient noise). Second, the multiple-message hypothesis states that complex signals may carry multiple messages, each component conveying a particular piece of information about the sender (Hebets and Papaj 2005). Animal coloration provides a good illustration for this hypothesis. Whereas carotenoid pigments in ornaments are thought to reflect physical condition, the melanin-based colors are thought to primarily signal social status (Candolin 2003 and references therein). One component of a complex signal may also serve to simply attract the general attention of the receiver, while another component signals the sender's quality (Hebets and Papaj 2005). Adding to this complexity, the same signals or signal components may not necessarily convey the same information, depending on the sender's sexual identity. The brightness of the yellow bib signals health and survivorship in males of the common yellowthroat (*Geothlypis trichas*) but is associated with poor fecundity and low survivorship in females of the species (Dunn et al. 2010; Freeman-Gallant et al. 2014). Information content of a signal cannot therefore always be extrapolated from one sex to the other.

The dewlap of anole lizards is a complex signaling device that has received considerable attention during the last decades, especially in the male sex. It is characterized by an extendable flap of skin attached to the throat and is believed to be a sexually selected structure (Vanhooydonck et al. 2005a; Cook et al. 2013). The fact that the male dewlap has been added to a list of display organs that exhibit positive allometry (i.e., disproportionally large dewlap area in relation to body size) additionally confirms that sexual selection via female choice and/or male–male competition is the main selective force driving dewlap size (Echelle et al. 1978; Kodric-Brown et al. 2006). The throat fan is generally observed to be extended in a variety of contexts, including encounters with potential mates (Greenberg and Noble 1944; Jenssen 1970; Crews 1975; Sigmund 1983), rivals (Jenssen et al. 2000), members of related species (Rand and Williams 1970; Losos 1985), and even predators (Leal and Rodriguez-Robles 1995, 1997). However, it seems that Anole species and sexes differ

considerably in the contextual use of their dewlap, as well as in their dewlap design (Nicholson et al. 2007). In our study, we focus on the dewlap structure of the brown anole lizard (*Anolis sagrei*) and the messages it conveys. Males of this species are polygynous, highly territorial, and substantially larger than females (Schoener and Schoener 1980; Schwartz and Henderson 1991). The dewlap in *A. sagrei* has been shown to be sexually dimorphic with more conspicuous and larger throat fans being present in males. It typically consists of two distinctly colored regions in both sexes, i.e., bright yellow to reddish in the dewlap center and yellow along the edge. Carotenoids create the yellow color of both dewlap regions and interact with pterins to create the orange and red colors in the center (Macedonia et al. 2000; Steffen and McGraw 2007, 2009). The dewlap additionally reflects in the ultraviolet (UV). Besides dewlap design, sexual differences can also be found in dewlap use, as *A. sagrei* males extend their fans much more frequently than females do in response to different stimuli (Partan et al. 2011; Driessens et al. 2014). Nonetheless, it appears that both sexes primarily perform dewlap extensions (DE) in a social context and particularly during intersexual interactions. This may indicate that dewlap use in *A. sagrei* plays an important role during courtship and potentially during subsequent mating (Simon 2011; Driessens et al. 2014). Despite years of intensive research on this structure, the exact signaling function and significance of the dewlap extension in a context of courtship and mating behavior remain unclear for both sexes of *A. sagrei*.

In this study, we investigated what is signaled by assorted components of the dewlap in *A. sagrei* and whether diverse aspects of dewlap signaling provide additive information (redundant message hypothesis) or highlight different characteristics of the sender (multiple-message hypothesis). Therefore, we measured several dewlap components involving design (i.e., area, patterning, and color) and use (i.e., DE frequency during intersexual interactions), and linked these to information a sender may need to transmit in order to increase its fitness (i.e., sexual identity, individual quality, and social status). We used several performance (i.e., bite force, sprint speed, and clinging capacity) and health state parameters (i.e., immunocompetence, hematocrit, and swelling response) as a measure of individual quality, whereas mirror-motivated aggressiveness (i.e., aggression score) was used as a measure of social status. Under the redundant message hypothesis, different components of the dewlap should index the same aspect of the sender's sexual identity, quality, or aggressiveness. Under the multiple-message hypothesis, distinct elements should convey information on different aspects of the sender. Of course, not all aspects of the dewlap considered here necessarily carry a signal, and some aspects may even represent unreliable signals (Møller and Pomiankowski 1993; Takahashi et al. 2008). Due to their fundamentally divergent

reproductive roles, we expected males and females to differ with respect to what is signaled by the dewlap. We therefore first highlighted sexual differences in dewlap design and then performed separate analyses for males and females. For the male sex, we additionally distinguished between the color of the dewlap center and edge region. A recent study has shown that the edge of the dewlap plays the most important signaling role, especially during competition for mates or territories (Steffen and Guyer 2014). To our knowledge, no other studies have tested such a large array of dewlap traits in both sexes of *A. sagrei* so far, and therefore, our work may serve as a valuable complement to previous results.

Material and methods

Study animals

The 61 male and 38 female *A. sagrei* used in this study were caught in Florida and obtained through the pet trade (Fantasia Reptiles, Belgium, license HK51101419), in October 2011. Snout–vent length (SVL) upon arrival ranged from 40.83 to 61.47 mm (males) and from 39.55 to 50.65 mm (females), which is above the threshold size for sexual maturation in this species (males: >39 mm, females: >34 mm, Licht and Gorman 1970; Lee et al. 1989). The lizards were individually housed in specially designed, nontransparent cages at the University of Antwerp, Belgium. Each cage (30×22.5×27.5 cm) contained a thick layer of peat bedding (Spaghnum, Novobalt) covered with banana tree leaf litter, a wooden perch (ca 40-cm length, 2.5-cm diameter), and a light source (Philips SpotOne 30°, 25 W) set to a 12:12-h light–dark regime. Air temperature within the cages ranged from 26 °C at night to a maximum of 35 °C during the day. The relative humidity of the room ranged from 55 to 70 %. All of the lizards had continuous access to drinking water from a small dish in their cage and were hand sprayed with water every other day. We fed each lizard small crickets (*Acheta domestica*) every other day, and we occasionally added wax moth larvae (*Galleria mellonella*) to their diet. Both food types were dusted with a dietary supplement containing a 2:1 calcium/phosphorus ratio and vitamin D3 (Zoo Med Reptivite with D3, Fantasia Reptiles, Belgium).

Four months after arrival, measurements and tests were started and were performed during the lizards' natural activity period between 09:00 and 16:00 h. Successive tests on the same individual were performed with a rest interval of at least 10 days to minimize stress levels. Moreover, more invasive techniques (i.e., hematocrit and swelling response measurements) were performed toward the end of the study. Prior to each test, body size (i.e., SVL) and body mass were measured using an electronic caliper (Mitutoyo, Kawasaki, Japan; accuracy 0.01 mm) and electronic balance (Scout Pro SPU202, Ohaus Corporation, USA; accuracy 0.01 g) respectively, to

determine body condition. Individuals in a poor condition or those clearly in bad health were excluded from any further testing. For this reason, sample sizes vary slightly among experiments and measurements.

“Dewlap” measurements

Area

To estimate dewlap area, we positioned the lizard sideways along a 1-cm squared grid paper and gently pulled the base of the ceratobranchial forward with a pair of forceps, until the dewlap was fully extended (see Bels 1990). We then photographed the dewlap using a Nikon D70 camera mounted on a tripod. We used Adobe Photoshop CS3 extended software (AP CS3, version 10.0) to trace the outer edge of the dewlap on the digital images and to calculate its area. A similar method for measuring dewlap dimensions has produced highly repeatable results in a previous study (Vanhooydonck et al. 2005a).

Patterning

In *A. sagrei* dewlaps, at least one and a maximum of two distinct colors, i.e., red and yellow create a degree of “patterning” that differs among individuals. On digital images we traced the total dewlap area that was colored (whitish background was excluded) and the area covered by the main color only (red in most individuals), using the “color range tool” (AP CS3 software, version 10.0). Then we calculated the ratio of the area covered by the main color to total color area and used this as an index of the degree of patterning. This continuous index corresponds well to the distinct dewlap pattern categories previously described by Nicholson et al. (2007). In this study, *A. sagrei* dewlaps with an index of 1 correspond to the “solid” category, those with an index >0.75 and <1.00 to the “marginal” class and those with an index <0.75 to the “spotted or marginal with spots” category.

