



Faculteit Wetenschappen

Departement Biologie

**Visual communication from a functional
and evolutionary perspective:
what does the *Anolis dewlap* say?**

-

**Visuele communicatie bekeken vanuit
een functioneel en evolutionair perspectief:
wat vertelt de *Anolis* keelvlag ons?**

Proefschrift voorgelegd tot het behalen van de graad Doctor in de Wetenschappen

aan de Universiteit Antwerpen

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*Anolis sagrei on Cuba,
photo taken by S. De Decker*

Biology gives you a brain, life turns it into a mind!

JEFFREY EUGENIDES - NOVELIST

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Summary

Animals communicate using a variety of signals that can differ dramatically among and even within species. The astonishing signal diversity found in the lizard genus *Anolis* has attracted considerable attention. Anoles are distributed throughout the Neotropics and communicate primarily by means of conspicuous visual displays consisting of head-nods (HN), push-ups (PU) and dewlap extensions (DE). The latter display type involves the extension and retraction of the dewlap, an extendible brightly-coloured flap of skin attached to the throat. Dewlap displays are observed in a variety of contexts, including encounters with mates, rivals, members of related species, and even predators. Yet, dewlap use and design (size, colour and pattern) vary considerably in *Anolis*, raising several questions about the exact function and complexity of this visual communication system. In addition, it remains poorly understood which evolutionary processes shape this extensive diversity in dewlap use and design. Although females of nearly all anole species have a dewlap, most of the studies have exclusively focused on males.

In this PhD dissertation, I used the brown anole lizard (*Anolis sagrei*) to study the signalling functions of the dewlap, as well as the evolutionary origins of geographical variation in this signalling device. Complementary to previous studies, I investigated these topics in males and females.

To study the signalling functions of the dewlap (Part I: chapters 2 to 3), I took an experimental approach by testing wild-caught *A. sagrei* lizards under controlled laboratory conditions.

Firstly, I aimed to better understand the context in which each display type (HN, PU, and particularly DE) is used by repeatedly exposing *A. sagrei* males and females to cues of predators and conspecific males and females (chapter 2).

I found that overall males have higher signalling rates than females across all contexts. In addition, results showed that lizards of both sexes exhibited higher display rates in the presence of conspecifics than when confronted with a predator. This indicates that HN, PU and DE in *A. sagrei* function primarily in intraspecific communication and not, in predator deterrence. I detected pronounced intersexual differences for HN and PU rates during social interactions. Yet, in both sexes, dewlap displays are primarily used in a courtship context.

Secondly, I studied the messages conveyed by assorted facets of the male and female dewlap (chapter 3). Additionally, I examined whether diverse facets of dewlap signalling provide additive information (redundant messages) or highlight different characteristics of the sender (multiple messages). To do this, several dewlap components were linked to sexual identity, estimates of individual quality (performance and health status), and social status. I found that body size together with relative dewlap size and dewlap colouration act as redundant messages in the advertisement of sexual identity. Further, my results revealed that dewlap colour in both the centre and edge region signals aspects of individual quality (specifically health state), but only in males. Whereas different colour components convey multiple messages about different aspects of health state in males (body condition, immune response, haematocrit), the dewlap centre and edge region mostly transfer redundant information on individual quality. For females, none of the measured dewlap characteristics were related to estimates of individual quality or social status. Nevertheless, I observed significant correlations between female dewlap design and dewlap display rates in an intersexual context, hinting again at a signalling function of the female dewlap during courtship.

In my studies on dewlap diversity and the evolutionary processes underlying it, I took a comparative approach using 17 *A. sagrei* populations distributed across several islands in the Caribbean (Part II: chapters 4 to 6). Notably, I extended an

existing dataset by sampling ten extra island populations and by measuring additional dewlap components (colour and display).

In chapter 4, I focused only on *A. sagrei* males and in particular, studied whether dewlap size signals bite force, an important determinant of combat outcome, in each of the study populations. Moreover, I examined whether the degree of intrasexual selection underlies variation in this honest signal size – fighting capacity relationship among populations. Regression analyses revealed that absolute dewlap size is an excellent predictor of bite force in nearly all populations. However, relative male dewlap size (independent of body size) is only a direct signal of bite force in a few populations. I suggest that dewlap size in *A. sagrei* males acts primarily as a redundant signal for body size in the advertisement of fighting capacity and only in certain cases as an honest, direct signal of bite force. Surprisingly, the degree of intrasexual selection (SSD and display intensity) could not explain the level of signal honesty found among the *A. sagrei* populations; other factors such as resource availability probably can. Overall, this study clearly shows that the link between signal size and performance traits can differ substantially within a single species.

For nearly all male and female dewlap components (size, colour, pattern, displays), I observed significant differences among *A. sagrei* populations. In chapter 5, I have tried to replicate and extend previously reported results on the contribution of various biotic selective factors (predation pressure, sexual selection and species recognition) in explaining these interpopulational differences in dewlap design and displays. Unexpectedly, in neither males nor females estimates of predation pressure or sexual selection could explain dewlap diversity. Yet, I found indirect support for the species recognition hypothesis, as *A. sagrei* males (but not females) were more likely to have a ‘spotted’ dewlap pattern when co-occurring with a higher number of syntopic *Anolis* species. Overall, I could only find limited support for the idea that dewlap diversity in *A. sagrei* is mediated by variation in these biotic selective factors. Therefore, in chapter 6, I tested whether climate-related environmental conditions could help to explain interpopulational variability in dewlap characteristics. My results showed

evidence for a strong relationship between dewlap design and prevailing environmental conditions, which may derive from differential selection on signal efficacy ('sensory drive'). More precisely, I found that males and females occurring in 'xeric' environments had a higher proportion of solid dewlaps with higher UV-reflectance. Lizards inhabiting 'mesic' environments had primarily marginal dewlaps showing high reflectance in red. For dewlap display, a correlation with environment was only observed in males. These results indicate that *A. sagrei* males increase dewlap displays in environments characterized by lower visibility and complex habitat structures, probably to enhance signal transmission.

Overall, my PhD dissertation shows that the dewlap is a complex multi-component signalling device used for exchanging both redundant and non-redundant information during social interactions and especially, during courtship. Nevertheless, the information conveyed by dewlap components can vary greatly, even within a single species. I found a remarkable dewlap diversity among *A. sagrei* populations that can partially be explained by variation in climate-related environmental conditions and the number of syntopic *Anolis* species. To conclude, my PhD dissertation may provide groundwork for future research on the function and evolution of the *Anolis* dewlap, and may additionally stimulate an increased interest in the so far 'scarcely studied' female ornaments.

Samenvatting

Dieren communiceren via diverse signalen, die sterk kunnen verschillen tussen en zelfs binnen soorten. De verbazingwekkende signaaldiversiteit die te vinden is in het hagedisgeslacht *Anolis*, heeft reeds veel aandacht getrokken. Anolissen komen voor doorheen de Neotropen en communiceren voornamelijk door middel van opvallende visuele vertoningen (displays), bestaande uit het kopknikken (HN), opduwbewegingen (PU) en keelvlaggen (DE). Dit laatste display type omvat de extensie en retractie van de keelvlag, een uitklapbare felgekleurde huidflap die vastgehecht is aan de keel. Keelvlaggebruik kan worden waargenomen in verschillende contexten, waaronder ontmoetingen met partners, rivalen, verwante soorten en zelfs predatoren. Toch variëren keelvlaggebruik en keelvlagdesign (d.i. grootte, kleur en patroon) aanzienlijk in *Anolis*, wat vragen doet rijzen over de precieze functie en complexiteit van dit visueel communicatiesysteem. Bovendien is het nog steeds onduidelijk welke evolutionaire processen vorm geven aan deze verbazingwekkende diversiteit in keelvlaggebruik en design. Hoewel vrouwtjes in bijna alle *Anolis* soorten ook een keelvlag bezitten, zijn de meeste keelvlagstudies uitsluitend gericht op de mannetjes.

In dit proefschrift heb ik de bruine anolis (*Anolis sagrei*) gebruikt om zowel de signaalfuncties van de keelvlag als de evolutionaire oorsprong van geografische variatie in dit communicatiesysteem, te bestuderen. In aanvulling op eerdere studies onderzocht ik deze thema's in zowel mannetjes als vrouwtjes.

Voor het bestuderen van de signaalfuncties van de keelvlag (deel I: hoofdstukken 2-3) koos ik een experimentele aanpak, waarbij wildvang *A. sagrei* hagedissen getest werden onder gecontroleerde laboratoriumomstandigheden.

Mijn eerste studie was gericht op het onderzoeken van de specifieke context waarin *A. sagrei* mannetjes en vrouwtjes de diverse display types gebruiken (HN, PU and DE in het bijzonder; hoofdstuk 2). Hiervoor werden bruine anolissen herhaaldelijk blootgesteld aan stimuli van predatoren en soortgenoten (intraseksuele en interseksuele interacties). Mijn resultaten toonden dat mannetjes aan een hogere intensiteit displayen dan vrouwtjes in alle contexten. Daarnaast constateerde ik dat beide seksen meer displayen in de aanwezigheid van soortgenoten dan wanneer ze geconfronteerd worden met een predator. Dit resultaat geeft aan dat HN, PU en DE in *A. sagrei* voornamelijk functioneren in intraspecifieke communicatie en niet in het afschrikken van predatoren. Verder vond ik uitgesproken interseksuele verschillen voor de intensiteit aan HN en PU tijdens de diverse sociale interacties. De DE blijken evenwel in beide seksen vooral gebruikt te worden tijdens hofmakerij.

In een tweede studie heb ik specifiek gekeken naar de ‘informatie’ die vervat zit in diverse componenten van de mannelijke en vrouwelijke keelvlag (hoofdstuk 3). Ik onderzocht bovendien of deze keelvlagcomponenten redundante informatie bevatten of verschillende karakteristieken van de zender communiceren. Hiervoor heb ik verscheidene keelvlagcomponenten gekoppeld aan seksuele identiteit, maten voor individuele kwaliteit (atletisch vermogen en gezondheidsconditie) en sociale status. Ik vond dat lichaamsgrootte samen met relatieve keelvlaggrootte en keelvlagkleur redundant zijn in het adverteren van seksuele identiteit. Voorts toonden mijn resultaten dat kleurcomponenten in het centrum en de periferie van de keelvlag aspecten van individuele kwaliteit communiceren (specifiek gezondheidsconditie), maar enkel in mannetjes. Terwijl diverse keelvlagkleurcomponenten informatie transfereren over verschillende aspecten van de gezondheidsconditie in mannetjes (lichaamsconditie, immuunrespons, hematocriet; ‘*multiple message hypothesis*’), bevatten het centrum en de periferie van de keelvlag redundante informatie over deze gezondheidsparameters (‘*redundant message hypothesis*’). In vrouwtjes daarentegen, waren geen van de gemeten keelvlagkenmerken gecorreleerd met maten van individuele kwaliteit of sociale status. Toch vond ik hier significante

correlaties tussen keelvlagkleur/grootte en keelvlaggebruik in een interseksuele context, wat opnieuw kan duiden op een signaalfunctie van de vrouwelijke keelvlag tijdens hofmakerij.

In mijn studies over keelvlagdiversiteit en de evolutionaire processen die aan de basis liggen, koos ik voor een comparatieve benadering (deel II: hoofdstukken 4-6). Hiervoor gebruikte ik data van 17 *A. sagrei* populaties verspreid over diverse eilanden in de Caraïben. Met dit onderzoek bouwde ik een reeds bestaande dataset uit door de bemonstering van tien extra eilandpopulaties en door het meten van additionele keelvlagcomponenten (keelvlaggebruik en keelvlagkleur).

In hoofdstuk 4 heb ik me enkel toegespitst op de *A. sagrei* mannetjes, om specifiek te onderzoeken of keelvlaggrootte een betrouwbaar signaal is voor bijtkracht, een belangrijke determinant van succes bij mannelijke gevechten. Ik heb dit getest voor elke populatie apart, en nadien onderzocht of de mate van intraseksuele selectie een verklaring kan bieden voor de variatie in deze signaalgrootte – vechts capaciteit verhouding tussen populaties. Uit mijn regressie analyses bleek dat absolute keelvlaggrootte een uitstekende indicator is voor bijtkracht in bijna alle populaties. Echter, relatieve keelvlaggrootte (onafhankelijk van lichaamsgrootte) is een eerlijk signaal voor bijtkracht in slechts enkele populaties. Ik suggereer dat keelvlaggrootte in *A. sagrei* mannetjes voornamelijk fungeert als een redundant signaal voor lichaamsgrootte in het verkondigen van vechtcapaciteit, en slechts in bepaalde gevallen als een eerlijk direct signaal voor bijtkracht. Verrassend genoeg kon de intensiteit van intraseksuele selectie (seksuele dimorfie in lichaamsgrootte en display intensiteit) geen verklaring bieden voor de variatie in het niveau van signaaleerlijkheid tussen de populaties; mogelijk kunnen andere factoren zoals voedselbeschikbaarheid dat wel. Algemeen toont deze studie aan dat de relatie tussen signaalgrootte en atletisch vermogen substantieel kan verschillen binnen een enkele soort.

Voor bijna alle keelvlagcomponenten (grootte, kleur, patroon, displays) documenteerde ik significante verschillen tussen *A. sagrei* populaties in mannetjes, maar ook in vrouwtjes. In hoofdstuk 5 heb ik getracht om eerder gerapporteerde resultaten over de bijdrage van biotische selectieve factoren

(predatiedruk, seksuele selectie, soortherkenning) in het verklaren van deze interpopulationale keelvlagvariatie, te valideren en uit te breiden. Verrassend genoeg kon noch predatiedruk, noch seksuele selectie de exceptionele keelvlagdiversiteit in *A. sagrei* verklaren. Ik vond echter wel indirect bewijs voor een bijdrage van soortherkenning (*'species recognition hypothesis'*), daar een hoger percentage aan *A. sagrei* mannetjes (maar niet vrouwtjes) met een gespot kleurpatroon correleerde met het voorkomen van een hoger aantal syntopische *Anolis* soorten. Over het algemeen kon ik in deze studie slechts weinig bewijs vinden voor het idee dat keelvlagdiversiteit in de bruine anolis gemedieerd wordt door variatie in de besproken biotische selectiedrukken. Daarom heb ik in hoofdstuk 6 getest of klimaat-gerelateerde omgevingscondities mogelijk een meer uitgesproken verklaring kunnen bieden voor die verbazingwekkende geografische variatie in de *A. sagrei* keelvlag. Mijn resultaten leverden bewijs voor een sterke relatie tussen keelvlagdesign en heersende omgevingscondities, mogelijk het gevolg van differentiële selectie voor signaalefficiëntie (*'sensory drive hypothesis'*). Specifiek kon ik aantonen dat mannetjes en vrouwtjes die voorkomen in *'xerische'* omgevingen vaker een uniform gekleurde keelvlag hebben met hoge reflectie in het UV. Anderzijds, hagedissen die leven in *'mesische'* omgevingen hebben voornamelijk keelvlaggen met een perifeer kleurpatroon en een hoge reflectie in rood. Voor keelvlaggebruik vond ik enkel een correlatie met omgevingscondities bij de mannetjes. Deze resultaten geven duidelijk weer dat *A. sagrei* mannetjes hun keelvlaggebruik verhogen in omgevingen met een verlaagde zichtbaarheid en complexe habitatstructuren, waarschijnlijk om de signaaltransmissie te optimaliseren.

Algemeen blijkt uit mijn proefschrift dat de *A. sagrei* keelvlag een complex multi-component signaalsysteem is, dat gebruikt wordt voor het uitwisselen van zowel redundante als niet-redundante informatie tijdens sociale interacties en in het bijzonder, tijdens het evalueren van partners. Toch kan de informatie die vervat zit in de keelvlagcomponenten sterk variëren, zelfs op soortniveau. Ik vond een verbazingwekkende keelvlagdiversiteit tussen *A. sagrei* populaties die tot op

zekere hoogte kan verklaard worden door variatie in klimaat-gerelateerde omgevingscondities en het aantal aanwezige syntopische *Anolis* soorten. Tot slot hoop ik dat mijn proefschrift een basis kan bieden voor toekomstige studies over de functie en de evolutie van de *Anolis* keelvlag, maar bovendien een toenemende belangstelling aanwakkert voor meer onderzoek naar de vaak vergeten vrouwelijke ornamenten.

CHAPTER 1

General Introduction

The scientist is not a person who gives the right answers, he's one who asks the right questions.

CLAUDE LÉVI-STRAUSS - ANTHROPOLOGIST

1.1 Animal communication and signals

Who is not baffled by the ‘Moonwalk’ dance of Red-Capped Manakin birds during courtship (Skutch 1949), the inaudible ultrasonic echolocation cries of bats (Griffin 1958), and the low-voltage electrical signals emitted and detected by some fish (Lissmann 1958)? These are just a few examples of an indispensable process in life, called ‘communication’. Here, I define ‘communication’ simply as ‘the provision of information from a sender to a receiver’ (Fig. 1.1; but see Scott-Phillips 2008, Rendell et al. 2009 for discussions on definitional and semantic disagreement). Animals use a variety of sensory modalities to ‘exchange’ information, e.g. vision, audition, smell/taste (Bradbury and Vehrencamp 1998). Yet, most animal groups focus only on a subset; for example, social insects primarily use the chemical modality (Billen and Morgan 1998), whereas anurans prefer auditory communication (Gerhardt and Huber 2002). Overall, animal communication functions in a wide variety of contexts (e.g. predator-prey interactions, heterospecific interactions), however, the majority of information exchange is certainly found within-species (e.g. inter- and intrasexual interactions; West-Eberhard 1983).

Following Irschick et al. (2015), I define ‘signal’ as ‘a trait that is produced by the sender, which transmits the information through the environment and which helps the receiver decide if and how to respond’. To illustrate, in the context of visual communication, the colour of a structure may transmit information that influences the behaviour and decision-making of the receiver, once the information has been interpreted (Fig. 1.1). Signals can, therefore have important fitness implications for both individuals, if the influenced behaviour of the receiver involves responses, such as choosing to mate with the sender or deciding whether or not to engage in a physical fight (Guilford and Dawkins 1991). Information exchange might occur via a single signal component (e.g. only armament size) or through a combination of multiple signal components (e.g. armament size in combination with behavioural displays). Multicomponent signals are far more common, especially for courtship and aggressive displays,

warning signals, and begging signals (Guilford and Dawkins 1991; Rowe 1999). In the context of multicomponent signalling, different signal components may provide the same information and thus act as back-ups to one another (e.g. Zuk et al. 1992; Johnstone 1996; Candolin 2003). The use of ‘redundant’ messages improves the detection, recognition, discrimination, and memorability of signals by receivers (evidence reviewed in Rowe 1999). Alternatively, differential signal components may each convey a different message, thereby broadening the content of information (e.g. Johnstone 1996; Candolin 2003; Hebets and Papaj 2005). For instance, different aspects of the senders’ quality (e.g. developmental quality, health state, physical condition, social status) can be provided at the same time (Candolin 2003; Locatello et al. 2012).

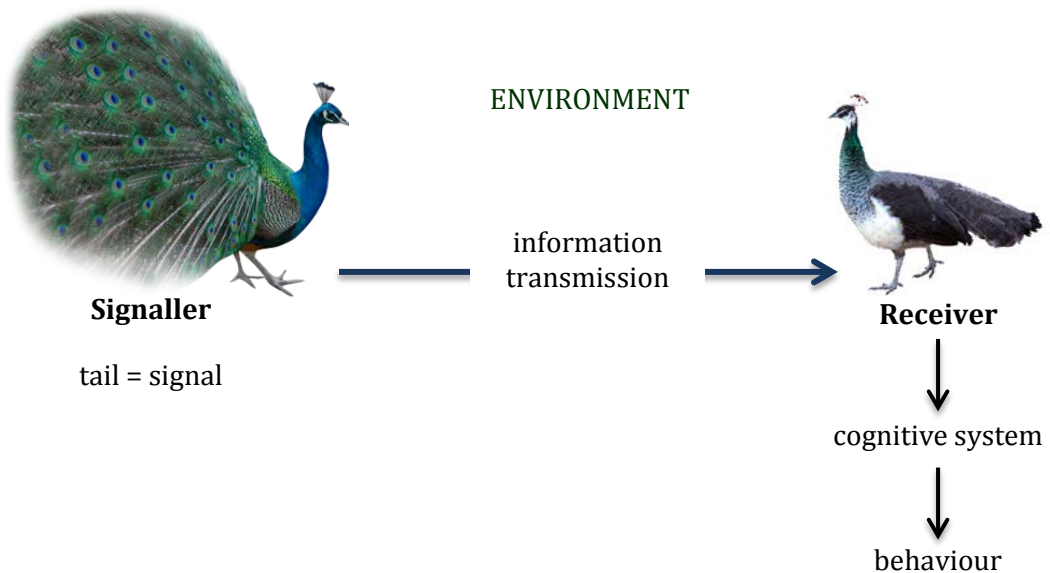


Fig. 1.1 Schematic representation of animal communication. Signals are sent by a signaller through the environment and then picked up by a receiver, who may change its behaviour accordingly; figure adjusted from Stevens 2013.

Within the field of animal communication, ‘honest signalling’ is a hotly debated topic and defined as the provision of reliable information by the sender (Bradbury and Vehrencamp 1998). Honest signalling assumes a correlation between an observable signal and an unobservable quality. How this correlation is exactly effectuated, remains a matter for debate. Yet, recent reviews agree that honesty

may persist thanks to mutual interests, constraints or signal costs (Vehrencamp 2000; Hurd and Enquist 2005; Searcy and Nowicki 2005).

Mutual interest – when sender and receiver share interests, both parties will benefit from the exchange of reliable information; there is no reason for ‘cheating’ (i.e. provision of unreliable information) in such a context. Examples that fall into the category of shared interests are mate recognition calls, alarm calls or calls maintaining group cohesion (Maynard Smith 1991).

Constraint – physical constraints may impose a mechanistic link (physical connection) between the signal intensity and a given trait, resulting in an honest signal whose form is causally related to its quality. Constraints typically involve body size, strength or age (Bradbury and Vehrencamp 1998). For example, the vibration frequency of the funnel-web building spider (*Agelenopsis aperta*) is determined by its own body mass and is as such an honest signal that cannot be faked (Riechert 1987). Or, red deer stags (*Cervus elaphus*) of a larger body size are able to perform calls of acoustic quality that are impossible for males with a smaller body size to produce (reviewed in Reby and McComb 2003).

Signal cost – the ‘handicap principle’ is widely accepted and cited as the main mechanism behind the honesty of signals under conflicting interests. This principle was originally proposed by Zahavi (1975) and states that only individuals of superior quality are able to maintain high-quality signals, because of the high costs associated with these signals. The elaborate tail of the peacock (*Pavo cristatus*) is perhaps the best-known example of a costly signal that serves as an honest indicator of male quality, because only males of high quality can allocate resources to the development of such an ornament that is dangerously conspicuous too (e.g. Zahavi 1975, 1977; but see Grose 2011). Another reputed example of handicap signalling involves the stotting behaviour of a threatened gazelle (*Eudorcas thomsonii*) in a prey-predator context. The gazelle will jump up and down to deter its coursing predator, while at the same time trying to escape from it. Because high rates of stotting behaviour lead to less distance between prey and predator, this type of costly signal can only be performed by individuals with the capacity to run at very high speeds (Zahavi 1975; FitzGibbon and

Fanshawe 1988; but see Számadó 2003 and Grose 2011). Although Zahavi (1975, 1977) and Grafen (1990) state that signals have to be costly in order to be honest, theoretical models have recently revealed that it is not the cost paid by honest signalers that maintains honesty, but rather the potential cost of cheating (Számadó 1999; Lachmann et al. 2001; more details on potential misconceptions of the handicap principle are provided in Számadó 2011).

1.2 Evolutionary processes shaping signal diversity

A staggering diversity of signal types exists across the animal kingdom, ranging from bright colours in birds and butterflies, to the calls of frogs and crickets, from pheromones released by ants and moths, to aggressive displays in lizards and fish. Perhaps less obvious to many of us, is the large variation in signal design observed on a smaller scale, i.e. within genera and even within species (Johnstone 1997).

One of the goals of comparative evolutionary biology is to identify the micro-evolutionary processes underlying this astonishing signal diversity. Over the last decades, an array of non-mutually exclusive processes – selective and stochastic – that may produce signal divergence has been put forward (e.g. Endler 1992; Andersson 1994; Foster and Endler 1999; Seddon 2005).

1.2.1 Sexual selection

Signals can vary due to differences in the ‘intensity’ or the ‘direction’ of intrasexual (competition) and intersexual (mate choice) selection (Darwin 1871). Intensity can vary among populations because of operational sex ratios. Direction, on the other hand, can vary due to a myriad of possibilities to advertise condition or ‘good genes’ (reviewed in West-Eberhard 1983). In the absence of ecological differences, the capacity of sexual selection to drive signal divergence among populations arises from runaway selection via mate choice (Fisher 1930), the efficacy of novel signals, and the absence of a definite limit to change (Darwin 1871; West-Eberhard 1983). According to orthodox Darwinian ideas on sexual

selection, the male sex has greater benefits from multiple mating, because sperm is cheaper to produce compared to eggs and because males generally invest less in parental care compared to females (Bateman 1948; Darwin 1871). Therefore, sexual selection typically results in male-biased sex-differences with elaborately ornamented males. As a consequence, most studies on signal divergence have centred on male signals (e.g. Farr 1977; Johansson and Jones 2007; Wilkins et al. 2013). To illustrate, acoustic signal divergence in males between subspecies of greenish warblers (*Phylloscopus trochiloides*; Irwin et al. 2008) and also among populations of dwarf frogs (*Engystomops petersi*; Boul 2007), has recently been shown to result from female preference for greater signal complexity. Luckily, a growing awareness of the existence of intrasexual competition in females as well as the occurrence of mutual or male mate choice (e.g. Clutton-Brock 2007; Edward and Chapman 2011) has instigated researchers to consider sexual selection and trait elaboration in females as well.

1.2.2 Ecological selection

Signals vary among populations due to differences in ecological factors (i.e. type or intensity) that affect the survival of individuals, but not directly their mating success (adjusted from Wilkins et al. 2013). Biotic agents mediating this process may include:

Predators – dull and less conspicuous visual signals are often beneficial to avoid detection by predators (predator-avoidance hypothesis) and thus, to increase survival (Endler 1980). Trinidadian guppies (*Poecilia reticulata*) represent a well-documented example, where fish have evolved brighter more conspicuous colouration patterns when inhabiting low predation risk environments (Endler 1982). In contrast, prey may also use conspicuous displays to signal vigilance to the predator and thereby, discourage it from attacking (pursuit deterrence hypothesis, Hasson 1991; see example of stotting behaviour in Thomson's gazelle in previous section). Under pursuit deterrent signalling, more conspicuous signals can be expected to evolve in areas with higher predation pressure.

Prey – type and availability can greatly influence survival prospects (e.g. Mayer and Wahl 1997; Graeb et al. 2004). In bats, for instance, disruptive selection acts on echolocation frequency, in association with specialization on different prey size classes leading to acoustic signal divergence (Jones 1997). Further, signals can function as visual lures to attract prey, as is the case for the bright body colouration in orb weavers (*Leucauge magnifica*; Tso et al. 2006).

Congeners – the ability to correctly identify conspecifics is crucial to avoid interactions that can come at a high survival cost for both individuals (e.g. interspecific courtship, mating or aggression; e.g. Coyne and Orr 2004; Ord and Stamps 2009). Therefore, differences in signal design and/or behaviour may evolve to ensure a rapid and accurate identification of conspecifics (i.e. species recognition; e.g. Williams and Rand 1977; West-Eberhard 1984). If species recognition drives signal diversity, sympatric species are expected to possess more distinct and easily distinguishable signals than allopatric species (Butcher and Rohwer 1989; Price 1998). Seddon (2005) found support for this idea in antbirds, by showing that the songs of closely related sympatric pairs of species are more divergent than those of allopatric pairs. Many other studies demonstrating the value of species recognition are available in the literature (e.g. Ryan and Rand 1993; Marshall and Cooley 2000; Macedonia et al. 2013).

Conspecifics – besides competition for mates (sexual selection), individuals may compete for other resources during non-sexual interactions (e.g. foraging territories, nest sites, quality of parental care) by using the same signals (Amundsen 2000; LeBas 2006; Tobias et al. 2012). The concept of social selection (West-Eberhard 1983), considered here as the ‘non-sexual form’ of sexual selection has received considerable attention to explain the evolution of female ornamentation (Tobias et al. 2012; see last paragraph of this section for more details).

In real-world scenarios, a trade-off between sexual and ecological selection often underlies the diversification of signals (e.g. Endler 1983; Townsend 1989; Andersson 1994). Notably, sexual selection tends to favour greater elaboration,

whereas ecological selection often favours less conspicuous signals to avoid detection by predators and enjoy improved survival prospects. Although direct testing of this ‘selection trade-off’ remains challenging, empirical evidence for it has been provided, mostly in fish (e.g. Schwartz and Hendry 2007; Heinen-Kay et al. 2014).

1.2.3 *Sensory drive*

Variation in signals among populations may also arise in response to differences in the physical environment that effectively stimulate the receiver sensory system. Simply, sensory drive concerns the efficacy of signal transmission in different environments and this is regardless of the identity of the intended signal recipient (Endler 1992); receivers can be rivals and mates (see sexual selection), as well as other players (see ecological selection). Accordingly, sensory drive is considered by some evolutionary biologists as a framework (not as a selective process in itself), defining the amount of standing signal variation available for sexual and ecological selection in a given habitat (Wilkins et al. 2013). Examples for a role of sensory drive in shaping auditory and chemical communication systems are fairly scarce, however, much evidence has been provided for visual signals (reviewed in Boughman 2002). One outstanding example is the colour divergence in the East-African cichlids, due to differential water clarity and depth-mediated light gradients (Seehausen et al. 2008).

1.2.4 *Correlated selection*

Signals may vary as a consequence of differential selection on a genetically or functionally correlated trait that is not directly involved in communication (e.g. Dobzhansky 1951; Caspari 1952). For example, the differentiation of beak size for efficient seed extraction in Darwin’s finches had a correlated effect on song trill rate. Notably, finches with larger bills produce slower-paced trills (Podos 2001).

1.2.5 *Genetic drift and mutation-order (M-O)*

Lastly, signals may also vary among populations due to differences arising from

stochastic processes (Lande 1976; Mani and Clarke 1990). In comparison to the previously discussed adaptive processes, far less studies have focussed on the role of stochastic events in explaining signal diversity. Nevertheless, evidence for genetic drift in shaping signal diversity has been found across distinct animal groups, e.g. in singing mice (*Scotinomys teguina*; Campbell et al. 2010), in Amazonian frogs (*Allobates femoralis*; Amézquita et al. 2009), in greenish warblers (*Phylloscopus trochiloides*; Irwin et al. 2008), and recently even in weak electric fish (*Brachyhypopomus occidentalis*; Picq et al. 2016). Besides genetic drift, M-O has been put forward as a potentially important process underlying signal diversity. According to M-O, signal divergence may occur among populations exposed to similar selective pressures, due to the stochastic order in which beneficial traits arise (Mani and Clarke 1990). To illustrate, an increased crypsis, production of chemical deterrents and faster locomotion are all adaptive to reduce predation risk; however, one population may by chance evolve increased crypsis first, while another population may initially evolve faster locomotion capacities. Although Mani and Clarke (1990) suggested that in some circumstances (i.e. large populations and/or strong selection) M-O can be far more important than genetic drift in shaping divergence, evidence for M-O remains scarce. Martin and Mendelson (2012) concluded that M-O is a major driver of signal divergence in darter fish, but to my knowledge this is the only study so far that has provided indirect evidence for M-O in this context.