Color

We measured dewlap reflectance using an Avantes spectrometer (AvaSpec-2048-USB2-UA-50, range 250–1000 nm) and deuterium-halogen light source (AvaLight-DHS) fitted with a fiber-optic probe. The probe, which was mounted within a metal holder to ensure readings at a constant distance from the surface, was held perpendicular to the plane of the maximally extended dewlap. All measurements were expressed in relation to a white reference tile (WS2; Avantes). Following previous studies (Leal and Fleishman 2002, 2004; Fleishman et al. 2006; Steffen and McGraw 2007), we measured spectral reflectance at distinct locations on the dewlap in males: at the center (one unique spectral measurement) and along the

dewlap's edge (the average of three unique, nonoverlapping spectral measurements). For females, we could only obtain a single spectral measurement from the dewlap center, as their dewlap is much smaller in size. Reflectance data were collected for wavelengths from 300 and 700 nm, representing the lower range of photon absorption by UV-sensitive photoreceptor cones published for anoles (Fleishman et al. 1993). To summarize the color information obtained from the spectrometer, we performed principal component analysis (PCA). This method is widely used for objectively analyzing spectral reflectance, because variation in mean reflectance and spectral shape is captured without making assumptions about the receiver's visual system (Cuthill et al. 1999). Each of our reflectance spectra originally comprised 690 data points (0.59-nm reflectance intervals from 300 to 700 nm). These were first reduced to the means of 10-nm bins, resulting in 40 mean reflectance values per dewlap region for each subject (Cuthill et al. 1999). PCAs were run on the 10-nm bandwidth means retaining all components with eigenvalues >1.5.

Dewlap extension (DE) frequency

We observed the lizards in a staged encounter with a member of the opposite sex to obtain a measure of how often individuals performed dewlap displays in a courtship context. For each individual, the number of executed dewlap extensions was counted during the trial and expressed as a frequency variable (counts/min). All the observations were scored online and analyzed afterwards, using JWatcher event recorder software (version 1.0; Blumstein et al. 2000). For more details on the setup, we refer to the “male–female” and “female–male” interaction experiments described in Driessens et al. (2014). The behavioral experiments were performed between the end of June and start of October, which mostly corresponds with the timing of the breeding season of *A. sagrei*, distributed in Florida (Lee et al. 1989). Previous results have shown that both male and female *A. sagrei* mostly tend to flash their dewlaps in an intersexual context (Simon 2011; Driessens et al. 2014).

“Quality” measurements

Prior to experimentation and in between performance trials, lizards were placed in an incubator set at 30 °C for at least 40 min to attain physiologically optimal body temperatures (Losos and Irschick 1996). All performance trials were measured by the same researcher (TD) to improve consistency.

Bite force

Lizards were encouraged to bite on two metal plates connected to an isometric Kistler force transducer (type 9203) and charge amplifier (type 5995; see Herrel et al. 1999 for detailed

description of setup). Each lizard was subjected to a total of five trials with a rest of approximately 40 min in between. The highest value obtained from these five trials was used as an individual measure for maximal bite force capacity.

Sprint speed

Lizards were placed on the lower end of a wooden dowel (3.5-cm diameter) which was positioned against a 2-m racetrack, tilted to an angle of 40° (Van Berkum 1986; Losos 1990; Vanhooydonck et al. 2006). They were then chased to run over the dowel, thereby passing eight pairs of infrared photocells stationed at 25-cm intervals. The time elapsed between triggering two consecutive photocells was recorded using custom built software. Each lizard was subjected to a total of three trials with a rest of at least 1 h in between. The fastest speed over any 25-cm interval was used as a measure of maximal sprint performance (see Vanhooydonck and Van Damme 2001 for more details about standard procedures for quantifying maximal sprint speed).

Clinging ability

Lizards were placed with their front feet on an acetate transparency sheet attached to the surface of a force plate (Kistler, type z17097; dimensions 20×2×4.5 cm). Next, they were dragged horizontally at a constant speed (approximately 5 cm/s) across the force plate for 20 s. One-dimensional clinging forces parallel to the force plate were measured and the output was sent to an eight-channel charge amplifier (Kistler, type 9865) and A/D converter (Kistler, type 5606a). The digital signals were read into a Toshiba Tecra computer and the BioWare software (Kistler Bioware®, v.3.2.5, Kistler Instrument Corp., 2003) was used for smoothing and analysis. Each lizard had a total of five clinging trials with a rest of at least 1 h in between. The single greatest measurement was considered an individual's maximal clinging capacity. For a more detailed description of our clinging capacity protocol, see Elstrott and Irschick (2004).

Body condition

The residuals from a linear regression of log₁₀-transformed mass against log₁₀-transformed SVL were used as an index of body condition (Jakob et al. 1996). Previous studies have shown that body condition correlates with energy storage and general health in reptiles (Forsman and Lindell 1996; Van Sluys 1998).

Swelling response

We assessed the lizards' immune function using the phytohemagglutinin (PHA) skin-swelling test. A subcutaneous

injection of 20 μ l PHA solution (males) or 10 μ l (females) [L8754, Sigma-Aldrich; 6 mg PHA solved in 1 ml PBS] was administered in the left hind foot. An equal amount of pure phosphate-based saline (PBS) solution was injected in the right hind foot, serving as a control. We measured the thickness of each lizard's hind foot three times at a standardized location (between first and fifth digits) shortly before and 24 h after injection, using a pressure-sensitive calliper (Alpa S.p.A., Milano, cod. SM112, accuracy 0.01 mm). Medians of the three replicate measurements were used in subsequent analyses (repeatability of the foot thickness measurements proved to be very high: $r > 0.97$). The immune swelling response to PHA was then calculated as the change in thickness of the PHA-injected foot (left) minus the change in thickness of the control foot (right). PHA stimulates mitosis of the T-lymphocytes as well as a variety of other vertebrate cell types, and therefore, both the acquired and innate immunity contribute to the swelling (Kennedy and Nager 2006; Martin et al. 2006). The PHA assay should thus be considered a multifaceted index of cutaneous immune activity, rather than an unambiguous index of T-cell mediated immunity per se (Martin et al. 2006).

Hematocrit

Blood was collected twice from the lizards' postorbital sinus behind the eye using microhematocrit capillaries (Hirschmann Laborgeräte, Germany), with a rest interval of 14 days between sampling; see MacLean et al. (1973) for more details. Filled capillaries were centrifuged for 10 min at 1300 rpm (Cox et al. 2010). Immediately after centrifugation, the proportion of red blood cell volume to total blood volume was measured using a digital calliper (Mitutoyo, Kawasaki, Japan; accuracy: 0.01 mm; repeatability $>.99$); the buffy coat was negligible. The average hematocrit value of both blood samples was used in all subsequent analyses. The hematocrit may indicate the amount and efficiency of oxygen uptake and transfer to tissues (Ots et al. 1998) and can therefore be used to evaluate the physical condition and general health of an animal.

“Social status” measurements

Aggressiveness

Each lizard was given an “aggressiveness” score, depending on how it behaved toward its own mirror image (mimicking a size-matched opponent). The mirror experiment was performed during the *A. sagrei* breeding season (end of April–beginning of May); details of the setup are described in Driessens et al. (2014). We followed the ethograms of Leal and Rodriguez-Robles (1995; 1997) and McMann (1993) to categorize the anoles' behavior as “aggressive” or

“submissive.” Aggressive behaviors were assigned positive scores, whereas submissive behaviors received negative scores (Perry et al. 2004). Dewlap extensions, push-ups, head nods, as well as approaching the mirror image were each assigned a score of +0.5 per count/min. Biting and bouncing against the mirror were often performed simultaneously and therefore grouped together accounting for a score of +1 per count/min. Submissive behaviors comprised hiding or escape attempts and were each assigned a score of −1 per count/min. For each lizard, we summed all scores to obtain a single measure indexing aggressiveness toward opponents of the same sex.

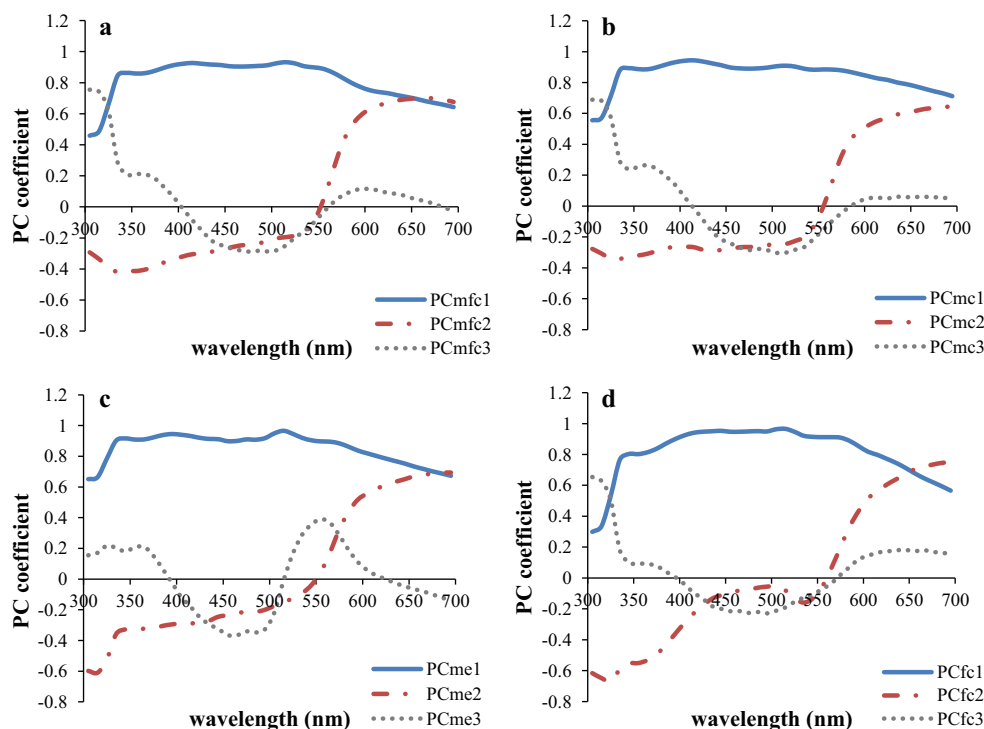
PCA on color variation

We performed four PCAs in total, all resulting in three combinatory variables (PC1, PC2, PC3) that together explain a considerable part of the original variation in reflectance. The first analysis was meant to compare males and females, and incorporated reflectance at the center of the dewlap of both sexes as input. We use the symbols PC_{mfc1} , PC_{mfc2} , and PC_{mfc3} to refer to the axes produced by this analysis. The second analysis was meant to summarize reflectance variation in color at the center of the dewlap among males (produced PC_{mc1} , PC_{mc2} , and PC_{mc3}). The third analysis was performed to summarize variation in reflectance near the dewlaps' edges (PC_{mc1} , PC_{mc2} , and PC_{mc3}). We chose not to combine information from the dewlap center and edge into one analysis because previous work has suggested that these areas may convey different types of information in males (Leal and Fleishman 2002, 2004; Fleishman et al. 2006; Steffen and McGraw 2007). Finally, a fourth analysis was run using the female reflectance data (central measurements only, produced PC_{fc1} , PC_{fc2} , and PC_{fc3}). To facilitate the exact interpretation of our principal component scores (PC), we calculated six commonly used reflectance indices and correlated these with our PC scores (Griggio et al. 2009): mean brightness [the mean percentage of reflectance from 300 to 700 nm], UV chroma ($R_{300-400}/R_{300-700}$), blue chroma ($R_{400-475}/R_{300-700}$), green chroma ($R_{475-550}/R_{300-700}$), yellow chroma ($R_{550-625}/R_{300-700}$), and red chroma ($R_{625-700}/R_{300-700}$) (Endler 1990; Montgomerie 2006).

Interpretation of PCs

In all separate PCAs, the first axis (PC1) can be considered an alternative index for brightness, whereas PC2 and PC3 typically represent color variation. PCA_{mfc} performed on the reflectance data for males and females jointly resulted in three significant axes that together explained 93.83 % of the total variation (Fig. 1a). PC_{mfc1} (67.93 % of variance) was highly correlated with mean brightness (Table 1). PC_{mfc2} (19.40 % of variance) represents variation in the relative amount of short

Fig. 1 Separate principal component analysis of **a** the dewlap center in both sexes (PCA_{mfc}), **b** the dewlap center in males (PCA_{mc}), **c** dewlap edge in males (PCA_{me}), and **d** dewlap center in females (PCA_{fc}). These panels show the association between PC factor-loading coefficients and wavelength intervals. For more details, see “PCA on color variation” and “Interpretation of PCs” in the “Materials and methods”



and medium (300–550 nm, UV–green) to long wavelengths (550–700 nm, yellow red), whereas PC_{mfc3} (6.50 % of variance) describes the variation in intermediate (400–550 nm, blue green) relative to short wavelength reflectance (300–400 nm, UV; Fig. 1a). The PCA_{mc} on the reflectance data measured at the center of the males’ dewlap (Fig. 1b) resulted in three composite variables (PC_{mc1} , 72.71 %; PC_{mc2} , 15.21 %; PC_{mc3} , 6.40 % of variance). PC_{mc1} again describes mean brightness. PC_{mc2} correlated positively with reflectance in the yellow and red parts of the spectrum and slightly negatively with reflectance in the UV (Table 1). PC_{mc3} correlated strongly and positively with reflectance in the UV-spectrum (especially in the lower UV spectrum 305–325 nm) and negatively with reflectance in the blue–green region (450–

550 nm; Fig. 1b, Table 1). A similar analysis on the reflectance data obtained from the dewlap edge (PCA_{me}) also produced three principal components (PC_{me1} , 73.92 %; PC_{me2} , 18.07 %; PC_{me3} , 4.92 % of variance; Fig. 1c). PC_{me1} correlated highly with reflectance values at all wavelengths (Table 1). PC_{me2} correlated negatively in the short and intermediate wavelength spectra (300–550 nm; UV green) with an absolute minimum in the UV range and positively in the long wavelength spectra (550–700 nm, yellow red) (Fig. 1c, Table 1). PC_{me3} was negatively related to reflectances in the blue and part of the green spectrum (445–500 nm) and positively with reflectances in the yellow spectrum (Fig. 1c, Table 1). Last, the PCA_{fc} on the reflectance values for the female dewlap center resulted in three component axes

Table 1 Pearson correlation coefficients between reflectance indices and PC scores (see “PCA on color variation” and “Interpretation of PCs” in the “Materials and methods” for more details)

Indices	Male and female center ($N=99$)			Male center ($N=61$)			Male edge ($N=61$)			Female center ($N=38$)		
	PC_{mfc1}	PC_{mfc2}	PC_{mfc3}	PC_{mc1}	PC_{mc2}	PC_{mc3}	PC_{me1}	PC_{me2}	PC_{me3}	PC_{fc1}	PC_{fc2}	PC_{fc3}
Mean brightness	0.98	0.20	0.02	0.98	0.19	0.01	0.98	0.18	0.03	0.99	0.14	0.05
UV chroma	0.33	−0.81	0.46	0.33	−0.76	0.54	0.48	−0.82	0.22	0.20	−0.94	0.24
Blue chroma	0.50	−0.74	−0.34	0.40	−0.81	−0.29	0.43	−0.71	−0.52	0.60	−0.54	−0.42
Green chroma	0.50	−0.65	−0.45	0.35	−0.72	−0.53	0.68	−0.59	0.03	0.66	−0.45	−0.48
Yellow chroma	−0.22	0.73	0.13	−0.08	0.72	−0.06	−0.07	0.77	0.49	−0.19	0.58	0.06
Red chroma	−0.55	0.77	0.01	−0.50	0.80	0.10	−0.70	0.65	−0.17	−0.55	0.79	0.16

Highly significant correlations are shown in **bold** ($p < 0.005$)

(PC_{fc1}, 68.99 %; PC_{fc2}, 20.42 %; PC_{fc3}, 4.84 % of variance) that together captured 94.25 % of the original variation (Fig. 1d). PC_{fc1} exhibited high loadings for reflectance at all wavelength bins (Table 1). Factor loadings for PC_{fc2} were highly negative for reflectance in the UV-region, and positive in the yellow–red color range (Fig. 1d). Pearson correlations confirmed a strong negative correlation with reflectance in the UV region, and positive correlations with yellow and red chromas (Table 1). The third axis, PC_{fc3} described the variation in intermediate (400–550 nm; blue green) relative to the short-wavelength (300–400 nm; UV) and long-wavelength spectra (575–700 nm; yellow red) (Fig. 1d). Only a strong negative correlation with PC_{fc3} and reflectance values in the green spectrum was found (Table 1).