1.2.6 Often forgotten females

Most research on signal diversity and underlying adaptive processes has focused on males; this is particularly true for sexual selection. This bias is unjustified because, at least in some species, females also compete for mates and engage in advertising their quality (Clutton-Brock 2009). Recently, a handful of studies have considered the evolution of ornamentation and weaponry in females, resulting in three major hypotheses. Firstly, the **genetic byproduct** hypothesis states that ornamental traits in females arise from selection acting on males and are as such the result of a correlated inheritance (Lande 1980). Secondly, females are subject

to **sexual selection** similar to those shaping trait evolution in males. This second hypothesis applies particularly to females in reversing sex-roles, as well as to polyandrous species. Thirdly, females compete for ecological resources rather than matings, i.e. **social selection**. Female traits are, therefore, shaped by non-sexual forms of intraspecific social competition (LeBas 2006; Kraaijeveld et al. 2007). These three hypotheses have each received theoretical and empirical support (reviewed in Tobias et al. 2012); examples are provided in figure 1.2. Yet, there is currently no consensus about which of these explanations plays a dominant role in females. A broader sampling of taxa, including species with exaggerated versus cryptic female traits and a range of different mating systems (e.g. monogamous, polyandrous, polygynous), is required to better understand how evolution exactly shapes signal design in females.

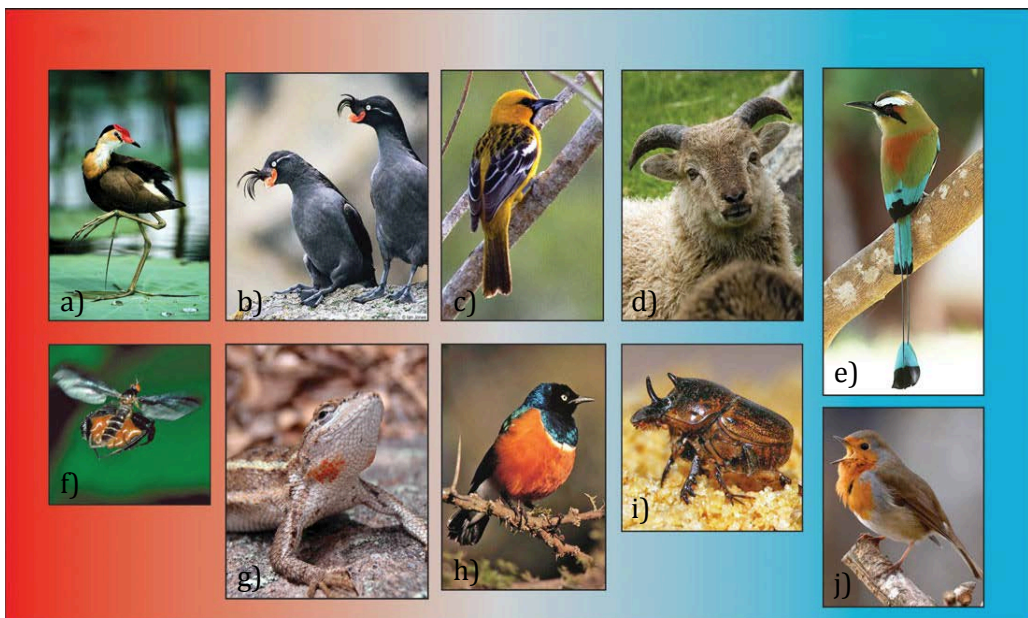


Fig. 1.2 Female traits can evolve under pure Darwinian sexual selection over matings (left, red), under social selection over ecological resources like foraging territories and nest sites (right, blue), as well as under predation pressure. This can be represented as a continuum, running from ornaments (on the left in this figure) to weapons and anti-predator signals (on the right). However, some traits may have multiple functions at different points, along this sexual – non-sexual continuum. The exact placement of certain species in this figure requires therefore a cautionary note. The phenotypic female trait and associated fitness components are shortly mentioned for each represented species: a) Comb-crested Jacanas (*Irediparra gallinacean*): females compete for mates, but no

literature available on the specific phenotypic traits involved (Mace 2000); b) Crested auklet (*Aethia cristatella*): crest size, competition for mates and egg or nest site (Jones and Hunter 1993, 1999); c) Streak-backed oriole (*Icterus pustulatus*): plumage colouration, competition for breeding and non-breeding territories (Price et al. 2008; Murphy et al. 2009); d) Domestic sheep (*Ovis aries*): horns, competition for food related to reproduction (Robinson and Kruuk 2007); e) Turquoise-browed motmot (*Eumomota superciliosa*): tail ornament, anti-predator context (i.e. pursuit deterrence signal; Murphy 2006); f) Long-tailed dance fly (*Rhamphomyia longicauda*): wings and tibia, competition for mates (Bussiere et al. 2008); g) Striped Plateau Lizard (*Sceloporus virgatus*): colour, competition for mates (Weiss 2002); h) Superb starling (*Lamprotornis superbus*): potentially competition for mates, reproductive opportunities or territories, no direct evidence for the exact phenotypic traits involved (Rubenstein and Lovette 2009); i) Dung beetle (*Onthophagus Sagittarius*): horns, competition for food related to reproduction (Watson and Simmons 2010a, b); j) European robin (*Erithacus rubecula*): vocalization, competition for non-breeding territory (Kriner and Schwabl 1991). This figure was taken and adjusted from Tobias et al. 2012.

1.3 *Anolis* lizards

Anolis lizards are distributed throughout the Neotropics, including the Caribbean islands and the mainland Americas (i.e. Central and northern South America, and the southeastern United States; Losos 2011). With over 400 species in the genus and with an exceptional degree of diversity in a broad range of characteristics (i.e. morphological, ecological and behavioural traits), *Anolis* presents an excellent system for studying biological diversity (Losos 2009). In addition, *Anolis* lizards provide a model system to study evolutionary processes, because they exhibit rapid trait diversification, which can lead to high rates of speciation (Losos 2009). The adaptive radiation of Caribbean Anoles represents a textbook example in this respect. On each island of the Greater Antilles (i.e. Cuba, Hispaniola, Jamaica and Puerto Rico), lineages have diversified to produce a suite of species adapted to use different habitats. Notably, the same set of microhabitat specialists, termed ecomorphs, has independently evolved similar morphologies and behaviours on each of these four islands (Williams 1972; Losos 2009; Mahler et al. 2013). Islands systems, including those within the Caribbean archipelago, are typically characterized by distinct boundaries, a relatively small size, simplified ecosystems and high population densities in comparison to the mainland, which makes them

ideal to evaluate evolutionary patterns (Whittaker and Fernandez-Palacios 2007; Losos 2009; MacArthur and Wilson 2015).

Anoles are visually oriented lizards that communicate by means of conspicuous displays. These displays typically consist of ‘head-nods’ (HN, up-and-down movement of the head), ‘push-ups’ (PU, up-and-down movement of the body and tail caused by flexion of the legs) and ‘dewlap extensions’ (DE, pulsing of the dewlap, i.e. a flap of skin below the chin supported by the second ceratobranchial cartilage; Bels 1990; see Fig. 1.3). In particular, dewlap displays have received considerable attention in the literature. Dewlap displays are observed in a variety of contexts, including encounters with mates (e.g. Greenberg and Noble 1944; Crews 1975), rivals (e.g. Jenssen et al. 2000), members of related species (e.g. Rand and Williams 1970; Losos 1985), and even predators (e.g. Leal and Rodriguez-Robles 1995, 1997a). Yet, the contextual use and design (size, colour and pattern) of the dewlap can differ considerably (Nicholson et al. 2007; Losos 2009), raising several questions about the exact function and complexity of this visual communication system. Besides, it remains poorly understood which evolutionary processes underlie the dewlap diversity found in *Anolis*. In fact, the dewlap has many characteristics typical of a Darwinian sexually selected trait; e.g. highly conspicuous, more elaborate in males than in females, and often displayed in a context of male territorial defence and courtship. However, there is mixed evidence for **sexual selection** on dewlaps. Evidence for intrasexual selection has been shown by the observation of high dewlap display rates during male-male territorial disputes (e.g. Gorman 1968; Trivers 1976; Scott 1984) and indirectly, by the positive correlation between male dewlap size and bite force observed in several territorial anole species (Irschick et al. 2006; Lailvaux and Irschick 2007; Lailvaux et al. 2012). However, *A. carolinensis* males with reduced versus normal dewlap size showed equal probabilities to win territorial disputes, contradicting the idea that intrasexual selection is an important process in explaining dewlap evolution (Henningesen and Irschick 2012). For intersexual selection, evidence is provided by the observations that sexually receptive *A. carolinensis* females prefer

to mate males with functional dewlaps (Greenberg and Noble 1944) and that they prefer the naturally-coloured, pink dewlaps over green dewlaps in males of this species (Sigmund 1983). Yet, other studies have failed to demonstrate female preference for or against a naturally occurring white-dewlap variant of *A. carolinensis* found in Southwestern Florida (MacDonald and Echternacht 1991). Further, two other studies showed that gene flow between populations with different dewlap colours is high, suggesting the lack of assortative mating driven by female preference in *A. distichus* (Ng and Glor 2011) and *A. apletophallus* (Stapley et al. 2011). Aside from sexual selection, **species recognition** has been proposed to act on the dewlap (e.g. Rand and Williams 1970; Webster and Burns 1973). The most direct evidence comes from experiments showing that *A. cybotes* males are more aggressive to closely related sympatric *A. marcanoii* males when their dewlap is painted in the same colour and pattern as the dewlap of *A. cybotes* itself (Losos 1985). Also Macedonia et al. (2013) showed that *A. grahami* males displayed more towards robotic models with their naturally-coloured orange dewlap, than to the differently coloured blue-dewlap models. More indirect support for species recognition is suggested by the observation that sympatric species nearly always differ in dewlap colour and pattern (Rand and Williams 1970; Nicholson et al. 2007). Next, an array of studies has also addressed the contribution of **predation pressure** in shaping display behaviour. Most studies have provided support for the predator-avoidance hypothesis, by showing that anoles decrease the intensity of displays and/or the amplitude of their signals in the presence of predatory cues (e.g. Lister and Aguayo 1992; Cantwell and Forrest 2013; Steinberg et al. 2014). Yet, also the pursuit deterrence hypothesis has been supported in *Anolis*, by direct observations of *A. cristatellus* males that display in response to a simulated approach of a predator (Leal and Rodriguez 1995, 1997a; Leal 1999). Finally, **sensory drive** has been proposed as an important mechanism to explain variation in dewlap colour and displays (e.g. Fleishman 1992, 2000). Here too, evidence is rather mixed. For both *A. cristatellus* and *A. distichus*, a correlation between dewlap colour and habitat conditions was found. Leal and Fleishman (2004) revealed that *Anolis cristatellus* populations occupying dark

signalling habitats (mesic forest) exhibited brighter and more UV-reflecting dewlaps than populations inhabiting brighter signalling habitats (xeric forests), which were shown to have relatively dull dewlaps with low UV-reflectance and red appearance. Instead, Ng et al. (2013a) found that *Anolis distichus* populations occurring in mesic habitats exhibited less bright and more orange dewlaps in comparison to populations occupying drier environments, which were characterized by brighter and more yellow dewlaps. Furthermore, Fleishman et al. (2009) did not find any support for sensory drive in explaining dewlap colour divergence across 4 closely related anole species. Lastly, when considering displays, Ord et al. (2007, 2010) found that anole lizards inhabiting poorly lit or windy environments compensate by enhancing the speed of display movements or by extending the duration of their displays.

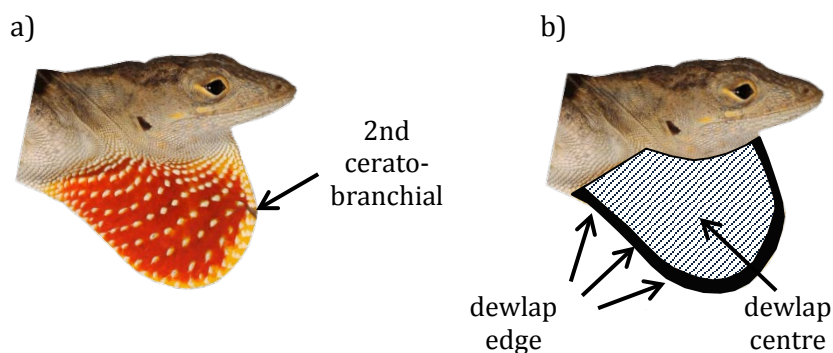


Fig. 1.3 The *Anolis* dewlap. a) Male *A. sagrei* lizard with dewlap in extended form; b) schematic representation of two dewlap regions, i.e. centre (shaded black) and edge (full black), that are often separately considered in dewlap studies. The dewlap centre is relatively invisible when the dewlap is retracted, whereas the dewlap edge is visible in both extended and retracted form. Photo taken by T. Driessens.

The bias towards research on male traits also applies to the genus *Anolis*; results reported in the previous paragraph were, therefore, exclusively obtained for the male sex. Nevertheless, in nearly all anole species, females also possess a dewlap structure (though, mostly far less elaborate; Losos 2009). To my knowledge, information regarding female dewlap use and function has only been reported for three anole species. Notably, *A. carolinensis* females rarely display their dewlap during intersexual interactions (Jenssen et al. 2000) as opposed to *A. valencienni*

females, who apparently use their dewlap to discourage courting males (Hicks and Trivers 1983). On the other hand, both *A. carolinensis* and *A. bahorucoensis* females use dewlap displays during intrasexual interactions (Orrell and Jensen 1998, 2003), which suggests that the female dewlap may have a signalling function in competition for resources, such as food or nest sites (West- Eberhard 1983). Recently, one study also considered the evolutionary processes that may shape dewlap size in females. By using comparative methods, Harrison and Poe (2012) looked for a correlated evolution between female dewlap size and several morphological and ecological variables, on the interspecific level. Results showed that variation in dewlap size reflected differences in habitat use, i.e. female dewlap size is larger in species that use more arboreal habitats, providing indirect evidence for **sensory drive**. In addition, they found that female dewlap size is larger in species where the sexes are less dimorphic. This suggests that **social selection** in the form of male mate choice or female-female competition for territory, may shape dewlap size in females.

To summarize, a vast number of studies have tried to unravel the signalling functions and evolution of the *Anolis* dewlap. However, the conclusions reached so far, almost exclusively apply only to males and appear to be species-specific rather than generally applicable. Taken together, I believe that examining the signalling functions and evolution of the dewlap on an intraspecific level and in females too, may be most fruitful to gain a better understanding of this fascinating structure.

1.4 The brown anole (*Anolis sagrei*)

1.4.1 Species description and ecology

The brown anole lizard (*Anolis sagrei*) is native to Cuba, the Bahamas and other islands of the West Indies (Schwartz and Henderson 1991). Yet, over the last years, its distribution has expanded to the American mainland as well (i.e. Florida, Texas, Louisiana, Georgia, South Carolina and parts of Central America), predominantly by jump dispersal (Means 1990; Platt and Fontenot 1994;

Campbell and Hammontree 1995; Turnbow 2006). *Anolis sagrei* prefers relatively open vegetation in urbanized areas and spends most of its time on the ground and on low vegetation and tree trunks (trunk-ground ecomorph; Rand and Williams 1969). This species is highly dimorphic in size (Schwartz and Henderson 1991); adult males can reach a snout-to-vent length (SVL) up to 70 mm (54 mm on average), whereas females are mostly no larger than 50 mm SVL (43 mm on average). Although in other anole species, distinct life-stage male morphs are observed within populations (e.g. 'lightweight' versus 'heavyweight' males in *A. carolinensis*; Lailvaux et al. 2004), no such bimodal distribution has been reported for adult *A. sagrei* males. The body colouration of brown anole lizards ranges from light grey to brown to almost black (large within and among-population variation; e.g. Schwartz and Henderson 1991; Rodriguez Schettino 1999). Both sexes possess a mustard yellow to deep red dewlap that varies extensively in morphology and colour aspects between sexes and among populations (Schwartz and Henderson 1991; Vanhooydonck et al. 2009). Like many other anoles, *A. sagrei* is a highly territorial and polygynous species. Sub-adult males and adult females have more or less exclusive home ranges, whereas adult males have a separate system superimposing layers of territories. To clarify, a large adult male can have up to six female territories lying within its own home range (Rand 1967; Schoener and Schoener 1980a). The reproduction of *A. sagrei* is mostly seasonal (March – September; Lee et al. 1989). In males, spermatozoa production normally begins in January and continues until September when testes begin to regress (Licht and Gorman 1970). During the breeding season, females lay 1 or 2 eggs at a time under decaying vegetation on the ground and this, on a weekly or two-weekly basis (15-18 eggs per breeding season; Gordon 1956; Tokarz 1998). The eggs will hatch independently after an incubation time of approximately 6-8 weeks and hatchlings will reach sexual maturity before their first breeding season the following summer (Duellman and Schwartz 1958). In some tropical areas, *A. sagrei* may reproduce year-round due to stabilized warm weather, which is needed for breeding and egg production (Sexton and Brown 1977).

The natural diet of *A. sagrei* typically consists of arthropods, worms and molluscs (Schwartz and Henderson 1991). Besides, cannibalism of adult anoles on their own hatchlings is documented and may be prevalent in *A. sagrei* (Cochran 1989; Nicholson et al. 2000). A study conducted by Schoener and Schoener (1980a) showed that *A. sagrei* population densities vary largely, ranging from 0.08 m⁻² (Bimini) up to 0.97 m⁻² (Abaco). In comparison to other *Anolis* species, *A. sagrei* densities are very high, and especially at sites of intermediate insolation (Schoener and Schoener 1980a). Large year-to-year fluctuations in population densities were observed for some but not all Bahamian islands. Besides, sex ratios can vary highly among sites. Notably, Schoener and Schoener (1980a) observed more females than males in ‘good’ habitats (in terms of high density) and more males than females in the ‘poor’ habitats. The life span of brown anole lizards in the wild is estimated at 12 months in males and 18 months in females (up to 7 years in captivity; Schwartz and Henderson 1991).

1.4.2 An excellent study subject

Due to their high densities, brown anoles are relatively easy subjects to observe and/or catch in the field (Losos 2009). In addition, brown anoles display readily in the field, as well as in the laboratory (Tokarz et al. 2003). Not surprisingly, anole researchers have been attracted to work with this ‘easy-accessible’ species, resulting in a myriad of studies on *A. sagrei*. These studies have mostly addressed the role of the dewlap in a context of territory defence and courtship (Scott 1984; McMann 2000; Paterson 2002; Tokarz 2002; Tokarz et al. 2003, 2005; Paterson and McMann 2004; Simon 2011; Steffen and Guyer 2014), with equivocal results. Notably, Scott (1984) found that encounters with male opponents and potential mates elicited similar dewlap extension rates in *A. sagrei* males, indicating the importance of the dewlap during both intraspecific competition and courtship. On the other hand, Tokarz et al. (2003) showed that males with disabled dewlaps were just as likely to maintain their territories as control males with normal dewlaps, contradicting Scott’s earlier finding that the *A. sagrei* dewlap is important in territory defence (Tokarz et al. 2003). The same authors additionally

noted that males with disabled dewlaps mated at the same rate as normal males, indicating that the dewlap is not required to obtain matings either. Simon (2011), then, showed with other field experiments that *A. sagrei* males extended their dewlaps far more frequently during male–female than during male–male interactions, suggesting that the dewlap is used in courtship and not necessarily in territorial defence. In yet another study, Guyer and Steffen (2014) showed that both dewlap colour and displays in *A. sagrei* males are important determinants of combat outcome, indicating that the dewlap does function in territorial defence in this species. In conclusion, one can clearly state that the role of the *A. sagrei* dewlap in a context of intraspecific competition and courtship is highly inconclusive, despite the vast number of conducted studies. Moreover, only a few studies have looked at the potential role of the *A. sagrei* dewlap in other contexts (i.e. predator, heterospecific interactions) and no studies have been published on contextual dewlap displays in *A. sagrei* females.

Besides being an ‘easy-accessible species’, brown anoles are exceptional for their high degree of intraspecific variation in dewlap characteristics. They occur on several islands in the Caribbean Archipelago characterized by a broad range of environmental conditions, e.g. presence of predators, number of sympatric species, habitat structure and light conditions. This provides an excellent, natural study system to evaluate the evolutionary processes shaping dewlap divergence within a single species and potentially even, for gaining insights into how communication can contribute to speciation events. Vanhooydonck et al. (2009) started a first evolutionary study on the remarkable intraspecific dewlap diversity in *A. sagrei* males and females. Basically, they compared dewlap size and pattern from seven populations on different islands in the Bahamas and linked these to estimates of predation pressure (presence or absence of the predatory curly-tailed lizard, *Leiocephalus carinatus*; tail break frequency and island area), sexual selection (sexual size dimorphism, SSD), and species recognition (number of sympatric *Anolis* species). Their results indicated that species recognition is the major process underlying divergence in dewlap colour patterning in both males

and females, with more complex dewlap patterns occurring on islands with more congeneric species. They further suggested that relative dewlap size is under natural and/or sexual selection. Notably, they found indirect evidence for the role of the dewlap as a pursuit-deterrence signal in both males and females, because relative dewlap size was larger on islands where *A. sagrei* occurred sympatrically with the predatory *Leiocephalus* lizard. Additionally, in males relatively large dewlaps seemed to be selected for in a sexual context, as a positive association between relative dewlap size and SSD was observed. In females, however, natural selection appeared to constrain relative dewlap size, probably by other predators than *Leiocephalus* lizards. Overall, this study on intraspecific dewlap diversity in *A. sagrei* males and females nicely illustrates that selective processes acting on dewlap design can be similar as well as different between both sexes. Important to note is that the dataset used in this study included ‘only’ seven datapoints (i.e. populations) and caution is, therefore, necessary when interpreting their results. Nevertheless, this study provides an excellent basis to further test the importance of both the same and additional evolutionary processes with more *A. sagrei* populations, whilst optimizing methods to estimate the strength of the involved selective processes. Furthermore, although dewlap colour and display repertoire have been suggested to play a critical role in anole speciation, these two dewlap components were not considered in the study of Vanhooydonck et al. (2009), leaving a gap for further research.

1.5 Thesis outline

In this thesis, I aim to understand the signalling functions of the dewlap, as well as the evolutionary origins underlying dewlap diversity, in both sexes of the brown anole lizard. In ‘Part I’ (chapters 2 and 3), I take an experimental approach to study the signalling function of the dewlap under controlled laboratory conditions. I use wild-caught *A. sagrei* lizards and particularly look at the individual level, by comparing variation within and between individuals. In Part II (chapters 4 to 6), I broaden the scope to a population level, to study geographical variation in dewlap characteristics and the evolutionary processes behind it. Here,

I take a comparative approach using 17 *A. sagrei* populations distributed across several islands in the Caribbean.

Part I: signalling functions of the dewlap

In *Chapter 2*, I examine contextual display use by exposing both male and female *A. sagrei* lizards to cues of a predator, non-predator and conspecifics (intra- and intersexual interactions). I particularly assess the frequency with which display types (HN, PU and DE) are performed in each of the described contexts and predict to find different display rates in males and females. With this study, I aim to improve our understanding of the function of each display type (particularly, dewlap displays) in both sexes of the brown anole lizard.

In *Chapter 3*, I study the information content conveyed by assorted facets of the male and female dewlap. To do this, I link dewlap characteristics to sexual identity, estimates of individual quality (performance and health status parameters), and social status (aggression). I additionally assess whether diverse components of the dewlap signal additive information (redundant messages) or highlight different characteristics of the sender (multiple messages). Due to their fundamentally divergent reproductive roles, I expect males and females to differ with respect to the information conveyed by the dewlap.

Part II: dewlap diversity and underlying evolutionary processes

In *Chapter 4*, I examine whether male dewlap size is an honest signal of bite force, which is an important determinant of male combat outcome, in 17 *A. sagrei* populations distributed across Caribbean islands. Next, I link the relative dewlap size – bite force relationship within each population to variation in the degree of intrasexual selection among populations, using sexual size dimorphism (SSD) and male display intensity. I expect differences in the relationship between male dewlap size and bite force among populations, and further hypothesize intrasexual selection to be a major evolutionary process driving this variation. In this study, the aim is to better understand how sexual selection may shape the honest signal size – fighting capacity relationship within a single species.

In *Chapter 5*, my focus changes to the potential contribution of predation pressure, sexual selection and species recognition in shaping geographic variation in the dewlap design and display behaviour of *A. sagrei* males and females. Notably, I attempt to both replicate and extend results from a previous study on interpopulational variation in *A. sagrei* using an enlarged dataset (ten additional datapoints or populations, and two extra dewlap characteristics). Based on that study, I expect each of the proposed selective pressures to be important in explaining interpopulational variability in distinct dewlap characteristics (size, colour, pattern and use).

In *Chapter 6*, I broaden my scope to the largely unexplored role of climate and habitat conditions in driving *A. sagrei* dewlap design and display behaviour. First, I briefly document the degree of interpopulational variation in all studied dewlap components of both males and females. Second, I assess whether the observed interpopulational differences in dewlap size, pattern and colour, as well as in display behaviour correlate to variation in climate-related environmental conditions (i.e. temperature, precipitation, incoming radiation and vegetation cover). I expect differences in environmental conditions to be important in shaping dewlap divergence among populations, in order to optimize signal visibility.

General discussion

Finally, in *Chapter 7*, I link all of the obtained results presented in chapters 2 to 6. Notably, I draw general conclusions about the signalling functions and evolution of the *A. sagrei* dewlap, I describe some perils and pitfalls of the applied methods used in this dissertation and in the broader context of evolutionary biology, and I suggest avenues for future research.

PART I

Signalling functions of the dewlap



*Anolis allisoni on Cuba,
photo taken by S. De Decker*

*Reason, observation, and experience; the whole
trinity of science.*

ROBERT GREEN INGERSOLL - POLITICIAN

CHAPTER 2

Deterring predators, daunting opponents or drawing partners? Signalling rates across diverse contexts in the lizard *Anolis sagrei*.

Tess Driessens, Bieke Vanhooydonck, Raoul Van Damme

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Although the visual display behaviour in *Anolis* lizards has received ample attention, the function of dewlap extensions (DE), push-ups (PU) and head-nods (HN) in general, and in *Anolis sagrei* in particular, remains highly equivocal. Therefore, our primary goal was to quantify the display rates of these visual signal types (DE, PU and HN) in a variety of contexts, using *A. sagrei* as study species. To our knowledge, this is the first study to test individuals of both sexes in a repeated-measures design across multiple contexts, including predator, non-predator and social interactions (mirror, male-male, male-female, female-male). We found that males have an overall higher signalling rate than females across all contexts. In addition we found that lizards of both sexes exhibited higher display rates in the presence of conspecifics than when confronted with a predator or non-predator, suggesting that DE, PU and HN function in intraspecific communication, not in predator deterrence. Whereas females did not significantly raise display rates in a consensual and heterosexual context with respect to subject-alone context, males did. The PU signal type only appears to play a major role for *A. sagrei* males during aggressive encounters. During heterosexual

interactions, increased frequencies of all signal types suggest that DE, PU and HN are essential for male courtship. Finally, we suggest that intersexual selection is probably a driving force for frequency-related dewlap use in both sexes. In contrast, pronounced intersexual differences were detected for PU and HN rates within a social context.

Key words: *Anolis sagrei*, display behaviour, predator context, social context

2.1 Introduction

Animal communication is defined as the transfer of information between a sender and receiver via ritualized signals, involving a variety of sensory modalities (Bradbury and Vehrencamp 1998). Most animal groups focus primarily on a subset of possible modalities. Social insects, for instance, mainly use chemical signals, whereas anurans emphasize auditory signals (Gerhardt and Huber 2002). In the genus *Anolis*, a diverse group of neotropical, polychrotid iguanian lizards, visual displays are the primary mode of communication (Jenssen 1977; Carpenter 1978). These displays consist of conspicuous behaviours that are known to be used in multiple contexts, such as exhibiting territory ownership and territory defence (Greenberg and Noble 1944; Carpenter 1978), mate attraction and female receptivity (Greenberg and Noble 1944; Jenssen 1970a; Crews 1975; Sigmund 1983), species recognition (Rand and Williams 1970; Losos 1985), and predator deterrence (Leal and Rodriguez-Robles 1995, 1997a; Leal 1999). The anole display repertoire typically consists of three major visual signal types that differ in their motion and conspicuousness: ‘head-nods’ (up-and-down movement of the head), ‘push-ups’ (up-and-down movement of the body and tail caused by flexion of the legs) and ‘dewlap extensions’ (pulsing of the often-colourful throat fan or dewlap) (referred to by slightly different names in Scott 1984). Although each visual signal type can be displayed in isolation, it is frequently used in conjunction with other signal types, resulting in a stereotyped display pattern that tends to be species-specific, but nevertheless shows considerable inter- and intraspecific variation (Jenssen 1977, 1978; Scott 1984; Fleishman 1992). It has been reported that all three signal types can appear across diverse contexts in both sexes of

Anolis lizards (Jenssen 1970a, b; Hicks and Trivers 1983; DeCourcy and Jenssen 1994; Nunez et al. 1997). However, no study has compared the respective display rates across a wide range of contexts using the same individuals. Also, previous studies have focused primarily on male signalling; female display behaviour has received far less attention.

The brown anole (*Anolis sagrei*) has become a model for studying a variety of topics in ecology, animal behaviour and evolution. The species is native to Cuba and the Bahamas, but has successfully been introduced in many other areas (Campbell 1996). Males are polygynous, highly territorial and substantially larger than females, but both sexes possess a bright yellow-to-reddish dewlap (Schoener and Schoener 1980a; Schwartz and Henderson 1991). The general display repertoire includes dewlap extensions, push-ups and head-nods, but the frequency with which each signal type occurs varies considerably (Scott 1984; Partan et al. 2011). *A. sagrei* is also known to display readily under field and laboratory conditions, and across diverse social contexts (e.g. Scott 1984; Tokarz 2002; Tokarz et al. 2003, 2005; Simon 2007, 2011), which makes it an appropriate study species for examining display structure and behaviour. Earlier studies on *A. sagrei* have primarily addressed the males' display behaviour during territory defence and courtship (Scott 1984; McMann 2000; Paterson 2002; Tokarz 2002; Tokarz et al. 2003; Paterson and McMann 2004; Tokarz et al. 2005; Simon 2011), with equivocal results. Scott (1984) concluded that encounters with rival males and potential mates elicited similar dewlap extension rates in males, suggesting that the signal functions in both hetero- and consensual contexts. However, Tokarz and co-workers found that male *A. sagrei* with inoperative dewlaps mated at the same rate as normal males, suggesting that dewlapping is not necessary for obtaining matings (Tokarz 2002; Tokarz et al. 2005). In addition they noted that males with inoperative dewlaps were just as likely to maintain their territories as normal males, indicating that dewlap signalling has no fundamental role in territory defence either (Tokarz et al. 2003). Simon (2011), on the other hand, found that males flashed their dewlaps more frequently during

male-female than during male-male interactions, suggesting that dewlaps are used primarily in courtship. To our knowledge, only two studies have so far addressed the display rates of *A. sagrei* in response to predatory cues (Simon 2007; Elmasri et al. 2012). Although both studies used a similar predator (a kestrel model), Simon (2007) found a significant decrease in the signal rate of the high-amplitude push-ups after exposure to the visual predatory cues, whereas Elmasri et al. (2012) failed to find any change in display behaviour of male *A. sagrei* to both visual and acoustic predatory cues. In short, the function of dewlap extensions, push-ups and head-nods in the behaviour of *Anolis* lizards in general, and *A. sagrei* in particular, remains highly equivocal. Therefore, our primary goal was to quantify the use of these visual signal types in a variety of contexts. In contrast to previous studies, we tested both sexes and used a repeated-measures design, exposing a large number of individuals to diverse contexts, including predator (*Leiocephalus carinatus*), non-predator (*Uromastyx ocellatus*) and social interactions (mirror, male-male, male-female, female-male). All the encounters were staged in laboratory conditions. In this way, the same subjects could be tested repeatedly and in isolation across several contexts under fully controlled testing conditions. Rather than examining display structure, we focused on the frequency with which each individual signal type was performed; previous field studies have shown that predator and social context can affect the relative signal frequencies exhibited by *A. sagrei* (McMann 2000; Simon 2007, 2011). If the individual signal types serve an intraspecific communication function, we expect that lizards will primarily employ them in social contexts, including both hetero- and consensual interactions. In contrast, when they function in a predator deterrence communication system, we expect higher usage in the presence of a predator. We further predicted we would find a different role of the signal types in male and female *A. sagrei*, as selective pressures on signalling traits often differ between sexes (Blanco and De La Puente 2002; Vanhooydonck et al. 2005b, 2009). Finally, we expected to improve our general understanding of the role of each visual signal type in *A. sagrei* by testing many individuals of both sexes.