Other statistical analyses

All continuous variables were log10-transformed to ensure normality (evaluated by the Shapiro–Wilks test), except for dewlap extension (DE) frequency. To point out the sexual differences in dewlap design, we first evaluated differences in allometry between male and female dewlaps using analysis of covariance (ANCOVA), with SVL entered as the covariate. Reduced major axis regression was then used to calculate the scaling factor for dewlap area in both sexes. Second, we tested intersexual differences in dewlap coloration by comparing the scores on PC_{mfc1}, PC_{mfc2}, and PC_{mfc3} between sexes using *t* tests. Last, differences in the degree of patterning between males and females were also evaluated using a *t* test (pattern ratio) and using Fisher’s exact probability test (pattern categories).

To test whether the measured dewlap design variables play a role in signaling sexual identity, we used forward stepwise discriminant analysis. This analysis builds a model that predicts group membership (sex), using linear combinations of predictive variables (SVL, relative dewlap area, pattern ratio, and PC_{mfc1–3}). The procedure started with the best discriminating predictor and added subsequent variables only if they improved the discriminatory power of the function (judged from Wilk’s lambda). We compared the power of the functions obtained to that of a model based solely on SVL by looking at the number of individuals misclassified.

To test whether assorted components of the dewlap convey information about quality and social status of the sender, dewlap design components were linked to an array of quality indices and to aggressiveness, using a series of multiple backward stepwise linear regression analyses. These analyses were run separately per sex and per dewlap region for the males. We further corrected for body size in all variables that significantly correlated with SVL (i.e., for males: dewlap area, PC_{mfc1}, PC_{mfc1–3}, DE frequency, and bite force; for females: dewlap area and bite force) by regressing those against log10-transformed SVL and calculating unstandardized residuals.

The multiple linear regression procedure started with building a “full” model, containing the dewlap design measurements (relative dewlap area, pattern ratio, and PC_{mfc1–3} or PC_{fc1–3}), to predict a given “quality” measure (relative bite force, sprint speed, clinging capacity, body condition, swelling response, or hematocrit) or to predict mirror-motivated aggressiveness (aggression score). Then predictor variables were removed from the model successively, until further removal resulted in significantly worse fit (backward stepwise elimination; probability of *F* set to 0.05 for entry and 0.07 for removal). Multicollinearity between predictor variables was assessed using collinearity diagnostics and found to be within acceptable levels for all analyses (all VIF <1.95 for analyses in males, all VIF <1.19 for analyses in females). We further tested whether measures of individual quality (relative bite force, sprint speed, clinging capacity, body condition, swelling response, and hematocrit) intercorrelate or show any relationship with aggressiveness using bivariate Pearson correlation analyses.

Besides dewlap design, we also tested predictions concerning dewlap use in relation to quality and aggressiveness. We ran linear regressions for males incorporating relative DE frequency as the predictor variable and the same response variables (see description above). For females, simple correlations (Spearman’s rho) were used instead, because DE frequency could not be transformed to produce a normal distribution. We additionally assessed the relationships between dewlap use and dewlap design via bivariate Pearson and Spearman’s rho correlations in males and females, respectively.

All statistical analyses were conducted with the statistical software package SPSS version 20 (SPSS, Chicago, IL, USA) and a value of *p* < 0.05 was considered as statistically significant.

Results

Sexual differences in dewlap design

On average, the males in our dataset were larger than the females (Table 2, $t_{95.69} = 14.70$, $p < 0.001$). Dewlap area increased with SVL in both sexes in a similar way (ANCOVA, comparison of slopes: $F_{1,94} = 2.37$, $p = 0.127$), but males had larger dewlaps at any given SVL (ANCOVA, comparison of intercepts: $F_{1,95} = 497.37$, $p < 0.001$). Independent *t* tests comparing relative (size-adjusted) dewlap area between sexes resulted in a *p* value < 0.0001 ($t_{96} = 6.27$). Investigating the scaling of dewlap area showed a positive allometry in both males and females (RMA slope and 95 % confidence interval for males: $B = 3.42$, $CI_{95} = 2.99–3.85$; for females: $B = 3.21$, $CI_{95} = 2.16–4.26$). The color reflectance curves for the dewlap center in males and females demonstrated the presence of

Table 2 Descriptive statistics for the measured traits in males and females

Traits	Males			Females		
	N	Mean	SE	N	Mean	SE
Dewlap area (cm ²)	60	1.32	0.04	38	0.22	0.01
Dewlap pattern ratio	61	0.81	0.02	38	0.77	0.02
Dewlap color	61	−0.16	0.13	38	0.27	0.16
	61	0.32	0.12	38	−0.51	0.15
	61	0.20	0.12	38	−0.32	0.16
DE frequency (counts/min)	27	2.19	0.35	17	0.24	0.10
Bite force (N)	60	4.00	0.14	37	2.09	0.07
Sprint speed (mm/s)	41	98.26	4.78	22	83.10	5.57
Clinging ability (N)	53	1.21	0.06	22	1.00	0.06
Mass (g)	61	4.83	0.15	38	2.29	0.07
SVL (mm)	61	55.67	0.55	38	45.49	0.41
Swelling response (mm)	24	0.27	0.03	10	0.13	0.03
Hematocrit (%)	27	60.46	2.70	12	52.34	4.85
Aggression score	36	2.39	0.49	18	0.72	0.19

Means and SE are given for the raw data (transformed variables or residuals corrected for size are not shown)

sexual color dichromatism (Fig. 2). Overall, females tended to have higher reflectance values in the UV, blue, and green wavelength spectra compared to males, but lower reflectance in the yellow and red regions. Females scored higher on PC_{mfc}1 (mean±SE=0.27±0.16, $N=38$, Table 2) than males did (−0.17±0.13, $N=61$, Table 2; $t_{97}=-2.13$, $p=0.036$). On the other hand, males scored much higher on PC_{mfc}2 (0.32±0.12, Table 2) than females did (−0.52±0.15, Table 2; $t_{97}=4.41$, $p<0.001$). For PC_{mfc}3, males scored again higher (0.20±0.12, Table 2) than females did (0.32±0.16, Table 2; $t_{97}=2.59$, $p<0.011$). Lastly, the color pattern ratio also differed between the sexes ($t_{96}=2.17$, $p=0.033$; Table 2). Accordingly, the distribution of dewlaps across the four

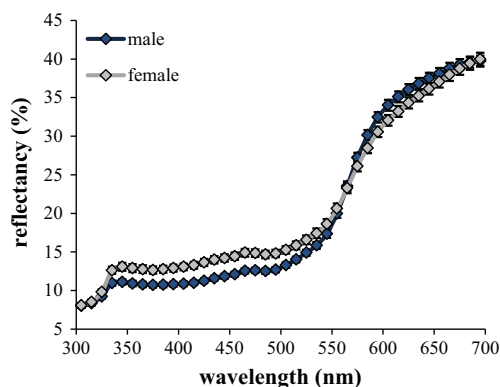


Fig. 2 Mean reflectance curves (%) of the dewlap center in *Anolis sagrei*. Color data for males ($N=61$) and females ($N=38$) are displayed separately; error bars represent SE

classes differed between males (solid: 0, marginal: 41, marginal+spotted: 20, spotted: 0) and females (solid: 1, marginal: 21, marginal+spotted: 12, spotted: 4; Fischer exact probability test, $p=0.033$).

Dewlap design and sexual identity

We performed a forward stepwise discriminant analysis, with SVL, relative dewlap area, PC_{mfc}1–3, and pattern ratio as possible predictors of sex. The final model included SVL ($F_{1,95}=183.59$), relative dewlap area ($F_{2,94}=784.50$), PC_{mfc}1 ($F_{3,93}=571.43$), and PC_{mfc}2 ($F_{4,92}=455.20$) (in this order, all $p<0.0001$) and attributed the correct gender to 100 % of the individuals. A model with SVL as the only predictor classified 89.8 % correctly.

Dewlap design, use, and quality

Of the six quality indices measured on males (relative bite force, sprint speed, clinging capacity, body condition, immune response, and hematocrit), only clinging capacity and immune response were significantly intercorrelated ($r=0.47$, $N=24$, $p=0.022$; other pairwise correlation coefficients varied between −0.18 and 0.32, all $p>0.08$). However, relative bite force in males also tended to correlate positively with body condition (Pearson correlation test, $r=0.25$, $p=0.055$, $N=60$).

Multiple backward stepwise regression analyses starting with a full model containing relative dewlap area, pattern ratio, and color as predictor variables for quality, resulted in only retaining dewlap design variables for quality measurements reflecting health state. Specifically, we found that several aspects of color in both the dewlap center and edge of males are important predictors of body condition, immune response, and hematocrit (Table 3, all $p<0.03$). Using body condition as response variable, we obtained significant results for PC2 in both dewlap regions (PC_{mc}2, $B\pm SE=0.013\pm 0.005$; PC_{me}2, $B\pm SE=0.017\pm 0.005$). Thus, individuals with a higher reflectance in yellow and red relative to green, blue, and UV seem to have a higher body condition index. For immune response, PC1 in both dewlap regions appeared to be a good predictor in our regression model (PC_{mc}1, $B\pm SE=-0.166\pm 0.066$; PC_{me}1, $B\pm SE=-0.156\pm 0.061$). The negative regression coefficients indicate that individuals with brighter dewlaps show a smaller swelling response after injection with PHA. The information contained in dewlap color is therefore similar for both dewlap regions with regard to body condition and swelling response. For hematocrit, on the contrary, only the color component for dewlap edge was a significant predictor (PC_{mc}3, $B\pm SE=0.050\pm 0.021$). Individuals possessing a dewlap edge with higher reflectance in the yellow and UV relative to the blue range show higher hematocrit values in the blood. Surprisingly, multiple backward regression analyses of the quality measurements reflecting individual performance (i.e.,

Table 3 Results of stepwise multiple linear regressions of dewlap design components as predictors for general health parameters in males

Model				Predictors (dewlap design)			
Response variable	<i>N</i>	<i>R</i> ²	<i>F</i>	Predictor variable	β	<i>t</i>	<i>p</i> Value
Body condition	60	0.103	6.637	PC _{mc} 2	0.320	2.576	0.013
	60	0.146	9.887	PC _{mc} 2	0.382	3.144	0.003
Swelling response	24	0.222	6.262	PC _{mc} 1	−0.471	−2.502	0.020
	24	0.227	6.476	PC _{mc} 1	−0.477	−2.545	0.018
Hematocrit	27	0.186	5.701	PC _{mc} 3	0.431	2.388	0.025

The following independent variables were incorporated in all regressions: relative dewlap area, pattern ratio, and color including PC_{mc}1–PC_{mc}3 or PC_{mc}1–PC_{mc}3 (separate analyses for dewlap center and edge). Only significant models and predictors are presented in this table ($p < 0.05$). For some characters, residuals treated as size-corrected variables were used in the regressions (see “Other statistical analyses” in “Materials and methods” for details)

relative bite force, sprint speed, and clinging capacity) retained none of the predicting dewlap design variables in males.

Dewlap use in males during intersexual interactions ranged from 0 to 6.48 DE/min (Table 2). Separate linear regressions showed that relative DE frequency was not a predictor for any of the six tested quality measurements in males (all $p > 0.08$). Furthermore, we could not find any relationship between relative DE frequency and our design variables including relative dewlap area, pattern ratio, and color parameters for both dewlap center and edge (i.e., PC_{mc}1–3 and PC_{mc}1–3; Pearson's correlation tests, $N=27$, pairwise correlation coefficients varied between −0.26 and 0.25, all $p > 0.150$).

In females, body condition correlated with relative bite force ($r=0.49$, $p=0.002$, $N=37$) and with maximal sprint speed ($r=0.54$, $p=0.009$, $N=22$). Pairwise correlations between all other performance variables were nonsignificant ($p > 0.15$).

None of the dewlap design variables quantified in females were a predictor for relative bite force ($N=37$), sprint speed ($N=22$), clinging capacity ($N=22$), body condition ($N=38$), swelling response ($N=9$), and hematocrit ($N=12$); no independent variables were retained in the stepwise multiple regressions.

The DE frequency in females in an intersexual context varied between 0 and 1.58 extensions/min (Table 2). Spearman's rho correlations showed no relationships between female dewlap use in an intersexual context and any of the six tested quality measurements (all $p > 0.1$). However, we did find significant correlations between female dewlap use and two dewlap design variables. DE rate in females was positively correlated with relative dewlap area (Spearman's rho test, $\rho=0.56$, $p=0.020$, $N=17$) and negatively with dewlap color PC_{fc}1, which corresponds to brightness ($\rho=-0.49$, $p=0.048$, $N=17$).

Dewlap design, use, and social status

Aggression scores in males varied between −0.70 and 10.55 (mean \pm SE=2.39 \pm 0.49, $N=36$). We found no evidence for a relationship between mirror-motivated aggression and any of

the quality measurements (Pearson correlation tests, pairwise correlation coefficients varied between −0.07 and 0.22, all $p > 0.200$). It was not possible to predict the level of mirror-motivated aggression in males from relative dewlap area, pattern ratio or dewlap color (multiple regression analysis retained no predictor variables), nor from relative DE frequency (linear regression, $r=0.07$, $F_{1,26}=0.13$, and $p=0.720$).

For females, aggression scores varied between −0.35 and +2.30 (mean \pm SE=0.72 \pm 0.19, $N=18$). As in males, we found no evidence for a relationship between mirror-motivated aggression and any of the dewlap design variables (no predictor variables were retained via multiple backward stepwise regression analyses). Besides, more aggressive females did not use their dewlaps more than less aggressive females (Spearman's rho test, $\rho=0.19$, $p=0.493$, $N=15$).

Discussion

Sexual differences in dewlap design

Intersexual differences in dewlap design were clearly observed for all tested components (i.e., dewlap area, patterning, and color), which corresponds to earlier findings (Jenssen et al. 2000; Vanhooydonck et al. 2005b, 2009; Steffen and McGraw 2009). The male dewlap of several *Anolis* species has previously been shown to exhibit a positive allometry, but female dewlaps were never considered in those studies (Rensch 1959; Echelle et al. 1978). Given the high degree of sexual dimorphism present in the study species, coupled with the importance of competition for territories and mates (especially males), we expected dewlap area to increase faster with SVL in *A. sagrei* males than females (Vanhooydonck et al. 2005a). Surprisingly, we found a similar, highly positive allometry for dewlap area (RMA slopes > 3.0) in the female sex, suggesting that this trait is sexually selected in *A. sagrei* females as well. In line with our result, Harrison and Poe (2012) recently revealed that the evolution of anole dewlap size in

females is supported by the social selection and sensory drive hypotheses, more than by genetic correlations between sexes or species recognition.

Dewlap design and sexual identity

Secondary sexual characters are known to contribute to the advertisement of sexual identity to conspecifics in a range of animal taxa (Andersson 1994; Bradbury and Vehrencamp 1998). Given the costs of responding to inappropriate mates, efficient systems signaling sexual identity seem to be required to enhance optimal detection, recognition, and eventually, reproductive success (reviewed in Rowe 1999; Partan and Marler 2005). Obviously, body size (SVL) is an important component for signaling sexual identity in *A. sagrei*, a species that exhibits a high degree of sexual size dimorphism (Schoener and Schoener 1980; Schwartz and Henderson 1991). But additionally, different components of dewlap design involving relative dewlap area and color (i.e., particularly brightness and reflectance in the yellow red relative to UV green spectrum) raise the accuracy to 100 %, when discriminating between sexes. Our results therefore support the redundant message hypothesis, as several dewlap components in *A. sagrei* broadcast the same property of the sender, i.e., its sexual identity. Depending on the distance between signaler and receiver and prevailing environmental conditions, recognizing a potential mating partner based on the estimation of its SVL only may be a hard task to fulfill. We therefore suggest that repeating the same message in different ways using body size together with dewlap traits is a highly appropriate strategy to get information about sexual identity efficiently and accurately across, even under nonoptimal environmental conditions.