2.2 Materials and methods

2.2.1 Animals

In this study, a total of 68 captive *Anolis sagrei* were tested, including 46 males and 22 females. All individuals were adults originally caught in Florida, and obtained via the pet trade (Fantasia Reptiles, Belgium, license HK51101419) in October 2011. The snout-vent length (SVL) of male and female individuals measured upon arrival ranged from 40.83 - 61.47 mm and 39.55 - 50.65 mm, respectively; the threshold size at which sexual maturation occurs in *A. sagrei* is considered 39 mm for males and 34 mm for females (Licht and Gorman 1970; Lee et al 1989). Outside experimentation, all anoles were housed individually in specially designed cages at the University of Antwerp, Belgium. Each cage (30 cm L x 22.5 cm W x 27.5 cm H) contained a thick layer of peat bedding (Spagnum, Novobalt) covered with banana tree leaf litter, a wooden perch (ca 40 cm L, 2.5 cm diameter) placed diagonally at a 40° angle, and a light source (Philips SpotOne 30°, 25 W, 230 V) set to a 12:12 h light-dark regime; cages had non-transparent walls to avoid any visual contact and social interactions among the anole lizards. The temperature within the cages ranged from 26 °C during dark regime to a max of 35 °C under the light source during light regime; the relative humidity of the animal room ranged from 55-70 %. All anole lizards had ad libitum access to water from a small dish in their cage and were hand sprayed with water every other day. We fed each lizard every two days with 3 to 5-week-old crickets (*Acheta domestica*) and occasionally added wax moth larvae (*Galleria mellonella*) to their diet; both food types were dusted with a dietary supplement containing a 2:1 calcium-phosphor ratio and vitamin D3 (Zoo Med Reptivit with D3, Fantasia Reptiles, Belgium).

We additionally housed a male *Leiocephalus carinatus* (curly-tailed lizard) and *Uromastyx ocellata* (ocellated spiny-tailed lizard) during the course of the 'predator' and 'non-predator' experiments, respectively. Both animals were obtained via the pet trade, and kept for a maximum of 4 weeks (Fantasia Reptiles, Belgium, license HK51101419 and CITES 12NL120140/11). The two lizards were

separately housed in a large terrarium (100 cm L x 40 cm W x 50 cm H) with appropriate environmental conditions, ad libitum water access, and the necessary dietary requirements (Schwartz and Henderson 1991; Schleich et al. 1996).

2.2.2 Experimental procedure

Male subjects were exposed to a total of five different experiments in the following order: 1) 'predator (PR)', 2) 'mirror (MI)', 3) 'male-male interaction (MM)', 4) 'non-predator (NP)', and 5) 'male-female interaction (MF)' experiment. Females were subjected to four experiments: 1) 'predator (PR)', 2) 'mirror (MI)', 3) 'female-male interaction (FM)' and 4) 'non-predator experiment (NP)'. Experiments were generally conducted between March and September 2012, which is considered the breeding season for *A. sagrei*. Due to technical failure, one experiment (i.e. MF) was postponed and carried out between the second half of September and start of October 2012. All experiments were performed during the natural activity period of the anole lizards, i.e. between 9:00 and 16:00. Staged encounters always involved interactions between unfamiliar subjects, as familiarity and prior physical contact can affect display and courtship behaviour in the brown anole (Tokarz 2006; McMann and Paterson 2012). In principle, each individual was used only once per experiment (for exceptions: see section MM and MF experiment). Different experiments were carried out with a rest interval in between of at least two weeks to minimize disturbing the lizards and any carry-over effects. Only healthy individuals were selected for testing, resulting in a smaller sample size for experiments conducted at a later stage.

The following procedure was used in all experiments. A subject was taken from its home cage, and kept in an incubator for 40 min at 30 °C to ensure a similar body temperature in all lizards. Then the lizard habituated to the experimental cage for a period ranging from 20 to 45 min. No measurements were carried out during this period. After habituation, the subject's display behaviour was scored for the entire treatment duration. During all experiments, the temperature of the peat layer in the centre of the terrarium was kept at $26 \pm 1^\circ\text{C}$, via an external electrical

heater (SuperSer, model GL-15, 1350 W). A high level of humidity (55 – 70 %) was maintained by hand spraying the terrarium before each habituation period. All sides of the experimental terrarium, except one (i.e. observer's side), were covered in white paper to prevent any distraction from outside. The observer's side was transparent and enabled online behavioural scoring by an observer sitting still at a distance at least 2 m away from the terrarium. At the end of every experiment, cages were thoroughly cleaned.

Predator experiment

During the predator experiment (PR) each subject was confronted with a single male curly-tailed lizard. As *L. carinatus* is widely distributed in Florida and known to predate on brown anoles (Schoener et al. 2002), the species constitutes a good predator model for our experimental setup. The PR experiment was conducted in a large glass terrarium (100 cm L x 40 cm W x 50 cm H) closed with a two-inlet lid on top, which allowed some airflow. The terrarium was provided with a thick layer of peat substrate partly covered by a banana leaf and with a branch placed on top of the ground layer (Fig. 2.1a). Both the banana leaf and branch were taken from the home cages of the tested individuals. Two light sources (Philips SpotOne 90°, 40 W) were mounted symmetrically on the inside of each inlet, i.e. left and right-hand side of terrarium, providing an optimal temperature for testing. To confront our subjects with the predator, a small glass tank (20 cm L x 20 cm W x 20 cm H) containing the living *L. carinatus* individual was placed inside the large terrarium providing indirect contact between predator and prey. The 'predator tank' containing the *L. carinatus* was fully transparent. Its base was fitted with a thin layer of peat and the top was covered with a mesh. Thus the set-up provided the visual, auditory and chemical cues of a predator. During habituation, individuals were exposed to a 'control tank' inside the large terrarium, i.e. an identical tank as described above, but without the predator (Fig. 2.1a). Then the actual experiment started with two consecutive treatments per subject: a control treatment (C, with an empty tank) and a predator treatment (PR, with the predator in the tank). Each treatment lasted 20 min, resulting in a total

behavioural observation time of 40 min per subject. During the whole experiment, subjects stayed inside the large terrarium and the appropriate tanks were changed via one of the upper inlets, with minimal disturbance. To rule out any confounding effects, control and predator tanks were placed alternatively on the left and right side of the terrarium with the same subject having the appropriate tank on the same side for both treatments. We followed a balanced, randomized test protocol to determine the position of the stimulus tank (i.e. left or right) and to define the order in which subjects were tested in the C en PR treatment. The *L. carinatus* individual behaved in a similarly passive way during all PR treatments thereby excluding possible effects caused by predator behaviour and movement.

Non-predator experiment

Set-up and design of the non-predator experiment (NP) were similar to that of the PR experiment (Fig. 2.1a). However, instead of a predatory lizard, an herbivorous heterospecific lizard was used. *Uromastix ocellata* inhabits rocky areas in the arid zones of northeastern Africa (Schleich et al. 1996) and is, from its dietary perspective (90 % herbivorous) harmless to our anole subjects. The idea of incorporating the NP experiment, was to test whether the response to the *L. carinatus* subject during the PR treatment is indeed a predator-specific response or rather a generic response to any heterospecific subject of similar size. We selected a male *U. ocellata* with SVL and weight similar to that of the *L. carinatus* individual used in the PR experiment. As with the PR experiment, this experiment consisted of a control treatment (C, empty tank) and a non-predator treatment (NP, with the *U. ocellata* inside the tank). Choice of the position of the stimulus tank and treatment order was as in the PR experiment. The *U. ocellata* also behaved passively during all treatments, ruling out any possible effects of change in behaviour and movement.

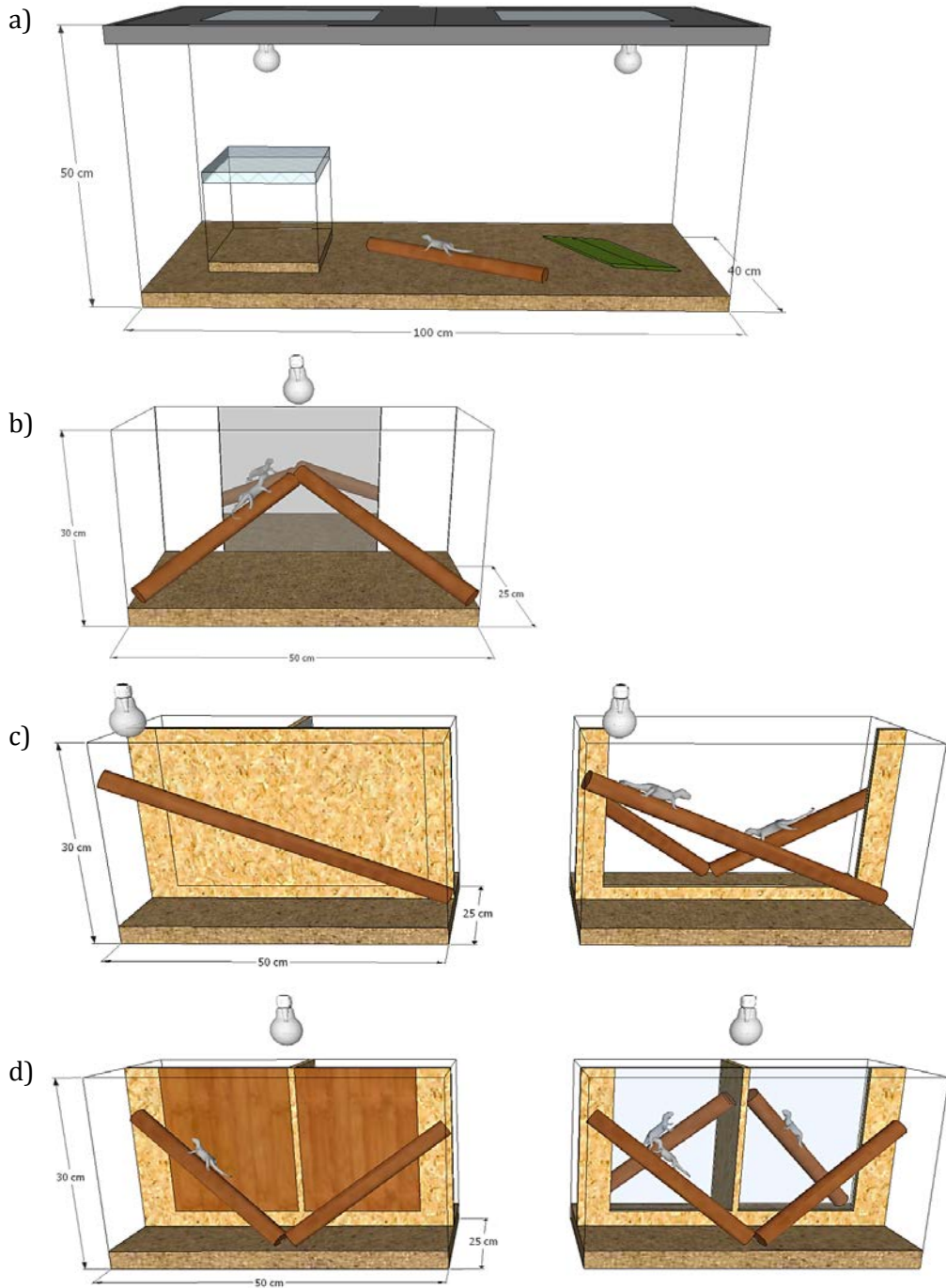


Fig. 2.1 Experimental set-ups. a) Predator (PR) and Non-predator (NP) experiment: Control (C) treatment, b) Mirror (MI) experiment, c) Male-male (MM) and Male-Female interaction (MF) experiment: habituation (left) and MM/MF treatment (right), d) Female-female interaction (FM) experiment: habituation (left) and FM treatment (right). For more details, see materials and methods section.

Mirror experiment

The mirror experiment (MI) was conducted to investigate the display behaviour of brown anoles when a subject is exposed to its mirror image, and thus to matching display behaviour. For this experiment, a terrarium (50 cm L x 25 cm W x 30 cm H) was fitted with a peat layer and two equally-sized branches positioned in a triangular way (Fig. 2.1b). A light source (Philips SpotOne 90°, 40 W) was placed 20cm above the intersection of the two branches (i.e. highest perch site) and created therefore an ideal common resource to compete for. A perforated wooden lid closed the terrarium. After habituation in the described set-up, a mirror (30 cm L x 30 cm H) was placed against the exterior side of the terrarium wall (Fig. 2.1b). Next, one of us (TD) opened the wooden lid and, using a stick, gently directed the subject towards the mirror, to make it aware of the visual presence of a matching opponent. To avoid the possible effects of disturbance, observations of display behaviour started only two minutes after the wooden lid had been closed. Observations in the MI treatment lasted 10 min per subject.

Male-male interaction experiment

For the male-male interaction experiment (MM), we matched 18 pairs of males for body mass and SVL because size affects dominance in anole lizards (Leuck 1995; Perry et al. 2004). The mean differences between members within pairs (\pm SE) were 0.26 ± 0.04 mm for SVL and 0.32 ± 0.08 g for mass. To fulfill the matching requirements, five males had to be part of two different pairs. For those five males, only data obtained during exposure to the first MM treatment were used in the analyses. For the MM experiment, a terrarium (50 cm L x 25 cm W x 30 cm H) was divided into three compartments using a wooden panel; two equally-sized smaller compartments at the rear and one larger compartment at the front (Fig. 2.1c). All compartments were provided with a peat layer and a diagonally positioned branch (rear compartments: branch of 25 cm length and 1.5 cm diameter; front compartment: branch of 55 cm length and 2.5 cm diameter). A light source (Philips SpotOne 30°, 25 W, 230 V) was placed above the end of the highest branch in the front compartment, creating a resource for competitive

interactions (Fig. 2.1c). Males were randomly assigned to one of the rear compartments and allowed to become accustomed to the new environment. We then removed the wooden panel, so that both males gained access to the larger front compartment containing the common resource. Observations started 5 min later and continued for 50 min. The display behaviour of both males was assessed simultaneously. We were able to distinguish between individual males due to recognizable differences in colour pattern and head shape. Display frequencies performed by matched males during the MM treatment, were not correlated within pairs (non-parametric Spearman's rho test per signal type, 2-tailed: all $P > 0.6$).

Male-female interaction experiment

The experimental design and set-up of the male-female experiment (MF) was analogous to that of the MM experiment (Fig. 2.1c), but involved the staging of 27 male-female couples. Couples consisted of a male and female subject that had no previous contact. As we retained a higher number of male subjects, some randomly selected females were assigned to two couples and thus used more than once. We think this redundancy should not constitute a problem, as only the males' display behaviour was scored in this experiment. Some couples copulated during the observation period ($N = 7$ out of 27 couples). In these cases, any displays exhibited by the male during the copulation were disregarded. Tallying of the displays recommenced as soon as the animals stopped copulating. Data obtained before and after copulation were later pooled for those 7 subjects, as no significant difference was found for any signal type (non-parametric Wilcoxon signed rank test per signal type, before and after copulation, 2-tailed: all $P > 0.4$).

Female-male interaction experiment

In the female-male interaction experiment (FM), we scored the display behaviour of a female in the presence of two males located behind a transparent panel. The same size-matched pairs as for the MM treatment were used, and females were assigned to a certain pair based on their SVL, i.e. the largest females were

confronted with the largest males. In all cases, females had never previously been in contact with either of the males. The set-up of the FM experiment resembled that of the MM and MF experiments, but after removal of the wooden panel, the rear and front compartments remained divided by a transparent Plexiglas sheet (Fig. 2.1d). As a consequence, the female in the front compartment was able to see the males in the rear compartments and vice versa, but could not reach them. The males in the rear compartments could not see each other. In each rear compartment, a branch (25 cm length, 2 cm diameter) was diagonally positioned at a $\pm 45^\circ$ angle with the highest branch end pointing to the terrarium centre. In the front compartment, two equally-sized branches (30 cm length, 2.5 cm diameter) were positioned in a V-shape, leaning against the terrarium at the observer's side. A light bulb was positioned 10cm above the terrarium centre (Philips SpotOne 30°, 25 W, 230 V). Only the display behaviour of the female subject was observed for a total amount of 50min, consisting of five 10-min observation bouts with a one hour interval between observation sets. During all trials, at least one of the male subjects showed display behaviour towards the female.

2.2.3 Data acquisition and behavioural analysis

An observer was present to score the subjects' display behaviour online during all trials. We additionally made camera recordings (Sony handycam HDRCX 260) to facilitate off-line analysis at a later stage, in cases of unclear online observations. All the observations were scored and analyzed using JWatcher eventrecorder software (version 1.0; Blumstein et al. 2000). We distinguished between three visual signal types: dewlap extensions (DE), push-ups (PU) and head-nods (HN). A dewlap extension was defined as one complete extension and retraction of the dewlap, a push-up as one single up and down movement of the whole body caused by flexion of only the front legs or all four legs, and a head-nod as one single up and down movement that involved only the head. For each subject, the number of executed displays was counted during the described treatments and expressed as a frequency variable (counts/minute). We inspected plots of display

frequencies against observation time (averages per sex) for all experiments and found no obvious trends in time. Therefore, we could standardize the obtained counts over time to enable us to compare the signal type variables among treatments with different durations. Treatments involving the interaction of conspecifics (i.e. MI, MM, MF and FM) are considered as ‘social context’ experiments within this study, whereas the PR and NP treatments are categorized under ‘predator context’. The C treatment, which can be regarded as ‘subject-alone context’ characterized by the advertisement of undirected signals, serves to assess a general baseline of the subject’s display behaviour.

2.2.4 Statistical analyses

We used generalized estimating equations (GEE) with repeated measures to test for the effect of experimental treatment on display frequencies. We assumed that each display variable (DE, PU, HN) followed a tweedie distribution and used a log link as model type. We further assumed an independent working correlation matrix, which is recommended when prior information about the correlation between measurements of the same individual is lacking (Pan 2002). GEE analyses were run separately for each sex and display variable, with ‘treatment’ taken as predictor variable (C, PR, NP, MI, MM, MF for males; C, PR, NP, MI, FM for females) and ‘DE’, ‘PU’ and ‘HN’ as dependent response variables. The obtained GEE parameter estimates were always calculated with respect to the C treatment. We decided only to include data of the first C treatment (PR experiment), because C treatments of PR and NP experiment did not differ significantly for any signal type variable (non-parametric Wilcoxon signed rank test for both sexes separately, 2-tailed, all $P > 0.1$). Differences in signalling frequencies between any two treatments were examined using pairwise comparisons of estimated marginal means, applying sequential Bonferroni adjustments to correct P -values for multiple testing. 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 statistically significant.

2.3 Results

Even though statistical analyses were run separately for sex, we still provide descriptive information regarding the magnitude of the signal type frequencies in males versus females across diverse contexts (Fig. 2.2). Overall, males signal at higher rates than females, with a distinct difference being found for the DE signal type. Males tend to dewlap much more than females across all contexts (2nd order mean \pm SE for DE frequency including all treatments: 1.14 ± 0.23 counts/min in males versus 0.07 ± 0.04 counts/min in females). Males perform more PU than females in both consensual and heterosexual interactions (2nd order mean \pm SE for PU frequency including all treatments: 1.42 ± 0.62 counts/min in males versus 0.25 ± 0.09 counts/min in females). The sexes differ less in their HN frequency (2nd order mean \pm SE for HN frequency including all treatments: 0.54 ± 0.11 counts/min in males versus 0.31 ± 0.12 counts/min in females).

The effect of treatment on signalling frequency in males was highly significant for each individual signal type (GEE, DE: Wald $\chi^2 = 27.78$, PU: Wald $\chi^2 = 41.81$, HN: Wald $\chi^2 = 24.21$, all $P < 0.001$). We observed an overall lower signalling rate in males for all signal types in the 'predator context', i.e. both PR and NP treatment (Fig. 2.3a-c). However, a statistically significant decrease in frequency between the predator (PR and NP) and social context treatments (MI, MM and MF) was only found for the PU signal type. When confronted with male stimuli (MI and MM), males performed more PU, but not DE and HN compared to subject-alone and predator context (Fig. 2.3a-c). Last, males increased their DE, PU, and HN frequencies simultaneously when exposed to cues of conspecific females (MF) (Fig. 2.3a-c).

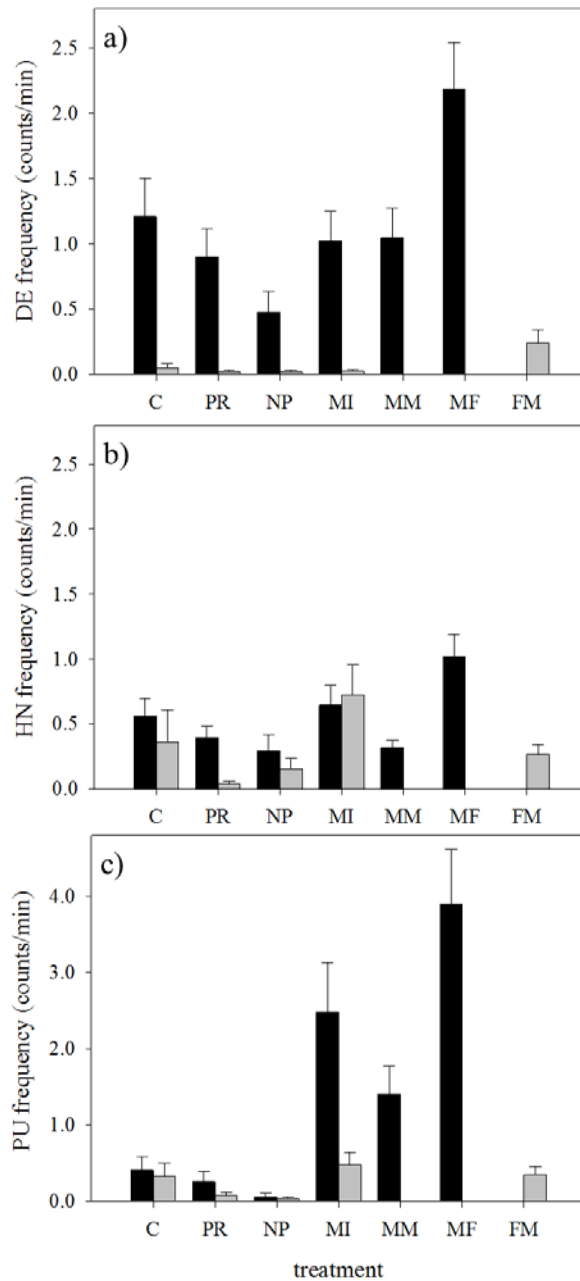


Fig. 2.2 Signal type frequencies across diverse treatments, in males versus females. Males (black bars) were exposed to a total of six treatments (C, PR, NP, MI, MM, MF) and females (gray bars) to five (C, PR, NP, MI, FM). Each signal type is represented in a separate panel: a) Dewlap extensions (DE), b) Push-ups (PU), and c) Head-nods (HN). In all panels, 1st order means and SE are shown. Treatment abbreviations: C = control, PR = predator, NP = non-predator, MI = mirror, MM = male-male interaction, MF = male-female interaction, and FM = female-male interaction treatment. Sample size per treatment and sex (M = male, F = female): C and PR, $N = 46$ M and 22 F; NP, $N = 30$ M and 16 F; MI, $N = 36$ M and 21 F; MM, $N = 31$ M; MF, $N = 27$ M; FM, $N = 17$ F.

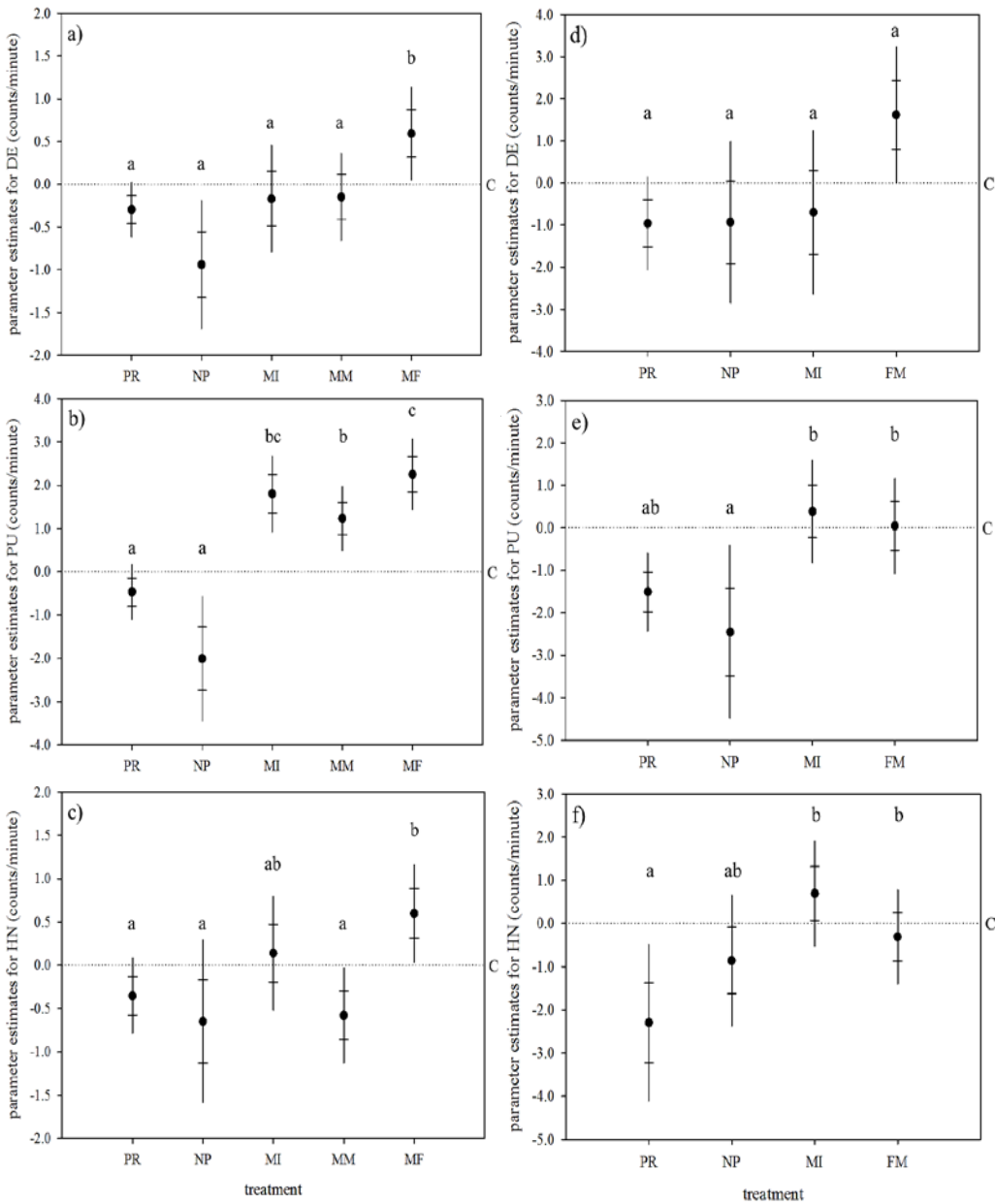


Fig. 2.3 GEE parameter estimates for signal type frequencies in *A. sagrei*, shown for multiple treatments with respect to control treatment (C, dotted line). Columns of panels represent different sexes: a-c) results for males and d-f) results for females. Rows of panels represent individual signal types: a, d) Dewlap extensions (DE), b, e) Push-ups (PU), and c, f) Head-nods (HN). Separate GEE analyses were run per sex and signal type testing treatment main effects; displayed values are mean (black dot), SE and 95 % CI. Different letters denote significant differences between treatments with $P < 0.05$ (pairwise contrasts between estimated marginal means adjusted for multiple comparisons using sequential Bonferroni). For treatment abbreviations and sample size, see legend Fig. 2.2.

In females, treatment had a strong effect on signalling frequency for the PU and HN, but not the DE signal type (GEE, DE: Wald $\chi^2 = 8.38$, $P = 0.079$; PU: Wald $\chi^2 = 14.16$, $P = 0.007$; HN: Wald $\chi^2 = 15.31$, $P = 0.004$). Like males, females decreased their overall display frequency in the presence of a predator and non-predator compared to the subject-alone and social context (Fig. 2.3d-f). Here, the decrease was most pronounced for PU during the NP treatment and HN during the PR treatment (Fig. 2.3e, f). With regard to the consensual context (MI), females showed no clear changes in behaviour in response to their mirror image (Fig. 2.3d-f). Last, when confronted with conspecific males (FM), no significant increase in display rate was found for any of the signal types (Fig. 2.3d-f). However, there was a strong trend showing that females performed more DE in the heterosexual context compared to all other treatments (all $P < 0.05$ without sequential Bonferroni correction) (Fig. 2.3d).

2.4 Discussion

2.4.1 Signalling in a predator context

When confronted to the predatory cues of a living curly-tailed lizard (*L. carinatus*), both sexes showed an overall decrease in the signalling rate of all three signal types in comparison to the subject-alone and social context. The drop in signalling frequency was particularly prominent for PU in males and HN in females. In a field study Simon (2007) demonstrated that male *A. sagrei* individuals marginally decrease their DE rate and exhibit significantly less PU in response to a simulated predatory attack by a kestrel model (PU were defined as “four-legged bobbing displays”). Two other recent studies on *A. sagrei* found no effect of imminent predation on display rates and times (Elmasri et al. 2012: visual and acoustic cues of bird predators; Yee et al. 2013: rubber snake model). In conclusion, our results and those of previous studies on a variety of predators (Simon 2007; Elmasri et al. 2012; Yee et al. 2013) strongly suggest that DE, PU and HN do not function as a pursuit deterrence signal (i.e. prey communicate their vigilance to a predator, thereby discouraging it from attacking; Hasson 1991).

This conclusion is in contrast with findings by Leal and Rodriguez-Robles (1995, 1997a), who demonstrated that in *Anolis cristatellus*, several signals used during social interactions (e.g. number of DE and PU; the latter defined as “four-legged bobbing displays”), were also used as deterrent signals in response to a natural predatory snake. It follows that the use and role of display signal types can differ strongly among closely-related species. Vanhooydonck et al. (2009) found that *A. sagrei* males and females tend to have larger dewlaps on islands where *L. carinatus* occurs in sympatry, and suggested that the dewlap serves a pursuit-deterrence role. As we observed an opposite result for dewlap use in a predator context with *L. carinatus*, we conclude that selective pressures acting on dewlap size and use can act in a different way.

In an attempt to complement those previous studies on *A. sagrei*, we incorporated a NP treatment to enable us to test for a predator-specific response rather than a general response to any kind of heterospecific lizard. As our subjects reacted in a similar way during the PR and NP treatment, we could not find evidence for a predator-specific response towards the curly-tailed lizard. The observed reduction in signalling rate during the PR treatment can thus either be explained in a risky predator context where minimum visibility would be beneficial to avoid predators (Stuart-Fox et al. 2003), or in a harmless heterospecific context where signalling to inappropriate mates and opponents would result in unnecessary energy expenditure (Andersson 1994).

2.4.2 Male signalling in a social context

We examined male signalling in a consensual context in two types of experiments. In the MI experiment males were exposed to their mirror images (only visual cues) and in the MM experiment males were exposed to real size-matched male opponents. We found overall similar display rates (DE, PU, HN) for both consensual experiments, which confirms the earlier finding that *Anolis* lizards use visual rather than chemical signals during social interactions (Jenssen 1977; Fleishman 1992; Jenssen et al. 1995; Forster et al. 2005). We further observed that *A. sagrei* males increased their PU frequency, but not their DE and HN rate during staged

consexual interactions. The role of DE and HN in male aggressive encounters seems thus limited in *A.sagrei*. Analogous to our DE findings, Simon (2011) showed that DE rates in male *A. sagrei* were lower during agonistic encounters than during courtship. Besides, Tokarz et al. (2003) revealed that males with inoperative dewlaps show the same ability to defend territories as normal males. Possibly, the benefits of dewlap use in a consensual context do not outweigh the potential predation cost associated with its use, as higher DE rates correspond to higher conspicuousness. Also, DE displays could be risky in a male-male context, because dewlaps are thin flaps of skin that could easily be damaged during escalated fights (suggested by Decourcy and Jenssen 1994). The PU signal type, on the other hand, seems to be of high importance during our staged male encounters conducted under laboratory conditions. Previous field studies have already shown that male *A. sagrei* produce higher bobbing rates (“bobbing” corresponds to PU following our terminology) in situations that may increase aggressive arousal, e.g. territory establishment and defence, when intruders are within close proximity, and when meeting unfamiliar rivals (e.g. McMann 2000; Paterson and McMann 2004; Simon 2011). Moreover, in *A. cristatellus* males, a positive correlation has been demonstrated between the number of bobbing displays and endurance capacity in a context of territorial defence and predator avoidance, suggesting that PU serves as an honest signal of endurance (Leal 1999; Perry et al. 2004). Thus males may be able to assess the endurance capacity of opponents, via their exhibited PU rates.

In the heterosexual context, tested by the exposure to an unfamiliar female, males showed higher signalling rates for all three signal types (DE, PU, HN). In accordance with our laboratory results for the DE signal type, Simon (2011) found increased DE rates for male *A. sagrei* during heterosexual interactions under natural conditions, suggesting that dewlapping indeed plays an important role in male courtship. However, the effect of DE on courtship success might be relatively subtle because several earlier studies found no significant differences in mating frequencies between males that were experimentally prevented from extending

the dewlap and sham-treated control males (Tokarz, 2002, Tokarz et al., 2005). Perhaps male DE function in sex recognition and/or stimulate female receptivity (Crews 1975; Stamps 1977; Orrell and Jenssen 2002; Simon 2011), but additional research is necessary to further investigate these proposed functions. Also the high PU frequencies we observed in males during heterosexual interactions, are in line with Simon's (2011) finding that high bobbing rates ("bobbing" corresponds to PU following our terminology) are associated with successful mating encounters. Although the PU signal type is considered to be an honest signal for endurance in a territorial and predator context (Leal 1999; Perry et al. 2004), it is unknown if this act in a similar way during courtship. There is no convincing evidence that female anoles make comparisons between males in making their mating choices (Tokarz 1998; Jenssen et al. 2001) and therefore, it is questionable whether high signal rates possibly associated with male quality will be advantageous during courtship. Similar to the DE signal type, HN as individual signal type seems to be extensively used during courtship only, and may, therefore, serve a particular function in this context. Finally, it should be noted that a simultaneous increase in all three signal types was seen only in the heterosexual context. By combining extensive signalling rates involving DE, PU and HN, displaying males appear more conspicuous during courtship than in any other context. Perhaps, the conspicuous male displays may advertise sexual receptivity to the female and act as a motivational component for copulation.

2.4.3 Female signalling in a social context

In the consensual context, female individuals did not explicitly increase any display rates in response to their mirror image. This suggests that none of the tested signalling types play an essential role during territorial and agonistic encounters in *A. sagrei* females. Previous studies on other anole species have however, shown that similar to males, females often defend exclusive territories (e.g. Rand 1967; Jenssen 1970a; Nunez et al. 1997; Orrell and Jenssen 1998). They may additionally benefit from signalling aggressively to other females by obtaining control over particular resources, such as food and nesting sites (West-Eberhard

1983). Yet, two studies on *A. carolinensis* have reported that consensual competition for resources has minimal effects on the female potential reproductive rates, indicating that the use of aggressive signals among females should be under minimal intrasexual selection (Nunez et al. 1997; Jenssen et al. 2000). With respect to our findings, it should be noted that we confronted females only with their mirror image and not with real adversaries presenting both visual and chemical cues. Besides, we used a similar resource to compete for (i.e. light source and highest perch site) in both sexes. Future work involving observations of real female encounters competing for other resources such as nesting sites and food, is required to further clarify the female display behaviour in a consensual context.

Also in the heterosexual context, females did not significantly change display frequencies compared to most other treatments. However, the strong increasing trend found for female dewlap use in the presence of a male cannot be ignored. Increased dewlap rates in females may be of importance in a context of male mating choice. Male preference for showy females has previously been demonstrated in guppies (Amundsen and Forsgren 2001) and some bird species (Amundsen 2000), but to our knowledge has not been reported in lizards. Besides, conspicuous DE exhibited by the female may also advertise sexual receptivity to males, prior to copulation. Sexual coercion imposed by males might be costly to both individuals and may additionally reduce female fecundity and survival (Clutton-Brock and Parker 1995; Le Galliard et al. 2005); advertising sexual receptivity may thus be beneficial for females.

2.4.4 Male versus female signalling

Generally, males signal (DE, PU, HN) at higher rates than females across all contexts, with a more pronounced intersexual difference being found for the DE signal type. This result is in accordance with previous studies on anoles, addressing male and female DE rates in subject-alone and differential social contexts in the field and under laboratory conditions (*A. carolinensis*: Jenssen et al.

2000; Orrell and Jenssen 2003; *A. sagrei*: Partan et al. 2011). For the overall PU and HN rates in males versus females, inconsistent use of terminology (see Partan et al. 2011 for more details) and a lack of data on those two signal types in females, hamper a comparison with other studies.

We further found that both male and female subjects exhibited higher display rates in the presence of conspecifics than when confronted with a predator or unknown heterospecific, indicating that DE, PU and HN function in intraspecific communication for both sexes, and not in predator deterrence. Within a social context, displaying of the dewlap in *A. sagrei* seems to play an essential role during the male courtship of females only. However, we found a strong trend that *A. sagrei* females use their dewlap far more in the presence of a male, suggesting that selective pressures on dewlap use act in a similar way for both sexes. Unlike dewlap displays, we found pronounced intersexual differences in the PU and HN use during social interactions. The PU signal type seems to be an essential key factor during aggressive encounters in males, but not in females. And increased HN rates were only found in the male sex during heterosexual interactions, indicating an important contribution of HN during male courtship. From these results, we suggest that within a social context, selective pressures acting on the PU and HN signal type differ between sexes.

We end with some cautionary notes on the generality of our findings. First of all, we only considered frequencies of three main individual signal types in our study. Even though a higher frequency of a certain signal type would indicate more functionality of that particular signal type within a certain context, rates of various display types including the combination of a particular set of signal types might also serve a crucial role in the respective contexts. Also, additional studies are required to assess the importance of more signal type parameters (e.g. speed, duration and absolute amplitude) and other signal types (e.g. crest erection, licking, yawning, ...) in the display behaviour of *A. sagrei*. Second, our study was conducted under fully-controlled laboratory conditions and subjects were tested separately and repeatedly. Although our results generally accord with findings

from field studies, we admit the difficulty of correctly interpreting display behaviour in nature on the basis of laboratory results. Third, all our experiments were tested in standardized lab terraria, providing only a framework for short-distance interactions. Earlier studies have shown that short versus long distances can affect the signalling behaviour across diverse contexts (Hasson et al. 1989; Decourcy and Jenssen 1994; McMann 2000), indicating that ‘distance’ should be accounted for in the general interpretation of the *A. sagrei* display behaviour.

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

CHAPTER 3

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

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Dewlaps of *Anolis* lizards are complex multi-component signalling 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 signalled by the dewlap in both sexes of *Anolis sagrei*. In addition, we investigate whether diverse facets of dewlap signalling provide additive information (redundant message hypothesis) or highlight different characteristics of the sender (multiple message hypothesis). To do so, components of dewlap design (area, colour, 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 colour act as redundant messages in the advertisement of sexual identity. Second, we found that dewlap colouration in the centre and edge region signals aspects of individual quality, specifically health state, but only in males.

The dewlap centre and edge acts primarily as redundant signals, at least for body condition and immune response. However, different colour components irrespective of dewlap region convey non-redundant 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 signalling functions of the female dewlap in a courtship context.

Key words: sexual identity, individual quality, social status, redundant messages, multiple messages

3.1 Introduction

For centuries, biologists have been fascinated by signals involved in sexual selection including the massive horn of rhinoceros beetles or the brilliantly coloured 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 behavioural 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 colouration 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 (*Salaria pavo*), for example, the size of the yellow head crest signals developmental quality, whereas its colour 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 multi-component 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 colouration provides a good illustration for this hypothesis. Whereas carotenoid pigments in ornaments are thought to reflect physical condition, the melanin-based colours 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 signalling 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. 2014). The fact that the male dewlap has been added to a list of display organs that

exhibit positive allometry (i.e., disproportionately 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 area (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 1970a; Crews 1975; Sigmund 1983), rivals (Jenssen et al. 2000; Vanhooydonck et al. 2005a), members of related species (Rand and Williams 1970; Losos 1985), and even predators (Leal and Rodriguez-Robles 1995, 1997a). 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 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 1980a; 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 coloured regions in both sexes i.e., bright yellow to reddish in the dewlap centre and yellow along the edge. Carotenoids create the yellow colour of both dewlap regions and interact with pterins to create the orange and red colour in the centre (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 signalling function and significance of the dewlap extension in a context of courtship and mating behaviour 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 signalling 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, colour) 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, haematocrit 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 colour of the dewlap centre and edge region. A recent study has shown that the edge of the dewlap plays the most important signalling 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.

3.2 Materials and methods

3.2.1 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: >39mm, females: >34mm, Licht and Gorman 1970; Lee et al. 1989). The lizards were individually housed in specially designed, non-transparent 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/phosphor ratio and vitamin D3 (Zoo Med Reptivit 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 hours. 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 towards 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.

3.2.2 '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 colours 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 coloured (whitish background was excluded) and the area covered by the main colour only (red in most individuals), using the 'colour range tool' (AP CS3 software, version 10.0). Then we calculated the ratio of the area covered by the main colour to total colour 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.

Colour — 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 fibre-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 centre (one unique spectral measurement) and along the dewlap's edge (the average of three unique, non-overlapping spectral measurements). For females, we could only obtain a single spectral measurement from the dewlap centre, 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 colour 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 300-700 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/minute). 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 set-up, we refer to the 'male-female' and 'female-male' interaction experiments described in Driessens et al. (2014). The behavioural 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).

3.2.3 '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. 1999a for detailed description of set-up). 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 infra-red 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 hour in between. The fastest speed over any 25-cm interval was used as a measure of maximal sprint performance (see Vanhooydonck et al. 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 x 2 x 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 hour 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 Slyk 1998).

Swelling response — We assessed the lizards' immune function using the phytohemagglutinin (PHA) skin swelling test. A subcutaneous injection of 20 µl PHA solution (males) or 10 µl (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 digit) 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).

Haematocrit — Blood was collected twice from the lizards' postorbital sinus behind the eye using microhaematocrit 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 > 0.99); the buffy coat was negligible. The average haematocrit value of both blood samples was used in all subsequent analyses. The haematocrit 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.

3.2.4 'Social status' measurements

Aggressiveness — Each lizard was given an 'aggressiveness' score, depending on how it behaved towards 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 set-up are described in Driessens et al. (2014). We followed the ethograms of Leal and Rodriguez-Robles (1995, 1997a) and McMann (1993) to categorize the anoles' behaviour as 'aggressive' or 'submissive'. Aggressive behaviours were assigned positive scores, whereas submissive behaviours 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 behaviours 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' towards opponents of the same sex.

3.2.5 *PCA on colour 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 centre 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 colour at the centre of the dewlap among males (produced PC_{mc1} , PC_{mc2} , PC_{mc3}). The third analysis was performed to summarize variation in reflectance near the dewlaps' edges (PC_{me1} , PC_{me2} , PC_{me3}). We chose not to combine information from the dewlap centre 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} , 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).

3.2.6 *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 colour 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. 3.1a). PC_{mfc1} (67.93 % of variance) was highly correlated with mean brightness (Table 3.1). PC_{mfc2} (19.40 % of variance) represents variation in the relative amount of short 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. 3.1a). The PCA_{mc} on the reflectance data measured at the centre of the males' dewlap (Fig. 3.1b) 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 3.1). 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. 3.1b, Table 3.1). 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. 3.1c). PC_{me1} correlated highly with reflectance values at all wavelengths (Table 3.1). 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. 3.1c, Table 3.1). 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. 3.1c, Table 3.1). Last, the PCA_{fc} on the reflectance values for the female dewlap centre resulted in three component axes (PC_{fc1}, 68.99 %; PC_{fc2}, 20.42 % ; PC_{fc3}, 4.84 % of variance) that together captured 94.25 % of the original variation (Fig. 3.1d). PC_{fc1} exhibited high loadings for reflectance at all wavelength bins (Table 3.1). Factor loadings for PC_{fc2} were highly negative for reflectance in the UV region, and positive in the yellow – red colour range (Fig. 3.1d). Pearson correlations confirmed a strong negative correlation with reflectance in the UV region, and positive correlations with yellow and red chromas (Table 3.1). The third axis, PC_{fc3} described the variation in intermediate (400–550 nm; blue – green) relative to the short (300–400 nm; UV) and long wavelength spectrum (575–700 nm; yellow – red) (Fig. 3.1d). Only a strong negative correlation with PC_{fc3} and reflectance values in the green spectrum were found (Table 3.1).

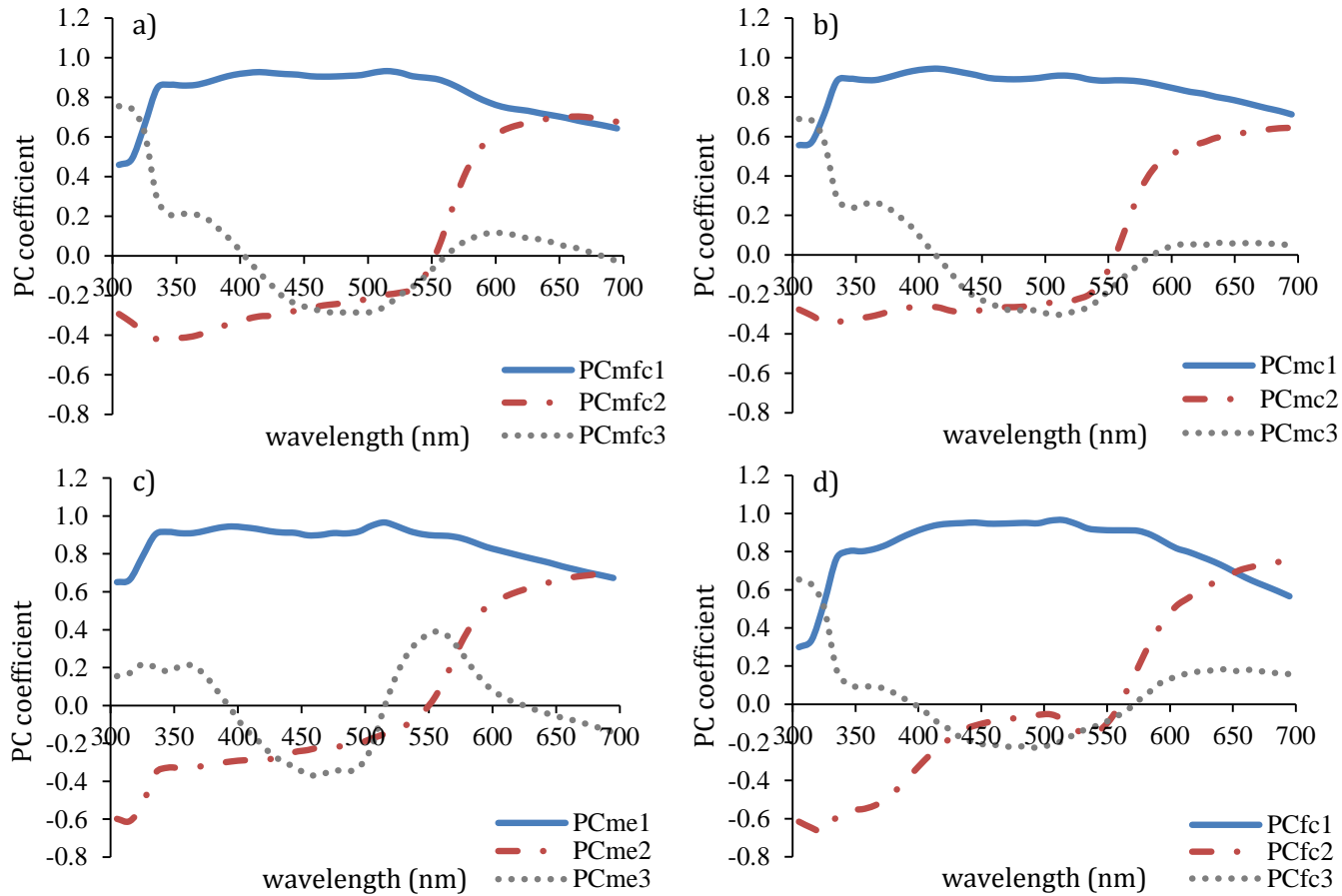


Fig. 3.1 Separate principal component analysis of the a) dewlap centre in both sexes (PCA_{mfc}), b) dewlap centre in males (PCA_{mc}), c) dewlap edge in males (PCA_{me}), and d) dewlap centre in females (PCA_{fc}). These panels show the association between PC factor-loading coefficients and wavelength intervals. For more details, see “PCA on colour variation” and “Interpretation of PCs” in the Materials and methods section.

Table 3.1 Pearson correlation coefficients between reflectance indices and PC scores (see “PCA on colour variation” and “Interpretation of PCs” in Materials and methods section for more details)

Indices	Male and female centre (<i>N</i> = 99)			Male centre (<i>N</i> = 61)			Male edge (<i>N</i> = 61)			Female centre (<i>N</i> = 38)		
	PC _{mfc} 1	PC _{mfc} 2	PC _{mfc} 3	PC _{mc} 1	PC _{mc} 2	PC _{mc} 3	PC _{me} 1	PC _{me} 2	PC _{me} 3	PC _{fc} 1	PC _{fc} 2	PC _{fc} 3
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 ($P < 0.005$).

3.2.7 *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, 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 colouration by comparing the scores on PC_{mfc}1, PC_{mfc}2 and PC_{mfc}3 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 signalling 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, PC_{mfc}1–3). 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_{mc}1, PC_{me}1–3, 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, PC_{mc}1–3 or PC_{me}1–3 or PC_{fc}1–3), to predict a given ‘quality’ measure (relative bite force, sprint speed, clinging capacity, body

condition, swelling response or haematocrit) 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 haematocrit) inter-correlate 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.

3.3 Results

3.3.1 Sexual differences in dewlap design

On average, the males in our dataset were larger than the females (Table 3.2, $t_{95,69} = 14.70$, $P < 0.001$). 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 colour reflectancy curves for the dewlap centre in males and females demonstrated the presence of sexual colour dichromatism (Fig. 3.2). Overall, females tended to have higher reflectance values in the UV, blue and green wavelength spectra compared to males, but lower reflectancy in the yellow and red regions. Females scored higher on PC_{mfc1} (mean \pm SE = 0.27 ± 0.16 , $N = 38$, Table 3.2) than males did (-0.17 ± 0.13 , $N = 61$, Table 3.2; $t_{97} = -2.13$, $P = 0.036$). On the other hand, males scored much higher on PC_{mfc2} (0.32 ± 0.12 , Table 3.2) than females did (-0.52 ± 0.15 , Table 3.2; $t_{97} = 4.41$, $P < 0.001$). For PC_{mfc3} , males scored again higher (0.20 ± 0.12 , Table 3.2) than females did (0.32 ± 0.16 , Table 3.2; $t_{97} = 2.59$, $P < 0.011$). Last, the colour pattern ratio also differed between the sexes ($t_{96} = 2.17$, $P = 0.033$; Table 3.2). Accordingly, the distribution of dewlaps across the four 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, $P = 0.033$).

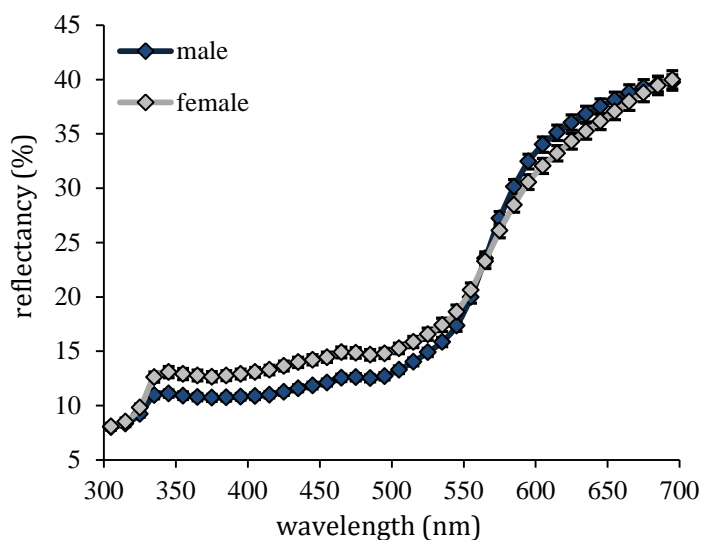


Fig. 3.2 Mean reflectance curves (%) of the dewlap centre in *Anolis sagrei*. Colour data for males ($N = 61$) and females ($N = 38$) are displayed separately; error bars represent SE.

3.3.2 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.

3.3.3 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 haematocrit), only clinging capacity and immune response were significantly inter-correlated ($r = 0.47$, $N = 24$, $P = 0.022$; other pair-wise 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 colour 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 colour in both the dewlap centre and edge of males are important predictors of body condition, immune response and haematocrit (Table 3.3, 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 reflectancy 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 colour is, therefore, similar for both dewlap regions with regard to body condition and swelling response. For haematocrit, on the contrary, only the colour component for dewlap edge was a significant predictor (PC_{me3} , $B \pm SE = 0.050 \pm 0.021$). Individuals possessing a dewlap edge with higher reflectancy in the yellow and UV relative to the blue range show higher haematocrit values in the blood. Surprisingly, multiple backward regression analyses of the quality measurements reflecting individual performance (i.e., relative bite force, sprint speed, clinging capacity) retained none of the predicting dewlap design variables in males.

Table 3.2 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 colour	PC _{mfc1}	61	-0.16	0.13	38	0.27	0.16
	PC _{mfc2}	61	0.32	0.12	38	-0.51	0.15
	PC _{mfc3}	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	
Haematocrit (%)	27	60.46	2.70	12	52.34	4.85	
Aggression score	36	2.39	0.49	18	0.72	0.19	

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

Dewlap use in males during intersexual interactions ranged from 0 to 6.48 DE/min (Table 3.2). 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 colour parameters for both dewlap centre and edge

(i.e., $PC_{mc}1-3$ and $PC_{me}1-3$; Pearson's correlation tests, $N = 27$, pair-wise 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 ($r = 0.54$, $P = 0.009$, $N = 22$). Pair-wise correlations between all other performance variables were non-significant ($P > 0.15$). 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 haematocrit ($N = 12$); 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 3.2). 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 colour $PC_{fc}1$, which corresponds to brightness ($\rho = -0.49$, $P = 0.048$, $N = 17$).

3.3.4 Dewlap design, use and social status

Aggression scores in males varied between -0.70 and 10.55 (mean \pm SE = 2.39 ± 0.49 , $N = 36$). We found no evidence for a relationship between mirror-motivated aggression and any of the quality measurements (Pearson correlation tests, pair-wise 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 colour (multiple regression analysis retained no predictor variables), nor from relative DE frequency (linear regression, $r = 0.07$, $F_{1,26} = 0.13$, $P = 0.720$).

For Females, aggression scores varied between -0.35 and +2.30 (mean \pm SE = 0.72 ± 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, $\rho = 0.19$, $P = 0.493$, $N = 15$).

Table 3.3 Results of stepwise multiple linear regressions of dewlap design components as predictors for general health parameters in males. Following independent variables were incorporated in all regressions: relative dewlap size, pattern ratio and colour including PC_{mc1} – PC_{mc3} or PC_{me1} – PC_{me3} (separate analyses for dewlap centre and edge). Only significant models and predictors are presented in this table ($P < 0.05$).

Response variable	Model			Predictors (dewlap design)			
	N	R ²	F	Predictor variable	β	t	P-value
Body condition	60	0.103	6.637	PC_{mc2}	0.320	2.576	0.013
	60	0.146	9.887	PC_{me2}	0.382	3.144	0.003
Swelling response	24	0.222	6.262	PC_{mc1}	-0.471	-2.502	0.020
	24	0.227	6.476	PC_{me1}	-0.477	-2.545	0.018
Haematocrit	27	0.186	5.701	PC_{me3}	0.431	2.388	0.025

Note: for some characters, residuals treated as size-corrected variables were used in the regressions (see “other statistical analyses” in Materials and methods section for details).

3.4 Discussion

3.4.1 Sexual differences in dewlap design

Intersexual differences in dewlap design were clearly observed for all tested components (i.e., dewlap area, patterning and colour), 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 area in females is supported by the social selection and sensory drive hypotheses, more than by genetic correlations between sexes or species recognition.

3.4.2 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 Veherencamp 1998). Given the costs of responding to inappropriate mates, efficient systems signalling 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 signalling sexual identity in *A. sagrei*, a species that exhibits a high degree of sexual size dimorphism (Schoener and Schoener 1980a; Schwartz and Henderson 1991). But additionally, different components of dewlap design involving relative dewlap area and colour (i.e., particularly brightness and reflectancy 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 non-optimal environmental conditions.

3.4.3 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 Veherencamp 1998). We found that in *A. sagrei*, dewlap colouration is primarily responsible for signalling aspects of individual quality, but only in the male sex. Our results particularly show that individual health state parameters are reflected in the colour components of both male dewlap centre and edge, and that multiple messages are conveyed by dewlap colour. 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 colour 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 colour 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 colour, 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). Although, 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 *A. brevirostris* lizards exhibited brighter dewlaps, indicating that these heavily parasitized individuals allocate more pigment resources to immune function to combat parasitism than to ornamental colouration. 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 colouration 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, 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 centre and edge transfer the same information, and thus act as redundant signals for body condition and immune response, only the dewlap edge signals an individuals' hematocrit. A recent study by Steffen and Guyer (2014) showed that UV, orange and red colouration in the dewlap edge is the region with a signalling function when it comes to male-male contest success in *A. sagrei*, and not the dewlap centre. The centre region of the dewlap can only be viewed during extension. In contrast, colour components of the dewlap edge can transfer information during extension as well as retraction mode. Thus, a dewlap edge region with signalling function involves less energy expenditures than a signalling dewlap centre, as information can still be transferred to a conspecific without extending the structure. Moreover, a signalling 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 signalling within a context of dewlap use. If a lizard extends its dewlap, the redundant message conveyed by dewlap centre 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 centre 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 colour components irrespective of the region, convey non-redundant 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 behaviour. 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 area of several anole species are honest indicators of maximum bite force (*A. grahmi* and *A. lineatopus*; Vanhooydonck et al. 2005a; *A. 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 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, signalling often involves the combination of ornamental design and behaviours that display it. For example, Steffen and Guyer (2014) showed that dewlap colour and display behaviour (a composite of head-bob, push-up, and DE) in *A. sagrei* have a combined signalling function in male-male combat that gives winners access to territories and to females. Cook et al. (2013) also found an association between dewlap colour and display behaviour (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 signalling male quality to females. The fact that we found no correlations between dewlap use and the colour components in the dewlap centre (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). 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 signalling 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 centres. 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 signalling 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 in the field are required, preferably with higher sample sizes and with the incorporation of dewlap colour in the edge region. Especially, the investigation of links between female dewlap traits (i.e., area, colour, patterning, 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 and steroid hormones, ...) should clarify whether females indeed use their dewlap to signal reproductive quality to males.

3.4.4 Conclusion

In conclusion, we can state that the dewlap signalling 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 centre and edge bear a signalling 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 signalling functions related to individual quality in females.

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

PART II

Dewlap diversity and underlying evolutionary processes



*Anolis maynardi on Little Cayman,
photo taken by S. Baeckens*

*It is not the strongest of the species that survive,
nor the most intelligent, but the one most
responsive to change.*

CHARLES DARWIN - BIOLOGIST

CHAPTER 4

Is male dewlap size a predictor of bite performance in *Anolis sagrei*? Interpopulational comparisons reveal high variation in this relationship.

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1. In many animals, male secondary sexual traits advertise reliable information on fighting capacity in an intrasexual context. Anole lizards have been extensively studied in this respect and evidence for a positive correlation between male dewlap size and bite capacity (an important determinant of combat outcome) has been provided for several territorial species. Intriguingly, earlier studies could not find this expected correlation (relative dewlap size – relative bite force) in the highly territorial brown anole lizard, *Anolis sagrei*.
2. In this study, we present data on the relationship between male dewlap size and bite force on the intraspecific level in *A. sagrei*. First, we assessed and compared the relationship between both variables in 17 island populations distributed throughout the Caribbean. Second, we linked the relative dewlap size – bite force relationship within each population to variation in the degree of intrasexual selection among populations, using sexual size dimorphism (SSD) and display intensity.
3. Our results showed that absolute dewlap size is an excellent predictor of maximum bite force in nearly all *A. sagrei* populations. However, relative dewlap size is only an honest signal of bite force in 4 out of the 17

populations. Surprisingly, the level of signal honesty did not correlate with the strength of intrasexual selection.

4. We suggest that dewlap size in *A. sagrei* males acts primarily as a redundant signal for body size in the advertisement of fighting capacity and only in particular cases as an honest, direct signal of bite capacity (independent of body size). We further discuss potential factors other than intrasexual selection that may explain differences in the relative dewlap size - bite force relationship among *A. sagrei* populations.
5. Our study shows that the link between signal size and performance traits can differ substantially within a single species. We therefore argue that caution is warranted when using single populations as representatives for entire species.

Key words: brown anole lizard, fighting capacity, male-male interactions, intrasexual selection, signalling, whole-organism performance.

4.1 Introduction

The evolution of male secondary sexual traits, such as the colossal antlers in deer or the giant horns in rhinoceros beetles, has fascinated biologists ever since Darwin (Darwin 1871; Andersson 1994; Bradbury and Vehrencamp 1998). These elaborate sexual traits can function as real weapons to overpower or even kill male opponents (e.g. mandibles of male fig wasps; Bean and Cook 2001), but also as reliable signals advertising 'fighting capacity' without playing a role during actual physical combats (e.g. red colouration in male mandrills; Setchell and Wickings 2005). Traits that honestly signal fighting capacity seem highly beneficial to predict contest outcomes and thereby avoid the costly interactions physical combats may impose (Rohwer 1975; Andersson 1994; Maynard-Smith and Harper 2003). This is especially true for species where actual fights between males can result in serious body damage and even in death (Andersson 1994; Maynard-Smith and Harper 2003). The idea that male secondary sexual signals communicate reliable information about 'quality' in an intrasexual context, has

been evidenced by a variety of studies showing a direct link between variation in signal design, especially size and colour, and the ability to win male contests (e.g. Jennions and Backwell 1996; Panhuis and Wilkinson 1999; Alonso-Alvarez et al. 2004). In many cases, the size of these sexual traits correlates strongly with overall body size (arguably the most important predictor of contest outcome, e.g. Clutton-Brock et al. 1979; Hughes 1996; Karsten et al. 2009; Hardy and Briffa 2013), and as such acts as a redundant or back-up signal (Zuk et al. 1992; Johnstone 1996; Candolin 2003) when advertising fighting capacity. However, in at least some cases, the size of secondary sexual traits reveals more than just the carrier's overall body size during agonistic interactions. Here, sexual signal size contains information on fighting capacity independent of overall body size (i.e. relative size), and can therefore be considered as a reliable signal in itself. In dung beetles, for example, relative male horn size accurately predicts pulling force and maximal exertion, two ecologically relevant performance measures associated with fighting success in beetles (Lailvaux et al. 2005). Also in lizards, male signals can act as size-free indexes of fighting capacity, quantified by endurance or bite force (e.g., Perry et al. 2004; Lappin and Husak 2005; Vanhooydonck et al. 2005a). *Anolis* lizards in particular have received considerable attention in this respect (e.g. Lailvaux et al. 2004; Vanhooydonck et al. 2005b; Lailvaux and Irschick 2007). They typically have an extendible throat fan, called dewlap. This sexually selected trait is generally far more elaborated in the male sex and exceptional for its high degree of interspecific variation in design (Nicholson et al. 2007). Besides, Anoles exhibit varying degrees of territoriality and male-male competition (Losos 2009), also reflected by their remarkable diversity in sexual size dimorphism (SSD; i.e. predominantly male-biased SSD) (Andersson 1994; Stamps et al. 1997). Does dewlap size indicate fighting capacity in *Anolis* lizards? The evidence is rather mixed. In highly territorial, sexually dimorphic (high-SSD) species (i.e. *A. carolinensis*, *A. cristatellus*, *A. evermanni*, *A. gundlachi* and *A. lineatopus*), relative dewlap size predicts bite force and thus seems to contain detailed information on fighting capacity (Vanhooydonck et al. 2005a; Lailvaux and Irschick 2007). However, no such relationship was found in less dimorphic (low-SSD) species

(i.e., *A. angusticeps*, *A. distichus* and *A. valencienni*; Vanhooydonck et al. 2005a; Lailvaux and Irschick 2007). The authors explain the lack of this relation in less dimorphic species by a low degree of territoriality mainly. Bite performance, in particular, might be far less important for males of species that do not actively defend territories or that do not experience a high degree of male-male competition associated with vigorous fights. Lailvaux and Irschick (2007) further corroborated this idea by showing that bite force predicted male combat success only in the high-SSD species and that the incidence of biting increased with SSD. Intriguingly, one species in their dataset defied this putative principle: *Anolis sagrei*, albeit clearly sexually dimorphic, did not show the expected positive correlation between relative dewlap size and bite performance (although, a significant relationship was found between absolute dewlap size and bite force). In accordance, we also failed to find such a relationship in wild-caught males from Florida, when looking at relative indexes (Driessens et al. 2015). Because of these unexpected results, we aimed to further explore the dewlap size – bite force relationship in this polygynous and highly territorial species (Schoener and Schoener 1980a; Tokarz 1998, 2002). Direct physical combats are commonly observed among brown anole males and primarily involve biting, jaw sparring and interlocking (Scott 1984; Tokarz 1985, 1987; McMann 2000; Steffen and Guyer 2014; pers. observation). *Anolis sagrei* has a yellow-to-reddish dewlap, that can show dramatic intraspecific variation in size, colour, pattern and even use (Vanhooydonck et al. 2009; Edwards and Lailvaux 2012). Adult males primarily use dewlap displays in combination with push-ups and head-bobs for territorial defence and/or for access to females (e.g. Scott 1984; Simon 2011; Driessens et al. 2014). Recently, display behaviour and dewlap colour have been reported to predict the outcome of staged contests between size-matched males (Steffen and Guyer 2014), further demonstrating the role of the *A. sagrei* dewlap in signalling ‘quality’ to opponents (but see Tokarz et al. 2003). Close-proximity contest experiments additionally revealed that *A. sagrei* males with enhanced biting capacities are at a competitive advantage for winning fights (Lailvaux and Irschick 2007), highlighting the importance of signalling bite capacity too, during agonistic

interactions.