Dewlap design and use linked to quality and social status

Besides sexual identity, ornamental traits can also convey valuable information about a signaler's individual quality and social status (Andersson 1994; Bradbury and Vehrencamp 1998). We found that in *A. sagrei*, dewlap coloration is primarily responsible for signaling aspects of individual quality, but only in the male sex. Our results particularly show that individual health state parameters are reflected in the color components of both male dewlap center and edge and that multiple messages are conveyed by dewlap color. Firstly, we found that individuals bearing dewlaps with higher amounts of yellow and red and lower amounts of UV show higher body condition indexes. As carotenoids cannot be synthesized by vertebrates and have to be acquired from the diet, carotenoid expression in traits is believed to act as an honest signal of an individual's nutritional state (Lozano 1994). The fact that our results show a strong positive relationship between body condition and yellow and red dewlap color

components, which are derived from both pterin and carotenoid pigments (Steffen and McGraw 2009), may serve to support this idea. However, recent evidence in male common lizards (*Lacerta vivipara*) suggest that color variability may be strongly influenced by differences in underlying reflective surfaces such as iridophores, rather than differences in pigment allocation alone (San-Jose et al. 2013). In addition, Steffen et al. (2010) found that *A. sagrei* males whose diets were supplemented with xanthophylls did not exhibit changes in dewlap color, indicating that xanthophylls do not communicate information about immediate nutritional states or foraging success. Besides nutritional state and foraging success, body condition in lizards may also correlate with male attractiveness and resource-holding potential leading to higher reproductive success (Hack 1997; Fitzstephens and Getty 2000; Jonart et al. 2007; Henningsen and Irschick 2012). However, further research is required to test whether these assumptions also apply for the amounts of red and yellow relative to UV chroma in the male dewlap of *A. sagrei*. Secondly, we found that male individuals with brighter dewlaps have lower immunocompetence, measured as PHA swelling response. Brightness is defined as a measure of light reflected of a surface, and pigments act in a subtractive manner by absorbing certain wavelengths; bright dewlaps may therefore suggest the presence of less light-absorbing pigments (McGraw and Ardia 2003; Cook et al. 2013). Less pigments in the dewlap may, in turn, indicate that more carotenoids are allocated to immune response and anti-oxidant capacity (McGraw and Ardia 2003). This energetic trade-off between pigment allocation to ornamentation and to physiological functions has been proven in several animal taxa including one *Anolis* species. Cook et al. (2013) found that heavily parasitized *Anolis brevirostris* lizards exhibited brighter dewlaps, indicating that these heavily parasitized individuals allocate more pigment resources to immune function to combat parasitism than to ornamental coloration. Their study confirms the handicap principle (Zahavi 1975) and suggests that the carotenoid-based dewlap in *A. brevirostris* acts as an honest signal of parasite resistance in adult males. We, on the other hand, could not find support for the energetic trade-off between pigment allocation to ornament coloration and immune function in *A. sagrei* males. Instead, we found a strong positive association, which may again lead us to the findings of San-Jose et al. (2013) that carotenoid-based ornaments do not exclusively reflect differences in integumentary carotenoid content and male qualities linked to carotenoid deposition (e.g., foraging success, immune function, and anti-oxidant capacity). Thirdly, we found that male individuals with more yellow and UV chroma in dewlap edge exhibit higher hematocrit values. Whereas the dewlap center and edge transfer the same information and thus act as redundant signals for body condition and immune response, only the dewlap edge signals an individual's hematocrit. A recent study by Steffen and Guyer

(2014) showed that UV, orange, and red coloration in the dewlap edge is the region with a signaling function when it comes to male–male contest success in *A. sagrei* and not the dewlap center. The center region of the dewlap can only be viewed during extension. In contrast, color components of the dewlap edge can transfer information during extension as well as retraction mode. Thus, a dewlap edge region with signaling function involves less energy expenditures than a signaling dewlap center, as information can still be transferred to a conspecific without extending the structure. Moreover, a signaling dewlap edge may additionally be beneficial in risky contexts where minimum visibility would be appropriate to avoid predators (Stuart-Fox et al. 2003). Yet, the fact that both dewlap regions together convey the same information about body index and immune response in our study also stresses the importance of redundant signaling within a context of dewlap use. If a lizard extends its dewlap, the redundant message conveyed by dewlap center and edge will be more likely to reach and inform the receiver about certain aspects of health state correctly (i.e., body index and immunity) and thus indirectly about male quality. The reason why for some parameters dewlap edge and center act as redundant signals, whereas for others this does not hold true, still remains unclear. We found no correlations among our three distinct health parameters (body condition, swelling response, and hematocrit), which further demonstrates that separate dewlap color components irrespective of the region, convey nonredundant information about different aspects of health state in *A. sagrei* males (multiple-message hypothesis). Besides, we found that individuals with high values for a certain health parameter do not necessarily show high scores for all physiological health measurements. This finding highlights the necessity to consider multiple parameters separately when assessing individual quality and additionally indicates the danger of extrapolating results about general health state based on only one measurement. Surprisingly, none of the tested components of dewlap design in *A. sagrei* males conveyed information on performance capacities and aggressive behavior. Previous studies on anole lizards have shown strong links between primarily sexual signal size and performance, as well as dominance within the framework of male–male competition. For example, relative dewlap size of several anole species acts as an honest indicator of maximum bite force (*Anolis grahami* and *Anolis lineatopus*; Vanhooydonck et al. 2005a; *Anolis carolinensis*; Henningsen and Irschick 2012). Although we found a strong positive correlation between absolute dewlap area and bite force in our *A. sagrei* subjects, this relationship does not hold true when correcting both variables for body size, indicating the importance of underlying allometry. In another study, Cox et al. (2009) showed that absolute measures of dewlap area and bite force were indeed positively correlated in *A. sagrei* males and that relative measures of size-corrected dewlap area and bite force were positively

correlated for pretreatment (winter) but not posttreatment (spring) tests. Their results together with our findings suggest that the link between relative dewlap area and bite force in *A. sagrei* may depend on seasonal changes and may be more complex than in other anole species.

To maximize transfer of valuable information about an individual's quality and social status, signaling often involves the combination of ornamental design and behaviors that display it. For example, Steffen and Guyer (2014) showed that dewlap color and display behavior (a composite of head-bob, push-up, and DE) in *A. sagrei* have a combined signaling function in male–male combat that gives winners access to territories and to females. Cook et al. (2013) also found an association between dewlap color and display behavior (DE only), both providing information on individual health state in *A. brevirostris* lizards. It is thus somewhat unexpected to find that dewlap use (DE frequency) in our *A. sagrei* males conveyed no information about an individuals' health status, nor about its performance and aggressiveness. Moreover, no single relationship was found between dewlap use in an intersexual context and different aspects of dewlap design. A previous study with the same *A. sagrei* individuals revealed that males increased their DE frequency significantly when confronted with females compared to other social and predator contexts, suggesting an important role of the male dewlap for courtship (Driessens et al. 2014). We consider that dewlap use in *A. sagrei* males may function in sex recognition and the advertisement of sexual receptivity to females to motivate copulation or to stimulate female receptivity (Crews 1975; Stamps 1977; Orrell and Jenssen 2002; Simon 2011), rather than in signaling male quality to females. The fact that we found no correlations between dewlap use and the color components in the dewlap center (PC_{mc1} and PC_{mc2}) that are shown to be important indicators of health state (i.e., body condition and immune response) may support this idea. Besides, no evidence for female mate choice based on male or territory quality has been convincingly shown in *A. sagrei* (Tokarz 1998; Flanagan and Bevier 2014). Additional experimental work is, of course, necessary to investigate the proposed functions of dewlap extensions in *A. sagrei* males, as well as to test the link between dewlap use and other measurements of male quality (e.g., survival rate, sperm quality, reproduction rate, etc.). Additionally, we stress that dewlap use in our study subjects was only observed in standardized lab terraria providing a framework for short-, but not long-distance interactions. Future experiments in the field, incorporating long-distance signaling, are necessary to further clarify the messages transferred during dewlap extensions.

In contrast to males, the messages conveyed by the female dewlap have received far less attention. For our study species, we found that neither dewlap design nor dewlap use in

females transfers information about the chosen performance, general health, and mirror-motivated aggression parameters. However, this result does not exclude the possibility that the female dewlap components may still act as reliable signals of other individual quality measurements. This may be especially true for dewlap area, as this trait showed strong positive allometry in our female subjects. In contrast to the males, we found correlations between dewlap design and use in the *A. sagrei* females. Individuals with larger dewlaps showed higher dewlap extension frequencies during intersexual interactions only, and the same is true for individuals with less bright dewlap centers. However, these results should be interpreted with caution as we only have a sample size of 17 individuals and a large variation in DE frequency among individuals. In a previous study with the same female subjects, we observed a strong trend for increased DE rates in the presence of males, suggesting an important role of female dewlap use in advertising sexual receptivity prior to copulation and/or in a context of male mate choice. Advertising sexual receptivity to males would be beneficial for females, as sexual coercion imposed by males may be costly to both individuals and may additionally reduce female fecundity and survival (Clutton-Brock and Parker 1995; Le Galliard et al. 2005). Regarding male mate choice, it has previously been shown that *A. sagrei* males discriminate among females as mating partners and use this ability to control mating decisions within their territories (preference for unfamiliar females, Tokarz 2006; Orrell and Jenssen 2002). Also in *A. carolinensis*, evidence for male mate choice was observed as the territorial males passed up 69 % of the mating opportunities with receptive females to search for a more suitable mate (Jenssen et al. 1995; Jenssen and Nunez 1998). For our study species, it seems that neither female size nor reproductive condition and experience are factors that can be used to explain a male's decision to mate with specific females (Tokarz 1998; Warner et al. 2013). But, to our knowledge, no study has addressed male preferences for dewlap characteristics in *A. sagrei* females. The correlations we found between female dewlap design and use during intersexual interactions fit into the context of male mate choice and may suggest that females want to impress males by potentially signaling reproductive quality in order to be chosen as suitable mating partner above other females. To confirm the proposed ideas, additional experiments in the lab and on the field are required, preferably with higher sample sizes and with the incorporation of dewlap color in the edge region. Especially, the investigation of links between female dewlap traits (i.e., area, color, patterning, and use) and qualities related to reproductive performance (e.g., egg size and quality, number of successive eggs produced, the quality of yolk involving nutritive content, steroid hormones, etc.) should clarify whether females indeed use their dewlap to signal reproductive quality to males.