The main goal of this study is to look in more detail at the relationship between male dewlap size and bite force, explicitly for *A. sagrei*. Therefore, we took a comparative approach, documenting and comparing this specific relationship in 17 *A. sagrei* island populations distributed across the Caribbean. Our study populations varied markedly in dewlap size and bite force, as well as in SSD and display intensity. The last two variables are often used as a surrogate for the degree of intrasexual selection, because of their positive correlation with the intensity of male-male competition (e.g. Stamps et al. 1997; Lailvaux and Ischick 2007; Steffen and Guyer 2014). We looked at the relationship between dewlap size and bite force, using absolute as well as relative indexes. Consistent with previous studies, we expected absolute dewlap size to be a good predictor of absolute bite force for each study population (Lailvaux and Ischick 2007; Cox et al. 2009; Driessens et al. 2015). However, due to large interpopulation differences in morphology, performance and territoriality, we expected relative dewlap size to predict bite force only in some study populations. Next, we linked the dewlap size – bite force relationship within each population to both SSD and display intensity among populations, taking into account phylogenetic relationships. Following Lailvaux and Irschick (2007), we here expected relative dewlap size to be a more relevant signal of bite force in populations exposed to a higher degree of intrasexual selection.

4.2 Materials and methods

4.2.1 Study animals

We sampled a total of 639 adult *Anolis sagrei* males from 17 populations distributed across the Caribbean. Sampling localities included Acklins, Andros, Chub Cay, Crooked Island, Grand Bahama, Pidgeon Cay, Staniel Cay (data collection for these seven populations occurred in April-May 2003), Jamaica (March 2012), Cuba (Santa Clara, Soroa 1, Soroa 2; April-May 2012), San Salvador (January 2013), Cayman Islands (Cayman Brac, Grand Cayman, Little Cayman;

March 2013), South Abaco and South Bimini (March 2015). Previous studies on *A. carolinensis* have reported a significant effect of season on dewlap size, bite force and display behaviour (Jenssen et al. 1995, 2001; Irschick et al. 2006; Lailvaux et al. 2015). Data were therefore collected during the *A. sagrei* breeding season (March - September, Lee et al. 1989), apart from one population (i.e. San Salvador) that was sampled in January. We caught 404 *A. sagrei* males by noose and kept them individually in plastic bags for maximum 48h, before releasing them back at the location of capture. For these individuals, we measured morphology, quantified dewlap size and carried out standard bite force measurements. Another 235 male individuals were video-recorded while behaving in their natural habitat.

4.2.2 Morphology

We measured the lizards' head length (HL) and snout-vent length (SVL) using digital calipers (Mitutoyo CD-15DC, accuracy 0.01 mm). For measuring dewlap size, lizards were first positioned on their left side against a 1-cm² gridded paper. We then gently pulled the base of the ceratobranchial forward with a pair of forceps until the dewlap was fully extended parallel to the grid (Bels 1990). Next, we photographed the dewlap, using a Nikon D70 camera mounted on a tripod. Last, Adobe Photoshop CS3 extended software (AP CS3, version 10.0) was used to trace the outer edge of the dewlap on the digital images and to calculate absolute dewlap area. This standard method for measuring dewlap dimensions has produced highly repeatable results in a previous study (Vanhooydonck et al. 2005a).

4.2.3 Bite force

Standard methods were used to measure maximum bite force. Briefly, we encouraged lizards to bite on two metal plates connected to an isometric Kistler force transducer (type 9203) and charge amplifier (type 5995); for detailed descriptions of setup and biting procedure, see Herrel et al. 1999a and Vanhooydonck et al. 2005b). Each individual was subjected to a total of five bite

trials with approximately 30 min in between (e.g., Herrel et al. 2001; Lailvaux et al. 2004; Irschick et al. 2006; Lailvaux and Irschick 2007). The highest of the five bite force measurements was then used as the maximal bite force capacity in each individual. The applied methodology has been widely used and shown to be effective for obtaining maximal bite forces in lizards (e.g., Herrel et al. 2001; Vanhooydonck et al. 2005b; Lailvaux and Irschick 2007). Temperature is known to affect bite performance (Bennett 1985; Herrel et al. 1999b; Anderson et al. 2008) and therefore lizards were warmed to their average field-active body temperature (30 ± 2 °C; Losos 2009) prior to every bite trial. Body temperature was verified using a cloacal thermometer (APPA51, K-type).

4.2.4 Sexual size dimorphism (SSD)

Consistent with Lailvaux and Irschick (2007, and references therein), we used SSD calculated as mean SVL in males divided by mean SVL in females. Values of SSD were calculated for each population, and only SVLs of mature males and females were included.

4.2.5 Display intensity

We recorded the natural behaviour of 20 - 30 males per population (10 study populations) for a timespan of 10 min, using a high-definition camera (Sony, HDR-CX260VE). First, we located lizards by walking slowly through their natural habitat until an apparently undisturbed individual was spotted. Next, we started filming the lizard's behaviour from ca 5-15 m using the camera zoom function (30x optical zoom), in order to minimize disturbances caused by our presence. Video recordings were only made during sunny or partly cloudy conditions to avoid possible confounding effects of weather on the lizard's activity level (Huey 1982; Hertz et al. 1993). All behavioural recordings were scored offline, using JWatcher 1.0 event-recorder software (Blumstein et al. 2000). For each focal individual, we noted the number and duration of three main display types: head-nods (up-and-down movement of the head), push-ups (up-and-down movement of the body and tail caused by flexion of the legs) and dewlap extensions (pulsing

of the often-colourful throat fan or dewlap). These displays can function in intrasexual communication and their intensity is typically inter-correlated in the sense that males that frequently perform one display type also exhibit the other types at a high rate (e.g. Scott 1984; McMann 2000; Driessens et al. 2014; Steffen and Guyer 2014). In the remaining, ‘PTD’ refers to the proportion of time that individuals spent displaying in their natural setting during the 10 minutes observation period (averaged per population).

4.2.6 Phylogeny

To account for the non-independency of our datapoints, we conducted our interpopulational analyses in an explicit phylogenetic context (Felsenstein 1985; Harvey and Pagel 1991). We constructed a hypothesis on the phylogenetic relationships among our study populations, based on mitochondrial DNA genes (ND2 and tRNA's) available in GenBank (<http://www.ncbi.nlm.nih.gov>). In particular, we used mtDNA haplotypes obtained by Kolbe et al. (2004), as they were previously proven effective in assessing interpopulational relationships in *A. sagrei*. The islands, Andros, Crooked, Grand Bahama, Grand Cayman, Little Cayman, San Salvador, Cuba, South Abaco, South Bimini and Staniel Cay have been sampled by Kolbe et al. (2004), and we assumed that our study populations occurring on these islands had the same genetic identity. No specific information was listed for Acklins, Chub Cay and Pidgeon Cay, and we therefore assumed that lizards of these study populations are closest related to specimens collected from the nearest islands, i.e. Crooked Island, Berry Islands and Staniel Cay, respectively (R.G. Reynolds, personal communication). Notably, for Acklins, we incorporated the genetic sequence of a specimen from Crooked Island and this specimen was different from the one we used to represent our Crooked Island study population; the same working strategy was adopted for Chub Cay and Pidgeon Cay (see appendix, Table A.1 for all details of the used *A. sagrei* specimens with corresponding GenBank accession numbers). On Jamaica, Kolbe et al. (2004) described the co-occurrence of two haplotypes and possible hybridization. We performed separate analyses with either of the haplotypes, which resulted in the

same phylogenetic tree. Consequently, we selected randomly one haplotype, as a representative for our Jamaican study population.

Sequences were aligned using MAFFT v7 (Kato and Standley 2013), and refined with Gblocks v0.91b (Castresana 2000; Talavera and Castresana 2007). Phylogenetic trees were built through Bayesian inference as implemented in MrBayes v3.2 (Ronquist et al. 2012); and the GTR+I+ Γ model was selected as the optimal model (jModelTest 2; Guindon and Gascuel 2003; Darriba et al. 2012). We performed 20×10^6 generation runs (Markov chain Monte Carlo), with trees sampled every 1000 generations, and a burn-in period of 4.0×10^6 generations. For a representation of the obtained Bayesian tree, see Supporting information (appendix, Fig. A.1).

4.2.7 Statistical analyses

Prior to statistical analyses, data on HL, dewlap size, bite force and SSD were log₁₀-transformed. Proportion data (i.e. PTD) were normalized via arcsin-square root transformation (Sokal and Rohlf 1995). In all cases, assumptions of normality were confirmed using Shapiro-Wilk tests.

All statistical tests involving dewlap size and bite force were done with absolute as well as relative (i.e. size-corrected) data. Consistent with Lailvaux and Irschick (2007), we used HL for removing effects of overall body size. This metric strongly correlated with dewlap size and bite force, and has previously proven to be most appropriate for calculating relative indexes of these two variables (Herrel and O'Reilly 2006; Vanhooydonck et al. 2005a).

We first ran a univariate general linear model (GLM) to test whether the relationship between dewlap size and bite force (independent and dependent variable, respectively) differed among our study populations. HL was then added to the model as a covariate, to assess the same effects after size correction. Both GLM analyses revealed significant dewlap size * population interaction effects on bite force, which impelled us to subsequently examine this relationship separately within populations. We therefore carried out linear regressions per population

with dewlap size as independent and bite force as dependent variable. Following Lailvaux and Irschick (2007), we obtained relative indexes by regressing dewlap size and bite force against HL and calculating the residuals for all individuals per population. We then ran a second set of linear regressions, this time with relative bite force against relative dewlap size (i.e. residuals; consistent with Vanhooydonck et al. 2005a and Lailvaux and Irschick 2007).

To test the idea that reliable information content of the dewlap in itself depends on the local intensity of intrasexual selection, we regressed the slope of the relative ‘dewlap size-bite force’ regression line for each population (i.e. coefficient b) against SSD and PTD, respectively. We here employed phylogenetic generalized least squares (pgls) regressions with incorporation of phylogenetic relationships on population level (caper package R, Orme et al. 2013; for a detailed description of the used phylogenetic tree, see ‘Phylogeny’ section in Materials and methods). This method uses maximum likelihood to simultaneously estimate the regression model and phylogenetic signal (Pagel’s λ) of the residual error (Garland and Ives 2000; Revell 2010), and has shown to do better than a priori tests of phylogenetic signal; especially when sample sizes are smaller than 20 (Blomberg et al. 2003; Revell 2010; Kamilar and Cooper 2013). Because data from one population (i.e. San Salvador) could only be collected outside the breeding season, we ran an additional set of the same pgls regression analyses excluding these particular data.

Consistent with Vanhooydonck et al. (2005a) and Lailvaux and Irschick (2007), we considered a P -value <0.05 as significant and thus, did not take a conservative approach to multiple comparisons such as Bonferroni corrections. Also, because we are concerned with the possibility of committing type II errors (see Nakagawa 2004 for more details). Statistical analyses were conducted in SPSS version 20 (SPSS, Chicago, IL, USA) and R version 3.2.1 (R Core Team 2015).

4.3 Results

Population means and standard deviations for tested variables are provided in Table 4.1. The relationship between dewlap size and bite force differed significantly among populations (dewlap size * population interaction, $F_{16,381} = 14.93$, $P < 0.0001$), also after correcting for body size (dewlap size*population interaction, $F_{16,380} = 9.36$, $P < 0.0001$). Within-population regression analyses revealed that absolute dewlap size is an excellent predictor of absolute bite force in nearly all study populations ($R > 0.65$, $P < 0.005$, Table 4.2); only for the population of Santa Clara the relationship failed to reach the conventional level of statistical significance ($R = 0.38$, $P = 0.054$, Table 4.2). However, after correcting for body size, in only four out of the seventeen tested populations, relative dewlap size still exhibited a significant positive relationship with bite force (Table 4.2, Fig. 4.1). We additionally observed that these results based on relative indexes, varied widely across populations with estimated slopes ranging from -0.353 in Little Cayman to +0.729 in South Abaco (Table 4.2). Overall, results of the population sampled outside the breeding season (i.e. San Salvador), did not deviate from the other study populations sampled during the reproductive cycle in *A. sagrei* (both absolute and relative indexes, Table 4.1-4.2, Fig. 4.1).

Phylogenetic generalized least squares (pgls) regression analyses revealed no significant association between the relative dewlap size-bite force relationship (i.e. slope coefficient b) and SSD ($R = 0.11$, $df = 16$, $P = 0.662$). Thus, in populations characterized by larger SSD, dewlap size in itself was not a more reliable signal of bite force than in populations characterized by lower SSD. The same applies to display intensity, as no significant correlation was found between the relative dewlap size-bite force relationship and PTD ($R = 0.23$, $df = 9$, $P = 0.532$). Excluding the population of San Salvador from the pgls regressions did not alter any of our results (results remained non-significant, SSD: $R = 0.12$, $df = 15$, $P = 0.657$ and PTD: $R = 0.13$, $df = 8$, $P = 0.747$).

Table 4.1 Descriptive statistics of the tested variables. Population means \pm standard deviations are presented for each population, with the exception of SSD (i.e., mean SVL males divided by mean SVL females). Sample sizes are provided between brackets for each variable separately; for SSD the number of implemented males and females is shown (left and right, respectively).

Population	HL (mm)	SVL (mm)	Dewlap size (cm ²)	Bite force (N)	SSD	PTD
Acklins	15.09 \pm 1.06 (10)	56.36 \pm 5.24 (10)	2.58 \pm 0.68 (10)	5.75 \pm 1.45 (10)	1.43 (10, 12)	–
Andros	12.81 \pm 0.87 (23)	46.37 \pm 3.25 (23)	1.21 \pm 0.33 (23)	1.90 \pm 0.51 (23)	1.23 (23, 18)	–
Cayman Brac	15.19 \pm 1.03 (28)	55.07 \pm 4.30 (28)	1.53 \pm 0.39 (28)	5.22 \pm 1.65 (28)	1.33 (28, 29)	0.01 \pm 0.03 (23)
Chub Cay	13.92 \pm 0.88 (20)	47.87 \pm 3.62 (20)	1.67 \pm 0.49 (20)	3.36 \pm 0.92 (20)	1.32 (20, 16)	–
Crooked Island	13.68 \pm 1.04 (23)	49.86 \pm 4.61 (23)	1.81 \pm 0.61 (23)	3.66 \pm 1.34 (23)	1.25 (23, 20)	–
Grand Bahama	12.82 \pm 1.43 (24)	46.78 \pm 6.34 (24)	1.59 \pm 0.41 (21)	2.26 \pm 1.39 (24)	1.33 (24, 11)	–
Grand Cayman	14.47 \pm 1.21 (27)	51.74 \pm 4.57 (27)	1.64 \pm 0.41 (27)	6.11 \pm 2.19 (27)	1.28 (27, 29)	0.07 \pm 0.11 (24)
Jamaica	13.92 \pm 1.00 (32)	48.60 \pm 3.98 (32)	1.17 \pm 0.27 (32)	6.90 \pm 2.17 (32)	1.24 (32, 23)	0.02 \pm 0.03 (22)
Little Cayman	15.17 \pm 1.06 (28)	53.46 \pm 4.35 (28)	2.00 \pm 0.56 (28)	5.22 \pm 1.57 (27)	1.29 (28, 27)	0.01 \pm 0.01 (23)
Pidgeon Cay	14.15 \pm 0.80 (16)	48.19 \pm 3.28 (16)	1.56 \pm 0.39 (16)	2.82 \pm 0.79 (16)	1.21 (16, 8)	–
San Salvador	16.27 \pm 1.52 (27)	58.13 \pm 5.85 (27)	1.96 \pm 0.75 (27)	7.99 \pm 2.24 (27)	1.35 (27, 14)	0.02 \pm 0.02 (24)
Santa Clara	15.80 \pm 0.82 (27)	55.21 \pm 2.97 (27)	2.06 \pm 0.36 (27)	7.68 \pm 1.78 (27)	1.33 (27, 24)	0.18 \pm 0.13 (24)
Soróa 1	14.84 \pm 1.35 (23)	51.10 \pm 4.44 (23)	1.91 \pm 0.45 (23)	6.63 \pm 1.94 (23)	1.24 (23, 21)	0.11 \pm 0.11 (24)
Soróa 2	15.50 \pm 1.03 (22)	55.45 \pm 4.46 (22)	2.27 \pm 0.46 (22)	7.53 \pm 2.00 (22)	1.32 (22, 24)	0.17 \pm 0.14 (30)
South Abaco	13.07 \pm 1.16 (26)	46.59 \pm 4.15 (26)	1.35 \pm 0.48 (26)	2.27 \pm 0.96 (25)	1.28 (26, 21)	0.02 \pm 0.04 (21)
South Bimini	14.91 \pm 1.38 (24)	53.66 \pm 4.60 (27)	1.62 \pm 0.45 (26)	4.04 \pm 1.13 (24)	1.30 (27, 23)	0.02 \pm 0.02 (20)
Staniel Cay	13.86 \pm 1.16 (26)	51.82 \pm 5.41 (26)	1.91 \pm 0.69 (26)	3.14 \pm 1.05 (26)	1.32 (26, 20)	–

Abbreviations: HL = head length, SVL = snout-to-vent length, SSD = sexual size dimorphism, PTD = proportion of time that individuals spent displaying.

Table 4.2 Univariate linear regression analyses of bite force (dependent variable) against dewlap size (independent variable) within population. Results are shown for regressions with absolute and relative variables, respectively.

<i>Population</i>	<i>R</i>	<i>F</i>	<i>df</i>	<i>coefficient b ± SE</i>	<i>P-value</i>
Absolute bite force against dewlap size					
Acklins	0.819	16.28	9	0.761 ± 0.189	0.004
Andros	0.806	38.81	22	0.758 ± 0.122	<0.001
Cayman Brac	0.652	19.28	27	0.792 ± 0.180	<0.001
Chub Cay	0.909	85.16	19	0.948 ± 0.102	<0.001
Crooked Island	0.810	40.09	22	0.828 ± 0.131	<0.001
Grand Bahama	0.723	20.82	20	1.503 ± 0.329	<0.001
Grand Cayman	0.784	39.87	26	1.156 ± 0.183	<0.001
Jamaica	0.740	36.31	31	0.933 ± 0.155	<0.001
Little Cayman	0.704	23.55	25	0.682 ± 0.141	<0.001
Pidgeon Cay	0.708	14.03	15	0.622 ± 0.166	0.002
San Salvador	0.904	112.0	26	0.637 ± 0.060	<0.001
Santa Clara	0.375	4.093	26	0.471 ± 0.233	0.054
Soróa 1	0.870	65.69	22	1.078 ± 0.133	<0.001
Soróa 2	0.795	34.45	21	1.254 ± 0.214	<0.001
South Abaco	0.762	31.89	24	0.936 ± 0.166	<0.001
South Bimini	0.729	23.77	22	0.670 ± 0.137	<0.001
Staniel Cay	0.799	42.48	25	0.651 ± 0.100	<0.001
Relative bite force against dewlap size					
Acklins	0.380	1.352	9	0.214 ± 0.184	0.278
Andros	0.413	4.328	22	0.420 ± 0.202	0.050
Cayman Brac	0.266	1.972	27	-0.303 ± 0.216	0.172
Chub Cay	0.490	5.679	19	0.635 ± 0.267	0.028
Crooked Island	0.108	0.249	22	0.089 ± 0.178	0.623
Grand Bahama	0.305	1.955	20	0.345 ± 0.246	0.178
Grand Cayman	0.153	0.603	26	0.185 ± 0.239	0.445
Jamaica	0.312	3.230	31	0.306 ± 0.170	0.082
Little Cayman	0.273	1.937	25	-0.353 ± 0.254	0.177
Pidgeon Cay	0.221	0.720	15	0.186 ± 0.219	0.411
San Salvador	0.166	0.707	26	0.112 ± 0.134	0.409
Santa Clara	0.212	1.177	26	0.251 ± 0.232	0.288
Soróa 1	0.623	13.36	22	0.639 ± 0.175	0.001
Soróa 2	0.335	2.523	21	0.451 ± 0.284	0.128
South Abaco	0.495	7.460	24	0.729 ± 0.267	0.012
South Bimini	0.243	1.318	22	0.301 ± 0.262	0.264
Staniel Cay	0.271	1.907	25	0.198 ± 0.144	0.180

Significant results ($P < 0.05$) are shown in bold font.

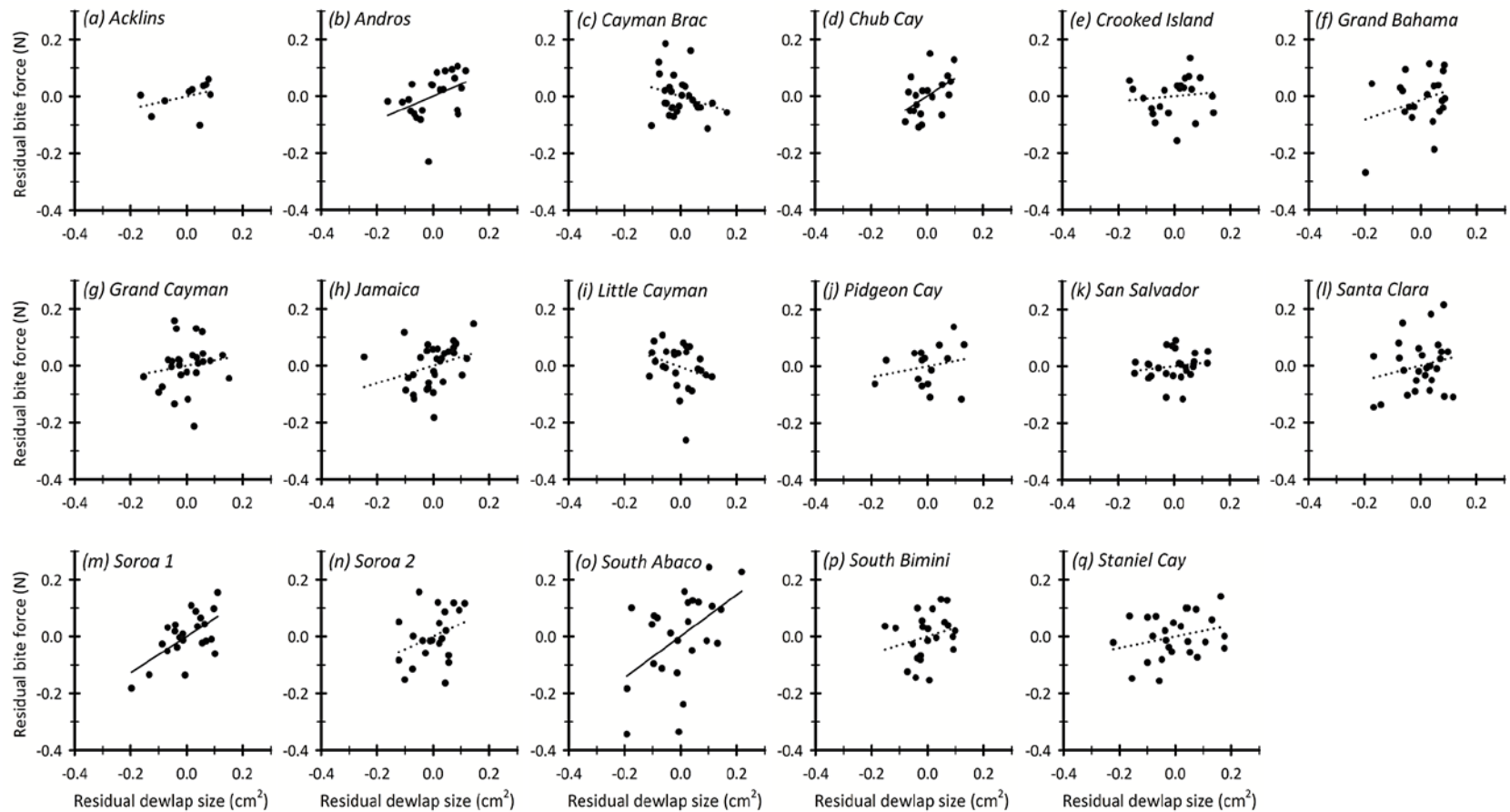


Fig. 4.1 Relative bite force regressed against relative dewlap size for each *A. sagrei* population, separately. Straight regression lines represent a significant correlation between both variables, i.e. (b) Andros, (d) Chub Cay, (m) Soroa 1 and (o) South Abaco; all P -value < 0.05. Dotted regression lines represent no significant relationship between relative dewlap size and bite force. Detailed statistics are provided in Table 4.2.

4.4 Discussion

We here present a first study on *A. sagrei* that looks into the relationship between sexual signal size and bite force on the intraspecific level, by examining a series of island populations. Following Lailvaux and Irschick (2007), we used absolute and relative indexes to assess the link between dewlap size and bite force, as both indexes can differ in the messages they convey.

4.4.1 Absolute dewlap size – bite force relationship

Our results revealed that dewlap size is an excellent predictor of bite force capacity in nearly all study populations. A strong association between absolute dewlap size and bite force in *A. sagrei* males has also been reported in all previous studies (Lailvaux and Irschick 2007; Cox et al. 2009; Driessens et al. 2015), emphasizing the generality of this finding. In many animal species, including *A. sagrei*, body size is the key predictor in determining combat outcome, with larger individuals having a substantial advantage over smaller ones (e.g. Tokarz 1985; Hughes 1996; Hardy and Briffa 2013). Gathering accurate information on the opponent's body size (assessment game) seems thus crucial to avoid costs associated with escalated fights (Andersson 1994; Maynard-Smith and Harper 2003). Yet, in reality, the accurate transmission of information is often impeded by ambient noise (e.g. precipitation, low light levels and windblown vegetation), and particularly when only one signal component is involved (e.g. Fleishman 1992; Lengagne and Slater 2002; Peters and Evans 2003; Leonard and Horn 2005). A commonly adopted signalling strategy to cope with such impeding factors, is to repeat the same message in different ways by using redundant signal components (e.g. Zuk et al. 1992, Møller and Pomiankowski 1993; Johnstone 1996). Within all our study populations, absolute dewlap size correlated strongly with overall body size and can as such, serve as a redundant signal component for body size to increase signal accuracy during the assessment game. Characterized by a brown to grayish body colour, *A. sagrei* is well camouflaged in the microhabitats it usually occupies (trunk-ground ecomorph, Schoener and Schoener 1982a; Losos 2009). In contrast, its bright yellow to reddish dewlap is

highly conspicuous, due to high colour and pattern contrasts with background vegetation (Endler 1992, 1993, 2012). Thus, by using the combination of a more cryptic body together with a conspicuous dewlap, males can transmit more accurate information on size and consequently, fighting capacity to opponents. The potential role of the *A. sagrei* dewlap as redundant signal for body size might be most prominent during the early stages of opponent assessment, when signalling still occurs over relatively long distances (more ambient noise), or perhaps during territorial advertisement in order to discourage unseen rival males from intruding (McMann 1998; Orrell and Jenssen 2003). Accordingly, Hennigson and Irschick (2012) showed in their study that surgically reducing the size of the dewlap did not change the outcome of staged close-proximity interactions between size-matched *A. carolinensis* males; bite force capacity in itself appeared to be more important in determining the outcome of these staged interactions. Based on their results, the authors suggested that dewlap size functions as a signal of bite force primarily during non-directed, long-distance territorial displays, whereas more direct means of assessing one another (e.g. jaw size, head size, body condition, push-ups) may be of higher importance during close-proximity aggressive interaction. In this respect, future behavioural experiments on *A. sagrei* testing the importance of absolute dewlap size as a redundant signal for size during long-distance versus short-distance male interactions, might be a valuable addition. Also because Tokarz et al. (2003) previously observed in the field that *A. sagrei* males with surgically impaired dewlaps could still defend their territories.

4.4.2 Relative dewlap size – bite force relationship

In addition to conveying information on body size, a sexual trait can function as direct, honest signal for advertising fighting capacity (e.g. Panhuis and Wilkinson 1999; Lailvaux et al. 2005). Evidence for a positive link between relative male dewlap size and bite force during the breeding season has been shown for several territorial anole species (Vanhooydonck et al. 2005a; Lailvaux and Irschick 2007). Surprisingly, earlier studies did not observe this correlation in the highly

territorial brown anole lizard (Lailvaux and Irschick 2007; Cox et al. 2009; Driessens et al. 2015). By examining a large set of island populations, we now also found support for a significant relationship between relative dewlap size and bite force within *A. sagrei*, though, only in four out of the seventeen tested populations. In contrast to our expectations, the degree of SSD and display intensity could not explain the observed variation in the relative dewlap size – bite force relationship found among our populations. Thus, populations where relative dewlap size appeared to be an honest signal of bite force were not per se characterized by a higher degree of intrasexual selection, which is inconsistent to earlier findings from Lailvaux and Irschick (2007) (at the species level). Standard errors of the estimated slopes for the relative dewlap size – bite force relationships fell within a relatively narrow range (0.134 – 0.284, Table 4.2), and we therefore believe that our failure to find an association between the slopes and SSD or display intensity is due to the actual among-population differences in variance. We may argue that SSD is a rather poor measure for estimating the degree of intrasexual selection, and especially for our study species. In a recent study on *A. sagrei*, for example, Bonneaud et al. (2016) reported that resource availability can highly influence the degree of SSD among insular populations distributed across the Bahamas. Furthermore, paternity studies on *A. sagrei* proved that sexual selection is not uniformly directional with respect to male size and therefore, fails to fully explain the observed male-biased SSD (Calsbeek and Sinervo 2004; Cox et al. 2007). Thus, the use of SSD here as metric for sexual selection, is disputable. Besides, display intensity may be a rather ‘gross’ proxy for the degree of intrasexual selection on each island population, because *A. sagrei* males may exhibit displays in various contexts (Driessens et al. 2014). Future studies linking the dewlap size – bite force relationship to more direct measures of intrasexual selection (e.g. operational sex ratios, amount of male-male interactions observed, duration of male-male agonistic events), can further improve our understanding.

Sexual size dimorphism and display intensity cannot explain differences in the relationship between relative dewlap size and bite force among populations, but

what other factors potentially can? One possible explanatory factor may involve intrapopulational variation in body size and the idea that relative indexes become particularly important in populations where opponents match more often in body size. Transferring information on body size is likely the first and most crucial step in the assessment game (e.g. Tokarz 1985; Hardy and Briffa 2013), as we already stated in the previous paragraph. However, when males of similar body size encounter each other, dewlap size might become the major signal for advertising fighting capacity. In support of this idea, we would expect relative dewlap size to become a more reliable signal of bite force when variation in body size decreases across populations. We could simply test this prediction with available data by regressing the slope of the relative dewlap size – bite force relationship against variance in body size across populations. Our data did not support the proposed idea (Pgl's regression: coefficient b – variance SVL, $R = 0.26$, $df = 16$, $P = 0.317$), perhaps because encounters between size-matched opponents may not occur that frequently. Moreover, previous studies have shown that when opponents are more similar in size, fights are more likely to escalate (as opposed to merely opponent assessment) and the outcomes harder to predict (Rand 1967; Molinjaborja et al. 1998; Panhuis and Wilkinson 1999). This might challenge the view that honest signals play a major role in the advertisement of fighting capacity during agonistic encounters between size-matched males.

Another factor that has recently been reported to affect the relationship between relative dewlap size and bite force, is resource availability. Particularly, Lailvaux et al. (2012) showed that under limiting resource conditions, the honest dewlap size – bite force relationship in *A. carolinensis* got disrupted. To put this idea to the test, we assessed whether variation in body condition (an estimate for resource availability) could explain the variation in the relative dewlap size – bite force relationship observed within *A. sagrei*. Indeed, we obtained a significant association with body condition (Pgl's regression: coefficient b – body mass normalized for SVL, $R = 0.62$, $df = 16$, $P = 0.009$). However, the correlation was negative and therefore, opposes the findings reported by Lailvaux et al. (2012). We found that for *A. sagrei* males, dewlap size in itself becomes a more reliable

signal of bite force in populations where males are in worse body condition (the relationship with body condition was not significant when using the absolute dewlap size - bite force relationships, $P = 0.575$). Overall, we suggest that body size remains, independent of resource availability, the key predictor during opponent assessment. Yet, when males of similar body size encounter each other, the use of dewlap size to honestly signal fighting capacity might be particularly important for *A. sagrei* males in poor body condition. We believe that males in poor body condition will suffer more from the exhaustion and injuries related to physical fights than *A. sagrei* males in normal or good body condition. Accordingly, in populations where males have a low body condition, the strong need to avoid escalated fights and thus, to precisely assess a size-matched opponent, might be higher (Andersson 1994; Maynard-Smith and Harper 2003). This may explain why dewlap size becomes a more reliable predictor of bite force in such populations. In contrast, males under high resource conditions might directly engage in physical fights when encountering a size-matched opponent (Rand 1967; Molinja-Borja et al. 1998; see also discussion on the variance in body size). Off course, future experiments are needed to confirm our suggestions and to provide additional evidence that resource availability indeed influences the correlation between relative dewlap size and bite force in *A. sagrei*. Last, several other factors have been found to explain variation only in dewlap size and can as such, also affect the relation between signal size and performance trait. For example, Vanhooydonck et al. (2009) revealed that *A. sagrei* males had relatively larger dewlaps in populations where curly-tailed lizards (*Leiocephalus carinatus*), known to predate on anoles, are present. In that same study was also reported that relative dewlap size increased with SSD. Also hormone levels (i.e. testosterone) are proven to change dewlap size in *A. sagrei* males (Cox et al. 2009) and can, due to fluctuating levels, affect the relationship between dewlap size and bite force throughout seasons. In accordance, a previous study on *A. carolinensis* has shown that dewlap size is only a reliable signal of bite force during the breeding season, and not during winter (Irschick et al. 2006). Following Lailvaux and Irschick (2007), we sampled our *A. sagrei* populations during the breeding

season, with the exception of one (i.e. population from San Salvador). Results from that latter population did not markedly deviate from the other study populations, indicating that the dewlap-bite force relationship in *A. sagrei* might not be significantly affected by season. Yet, experiments assessing the link between dewlap size and bite force in the same *A. sagrei* individuals throughout the year, are needed to accurately assess seasonal effects.

4.4.2 Conclusion

To our knowledge, this is the first study showing evidence for a link between relative dewlap size and bite force within *A. sagrei*, during the breeding season. Based on our results, we suggest that dewlap size in *A. sagrei* males is in general a redundant signal for body size in the advertisement of fighting capacity (absolute indexes), but only in particular cases a direct signal of bite force (relative indexes). Our study makes an important contribution by showing that the relationship between signal size and performance trait can differ substantially within one species. We therefore suggest that the use of only one population is not sufficient to draw general conclusions for a whole species, in this respect. Several factors (e.g. degree of territoriality, resource availability, season) are already known to affect the correlation between dewlap size and bite force, however, additional research is needed to shed more light on how these factors exactly affect this relationship.

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The Ethical committee on animal experimentation approved protocols for the use of live animals in this study (ECD 2011-64).

The authors declare that they have no conflict of interest.

CHAPTER 5

The brown anole dewlap revisited: do predation pressure, sexual selection and species recognition shape interpopulational signal diversity?

Tess Driessens, Raoul Van Damme

Animal signalling structures are amongst the most variable characteristics, as they meet a mishmash of selection pressures. A well-known example of a diverse signalling system is the dewlap of *Anolis* lizards. Dewlap characteristics can vary remarkably among and within species, and also between sexes. Although a considerable amount of studies have attempted to disentangle the staggering dewlap diversity in *Anolis*, the exact underlying evolutionary processes remain elusive. In this study, we focus on the contribution of biotic selective pressures in shaping geographic variation in dewlap design (size, colour and pattern) and dewlap use at the intraspecific level. Notably, we have tried to replicate and extend previously reported results hereof in both sexes of the brown anole lizard (*Anolis sagrei*). To do this, we used a dataset consisting of 17 *A. sagrei* island populations from the Caribbean and specifically tested whether predation pressure, sexual selection or species recognition could explain interpopulational variation in an array of dewlap characteristics. In neither males nor females estimates of predation pressure (island size, tail break frequency, model attack rate, presence of predatory *Leiocephalus* lizards) or sexual selection (sexual size dimorphism) could explain variation in dewlap design. We did find that *A. sagrei*

males from larger islands showed higher display intensities than males from smaller islands, but the direct connection with predation pressure remains ambiguous and demands further investigation. Last, we could show indirect support for species recognition only in males, as they are more likely to have a 'spotted' dewlap pattern when co-occurring with a higher number of syntopic *Anolis* species. In conclusion, we found overall limited support for the idea that the extensive interpopulational variability in dewlap design and use in *A. sagrei* is mediated by variation in their biotic environment.

Key words: *Anolis sagrei*, biotic environment, evolution, visual signalling system

5.1 Introduction

Even more than most other animal traits, signalling structures meet a difficult mishmash of selection pressures (Johnstone 1997; Smith and Harper 2003). To be effective, they have to be clear and conspicuous, often under a variety of environmental conditions (Endler 1992). A single signalling structure is often used to convey different messages to multiple receivers, and therefore must be capable of reaching several sensing systems (e.g. Loyau et al. 2005; Finkbeiner et al. 2014). At the same time, any form of transmission is prone to eavesdropping by predators or parasites, and thus signalling structures should not be *too* prominent (Roberts et al. 2001; Clark 2004; Bernal et al. 2006). Adding to the complexity, as there are often multiple ways in which the same message (e.g. good genes) can be conveyed (e.g. by an acrobatic display, a bright-red crest, a particular odour), signalling structures also seem more liable to the capriciousness of genetic drift (Richards-Zawacki et al. 2013; Clark et al. 2015) and mate choice (Møller and Pomiankowski 1993). It should not come as a surprise, then, that signalling structures are amongst the most variable animal characteristics (Zuk and Tinghitella 2008), and that understanding their evolution has proved particularly challenging.

The dewlap of *Anolis* lizards, i.e. an extendible flap of skin attached to their throat, is no exception. Both male and female anoles display this often brightly-coloured

throatfan in a variety of contexts and the resultant signal is said to function in social and sexual communication (e.g. Greenberg and Noble 1944; Jenssen 1970a; Crews 1975; Carpenter 1978), in species recognition (e.g. Rand and Williams 1970; Losos 1985) and in predator deterrence (e.g. Leal and Rodriguez-Robles 1995, 1997a, b). Dewlaps can differ greatly in size, shape, colour and patterning, among species, among populations within species, and between sexes (Nicholson et al. 2007). In 2009, Vanhooydonck and co-workers studied differences in dewlap characteristics among seven island populations of the brown anole, *Anolis sagrei*, from the Bahamas. They reported that dewlap pattern and size have evolved under different selection regimes. Notably, their data showed that diversity in dewlap pattern is best explained by the number of syntopic *Anolis* species (species recognition), whereas variation in relative dewlap size is primarily explained by the presence or absence of predatory *Leiocephalus* lizards (natural selection) and to some extent by sexual size dimorphism (sexual selection; in males only). Relative dewlap size in males and females appeared to be larger on islands where *A. sagrei* occurred in sympatry with *Leiocephalus* lizards. Based on this finding, the authors suggested that the *A. sagrei* dewlap functions as a pursuit-deterrence signal.

In this study, we have tried to replicate these results for males and females using an extended dataset. To the data from the seven Bahamian islands (i.e. Acklins, Andros, Chub Cay, Crooked Island, Grand Bahama, Pidgeon Cay, Staniel Cay) reported in Vanhooydonck et al. (2009), we added information of *A. sagrei* populations from Cayman Brac, Cuba, Grand Cayman, Jamaica, Little Cayman, San Salvador, South Abaco and South Bimini. Besides, we measured two additional dewlap characteristics (dewlap colour and use) that have been suggested to play a critical role in anole diversification and speciation (e.g. Sigmund 1983; Losos 1985; Ord et al. 2010; Macedonia et al. 2013). Using similar proxies for quantifying selective regimes as Vanhooydonck and co-workers (i.e. island size, tail break frequency, model attack rate, presence of predatory *Leiocephalus* lizards, number of syntopic *Anolis* species, sexual size dimorphism), we here test

whether interpopulational variation in *A. sagrei* dewlap characteristics (design and displays) can be explained by predation, species recognition, or sexual selection hypotheses.

5.2 Materials and methods

5.2.1 Animals

We collected data on adult *A. sagrei* lizards from nine populations in the Caribbean during the breeding seasons of 2012, 2013 and 2015 (March – September; Lee et al. 1989). Due to circumstances, data on one additional population (San Salvador) was collected outside the breeding season (January 2013). Details on the sampling locations of our populations are provided in the Supporting information (appendix, Table A.1). To measure aspects of dewlap design (see details below), we caught a total of 282 males and 245 females by noose (raw data for 143 males and 117 females were available from Vanhooydonck et al. 2009). To quantify dewlap use, we observed the behaviour of another 235 males and 189 females.

5.2.2 Dewlap measurements

Size — We used a technique outlined by Vanhooydonck et al. (2005a) to estimate the surface area of the dewlap in every lizard caught. In short, we held the lizard on its left side against a 1-cm² gridded paper and gently pulled the base of the ceratobranchial forward with a pair of forceps until the dewlap was fully extended parallel to the grid. 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 absolute dewlap area for all lizards (17 populations). To remove effects of overall size, we regressed log₁₀-transformed dewlap size against log₁₀-transformed SVL for males and females separately. The obtained unstandardized residuals of all individuals were then averaged per population and used as estimate of relative dewlap size.

Pattern — In most brown anoles, the alteration of red and yellow-coloured patches on the dewlap gives rise to a ‘pattern’ that can be categorized into three types (Nicholson et al. 2007; Driessens et al. 2015). ‘Solid’ dewlaps are uniformly coloured; ‘marginal’ dewlaps have an evenly reddish coloured centre and a yellowish margin; and ‘spotted’ dewlaps have yellowish spots scattered across the reddish centre, regardless of the presence of a margin. One of us assigned each of the 425 male and 362 female dewlaps from the 17 study populations to one of the pattern categories on the basis of high-quality digital photos. We then determined the percentage of individuals attributed for the respective categories, per sex and per population.

Colour — We measured dewlap reflectance at the centre of the dewlap, using an Avantes spectrometer (AvaSpec-2048 USB2-UA-50, range 250–1000 nm) and deuterium-halogen light source (AvaLight-DHS) equipped with a fiber-optic probe. The probe was mounted within a metal holder to ensure readings at a fixed distance from the surface and was held perpendicular to the surface of the maximally extended dewlap. All measurements were expressed in relation to a white reference tile (WS2, Avantes). Reflectance data were collected for wavelengths from 300 to 700 nm, including the lower range of photon absorption by UV-sensitive photoreceptor cones published for anoles (Fleishman et al. 1993). To investigate dewlap colour variation, we interpolated each spectrum to 1nm wavelength intervals and extracted four variables following Ng et al. (2013a): brightness, hue, relative reflectance in the UV (RF 365 nm) and in red (RF 655 nm) (Montgomerie 2006). We calculated brightness as the total area under the uncorrected spectral curve (300-700 nm) (Andersson et al. 1998; Smiseth et al. 2001). For the remaining three colour variables, we corrected each spectrum for brightness by making the area under the curve equal to 1 (Endler 1990). Hue was defined as the cut-on wavelength, i.e. the midpoint between baseline and maximum reflectance (Andersson et al. 1998; Keyser and Hill 2000; Saks et al. 2003; Cummings 2007). We decided to extract relative reflectance specifically in UV (365 nm) and red (655 nm), as the *A. sagrei* dewlap spectrum shows maxima and a high level of intraspecific variation at both wavelengths (Steffen and

McGraw 2007, personal observation). Spectral measurements were carried out for 242 males and 217 females in total, distributed across nine populations. We do not have spectral data for the seven populations sampled by Vanhooydonck et al. (2009), or for the population from Central Cuba (Santa Clara), due to technical problems with the spectrometer. All analyses of spectral data were run in R using the ‘pavo’ package (Maia et al. 2013).

Display behaviour — We observed each lizard ($N = 20$ – 30 males and $N = 8$ – 25 females per population; 10 populations in total) for 10 min, using a high-definition camera (Sony, HDR-CX260VE). We first located lizards by walking quietly through their natural habitat until an apparently undisturbed individual was spotted. We then videotaped the lizard from a distant location using the camera zoom function (30x optical zoom), to minimize any disturbance caused by our presence. Video recordings were only made during sunny or partly clouded weather and between 9:00–16:30 h to avoid possible confounding effects of weather and time on the lizard’s activity level (Huey 1982; Hertz et al. 1993). All behavioural recordings were scored offline, using JWatcher 1.0 event-recorder software (Blumstein et al. 2000). We calculated the dewlap extension (DE) rate, i.e. the number of times the dewlap was extended per minute. We then calculated the average DE rate per population. We also calculated the proportion of individuals per population that were seen dewlapping at least once (prop. DE). Calculations were performed separately for the two sexes. We combined these two measures for displaying intensity into a single measure by feeding them into a principal component analysis (PCA). The analysis produced a single component that explained $> 90\%$ of the variation in males and females, and was highly positive correlated with DE rate and prop. DE. We used this combined measure (PC dewlap display) to index the intensity of dewlap displaying behaviour in males and females of our study populations.

5.2.3 Selection indices

Predation pressure — As Vanhooydonck et al. (2009), we used island size, tail break frequency (TBF) and the presence/absence of *Leiocephalus* lizards as indices for predation pressure in the respective populations. Firstly, island size is a (very crude) estimator of predation intensity, because larger islands tend to house larger numbers of predators, like raptors and snakes (Losos and Schluter 2000; Ricklefs and Bermingham 2004). In the Bahamas, the survival rate of *Anolis sagrei* is substantially lower on larger islands with more bird species (Schoener and Schoener 1982). Information on island area was obtained from the literature (Losos et al. 1994; Vanhooydonck et al. 2009; Bradley and Rey-Millet 2013) or taken from websites (<http://islands.unep.ch/ISB.htm>; <http://www.geographia.com/bahamas/map.htm>). Secondly, we concur with Vanhooydonck et al. (2009) and many other authors (e.g. Schoener and Schoener 1980b; Turner et al. 1982; Fox et al. 1994) that tail break frequency (TBF) is a questionable measure of actual predation risk, and use it here for sake of conformity and in combination with other estimates of predation. This index was calculated as the ratio of the number of lizards with a regenerated tail to the total number of lizards captured for each population and separately per sex. Thirdly, the results of Vanhooydonck et al. (2009) suggest that the presence/absence of curly-tailed lizards (*Leiocephalus carinatus*) from their study islands in the Bahamas constitutes an important factor in the evolution of dewlap size. Curly-tailed lizards may exert their influence through competition for arthropod food or directly, by preying on anoles (Schoener et al. 1982, 2002). For the smaller islands (< 30 km²), we relied on data in the literature for deciding whether the *A. sagrei* populations were or were not syntopic with curly-tailed lizards. However, for the larger islands, we established a circle of 10 km radius (Dean et al. 2004) around our study sites and considered them ‘under *Leiocephalus* pressure’ only when a curly-tailed lizard was seen within that area by us or by consulted local herpetologists. Finally, in addition to the three indices of predation intensity used by Vanhooydonck et al. (2009), we tallied the number of clay models of *Anolis* lizards attacked by predators. This technique has been used successfully to

estimate predation rate (especially by birds) in other anole studies (e.g. Brodie 1993; Moore and Robinson 2004; Husak et al. 2006; Steffen 2009). We first constructed hundreds of models by pouring brown-coloured clay (Plastalina, Claytoon, Valencia) in a mould made from an *A. sagrei* specimen (Fig. 5.1a). On location, we placed approximately 120 clay models per sampling locality with a distance of 4 to 6 m in between. Models were randomly distributed on natural perches for *A. sagrei* lizards (trunk-ground ecomorph), including trunks, branches, stones and litter on the ground. After leaving the sampling site undisturbed for 48 hours, we recollected the clay models and scored for predator marks (i.e. clear attacks of birds, lizards and/or rodents, Fig. 5.1b). The proportion of attacked models was calculated as the number of recollected attacked models divided by the total number of recollected models, per population. Data on the proportion of attacked clay models could be collected for nine *A. sagrei* populations only.

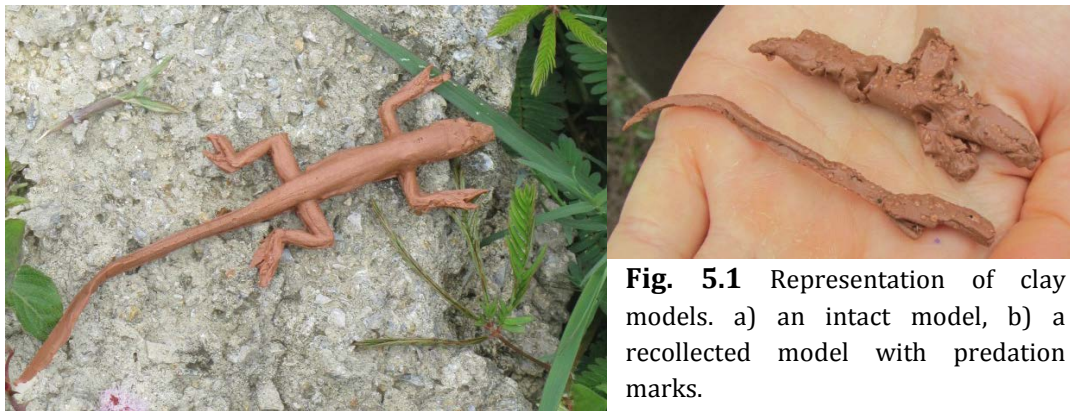


Fig. 5.1 Representation of clay models. a) an intact model, b) a recollected model with predation marks.

Sexual selection — We used sexual size dimorphism (SSD) as a proxy for the intensity of sexual selection in each of our study populations. SSD has been widely used to gauge the strength of sexual selection in lizards, and anoles in particular, because there is strong evidence that a large body size increases a male's competitiveness and thereby it's access to females (Stuart-Fox and Ord 2004; Ord and Martins 2006; and references therein). Following Vanhooydonck et al. (2009), we here defined SSD as mean SVL in males divided by mean SVL in females, per population.

Species recognition — To test the ‘species recognition’ hypothesis, we noted the presence of any other *Anolis* species within our sampling areas (Vanhooydonck et al. 2009; details on the exact sampling locations are provided in appendix, Table A.1).

5.2.4 Statistics

In this study, we considered interpopulational variation and therefore used population means and proportions of individuals per population as data points. We applied arcsine square root transformations to all proportion data (dewlap pattern, TBF, model attack rate) to meet normality assumptions (Sokal and Rohlf 1995).

Our statistical analyses differed from the ones adopted by Vanhooydonck et al. (2009) in two important ways. First, Vanhooydonck et al. (2009) adopted an information theoretic approach to compare seven plausible models of selection for dewlap size and pattern. In doing so, they tested which combination of multiple predictor variables best describes the variation in dewlap characteristics. We are reluctant to take this approach because of the limited number of data points (seven populations in their case, nine to seventeen in our extended dataset). Rather, we opted for univariate regression analyses, linking individual predictor variables to individual dewlap characteristics. This allowed us to make full use of the information available for a particular pair of predictor and response variable (not all variables could be measured in all populations). Second, in analysing the data here, we took phylogenetic relationships among the study populations into account. The phylogeny used in the comparative analyses is based on variation in mitochondrial genes (ND2 and tRNA’s) as reported in GenBank (<http://www.ncbi.nlm.nih.gov>). In particular, we used mtDNA haplotypes obtained by Kolbe et al. (2004). These authors sampled *A. sagrei* on Andros, Crooked, Grand Bahama, Grand Cayman, Little Cayman, San Salvador, Cuba, South Abaco, South Bimini and Staniel Cay and we assume that our study populations occurring on these islands had the same genetic identity. No specific

information was listed for Acklins, Chub Cay and Pidgeon Cay, and we therefore assumed that lizards of these study populations are closest related to specimens collected from the nearest islands, i.e. Crooked Island, Berry Islands and Staniel Cay, respectively (R.G. Reynolds, personal communication). Notably, for Acklins, we incorporated the genetic sequence of a specimen from Crooked Island and this specimen was different from the one we used to represent our Crooked Island study population; the same working strategy was adopted for Chub Cay and Pidgeon Cay (see appendix, Table A.1 for all details of the used *A. sagrei* specimens with corresponding GenBank accession numbers). For Jamaica, Kolbe et al. (2004) described the co-occurrence of two haplotypes and possible hybridization. We performed separate analyses with either of the haplotypes, which resulted in the same phylogenetic tree. Consequently, we selected randomly one haplotype, as a representative for our Jamaican study population.

Prior to analysis, sequences were aligned using MAFFT v7 (Kato and Standley 2013), and refined with Gblocks v0.91b (Castresana 2000; Talavera and Castresana 2007). Phylogenetic trees were built through Bayesian inference as implemented in MrBayes v3.2 (Ronquist et al. 2012); and the GTR+I+ Γ model was selected as the optimal model (jModelTest 2; Guindon and Gascuel 2003; Darriba et al. 2012). We performed 20×10^6 generation runs (Markov chain Monte Carlo), with trees sampled every 1000 generations, and a burn-in period of 4.0×10^6 generations. The Supporting information (appendix, Fig. A.1) provides a graphical representation of the obtained Bayesian tree.

Phylogenetic regression analyses were conducted using the `pgls()` function in the 'caper' package in R (Revell 2010; Orme et al. 2013). This method uses maximum likelihood to simultaneously estimate the regression model and the phylogenetic signal (Pagel's λ) of the residual error (Garland and Ives 2000; Revell 2010). Comparisons of dewlap characteristics between islands with and without *Leiocephalus* lizards were conducted using the `phylANOVA()` function in the 'phytools' package in R (Revell 2012).

Because data from one population (San Salvador) could only be obtained outside the breeding season (see materials and methods, section ‘Animals’), we have run all analyses with and without inclusion of this population. Results were nearly identical and we will therefore report results for the complete dataset only.

Raw P -values < 0.05 were corrected for multiple testing using the Benjamini-Hochberg (BH) procedure (Benjamini and Hochberg 1995). All statistical analyses were conducted in R version 3.2.1 (R Core Team 2015).

5.3 Results

The raw data on dewlap characteristics, island size, TBF, model attack rate, presence/absence of curly-tailed lizards, SSD and number of *Anolis* species can be found in Table 5.1a, b and 5.2.

None of the four measures assumed to index the intensity of predation in our study populations correlated significantly with any of the dewlap design characteristics (Table 5.3, all $P > 0.06$). Dewlap size, colour, and pattern also did not differ consistently between populations syntopic or not with the predatory curly-tailed lizard (Table 5.4, all $P > 0.13$). These results suggest that interpopulational differences in dewlap design characteristics have little to do with differences in predation intensity. We did find evidence for an effect of island size on dewlap display behaviour in males. Male anoles on larger islands scored higher on PC dewlap display, indicating that they used their dewlap more often than conspecifics on smaller islands ($P = 0.002$). All other indices for predation pressure (TBF, model attack rate, presence/absence of curly-tailed lizards) did not have a comparable effect on male display intensity (all $P > 0.15$). Neither did we find any relationship between predation intensity and female display rate (all $P > 0.20$).

In neither males nor females, differences in SSD significantly contributed to interpopulational variation in dewlap characteristics (Table 5.5; all $P > 0.13$). In populations exhibiting stronger size dimorphism, males nor females had dewlaps

that were consistently larger, brighter or of a more pure colour than in populations with limited SSD. Nor did they have dewlaps that reflected more in the UV or red region. We found also no evidence for a relationship between SSD and the proportion of different types of dewlap patterns (i.e. solid, marginal or spotted). Together, these findings strongly undermine the idea that differences in the intensity of sexual selection among the populations would contribute to among-island variation in dewlap design.

Finally, we found no significant relationship between the number of co-occurring *Anolis* species and relative dewlap size, brightness, hue, or reflectance in the UV or red regions (Table 5.6, all $P > 0.31$). We neither found an effect on dewlapping behaviour ($P > 0.58$). Interestingly, males –but not females– of populations with higher numbers of syntopic congeneric species were more likely to have a ‘spotted’ dewlap pattern ($P = 0.001$ in males).

Table 5.1a Descriptive statistics for dewlap measurements in males. Population means \pm standard deviation or proportions (prop.) are presented for each population. Sample size (N) can vary according to dewlap measurement and therefore, ranges are provided for some populations.

Population	N	Dewlap size size (cm ²)	Dewlap pattern			Dewlap colour				Dewlap display	
			prop. solid	prop. marginal	prop. spotted	brightness	hue	RF 365 nm	RF 655 nm	DE rate (counts/min)	prop. DE
<i>Males</i>											
Acklins	11	2.58 \pm 0.68	0.91	0.09	0.00	-	-	-	-	-	-
Andros	24	1.21 \pm 0.33	0.00	0.13	0.88	-	-	-	-	-	-
Cayman Brac	23-28	1.53 \pm 0.39	0.43	0.54	0.04	11353 \pm 1317	567.3 \pm 4.9	0.0015 \pm 0.0002	0.0048 \pm 0.0003	0.16 \pm 0.46	0.22
Chub Cay	23	1.67 \pm 0.49	0.00	0.04	0.96	-	-	-	-	-	-
Crooked Island	24	1.81 \pm 0.61	0.50	0.46	0.04	-	-	-	-	-	-
Grand Bahama	28	1.59 \pm 0.41	0.25	0.68	0.07	-	-	-	-	-	-
Grand Cayman	24-27	1.64 \pm 0.41	0.00	0.93	0.07	8746 \pm 1213	550.8 \pm 3.8	0.0011 \pm 0.0002	0.0053 \pm 0.0005	0.94 \pm 1.57	0.50
Jamaica	22-35	1.17 \pm 0.27	0.60	0.11	0.29	10439 \pm 1791	554.7 \pm 12.2	0.0017 \pm 0.0003	0.0043 \pm 0.0004	0.35 \pm 0.65	0.32
Little Cayman	23-29	2.00 \pm 0.56	0.45	0.52	0.03	8637 \pm 1449	555.6 \pm 8.7	0.0011 \pm 0.0001	0.0053 \pm 0.0003	0.04 \pm 0.11	0.13
Pidgeon Cay	17	1.56 \pm 0.39	0.82	0.06	0.12	-	-	-	-	-	-
San Salvador	24-27	1.96 \pm 0.75	0.59	0.41	0.00	7164 \pm 1138	558.6 \pm 7.7	0.0018 \pm 0.0002	0.0040 \pm 0.0004	0.08 \pm 0.14	0.33
Santa Clara	24-27	2.06 \pm 0.36	0.00	0.41	0.59	-	-	-	-	2.28 \pm 1.79	0.96
Soroa 1	23-24	1.91 \pm 0.45	0.00	0.75	0.25	10659 \pm 1418	550.3 \pm 5.0	0.0011 \pm 0.0001	0.0052 \pm 0.0003	2.15 \pm 2.24	0.67
Soroa 2	22-30	2.27 \pm 0.46	0.00	0.73	0.27	10006 \pm 1520	550.1 \pm 1.7	0.0011 \pm 0.0002	0.0052 \pm 0.0003	3.57 \pm 2.62	0.97
South Abaco	21-28	1.35 \pm 0.48	0.00	0.64	0.36	8374 \pm 1038	550.5 \pm 5.9	0.0015 \pm 0.0002	0.0042 \pm 0.0003	0.35 \pm 0.61	0.43
South Bimini	20-27	1.62 \pm 0.45	0.00	0.00	1.00	9081 \pm 1332	551.8 \pm 10.2	0.0016 \pm 0.0002	0.0042 \pm 0.0003	0.51 \pm 0.74	0.55
Staniel Cay	27	1.91 \pm 0.69	0.48	0.41	0.11	-	-	-	-	-	-

Abbreviations: DE = dewlap extension, '-' = missing data.

Table 5.1b Descriptive statistics for dewlap measurements in females. Population means \pm standard deviation or proportions (prop.) are presented for each population. Sample size (N) can vary according to dewlap measurement and therefore, ranges are provided for some populations.

Population	N	Dewlap size size (cm ²)	Dewlap pattern			Dewlap colour				Dewlap display	
			prop. solid	prop. marginal	prop. spotted	brightness	hue	RF 365 nm	RF 655 nm	DE rate (counts/min)	prop. DE
<i>Females</i>											
Acklins	12	0.33 \pm 0.06	1.00	0.00	0.00	-	-	-	-	-	-
Andros	19	0.25 \pm 0.05	0.21	0.68	0.11	-	-	-	-	-	-
Cayman Brac	25-29	0.28 \pm 0.03	0.38	0.62	0.00	13143 \pm 1339	563.3 \pm 4.9	0.0019 \pm 0.0002	0.0039 \pm 0.0003	0.01 \pm 0.04	0.08
Chub Cay	20	0.26 \pm 0.06	0.45	0.25	0.30	-	-	-	-	-	-
Crooked Island	18-19	0.30 \pm 0.05	0.68	0.21	0.11	-	-	-	-	-	-
Grand Bahama	23	0.21 \pm 0.04	0.22	0.78	0.00	-	-	-	-	-	-
Grand Cayman	22-29	0.30 \pm 0.05	0.00	0.86	0.14	10543 \pm 1277	547.2 \pm 3.0	0.0014 \pm 0.0002	0.0042 \pm 0.0005	0.03 \pm 0.13	0.05
Jamaica	14-15	0.27 \pm 0.04	0.64	0.36	0.00	12215 \pm 1690	557.3 \pm 18.2	0.0022 \pm 0.0003	0.0034 \pm 0.0004	0.00	0.00
Little Cayman	22-30	0.30 \pm 0.04	0.00	1.00	0.00	9821 \pm 1565	552.1 \pm 5.4	0.0014 \pm 0.0002	0.0043 \pm 0.0003	0.00	0.00
Pidgeon Cay	8	0.32 \pm 0.06	1.00	0.00	0.00	-	-	-	-	-	-
San Salvador	22-24	0.34 \pm 0.06	0.13	0.87	0.00	8929 \pm 1296	561.3 \pm 7.2	0.0016 \pm 0.0001	0.0044 \pm 0.0002	0.02 \pm 0.09	0.08
Santa Clara	15-24	0.30 \pm 0.05	0.13	0.71	0.17	-	-	-	-	0.02 \pm 0.04	0.13
Soroa 1	8-21	0.40 \pm 0.05	0.00	1.00	0.00	10386 \pm 1007	549.4 \pm 2.3	0.0013 \pm 0.0002	0.0047 \pm 0.0003	0.03 \pm 0.07	0.14
Soroa 2	17-24	0.39 \pm 0.06	0.04	0.92	0.04	10207 \pm 1294	549.6 \pm 1.9	0.0013 \pm 0.0001	0.0048 \pm 0.0003	0.1 \pm 0.24	0.18
South Abaco	20-25	0.28 \pm 0.05	0.00	0.40	0.60	11549 \pm 1887	548.2 \pm 8.8	0.0017 \pm 0.0002	0.0037 \pm 0.0003	0.08 \pm 0.15	0.25
South Bimini	22-24	0.37 \pm 0.05	0.46	0.21	0.33	10856 \pm 1267	547.0 \pm 8.6	0.0017 \pm 0.0002	0.0039 \pm 0.0003	0.00	0.00
Staniel Cay	20	0.26 \pm 0.06	0.81	0.19	0.00	-	-	-	-	-	-

Abbreviations: DE = dewlap extension, '-' = missing data.