Conclusion

In conclusion, we can state that the dewlap signaling device is a complex integrated system consisting of different components transferring redundant (sexual identity) as well as non-redundant information (individual quality). We found that both the dewlap center and edge bear a signaling function, but this was only tested in males. As expected, male and female dewlaps differ in the messages they convey and further research is necessary to clarify other possible signaling functions related to individual quality in females.

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Compliance with Ethical Standards Our research complies with current laws in Belgium; animal housing and behavioral testing were conducted under permit of the Ethical committee on animal experimentation (ECD 2011-64).

Conflict of interest The authors declare that they have no conflict of interest.

References

- Andersson M (1994) Sexual selection. Princeton University Press, Princeton
- Bels V (1990) The mechanism of dewlap extension in *Anolis carolinensis* (Reptilia: Iguanidae) with histological analysis of the hyoid apparatus. *J Morphol* 206:225–244
- Blumstein DT, Evans CS, Daniel JC (2000) JWatcher V1.0. An introductory user's guide, <http://www.jwatcher.ucla.edu>. Accessed March 2012
- Bradbury JW, Vehrencamp SL (1998) The principles of animal communication. Sinauer Associates, Sunderland
- Candolin U (2003) The use of multiple cues in mate choice. *Biol Rev* 78: 575–595
- Clutton-Brock TH, Parker GA (1995) Sexual coercion in animal societies. *Anim Behav* 49:1345–1365
- Cook EG, Murphy TG, Johnson MA (2013) Colorful displays signal male quality in a tropical anole lizard. *Naturwissenschaften* 100: 993–996
- Cox RM, Stenquist DS, Henningsen JP, Calsbeek R (2009) Manipulating testosterone to assess links between behavior, morphology, and performance in the brown anole *Anolis sagrei*. *Physiol Biochem Zool* 82:686–698
- Cox RM, Parker EU, Cheney DM, Liebl AL, Martin LB, Calsbeek R (2010) Experimental evidence for physiological costs underlying the trade-off between reproduction and survival. *Funct Ecol* 24:1262–1269
- Crews D (1975) Effects of different components of male courtship behavior on environmentally induced ovarian recrudescence and mating preferences in the lizard, *Anolis carolinensis*. *Anim Behav* 23: 349–356

- Cuthill IC, Bennett ATD, Partridge JC, Maier EJ (1999) Plumage reflectance and the objective assessment of avian sexual dichromatism. *Am Nat* 160:183–200
- Driessens T, Vanhooydonck B, Van Damme R (2014) Detering predators, daunting opponents or drawing partners? Signaling rates across diverse contexts in the lizard *Anolis sagrei*. *Behav Ecol Sociobiol* 68:173–184
- Dunn PO, Garvin JC, Whittingham LA, Freeman-Gallant CR, Hasselquist D (2010) Carotenoid and melanin-based ornaments signal similar aspects of male quality in two populations of the common yellowthroat. *Funct Ecol* 24:149–158
- Echelle AG, Echelle AA, Fitch HS (1978) Inter- and intraspecific allometry in a display organ: The dewlap of *Anolis* (Iguanidae) species. *Copeia* 1978:245–250
- Elstrott J, Irschick DJ (2004) Evolutionary correlations among morphology, habitat use and clinging performance in Caribbean *Anolis* lizards. *Biol J Linn Soc* 83:389–398
- Endler JA (1990) On the measurement and classification of color in studies of animal color patterns. *Biol J Linn Soc* 41:315–352
- Fitzstephens DM, Getty T (2000) Colour, fat and social status in male damselflies, *Calopteryx maculata*. *Anim Behav* 60:851–855
- Flanagan SP, Bevier CR (2014) Do male activity and territory quality affect female association time in the brown anole, *Anolis sagrei*? *Ethology* 120:365–374
- Fleishman LJ, Loew ER, Leal M (1993) Ultraviolet vision in lizards. *Nature* 365:397
- Fleishman LJ, Leal M, Sheehan J (2006) Illumination geometry, detector position and the objective determination of animal signal colors in natural light. *Anim Behav* 71:463–474
- Forsman A, Lindell LE (1996) Resource dependent growth and body condition dynamics in juvenile snakes: an experiment. *Oecologia* 108:669–675
- Freeman-Gallant CR, Schneider RL, Taff CC, Dunn PO, Whittingham LA (2014) Contrasting patterns of selection on the size and coloration of a female plumage ornament in common yellowthroats. *J Evol Biol* 27:982–991
- Greenberg G, Noble GK (1944) Social behavior of the American chameleon (*Anolis carolinensis* Voigt). *Physiol Zool* 17:392–439
- Grether GF, Kolluru GR, Nersissian K (2004) Individual color patches as multicomponent signals. *Biol Rev* 79:583–610
- Griggio M, Serra L, Licheri D, Campomori C, Pilastro A (2009) Molt speed affects structural feather ornaments in the blue tit. *J Evol Biol* 22:782–792
- Guilford T, Dawkins MS (1991) Receiver psychology and the evolution of animal signals. *Anim Behav* 42:1–14
- Hack MA (1997) Assessment strategies in the contests of male crickets, *Acheta domesticus* (L.). *Anim Behav* 53:733–747
- Harrison A, Poe S (2012) Evolution of an ornament, the dewlap, in females of the lizard genus *Anolis*. *Biol J Linn Soc* 106:191–201
- Hebets EA, Papaj DR (2005) Complex signal function: developing a framework of testable hypotheses. *Behav Ecol Sociobiol* 57:197–214
- Henningsen JP, Irschick DJ (2012) Manipulating dewlap size reveals that performance is more important than signal size in determining the outcome of staged dominance interactions in male green anole lizards. *Funct Ecol* 26:3–10
- Herrel A, Spithoven L, Van Damme R, De Vree F (1999) Sexual dimorphism of head size in *Gallotia galloti*: testing the niche divergence hypothesis by functional analysis. *Funct Ecol* 13:289–297
- Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: a comparison of body condition indices. *Oikos* 77:61–67
- Jenssen TA (1970) The ethoecology of *Anolis nebulosus*. *J Herpetol* 4:1–38
- Jenssen TA, Nunez SC (1998) Spatial and breeding relationships of the lizard, *Anolis carolinensis*: evidence of intrasexual selection. *Behaviour* 135:981–1003
- Jenssen TA, Greenberg N, Hovde KA (1995) Behavioral profile of free-ranging male lizards, *Anolis carolinensis*, across breeding and post-breeding seasons. *Herpetol Monogr* 9:41–62
- Jenssen TA, Orrell KS, Lovorn MB (2000) Sexual dimorphisms in aggressive signal structure and use by a polygynous lizard, *Anolis carolinensis*. *Copeia* 2000:140–149
- Johnstone RA (1995) Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. *Biol Rev* 70:1–65
- Johnstone RA (1996) Multiple displays in animal communication: “back-up signals” and “multiple messages”. *Philos T Roy Soc B* 35:329–338
- Jonart LM, Hill GE, Badyaev AV (2007) Fighting ability and motivation: determinants of dominance and contest strategies in females of a passerine bird. *Anim Behav* 74:1675–1681
- Kennedy MW, Nager RG (2006) The perils and prospects of using phytohaemagglutinin in evolutionary ecology. *Trends Ecol Evol* 21:653–655
- Kodric-Brown A, Nicoletto PF (2001) Female choice in the guppy (*Poecilia reticulata*): the interaction between male color and display. *Behav Ecol Sociobiol* 50:346–351
- Kodric-Brown A, Sibly RM, Brown JH (2006) The allometry of ornaments and weapons. *Proc Natl Acad Sci U S A* 103:8733–8738
- Le Galliard JF, Fitze PS, Ferriere R, Clobert J (2005) Sex ratio bias, male aggression, and population collapse in lizards. *Proc Natl Acad Sci U S A* 102:18231–18236
- Leal M, Fleishman LJ (2002) Evidence for habitat partitioning based on adaptation to environmental light in a pair of sympatric lizard species. *Proc R Soc Lond B* 269:351–359
- Leal M, Fleishman LJ (2004) Differences in visual signal design and detectability between allopatric populations of *Anolis* lizards. *Am Nat* 163:26–39
- Leal M, Rodríguez-Robles JA (1995) Antipredator responses of *Anolis cristatellus* (Sauria: Polychrotidae). *Copeia* 1995:155–161
- Leal M, Rodríguez-Robles JA (1997) Signaling displays during predator-prey interactions in a Puerto Rican anole, *Anolis cristatellus*. *Anim Behav* 54:1147–1154
- Lee JC, Clayton D, Eisenstein S, Perez I (1989) The reproductive cycle of *Anolis sagrei* in southern Florida. *Copeia* 1989:930–937
- Licht P, Gorman GC (1970) Reproductive and fat cycles in Caribbean *Anolis* lizards. *Univ Calif Publ Zool* 95:1–52
- Locatello L, Pizzolon M, Rasotto BM (2012) One trait, many signals: different information on male quality is enclosed within the same trait in a blenny fish. *Naturwissenschaften* 99:863–867
- Losos JB (1985) An experimental demonstration of the species recognition role of the *Anolis* dewlap color. *Copeia* 1985:905–910
- Losos JB (1990) Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol Monogr* 60:369–388
- Losos JB, Irschick DJ (1996) The effect of perch diameter on escape behavior of *Anolis* lizards: laboratory predictions and field tests. *Anim Behav* 51:593–602
- Lozano GA (1994) Carotenoids, parasites, and sexual selection. *Oikos* 70:309–311
- Macedonia JM, James S, Wittle LW, Clark DL (2000) Skin pigments and coloration in the Jamaican radiation of *Anolis* lizards. *J Herpetol* 34:99–109
- MacLean GS, Lee AK, Wilson KJ (1973) A simple method of obtaining blood from lizards. *Copeia* 1973:338–339
- Martin LB II, Han P, Lewittes J, Kuhlman JR, Klasing KL, Wikelski M (2006) Phytohemagglutinin-induced skin swelling in birds: histological support for a classic immunoeological technique. *Funct Ecol* 20:290–299
- McGraw KJ, Ardia DR (2003) Carotenoids, immunocompetence, and the information content of sexual colors: an experimental test. *Am Nat* 162:704–712