Table 5.2 Data on selection indices: island size, tail break frequency (TBF; for males and females, respectively), proportion of attacked clay models, presence/absence of *Leiocephalus carinatus* lizards (0 = absent, 1 = present), sexual size dimorphism (SSD) and total number of co-occurring *Anolis* species (Nr. *Anolis* species); ‘-’ represents missing data. Sample sizes (*N*) used to calculate TBF and SSD are provided in Table 5.1a, b.

Population	Island size (km^2)	TBF	Model attack rate	<i>L. cari- inatus</i>	SSD	Nr. <i>Anolis</i> species
Acklins	310.8	0.55, 0.09	-	1	1.43	2
Andros	5957	0.50, 0.45	-	0	1.23	4
Cayman Brac	38	0.29, 0.10	0.008	1	1.33	2
Chub Cay	15.76	0.35, 0.35	-	1	1.32	4
Crooked Island	238.28	0.46, 0.48	-	1	1.25	2
Grand Bahama	1373	0.56, 0.38	-	1	1.33	3
Grand Cayman	197	0.26, 0.10	0.070	1	1.28	2
Jamaica	10911	0.29, 0.29	0.073	0	1.24	3
Little Cayman	28	0.59, 0.43	0.034	1	1.29	2
Pidgeon Cay	0.019	0.47, 0.25	-	0	1.21	2
San Salvador	163	0.41, 0.48	0.067	1	1.35	2
Santa Clara	105006	0.67, 0.58	0.020	0	1.33	2
Soroa 1	105006	0.42, 0.38	-	0	1.24	3
Soroa 2	105006	0.50, 0.46	0.019	0	1.32	3
South Abaco	1145.9	0.30, 0.29	0.008	1	1.28	2
South Bimini	10.36	0.44, 0.36	0.000	1	1.30	4
Staniel Cay	5.18	0.37, 0.33	-	0	1.32	3

Details about the used variables can be found in the ‘Materials and methods’ section.

Table 5.3 Predation pressure: univariate pglS regression analyses of dewlap design and display versus estimates of predation intensity.

Dewlap variables	Island size				Tail break frequency (TBF)			Model attack rate			
	<i>N</i>	<i>b</i>	<i>SE</i>	<i>P-value</i>	<i>b</i>	<i>SE</i>	<i>P-value</i>	<i>N</i>	<i>b</i>	<i>SE</i>	<i>P-value</i>
<i>Males</i>											
relative dewlap size	17	3.7×10 ⁻⁷	4.2×10 ⁻⁷	0.392	0.241	0.131	0.086	9	-0.038	0.237	0.877
pattern - solid	17	-1.8×10 ⁻⁶	2.6×10 ⁻⁶	0.498	0.757	0.569	0.204	9	1.684	1.009	0.139
pattern - marginal	17	5.5×10 ⁻⁸	2.5×10 ⁻⁶	0.982	-0.587	0.525	0.281	9	1.475	1.411	0.331
pattern - spotted	17	1.5×10 ⁻⁶	2.4×10 ⁻⁶	0.558	-0.104	0.539	0.850	9	-3.006	1.183	0.351
colour - brightness	9	1.4×10 ⁻²	9.9×10 ⁻³	0.197	-2405	4044	0.571	8	-3709	4880	0.476
colour - hue	9	-5.0×10 ⁻⁵	4.2×10 ⁻⁵	0.275	-14.88	12.08	0.257	8	-2.11	22.03	0.927
colour - RF365nm	9	-3.6×10 ⁻⁹	2.1×10 ⁻⁹	0.135	-7.3×10 ⁻⁴	6.0×10 ⁻⁴	0.261	8	-1.3×10 ⁻⁴	1.2×10 ⁻³	0.917
colour - RF655nm	9	2.2×10 ⁻⁹	3.9×10 ⁻⁹	0.592	9.6×10 ⁻⁴	1.1×10 ⁻³	0.409	8	1.1×10 ⁻³	1.8×10 ⁻³	0.571
PC dewlap display	10	1.8×10⁻⁵	2.9×10⁻⁶	0.002*	2.587	1.656	0.157	9	-2.203	3.360	0.533
<i>Females</i>											
relative dewlap size	17	4.7×10 ⁻⁷	2.8×10 ⁻⁷	0.114	-0.002	0.073	0.980	9	-0.254	0.153	0.140
pattern - solid	17	-4.8×10 ⁻⁶	3.0×10 ⁻⁶	0.131	-0.946	0.548	0.105	9	-0.506	1.381	0.725
pattern - marginal	17	5.4 ×10 ⁻⁶	2.7×10 ⁻⁶	0.062	1.135	0.461	0.176	9	1.693	1.251	0.218
pattern - spotted	17	-1.9×10 ⁻⁷	1.8×10 ⁻⁶	0.916	0.253	0.437	0.571	9	-1.689	0.967	0.124
colour - brightness	9	6.1×10 ⁻³	1.0×10 ⁻²	0.577	-5349	2112	0.176	8	-4188	5199	0.451
colour - hue	9	-3.9×10 ⁻⁵	4.9×10 ⁻⁵	0.455	-3.61	13.18	0.792	8	18.18	25.05	0.495
colour - RF365nm	9	-3.6×10 ⁻⁹	2.0×10 ⁻⁹	0.115	-6.7×10 ⁻⁴	4.1×10 ⁻⁴	0.146	8	-5.1×10 ⁻⁵	1.1×10 ⁻³	0.965
colour - RF655nm	9	7.1×10 ⁻⁹	2.8×10 ⁻⁹	0.236	1.2×10 ⁻³	6.6×10 ⁻⁴	0.120	8	2.2×10 ⁻⁴	1.8×10 ⁻³	0.907
PC dewlap display	10	8.8×10 ⁻⁶	6.4×10 ⁻⁶	0.204	0.846	1.955	0.676	9	-2.285	4.065	0.591

Results are shown separately per sex; *b* indicates the regression coefficient and *SE*, its standard error. Significant results (BH-corrected *P*-values) are in bolt and marked with an asterisk. See 'statistical analyses' section for more details.

Table 5.4 Predation pressure: phylogenetic analysis of variance incorporating dewlap design and use versus presence or absence of predatory curly-tailed lizards.

Dewlap variables	<i>L. carinatus</i>		
	<i>N</i>	<i>F</i>	<i>P-value</i>
<i>Males</i>			
relative dewlap size	17	0.678	0.461
pattern - solid	17	0.686	0.457
pattern - marginal	17	0.004	0.961
pattern - spotted	17	0.779	0.458
colour - brightness	9	0.422	0.575
colour - hue	9	3.545	0.135
colour - RF365nm	9	2.979	0.137
colour - RF655nm	9	3.088	0.147
PC dewlap display	10	2.277	0.210
<i>Females</i>			
relative dewlap size	17	0.018	0.920
pattern - solid	17	0.004	0.950
pattern - marginal	17	0.106	0.746
pattern - spotted	17	0.635	0.487
colour - brightness	9	0.322	0.633
colour - hue	9	3.421	0.113
colour - RF365nm	9	0.924	0.399
colour - RF655nm	9	0.459	0.555
PC dewlap display	10	0.040	0.877

Results are shown separately per sex. No significant *P-values* were obtained.

Table 5.5 Sexual selection: univariate ppls analyses of dewlap design and display versus sexual size dimorphism (SSD).

Dewlap variables	Sexual size dimorphism (SSD)			
	<i>N</i>	<i>b</i>	<i>SE</i>	<i>P-value</i>
<i>Males</i>				
relative dewlap size	17	-0.014	0.323	0.966
pattern - solid	17	0.937	1.178	0.439
pattern - marginal	17	-0.250	1.094	0.822
pattern - spotted	17	-0.984	1.035	0.357
colour - brightness	9	-10549	12179	0.415
colour - hue	9	67.45	39.45	0.131
colour - RF365nm	9	0.001	0.002	0.701
colour - RF655nm	9	-0.001	0.003	0.741
PC dewlap display	10	8.327	5.290	0.154
<i>Females</i>				
relative dewlap size	17	-0.026	0.221	0.907
pattern - solid	17	1.354	1.641	0.422
pattern - marginal	17	-0.677	1.696	0.696
pattern - spotted	17	-0.447	1.324	0.740
colour - brightness	9	-6202	12159	0.626
colour - hue	9	65.08	49.10	0.227
colour - RF365nm	9	-0.001	0.002	0.751
colour - RF655nm	9	0.003	0.003	0.401
PC dewlap display	10	3.462	8.755	0.703

Results are shown separately per sex; *b* indicates the regression coefficient and *SE*, its standard error. For sexual selection, no significant *P-values* were obtained.

Table 5.6 Species recognition: univariate ppls regression analyses of dewlap design and display versus total number of co-occurring *Anolis* species.

Dewlap variables	Nr. <i>Anolis</i> species			
	<i>N</i>	<i>b</i>	<i>SE</i>	<i>P-value</i>
<i>Males</i>				
relative dewlap size	17	0.011	0.022	0.632
pattern - solid	17	-0.081	0.134	0.554
pattern - marginal	17	-0.168	0.116	0.168
pattern - spotted	17	0.471	0.094	0.001*
colour - brightness	9	564.9	652.9	0.416
colour - hue	9	-2.552	2.617	0.362
colour - RF365nm	9	1.1×10^{-4}	1.4×10^{-4}	0.473
colour - RF655nm	9	-2.2×10^{-4}	2.4×10^{-4}	0.388
PC dewlap display	10	0.243	0.421	0.580
<i>Females</i>				
relative dewlap size	17	0.015	0.015	0.349
pattern - solid	17	0.076	0.185	0.687
pattern - marginal	17	0.089	0.171	0.609
pattern - spotted	17	0.084	0.089	0.362
colour - brightness	9	63.93	664.9	0.926
colour - hue	9	-3.250	3.000	0.314
colour - RF365nm	9	7.9×10^{-5}	1.4×10^{-4}	0.595
colour - RF655nm	9	-6.0×10^{-5}	2.4×10^{-4}	0.811
PC dewlap display	10	-0.257	0.486	0.612

Results are shown separately per sex; *b* indicates the regression coefficient and *SE*, its standard error. Significant results (BH-corrected *P-value*) are in bolt and marked with an asterisk.

5.4 Discussion

Overall, we found limited support for the idea that the extensive among-population variability in dewlap characteristics in *A. sagrei* is mediated by variation in their biotic environment. Sexual size dimorphism, an index of sexual selection, varied considerably among our study populations, but did not correlate with any of the structural aspects of the dewlap considered, or with the intensity of displaying behaviour. Of the four proxies we used to assess relative predation intensity, none explained differences in dewlap design, and only one (island size) was associated with increased dewlap use. Our results do corroborate the hypothesis that the complexity of dewlap patterning functions in species recognition – at least in males. The relative size or colour characteristics of the dewlap, however, did not change consistently with the number of co-occurring congeneric species.

Vanhooydonck et al. (2009), using information on a subset of the populations studied here, concluded that predation pressure, especially the presence of predatory *L. carinatus* lizards, plays a significant role in the evolution of relative dewlap size. They argued that anoles may evolve larger dewlaps when in syntopy with these saurophagous lizards, because large size would benefit the pursuit deterrence function of the dewlap. The predator deterrence hypothesis holds that prey perform eye-catching displays to warn the predator that its presence has been detected and that pursuits are likely to be futile or even dangerous (Hasson 1991). It is a well-established fact that many lizard species will indeed engage in conspicuous displaying behaviour when confronted with a predator (reviewed in Greene 1988). However, in most cases, these displays involve tail vibrations, curling or waving, rather than dewlap extensions (Dial 1986; Hasson et al. 1989; Cooper 2001, 2007, 2010, 2011; Telemeco et al. 2011; York and Baird 2016), and they are more likely intended to deflect the predator's attack towards less vulnerable, expendable body parts, rather than to discourage pursuit. Leal and Rodriguez-Robles (1995, 1997a, b) have argued that dewlapping in *A. cristatellus* and *A. cuvieri* may act as a pursuit deterrence signal, but the evidence is weak. In

laboratory conditions, specimens of *A. cristatellus* were reported to dewlap ‘only rarely’ when a life native snake predator was introduced into their cage (Leal and Rodríguez-Robles 1995); in the field, specimens of the same species did not extend their dewlaps more often when a snake model was moved into their territory (Leal and Rodríguez 1997a). In their paper on *A. cuvieri*, Leal and Rodríguez-Robles (1997b) reported dewlap extensions in response to a snake model in only one individual out of a total of five. Moreover, several recent studies on *A. sagrei* found no evidence for increased dewlap extension rates in response to predatory birds (Simon 2007; Elmasri et al. 2011), snakes (Yee et al. 2013) or curly-tailed lizards (Driessens et al. 2014; Steinberg et al. 2014). For these reasons, we are sceptical about the role of the dewlap as a pursuit-deterrent and hence, about predation pressure as a driver for dewlap size evolution. Accordingly, our analyses show very little evidence for a link between the used indices of predation pressure and dewlap size – or any other structural aspect of the dewlap. We are fully aware of the difficulty of measuring predation pressure. Each of the methods we employed has been criticized. Firstly, tail break frequencies may reflect predator inefficiency, rather than intensity (Schoener and Schoener 1980b; Turner et al. 1982; Jaksic and Greene 1984; Fox et al. 1994). Secondly, stationary clay models do not adequately mimic natural organisms with respect to traits such as odour, anti-predator postures, movement et cetera (Rangen et al. 2000; Thompson and Burhans 2004; Cooper et al. 2008; Santos and Canatella 2011; Paluh et al. 2014). Moreover, our model attack rates estimated especially predation by birds, but other predators like snakes and lizards can impose high predation threats as well (e.g. Schoener et al. 1982; Henderson and Crother 1989; Rodríguez-Robles 1992; Rodríguez-Cabrera et al. 2016). Thirdly, island size and the presence/absence of *L. carinatus* can be considered as very crude estimates of predator pressure – at the best. Lastly, prompting even more caution, none of the four respective indices of predation intensity used in this study varied in concert (correlation analyses, all $R < 0.22$ and all $P > 0.24$). Perhaps the number of predatory species present on each island may provide more accurate information on the role of predation pressure in shaping dewlap

design. Yet, greater species richness does not necessarily translate into higher predation rates as each predatory species may be less abundant or may include anoles as a smaller part of the diet (Losos 2009). Combining the total number of predatory species with measures of their abundance and diet composition might be most appropriate, but is hardly feasible in the field. Admittedly, an accurate quantification of predation pressure in the field is very challenging. Our findings that predation pressure does not contribute to the evolution of dewlap design in the brown anole lizard remains therefore highly tentative and demands further research.

For dewlap displays, we did find that *A. sagrei* males from larger islands showed higher display intensities than males from smaller islands. Yet, part of this relationship might be driven by the fact that the three populations sampled on Cuba (three times the same 'large' value for island size) exhibited high display rates. Besides, it is highly questionable whether the positive correlation between island size and male display rate is truly because larger islands harbour more predators. Island size is known to influence many ecologically relevant variables (e.g. habitat complexity, community richness; Ricklefs and Lovette 1999; Losos and Schluter 2000) that were not considered here. Future studies are required to clarify and interpret our result showing that males use dewlaps more on larger islands (i.e. non-independent island size datapoints from Cuba, island size as accurate index of predation pressure).

The model that best explained the variation in male relative dewlap size in Vanhooydonck et al.'s dataset also contained sexual selection (SSD) as a predictor variable: on islands with high SSD (assumed to reflect high intensity of sexual selection), males tended to have larger dewlaps. However, in our extended dataset, we found no indication that differences in SSD among islands are reflected in relative dewlap size, or any other dewlap characteristic. We can think of three ways to explain this result. First, sexual selection is simply not acting on dewlap traits. This sounds highly improbable, because the dewlap has all the characteristics of a sexually secondary trait: it is highly dimorphic in adult

individuals, and exhibits the typical developmental pattern with sex-specific growth trajectories once the age of maturity is reached (Vanhooydonck et al. 2015). Several studies have shown that male brown anoles use their dewlap during territorial disputes (Scott 1984) and/or during courtship (Simon 2011; Driessens et al. 2014). At least in males, aspects of the dewlap reveal information on the individual signalling that is highly relevant in a sexual selection context (Driessens et al. 2015). Second, the effect of differences in the intensity of sexual selection among populations may be offset or overruled by some other factor. Natural selection may be counterbalancing or constraining any effects that divergent sexual selection is having on the among-island variation in dewlap characteristics (but see above). Dewlap traits may also be under differential selection for reasons not considered here. For instance, the climatic conditions and structural habitats in which our study populations live vary considerably. Physical aspects of the environment have been shown to influence the effectiveness of visual signals to a great extent in several animal species (Hebets and Papaj 2005; Stuart-Fox et al. 2007), including anoles (Leal and Fleishman 2004; Ord et al. 2007; Ng et al. 2013a). We will investigate the possibility that the among-population differences in dewlap size and other dewlap characteristics in brown anoles result from adaptation to features of the physical environment in a separate study. A third explanation for the apparent lack of a relationship between sexual selection and dewlap design, is methodological: SSD may simply not be a good proxy for the intensity of sexual selection. Although male-biased sexual size dimorphism in lizards is traditionally linked to intrasexual selection (Trivers 1976; Stamps 1983, 1999; Stamps et al. 1997), evolutionary shifts in male aggression, territoriality and (relative) home range size explain but a small proportion of evolutionary changes in SSD (Cox et al. 2003), suggesting that other factors may be at play. Recently, Bonneaud et al. (2016) presented evidence that sex-specific developmental plasticity may contribute to adult SSD in brown anoles: on six Bahamian islands, the amount of food biomass explained variation in male, but not female body size, giving rise to significant differences in SSD.

Clearly, future studies should invest in collecting information on more reliable estimates of the intensity of sexual selection.

Our analyses lend partial support to the finding that dewlap ‘patterning’ may play a role in species recognition. Male brown anoles are more likely to have a ‘spotted’ dewlap when co-occurring with several other *Anolis* species. Whereas Vanhooydonck et al. (2009) found this to be true in both sexes, the effect was only significant in males in our extended dataset. Perhaps males that can broadcast their species identity properly are less likely to be attacked by non-conspecific males. Indeed, male anoles can behave very aggressively towards conspecific males, while at the same time ignoring males of other *Anolis* species (Losos 1985). Females, on the other hand, may communicate their species identity in some other way (perhaps via head bobbing patterns).

Rand and Williams (1970) coined the species recognition hypothesis for explaining dewlap diversity in *Anolis* five decades ago. Subsequent behavioural studies on pairs of anole species have offered further support to the hypothesis (e.g. Losos 1985; Macedonia and Stamps 1994; Macedonia et al. 2013), but a broad-scale, phylogenetically-informed analysis could not provide statistical corroboration (Nicholson et al. 2007). The brown anole seems an interesting study animal to deeper explore the possibility of sexual character displacement, but this will require detailed data on the signals of the co-occurring species and behavioural experiments.

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CHAPTER 6

Climate-related variation in a visual signalling device: the male and female dewlap in *Anolis sagrei* lizards.

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Animals communicate using a variety of signals that differ dramatically among and within species. The astonishing dewlap diversity in Anoles has attracted considerable attention in this respect. Yet, the evolutionary processes behind it remain elusive and have mostly been explored for males only. Here, we considered *Anolis sagrei* males and females to study signal divergence among populations. First, we assessed the degree of variation in dewlap design (size, pattern, colour) and displays by comparing 17 populations distributed across the Caribbean. Second, we assessed whether the observed dewlap diversity is associated to variation in climate-related environmental conditions. Results showed that populations differed in all dewlap characteristics, with the exception of display rate in females. We further found that males and females occurring in 'xeric' environments had a higher proportion of solid dewlaps with higher UV-reflectance. Besides, lizards inhabiting 'mesic' environments had primarily marginal dewlaps showing high reflectance in red. For dewlap display, a correlation with environment was only observed in males. Our study provides evidence for a strong relationship between signal design and prevailing environmental conditions, which may result from differential selection on signal

efficacy. Moreover, our study highlights the importance of including females when studying dewlaps in an evolutionary context.

Key-words: brown anole, environmental conditions, geographic variation, local adaptation, signal diversity, throat fan

6.1 Introduction

Animals communicate to one another using a wide variety of signals that can differ dramatically among and even within species. A thorough understanding of the evolutionary processes giving rise to this signal diversity seems crucial, as it provides insights into how communication can contribute to population differentiation and speciation events (Boughman 2002; Smith and Harper 2003; Rundle and Nosil 2005). In the absence of long-term time series, within-species geographical variation provides one of the best tools in studying factors that shape the evolution of communication signals (Wilczynski and Ryan 1999). Differences in signalling systems among populations can arise through random genetic drift (Campbell et al. 2010), as a by-product of selection on other characteristics (e.g. Nevo and Capranica 1985; O'Neill and Beard 2011), or by direct differential selection. Populations may evolve alternative signals in the presence/absence of players advertising through the same sensory channel ('reproductive character displacement', e.g. Hobel and Gerhardt 2003), or when subject to different numbers of eavesdropping predators (Brandley et al. 2013). Sexual selection will have a diversifying effect on signal design if the criteria used in mate choice or for establishing male dominance relationships vary among populations. When signal expression is under Fisherian sexual selection, geographic variation can be expected to be quite erratic, with females preferring a particular design in one population and another in the next (Fisher 1958; Wilczynski and Ryan 1999). If the signal is reflecting 'good genes', differences among populations may still arise when factors that influence fitness vary geographically (Endler 1993a), or when the same message of quality can be conveyed in multiple ways. Finally, there is growing evidence that the effectiveness with which messages can be transmitted between the sender and

the receiver depends on climatic conditions (e.g. Möller 2011; Snell-Rood 2012; Martín and López 2013) and habitat characteristics (e.g. Boughman 2002; Seehausen et al. 2008). Therefore, local variation in these ecological factors may produce concordant changes in signal design.

With nearly 400 species, *Anolis* constitutes an extremely diverse lizard genus that is distributed throughout the Neotropics (i.e. Caribbean and mainland Americas) and often used as model system for studying ecology and evolutionary biology (Losos 2009). For communicating, anoles typically use an extendable flap of skin attached to the throat, called 'dewlap' (Jenssen 1977; Carpenter 1978). Although the exact functions are not fully comprehended, this visual signalling device is primarily used in a context of mating and territorial defence (Greenberg and Noble 1944; Jenssen 1970a; Crews 1975; Sigmund 1983; Jenssen et al. 2000), in species recognition (Rand and Williams 1970; Losos 1985) and even, in predator deterrence (Leal and Rodriguez-Robles 1995, 1997a). Anole species can differ dramatically in dewlap design and display behaviour, and therefore, these elements have frequently been used to delineate species boundaries (Schwartz and Henderson 1991 and references therein; Nicholson et al. 2007). Besides, dewlap characteristics can also show considerable variation among, and even within populations (Vanhooydonck et al. 2009). In attempt to disentangle the astonishing dewlap diversity and its role in speciation, anole researchers have proposed several non-mutually exclusive mechanisms including sexual selection (e.g., Greenberg and Noble 1944; Crews 1975; Sigmund 1983; Harrison and Poe 2012), species recognition (e.g., Rand and Williams 1970; Webster and Burns 1973; Losos 1985; Macedonia and Stamps 1994; Nicholson et al. 2007), predation pressure (Leal and Rodriguez-Robles 1997a; Vanhooydonck et al. 2009) and sensory drive (e.g., Leal and Fleishman 2002, 2004; Ord et al. 2010; Ord et al. 2011). Many of these authors have suggested that dewlap colour and display repertoire, in particular, may have played a critical role in anole speciation.

For several reasons, we are particularly interested in how interpopulational variation in dewlap design and display relates to climate and associated

environmental conditions, in both sexes of *Anolis sagrei*. First, interpopulational differences provide an excellent framework for investigating incipient stages of speciation (Foster et al. 1998). Examining them may thus help identifying the mechanisms leading to signal divergence and possibly speciation, even for entire radiations. Second, *Anolis sagrei* seems a highly appropriate species to examine dewlap diversity. In contrast to most anole species, which exhibit little intraspecific variation, *A. sagrei* is reported to show considerable interpopulational variation in several aspects of dewlap design (Vanhooydonck et al. 2009). Dewlap colour can for example, range from dull or bright ultraviolet (UV)-yellow to deep red, and the dewlap can additionally exhibit a variety of colour patterns. Both sexes of *A. sagrei* possess a dewlap, but the structure is more elaborated in males, i.e. larger and more conspicuously coloured dewlaps as well as higher display rates in comparison to females (Jenssen et al. 2000; Vanhooydonck et al. 2009; Steffen and McGraw 2009; Partan et al. 2011; Driessens et al. 2014; Driessens et al. 2015). Evolutionary processes driving divergence in dewlap design and display might therefore be sex-specific. In a previous study on *A. sagrei*, Vanhooydonck et al. (2009) have indeed shown that sexual selection acts differently on dewlap size in both sexes, whereas predation pressure and species recognition drive dewlap size and pattern, respectively, in the same direction. Unfortunately, research on anoline dewlap diversity has almost exclusively been conducted in males (e.g., Echelle et al. 1978; Losos and Chu 1998; Nicholson et al. 2007). Incorporating data on female dewlap design and display behaviour seems thus highly necessary, also because we already know that *A. sagrei* females use their dewlap (Driessens et al. 2014). Third, to our knowledge, the contribution of climate-related environmental conditions in explaining dewlap divergence has never been investigated within *A. sagrei*. The species is distributed across a large geographical area, characterized by a variety of environments, which provides an excellent framework for testing. Moreover, two anole studies addressing interpopulational signal divergence in relation to environmental heterogeneity revealed partially discordant results (Leal and Fleishman 2004; Ng et al. 2013a). Leal and Fleishman (2004) reported that *Anolis*

crisatellus populations occupying dark signalling environments (i.e. mesic forest) exhibited brighter and more UV-reflecting dewlaps than populations inhabiting brighter signalling environments (i.e. xeric forests), which were shown to have relatively dull dewlaps with low UV-reflectance and red appearance. The same authors additionally proved that the *A. crisatellus* populations were more detectable in their respective natural habitat. Instead, Ng et al. (2013a) found that *Anolis distichus* populations occurring in mesic habitats exhibited less bright and more orange dewlaps in comparison to populations occupying drier environments, which were characterized by brighter and more yellow dewlaps. They also noted that dewlaps were more orange in cooler environments with more tree cover. Testing associations between signal design and prevailing environmental conditions within additional anole species can improve our understanding of the directions in which dewlap colours may adaptively diverge.

In this study, we have two main objectives. First, we aim to document the occurrence and degree of variation in dewlap characteristics among *A. sagrei* populations distributed across islands in the Caribbean. Second, we assess whether the observed interpopulational variation in dewlap design and display behaviour correlates with variation in prevailing environmental conditions, studied on a macro-ecological scale. To do this, we linked dewlap data (i.e. size, pattern, colour and display) to climatic parameters (i.e. temperature, precipitation, incoming radiation and vegetation cover) from 17 *A. sagrei* island populations, while accounting for phylogenetic relationships. In complement to previous studies, we did not exclusively test males, but considered females too. We hypothesize differences in prevailing environmental conditions to be important in shaping dewlap diversity among populations for *A. sagrei* males, and potentially also for *A. sagrei* females.

6.2 Materials and methods

6.2.1 Animals

For this study, we used data of 17 *Anolis sagrei* populations distributed across the Caribbean (Fig. 6.1 and 6.3b). We incorporated data from seven populations in the Bahamas (Acklins, Andros, Chub Cay, Crooked Island, Grand Bahama, Pidgeon Cay and Staniel Cay; April-May 2003) published by Vanhooydonck et al. (2009). An additional ten populations were sampled on Jamaica (March 2012), Cuba (Santa Clara, Soroa 1, Soroa 2; April-May 2012), San Salvador (January 2013), Cayman Islands (Cayman Brac, Grand Cayman, Little Cayman, March 2013), South Abaco and South Bimini (March 2015). All data were collected during the breeding season (March - September, Lee et al. 1989), with exception of one population (i.e. San Salvador). A total of 425 male and 362 female individuals were caught by noose and kept individually in plastic bags for maximum 48h before releasing back at the location of capture. We measured snout-vent length (SVL) with callipers (Mitutoyo CD-15DC, accuracy 0.01 mm) and quantified dewlap design (i.e., size, pattern and colour) for each captured lizard. Of the latter ten populations, another 235 male and 189 female *A. sagrei* individuals were filmed to quantify dewlap use.

6.2.2 Dewlap

Size

We positioned each lizard on its left side against a 1 cm² grid and gently pulled the base of the ceratobranchial forward with a pair of forceps until the dewlap was fully extended parallel to the grid (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). We corrected for body size by regressing the log₁₀-transformed SVL against log₁₀-transformed dewlap size for males and females

separately. The obtained residuals were subsequently used as estimates of relative dewlap size.

Pattern

One or two distinct colours, i.e. red and yellow create a degree of patterning in the dewlap of *A. sagrei* that can vary among individuals. According to well-defined descriptions of dewlap pattern categories (Nicholson et al. 2007), we assigned dewlaps to one of the following pattern categories: solid (uniformly coloured, Fig. 6.2a), marginal (uniformly reddish-coloured centre with yellowish margin, Fig. 6.2b) or spotted (yellowish spots across the reddish centre, regardless of the presence of a margin, Fig. 6.2c). The same observer (T.D.) who had prior experience with classifying *A. sagrei* dewlap patterns (Driessens et al. 2015), assigned a total of 425 male and 362 female dewlaps (17 study populations) to one of the given pattern categories based on high-quality digital photos. The proportion of individuals classified under each of the three pattern categories was then calculated for every population.

Colour

We measured dewlap reflectance using an Avantes spectrometer (AvaSpec-2048 USB2-UA-50, range 250–1000 nm) and deuterium-halogen light source (AvaLight-DHS) equipped with a fibre-optic probe. The probe was mounted within a metal holder to ensure readings at a fixed distance from the surface and was held perpendicular to the surface of the maximally extended dewlap. All measurements were expressed in relation to a white reference tile (WS2, Avantes). For both males and females, spectral reflectance was measured at the dewlap centre region (more details are provided in Driessens et al. 2015). Reflectance data were collected for wavelengths from 300 to 700 nm, including the lower range of photon absorption by UV-sensitive photoreceptor cones published for anoles (Fleishman et al. 1993). To investigate dewlap colour variation, we interpolated each spectrum to 1nm wavelength intervals and extracted four variables following Ng et al. (2013a): brightness, hue, reflectance in

the UV (RF 365 nm) and in red (RF 655 nm) (Montgomerie 2006). We calculated brightness as the total area under the uncorrected spectral curve (300-700 nm) (Andersson et al. 1998; Smiseth et al. 2001). For the remaining three colour variables (i.e. hue, RF365 nm and RF 655 nm), we corrected each spectrum for brightness by making the area under the curve equal to 1 in order to allow the identification of differences in spectral shape independent of brightness (Endler 1990). Hue was defined as the cut-on wavelength, i.e. the midpoint between baseline and maximum reflectance (Andersson et al. 1998; Keyser and Hill 2000; Saks et al. 2003; Cummings 2007). We decided to extract reflectance specifically in UV (365 nm) and red (655 nm), as the *A. sagrei* dewlap spectrum shows maxima and a high level of intraspecific variation at both wavelengths (Steffen and McGraw 2007, Driessens et al. 2015). The spectral measurements were done for 242 males and 217 females in total, distributed across nine populations. We do not have spectral data for the seven populations sampled by Vanhooydonck et al. (2009), or for the population from Central Cuba (Santa Clara) due to technical problems with the spectrometer. All analyses of spectral data were run in R using the ‘pavo’ package (Maia et al. 2013).