- McMann S (1993) Contextual signaling and the structure of dyadic encounters in *Anolis carolinensis*. *Anim Behav* 46:657–668
- Møller AP, Pomiankowski A (1993) Why have birds got multiple sexual ornaments? *Behav Ecol Sociobiol* 32:167–176
- Montgomerie R (2006) Analyzing colors. In: Hill GE, McGraw KJ (eds) *Bird coloration: mechanics and measurements*. Harvard University Press, Cambridge, pp 90–147
- Nicholson KE, Harmon LJ, Losos JB (2007) Evolution of *Anolis* lizard dewlap diversity. *PLoS ONE* 2, e274
- Orrell KS, Jenssen TA (2002) Male mate choice by the lizard *Anolis carolinensis*: a preference for novel females. *Anim Behav* 63: 1091–1102
- Ots I, Murumagi A, Horak P (1998) Haematological health state indices of reproducing great tits: methodology and sources of natural variation. *Funct Ecol* 12:700–707
- Partan SR, Marler P (2005) Issues in the classification of multimodal communication signals. *Am Nat* 166:231–245
- Partan SR, Otovic P, Price VL, Scott SE (2011) Assessing display variability in wild brown anoles *Anolis sagrei* using a mechanical lizard model. *Curr Zool* 57:140–152
- Perry G, Levering K, Girard I, Garland T Jr (2004) Locomotor performance and dominance in male *Anolis cristatellus*. *Anim Behav* 67: 37–47
- Rand AS, Williams EE (1970) An estimation of redundancy and information content of anole dewlaps. *Am Nat* 104:99–103
- Rensch B (1959) *Evolution above the species level*. Columbia University Press, New York
- Rowe C (1999) Receiver psychology and the evolution of multicomponent signals. *Anim Behav* 58:921–931
- San-Jose LM, Granado-Lorencio F, Sinervo B, Fitze PS (2013) Iridophores and not carotenoids account for chromatic variation of carotenoid-based coloration in common lizards (*Lacerta vivipara*). *Am Nat* 181:396–409
- Schoener TW, Schoener A (1980) Densities sex ratios and population structure in four species of Bahamian *Anolis* lizards. *J Anim Ecol* 49:19–54
- Schwartz A, Henderson RW (1991) *Amphibians and reptiles of the West Indies: descriptions, distributions, and natural history*. University Press of Florida Press, Gainesville
- Sigmund WR (1983) Female preferences for *Anolis carolinensis* males as a function of dewlap color and background coloration. *J Herpetol* 17:137–143
- Simon V (2011) Communication signal rates predict interaction outcome in the brown anole lizard, *Anolis sagrei*. *Copeia* 2011:38–45
- Stamps JA (1977) The relationship between resource competition, risk, and aggression in a tropical territorial lizard. *Ecology* 58:349–358
- Steffen JE, Guyer CC (2014) Display behaviour and dewlap colour as predictors of contest success in brown anoles. *Biol J Linn Soc* 111: 646–655
- Steffen JE, McGraw KJ (2007) Contributions of pterin and carotenoid pigments to dewlap coloration in two anole species. *Comp Biochem Phys B* 146:42–46
- Steffen JE, McGraw KJ (2009) How dewlap color reflects its carotenoid and pterin content in male and female brown anoles (*Norops sagrei*). *Comp Biochem Phys B* 154:334–340
- Steffen JE, Hill GE, Guyer CC (2010) Carotenoid access, nutritional stress, and the dewlap color of male brown anoles. *Copeia* 2010: 239–246
- Stuart-Fox DM, Moussalli A, Marshall NJ, Owens IPF (2003) Conspicuous males suffer higher predation risk: visual modeling and experimental evidence from lizards. *Anim Behav* 66:541–550
- Takahashi M, Arita H, Hiraiwa-Hasegawa M, Hasegawa T (2008) Peahens do not prefer peacocks with more elaborate trains. *Anim Behav* 75:1209–1219
- Tokarz RR (1998) Mating pattern in the lizard, *Anolis sagrei*: implications for mate choice and sperm competition. *Herpetologica* 54: 388–394
- Tokarz RR (2006) Importance of prior physical contact with familiar females in the development of a male courtship and mating preference for unfamiliar females in the lizard *Anolis sagrei*. *Herpetologica* 62:115–124
- Van Berkum FH (1986) Evolutionary patterns of the thermal sensitivity of sprint speed in *Anolis* lizards. *Evolution* 40:594–604
- Van Sluys M (1998) Growth and body condition of the saxicolous lizard *Tropicurus itambere* in southeastern Brazil. *J Herpetol* 32: 359–365
- Vanhooydonck B, Van Damme R (2001) Evolutionary trade-offs in locomotor capacities in lacertid lizards: are splendid sprinters clumsy climbers? *J Evol Biol* 14:6–54
- Vanhooydonck B, Herrel A, Van Damme R, Irschick DJ (2005a) Does dewlap size predict male bite performance in Jamaican *Anolis* lizards? *Funct Ecol* 19:38–42
- Vanhooydonck B, Herrel A, Van Damme R, Meyers JJ, Irschick DJ (2005b) The relationship between dewlap size and performance changes with age and sex in a green anole (*Anolis carolinensis*) lizard population. *Behav Ecol Sociobiol* 59:157–165
- Vanhooydonck B, Herrel A, Van Damme R, Irschick DJ (2006) The quick and the fast: the evolution of acceleration capacity in *Anolis* lizards. *Evolution* 60:2137–2147
- Vanhooydonck B, Herrel A, Meyers JJ, Irschick DJ (2009) What determines dewlap diversity in *Anolis* lizards? An among-island comparison. *J Evol Biol* 22:293–305
- Warner DA, Kelly CD, Lovern MB (2013) Reproductive experience affects mating behavior, but does not impact parental allocation in a lizard. *Behav Ecol Sociobiol* 67:973–983
- Zahavi A (1975) Mate selection—a selection for a handicap. *J Theor Biol* 53:205–214