Display

We observed each lizard ($N = 20-30$ males and $N = 8-25$ females per population; 10 populations in total) for 10 min, using a high-definition camera (Sony, HDR-CX260VE). We first located lizards by walking quietly through their natural habitat until an apparently undisturbed individual was spotted. We then videotaped the lizard from a distant location using the camera zoom function (30x optical zoom), to minimize any disturbance caused by our presence. Video recordings were only made during sunny or partly clouded weather and between 9:00 - 16:30 h, to avoid possible confounding effects of weather and time on the lizard’s activity level (Huey 1982; Hertz et al. 1993). All behavioural recordings were scored offline, using JWatcher 1.0 event-recorder software (Blumstein et al. 2000). To quantify dewlap display, we calculated the dewlap extension (DE) rate, expressed in counts per minute; one DE was defined as the complete extension

and retraction of the throat fan. At the population level, we defined ‘prop. DE’ as the proportion of individuals that extended their dewlap at least once while they were filmed.



Fig. 6.1 Brown anole (*Anolis sagrei*) from Cuba. (a) Displaying male and (b) male and female (left and right, respectively) *Anolis* basking in the sun. Photographs by Steven De Decker (April 2012).

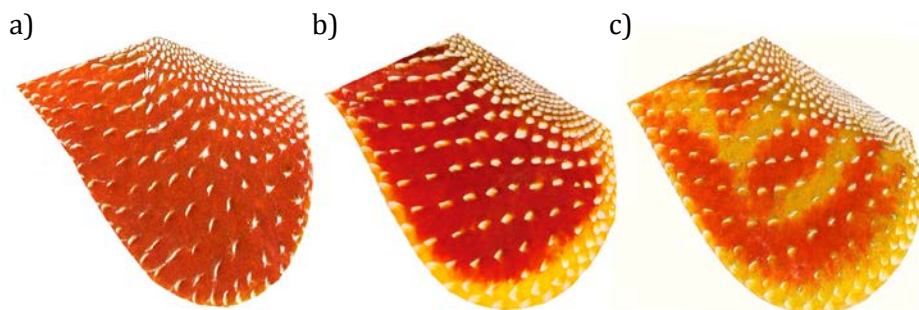


Fig. 6.2 Dewlap pattern categories used in this study, for both sexes of *A. sagrei*. (a) Solid, uniformly coloured (b) marginal, uniformly coloured centre with yellow margin and (c) spotted, yellow spots across a red centre regardless of the presence of a margin.

6.2.3 *Climate-related environmental conditions*

For each population, a set of environmental parameters was obtained from both climate (WorldClim, <http://www.worldclim.org>, Hijmans et al. 2005; Meteorological Archival and Retrieval System, <https://software.ecmwf.int>) and Moderate Resolution Imaging Spectroradiometer (NASA-MODIS/Terra data set, <http://modis.gsfc.nasa.gov>) databases. Consistent with Ng et al. (2013a), we extracted annual precipitation (BIO12) and land surface temperature (LST) (MODIS MOD11A2 product). We additionally obtained annual global incoming shortwave radiation data (MARS) and the normalized difference vegetation index (NDVI) (MODIS MOD13Q1 product), which is the most frequently used remotely sensed indicator of vegetation and land cover changes (Rouse et al. 1974). By using the ‘Global Subsetting Tool’ (available online at http://daacmodis.ornl.gov/cgi-bin/MODIS/GLBVIZ_1_Glb/modis_subset_order_global_col5.pl), MOD13Q1 provided 16-day NDVI time series at a 250-m spatial resolution and we extracted from this dataset a four-by-four pixel area centred at the sampling locality. The described environmental parameters (with exception of LST) can alter both the ambient light environment and background vegetation in a lizard’s habitat; two factors that are known to strongly influence signal visibility (Endler 1993a). Besides, the brown anole lizard is a territorial species, often moving and spending time in a variety of structural microhabitats within its home range (Schoener and Schoener 1982a; McMann 1998). Based on the above arguments, we believe that the set of environmental parameters extracted for each population (spatial resolution of ca. 1 km²) is reliable for estimating the prevailing environmental conditions in which *A. sagrei* populations occur (consistent to Ng et al. 2013a, using *A. distichus*).

6.2.4 *Phylogeny*

To account for the non-independency of our datapoints (population means), we conducted our analyses in an explicit phylogenetic context (Felsenstein 1985; Harvey and Pagel 1991). We constructed a hypothesis on the phylogenetic relationships among our study populations, based on mitochondrial DNA genes

(ND2 and tRNA's) available in GenBank (<http://www.ncbi.nlm.nih.gov>). In particular, we used mtDNA haplotypes obtained by Kolbe et al. (2004), as they were previously proven effective in assessing interpopulational relationships in *A. sagrei*. The islands, Andros, Crooked, Grand Bahama, Grand Cayman, Little Cayman, San Salvador, Cuba, South Abaco, South Bimini and Staniel Cay have been sampled by Kolbe et al. (2004), and we assumed that our study populations occurring on these islands had the same genetic identity. No specific information was listed for Acklins, Chub Cay and Pidgeon Cay, and we therefore assumed that lizards of these study populations are closest related to specimens collected from the nearest islands, i.e. Crooked Island, Berry Islands and Staniel Cay, respectively (R.G. Reynolds, personal communication). Notably, for Acklins, we incorporated the genetic sequence of a specimen from Crooked Island and this specimen was different from the one we used to represent our Crooked Island study population; the same working strategy was adopted for Chub Cay and Pidgeon Cay (see appendix, Table A.1 for all details of the used *A. sagrei* specimens with corresponding GenBank accession numbers). On Jamaica, Kolbe et al. (2004) described the co-occurrence of two haplotypes and possible hybridization. We performed separate analyses with either of the haplotypes, which resulted in the same phylogenetic tree. Consequently, we selected randomly one haplotype, as a representative for our Jamaican study population.

Prior to analysis, sequences were aligned using MAFFT v7 (Kato and Standley 2013), and refined with Gblocks v0.91b (Castresana 2000; Talavera and Castresana 2007). Phylogenetic trees were built through Bayesian inference as implemented in MrBayes v3.2 (Ronquist et al. 2012); and the GTR+I+ Γ model was selected as the optimal model (jModelTest 2; Guindon and Gascuel 2003; Darriba et al. 2012). We performed 20×10^6 generation runs (Markov chain Monte Carlo), with trees sampled every 1000 generations, and a burn-in period of 4.0×10^6 generations. A representation of the obtained Bayesian tree together with our sampling localities is provided in figure 6.3a, b.

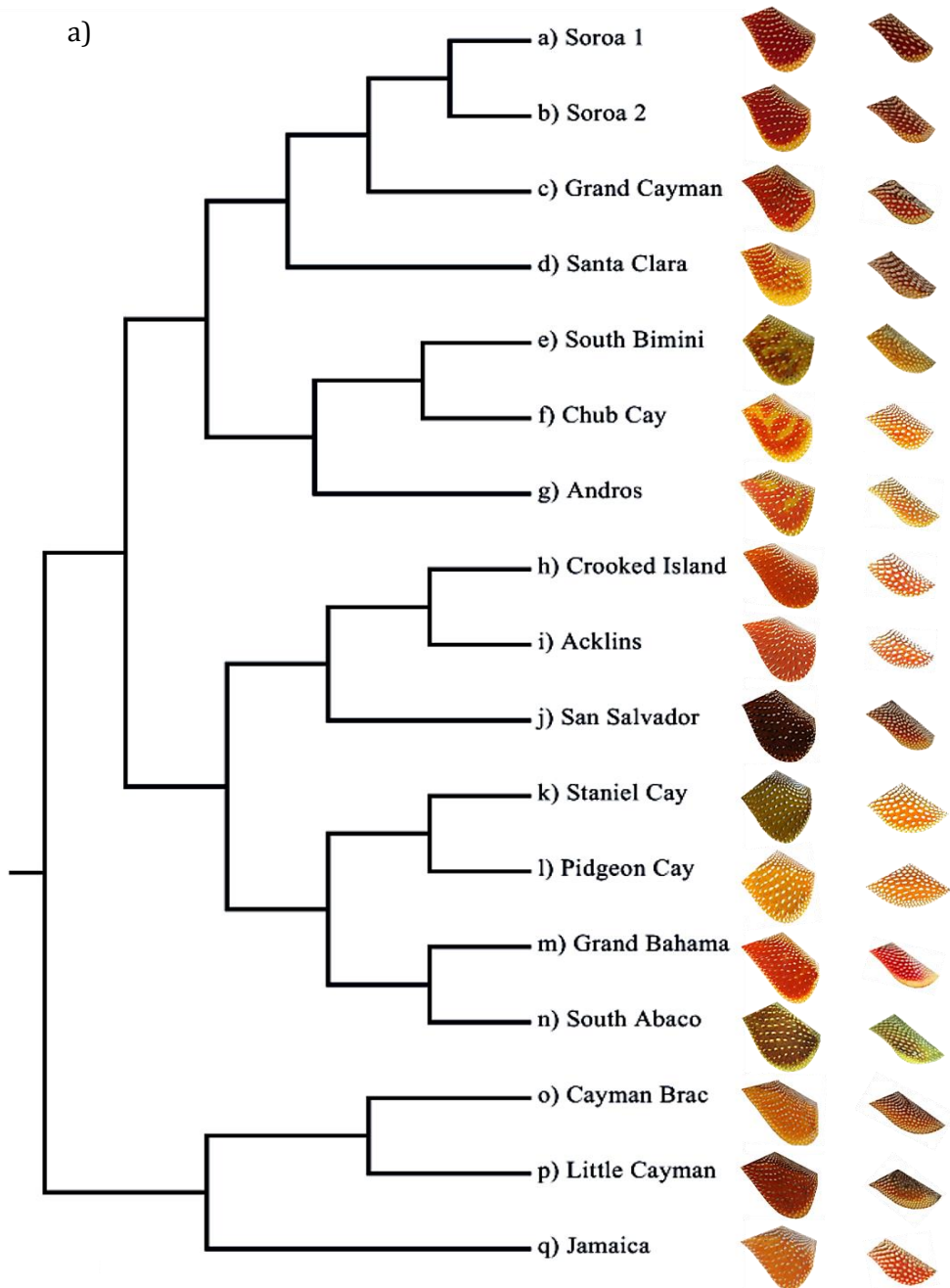


Fig. 6.3a Phylogenetic relationships among our *A. sagrei* study populations. The Bayesian tree is based on mtDNA haplotypes from Kolbe et al. (2004); details on the specimens incorporated and the methods used to construct this phylogenetic tree are provided in the Materials and methods section (6.2.4 Phylogeny). Photos represent male (left) and female (right) dewlaps of typical individuals from every population. The shown dewlap pattern corresponds with the patterning occurring in the largest proportion of individuals for a given population. Photos were scaled differently (especially female dewlaps) and therefore provide no information about dewlap size.

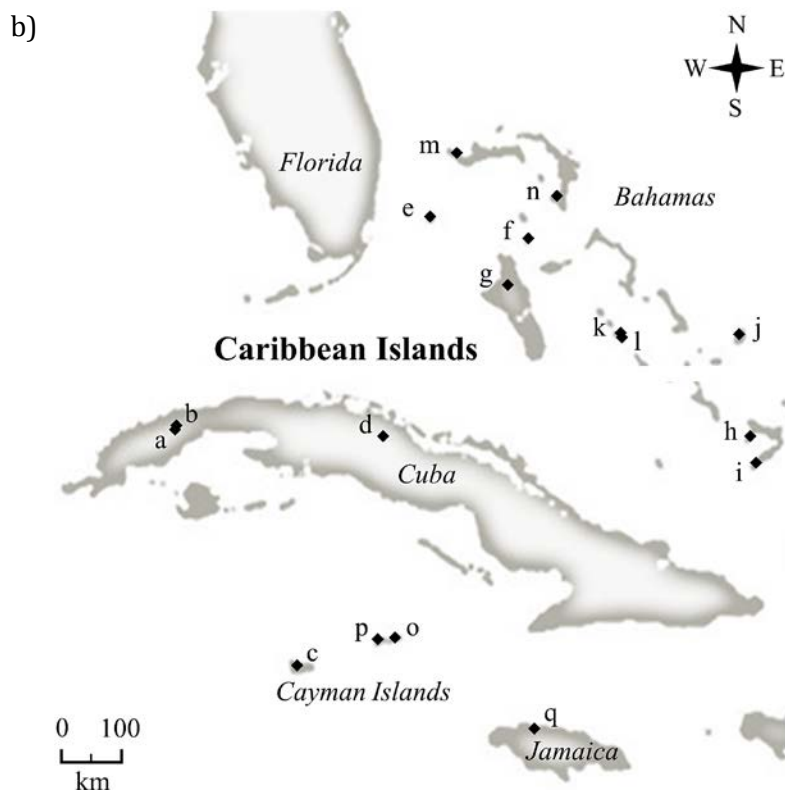


Fig. 6.3b Geographical locations of our *A. sagrei* populations distributed across the Caribbean. Letters on the map refer to the respective study populations presented in the phylogenetic tree (Fig. 6.3a). Exact coordinates of the sampling localities can be found in the Supporting information (appendix, Table A.1).

6.2.5 Statistics

All statistical analyses were run separately for males and females, due to a high degree of sexual dimorphism detected in most dewlap measurements.

To test for interpopulational differences in dewlap design, we ran separate one-way analyses of variance (ANOVAs), including population as factor; when the assumption of homogeneity of variances was not met, the Welch test was used instead. For dewlap pattern and display, Kruskal-Wallis tests were applied to deal with ordinal and not normally distributed variables, respectively.

For all further statistical analyses, we only considered interpopulational variation and therefore used population means and proportions of individuals per population as data points. We applied an arcsine square root transformation to

the proportion data (dewlap pattern) to meet normality assumptions (Sokal and Rohlf 1995). To reduce dimensionality and get rid of redundancy problems, we applied separate principal component analyses (PCAs) for dewlap display and the environmental parameters. We first assessed the validity of PCAs on our datasets by performing Bartlett tests of sphericity and the Kaiser-Meyer-Olkin Measure of Sampling Adequacy (MSA) (Bartlett 1950, 1954; Kaiser 1970; Dziuban and Shirkey 1974). Only PC axes with eigenvalues greater than one were interpreted and used in subsequent regression analyses (i.e. PC dewlap display and PC environment).

To test whether variation in climate-related environmental conditions (i.e. PC environment) might explain interpopulational differences in dewlap design and display, we ran a series of univariate regression analyses incorporating population means and phylogenetic relationships. Specifically, we applied the phylogenetic generalized least squares (PGLS) method described in Revell (2010) and implemented by the `pgls()` function in R (caper package, Orme et al. 2013). This method uses maximum likelihood to simultaneously estimate the regression model and phylogenetic signal (i.e. Pagel's λ) of the residual error (Garland and Ives 2000; Revell 2010). It has been shown to do better than a priori tests of phylogenetic signal to estimate the appropriateness of phylogenetically corrected tests; especially when sample sizes are smaller than 20 (Blomberg et al. 2003; Revell 2010; Kamilar and Cooper 2013). Because data from one population (San Salvador) could only be obtained outside the breeding season, we performed an additional set of the same regression analyses excluding these particular data. *P*-values resulting from the phylogenetic regression analyses were corrected for multiple testing using the Benjamini-Hochberg procedure, which is described as a powerful method based on adjustment of the false discovery rate (Benjamini and Hochberg 1995).

Statistical analyses were conducted in SPSS version 20 (SPSS, Chicago, IL, USA) and R version 3.2.1 (R Core Team 2015).

6.3 Results

6.3.1 *Interpopulational variation in dewlap design and display*

For males, we found substantial differences among populations for each dewlap characteristic we measured (ANOVAs or Welch tests: relative dewlap size $F_{16,140.29} = 18.55$, $N = 425$; brightness $F_{8,233} = 24.46$, $N = 242$; hue $F_{8,94.53} = 40.10$, $N = 242$; RF 365 nm $F_{8,95.14} = 92.22$, $N = 242$; RF 655 nm $F_{8,95.18} = 71.80$, $N = 242$, all $P < 0.001$; Kruskal-Wallis test: dewlap pattern $\chi^2(16) = 227.92$, $N = 428$; DE rate $\chi^2(9) = 108.89$, $N = 235$, all $P < 0.001$). Similar results were obtained for females with the exception of DE rate that did not differ among populations (ANOVAs or Welch tests: relative dewlap size $F_{16,112.17} = 20.41$, $N = 361$; brightness $F_{8,208} = 19.90$, $N = 217$; hue $F_{8,79.40} = 36.18$, $N = 217$; RF 365 nm $F_{8,208} = 47.69$, $N = 217$; RF 655 nm $F_{8,81.15} = 47.70$, $N = 217$, all $P < 0.000$; Kruskal-Wallis test: dewlap pattern $\chi^2(16) = 140.35$, $N = 364$, $P < 0.000$; DE rate $\chi^2(9) = 15.73$, $N = 189$, $P = 0.073$). Population means and standard deviations for all tested dewlap variables are provided in Table 6.1a, b.

6.3.2 *PCA analyses and interpretations*

Bartlett tests of sphericity and MSA yielded appropriate values, allowing us to perform valid PCAs on the population means. For dewlap display, PCAs were run separately for males and females incorporating mean DE rate and proportions of individuals showing DE (prop. DE). We obtained one significant axis per sex (PC dewlap display, males: eigenvalue = 1.92; females: eigenvalue = 1.83) explaining 95.90 % of the variation in males and 91.71 % in females. Higher values of PC dewlap display correspond in both sexes to more individuals showing DEs and a higher DE rate, which can be generally considered as more dewlap use (Table 6.2). For the PCA incorporating mean annual precipitation, radiation, NDVI and LST, also one PC-axis was obtained (PC environment, eigenvalue = 2.85), explaining 64.57 % of the variation. High values of PC environment correspond to relatively dark, dense green habitats with more rainfall (hereafter referred to as 'mesic' environments), whereas low values are

consistent with more open, less green sites characterized by a higher incoming radiation ('xeric' environments) (Table 6.2).

6.3.3 Dewlap design and display linked to climate-related environmental conditions

Excluding San Salvador from the ppls regressions did not alter our obtained results for males, and the presented results will therefore always include this particular population (Table 6.3). For females, however, results from both regression sets are reported because data of San Salvador influenced the results for some variables substantially (Table 6.3). No associations were found between relative dewlap size and PC environment in males, nor in females ($P > 0.4$, Table 6.3). For dewlap pattern, we found PC environment to be a major predictor for the proportion of individuals with a solid or marginal, but not with a spotted dewlap (Table 6.3). Our results for dewlap pattern variation were similar for both sexes and showed that for populations with a low PC environment value (i.e. xeric environments), a higher proportion of solid dewlaps were present. In contrast, populations characterized by high PC environment values (i.e. mesic environments) contained a higher proportion of individuals with marginal dewlap patterns (Table 6.3). We are aware of a possible constant sum issue with respect to the proportional dewlap pattern variables, and its implications for interpreting our results. We therefore report that no negative correlation between the proportion of individuals with solid and marginal dewlap pattern was obtained for the male sex (Spearman's correlation: $\rho = -0.34$, $P = 0.186$, $N = 17$). In females, however, a highly significant negative correlation was seen (Spearman's correlation: $\rho = -0.88$, $P = 0.000$, $N = 17$), presumably because of the low abundance of the spotted dewlap pattern in most of the study populations (Table 6.1b).

Dewlap colour was highly correlated with prevailing environmental conditions in males. In females, similar results were obtained as for males, only after excluding data of San Salvador (Table 6.3). Colour results specifically revealed a negative link between PC environment and the relative reflectance at UV (RF 365 nm)

(Fig. 6.4a, b). In addition, dewlaps from mesic environments (high values for PC environment) reflected more in red (RF 655 nm) (Fig. 6.4c, d). It is, however, important to note that RF 365 nm and RF 655 nm were strongly negatively correlated, in each sex (Pearson correlation: males, $r = -0.96$, $P < 0.001$, $N = 9$ and females, $r = -0.91$, $P < 0.001$, $N = 9$). Surprisingly, no significant relationships were found between environmental conditions and the other components of dewlap colouration, including brightness and hue (Table 6.3).

Lastly, we found that male lizards from populations with higher PC environment values use their dewlaps more frequently (Table 6.3). No such correlation between dewlap display and environment was seen in females.

Running standard regression analyses without the incorporation of phylogenetic relationships revealed similar results.

Table 6.1a Descriptive statistics for dewlap measurements in males. Population means \pm standard deviation or proportions (prop.) are displayed for each population. Sample size (N) can vary according to dewlap measurement and therefore, ranges are provided for some populations.

Population	N	Dewlap size size (cm ²)	Dewlap pattern			Dewlap colour				Dewlap display	
			prop. solid	prop. marginal	prop. spotted	brightness	hue	RF 365 nm	RF 655 nm	DE rate (counts/min)	prop. DE
<i>Males</i>											
Acklins	11	2.58 \pm 0.68	0.91	0.09	0.00	-	-	-	-	-	-
Andros	24	1.21 \pm 0.33	0.00	0.13	0.88	-	-	-	-	-	-
Cayman Brac	23-28	1.53 \pm 0.39	0.43	0.54	0.04	11353 \pm 1317	567.3 \pm 4.9	0.0015 \pm 0.0002	0.0048 \pm 0.0003	0.16 \pm 0.46	0.22
Chub Cay	23	1.67 \pm 0.49	0.00	0.04	0.96	-	-	-	-	-	-
Crooked Island	24	1.81 \pm 0.61	0.50	0.46	0.04	-	-	-	-	-	-
Grand Bahama	28	1.59 \pm 0.41	0.25	0.68	0.07	-	-	-	-	-	-
Grand Cayman	24-27	1.64 \pm 0.41	0.00	0.93	0.07	8746 \pm 1213	550.8 \pm 3.8	0.0011 \pm 0.0002	0.0053 \pm 0.0005	0.94 \pm 1.57	0.50
Jamaica	22-35	1.17 \pm 0.27	0.60	0.11	0.29	10439 \pm 1791	554.7 \pm 12.2	0.0017 \pm 0.0003	0.0043 \pm 0.0004	0.35 \pm 0.65	0.32
Little Cayman	23-29	2.00 \pm 0.56	0.45	0.52	0.03	8637 \pm 1449	555.6 \pm 8.7	0.0011 \pm 0.0001	0.0053 \pm 0.0003	0.04 \pm 0.11	0.13
Pidgeon Cay	17	1.56 \pm 0.39	0.82	0.06	0.12	-	-	-	-	-	-
San Salvador	24-27	1.96 \pm 0.75	0.59	0.41	0.00	7164 \pm 1138	558.6 \pm 7.7	0.0018 \pm 0.0002	0.0040 \pm 0.0004	0.08 \pm 0.14	0.33
Santa Clara	24-27	2.06 \pm 0.36	0.00	0.41	0.59	-	-	-	-	2.28 \pm 1.79	0.96
Soroa 1	23-24	1.91 \pm 0.45	0.00	0.75	0.25	10659 \pm 1418	550.3 \pm 5.0	0.0011 \pm 0.0001	0.0052 \pm 0.0003	2.15 \pm 2.24	0.67
Soroa 2	22-30	2.27 \pm 0.46	0.00	0.73	0.27	10006 \pm 1520	550.1 \pm 1.7	0.0011 \pm 0.0002	0.0052 \pm 0.0003	3.57 \pm 2.62	0.97
South Abaco	21-28	1.35 \pm 0.48	0.00	0.64	0.36	8374 \pm 1038	550.5 \pm 5.9	0.0015 \pm 0.0002	0.0042 \pm 0.0003	0.35 \pm 0.61	0.43
South Bimini	20-27	1.62 \pm 0.45	0.00	0.00	1.00	9081 \pm 1332	551.8 \pm 10.2	0.0016 \pm 0.0002	0.0042 \pm 0.0003	0.51 \pm 0.74	0.55
Staniel Cay	27	1.91 \pm 0.69	0.48	0.41	0.11	-	-	-	-	-	-

Abbreviations: DE = dewlap extension, '-' = missing data; more details about the variables can be found in the 'Materials and methods' section.

Table 6.1b Descriptive statistics for dewlap measurements in females. Population means \pm standard deviation or proportions (prop.) are displayed for each population. Sample size (N) can vary according to dewlap measurement and therefore, ranges are provided for some populations.

Population	N	Dewlap size size (cm ²)	Dewlap pattern			Dewlap colour				Dewlap display	
			prop. solid	prop. marginal	prop. spotted	brightness	hue	RF 365 nm	RF 655 nm	DE rate (counts/min)	prop. DE
<i>Females</i>											
Acklins	12	0.33 \pm 0.06	1.00	0.00	0.00	-	-	-	-	-	-
Andros	19	0.25 \pm 0.05	0.21	0.68	0.11	-	-	-	-	-	-
Cayman Brac	25-29	0.28 \pm 0.03	0.38	0.62	0.00	13143 \pm 1339	563.3 \pm 4.9	0.0019 \pm 0.0002	0.0039 \pm 0.0003	0.01 \pm 0.04	0.08
Chub Cay	20	0.26 \pm 0.06	0.45	0.25	0.30	-	-	-	-	-	-
Crooked Island	18-19	0.30 \pm 0.05	0.68	0.21	0.11	-	-	-	-	-	-
Grand Bahama	23	0.21 \pm 0.04	0.22	0.78	0.00	-	-	-	-	-	-
Grand Cayman	22-29	0.30 \pm 0.05	0.00	0.86	0.14	10543 \pm 1277	547.2 \pm 3.0	0.0014 \pm 0.0002	0.0042 \pm 0.0005	0.03 \pm 0.13	0.05
Jamaica	14-15	0.27 \pm 0.04	0.64	0.36	0.00	12215 \pm 1690	557.3 \pm 18.2	0.0022 \pm 0.0003	0.0034 \pm 0.0004	0.00	0.00
Little Cayman	22-30	0.30 \pm 0.04	0.00	1.00	0.00	9821 \pm 1565	552.1 \pm 5.4	0.0014 \pm 0.0002	0.0043 \pm 0.0003	0.00	0.00
Pidgeon Cay	8	0.32 \pm 0.06	1.00	0.00	0.00	-	-	-	-	-	-
San Salvador	22-24	0.34 \pm 0.06	0.13	0.87	0.00	8929 \pm 1296	561.3 \pm 7.2	0.0016 \pm 0.0001	0.0044 \pm 0.0002	0.02 \pm 0.09	0.08
Santa Clara	15-24	0.30 \pm 0.05	0.13	0.71	0.17	-	-	-	-	0.02 \pm 0.04	0.13
Soroa 1	8-21	0.40 \pm 0.05	0.00	1.00	0.00	10386 \pm 1007	549.4 \pm 2.3	0.0013 \pm 0.0002	0.0047 \pm 0.0003	0.03 \pm 0.07	0.14
Soroa 2	17-24	0.39 \pm 0.06	0.04	0.92	0.04	10207 \pm 1294	549.6 \pm 1.9	0.0013 \pm 0.0001	0.0048 \pm 0.0003	0.1 \pm 0.24	0.18
South Abaco	20-25	0.28 \pm 0.05	0.00	0.40	0.60	11549 \pm 1887	548.2 \pm 8.8	0.0017 \pm 0.0002	0.0037 \pm 0.0003	0.08 \pm 0.15	0.25
South Bimini	22-24	0.37 \pm 0.05	0.46	0.21	0.33	10856 \pm 1267	547.0 \pm 8.6	0.0017 \pm 0.0002	0.0039 \pm 0.0003	0.00	0.00
Staniel Cay	20	0.26 \pm 0.06	0.81	0.19	0.00	-	-	-	-	-	-

Abbreviations: DE = dewlap extension, '-' = missing data; more details about the variables can be found in the 'Materials and methods' section.

Table 6.2 Loadings of principal component analyses (PCAs) on dewlap display and climate-related environmental parameters with eigenvalues and variance (%).

Variables	PC (males)	PC (females)
<i>Dewlap display</i> ($N = 10$)		
Prop. DE	0.979	0.958
DE rate	0.979	0.958
Eigenvalue	1.92	1.83
Percentage variation	95.89	91.71
<i>Environment</i> ($N = 17$)		
Annual precipitation		0.957
Annual radiation		-0.902
Annual NDVI		0.730
Annual LST		0.565
Eigenvalue		2.58
Percentage variation		64.57

Mean population values were always included in the PCAs with exception of the proportion of individuals showing a dewlap extension (Prop. DE). PCAs were run separately per sex for dewlap display. For environment, one PCA was performed and the same PC values were used in both sexes. Abbreviations: DE = dewlap extension, NDVI = normalized difference vegetation index, LST = land surface temperature.

Table 6.3 Univariate phylogenetic linear regression analyses (pgls) of dewlap design and display versus prevailing environmental conditions.

Dewlap variables	PC environment				
	<i>N</i>	<i>b</i>	SE	<i>P</i> -value	<i>r</i> ²
<i>Males</i>					
relative dewlap size	17	0.017	0.018	0.408	0.055
dewlap pattern - solid	17	-0.320	0.087	0.011	0.471
dewlap pattern - marginal	17	0.287	0.090	0.014	0.402
dewlap pattern - spotted	17	0.012	0.116	0.921	0.001
dewlap colour - brightness	9	574.01	491.68	0.362	0.163
dewlap colour - hue	9	-2.515	2.072	0.362	0.174
dewlap colour - RF 365 nm	9	-2.97 × 10⁻⁴	4.79 × 10⁻⁵	0.004	0.846
dewlap colour - RF 655 nm	9	5.14 × 10⁻⁴	1.23 × 10⁻⁴	0.012	0.714
PC dewlap display	10	0.750	0.238	0.024	0.554
<i>Females</i>					
<i>(including San Salvador)</i>					
relative dewlap size	17	-0.002	0.013	0.929	0.002
dewlap pattern - solid	17	-0.381	0.088	0.005	0.555
dewlap pattern - marginal	17	0.338	0.086	0.006	0.505
dewlap pattern - spotted	17	0.059	0.071	0.533	0.045
dewlap colour - brightness	9	-47.14	513.27	0.929	0.001
dewlap colour - hue	9	-3.915	2.018	0.195	0.350
dewlap colour - RF 365 nm	9	-2.00 × 10⁻⁴	8.64 × 10⁻⁵	0.143	0.451
dewlap colour - RF 655 nm	9	2.83 × 10⁻⁴	1.54 × 10⁻⁴	0.195	0.326
PC dewlap display	10	0.352	0.334	0.483	0.122
<i>Females</i>					
<i>(excluding San Salvador)</i>					
relative dewlap size	16	-0.005	0.0148	0.733	0.009
dewlap pattern - solid	16	-0.454	0.078	<0.001	0.707
dewlap pattern - marginal	16	0.444	0.079	<0.001	0.692
dewlap pattern - spotted	16	0.045	0.077	0.638	0.024
dewlap colour - brightness	8	-811.03	472.47	0.246	0.329
dewlap colour - hue	8	-1.758	2.845	0.638	0.060
dewlap colour - RF 365 nm	8	-3.35 × 10⁻⁴	8.01 × 10⁻⁵	0.013	0.745
dewlap colour - RF 655 nm	8	5.41 × 10⁻⁴	1.18 × 10⁻⁴	0.011	0.777
PC dewlap display	9	0.504	0.431	0.42	0.164

Results are shown separately per sex. For females, we additionally provided results from regression analyses excluding the population of San Salvador. *P*-values were corrected for multiple testing using the Benjamini-Hochberg method and significant results ($P < 0.05$) are shown in bold font.

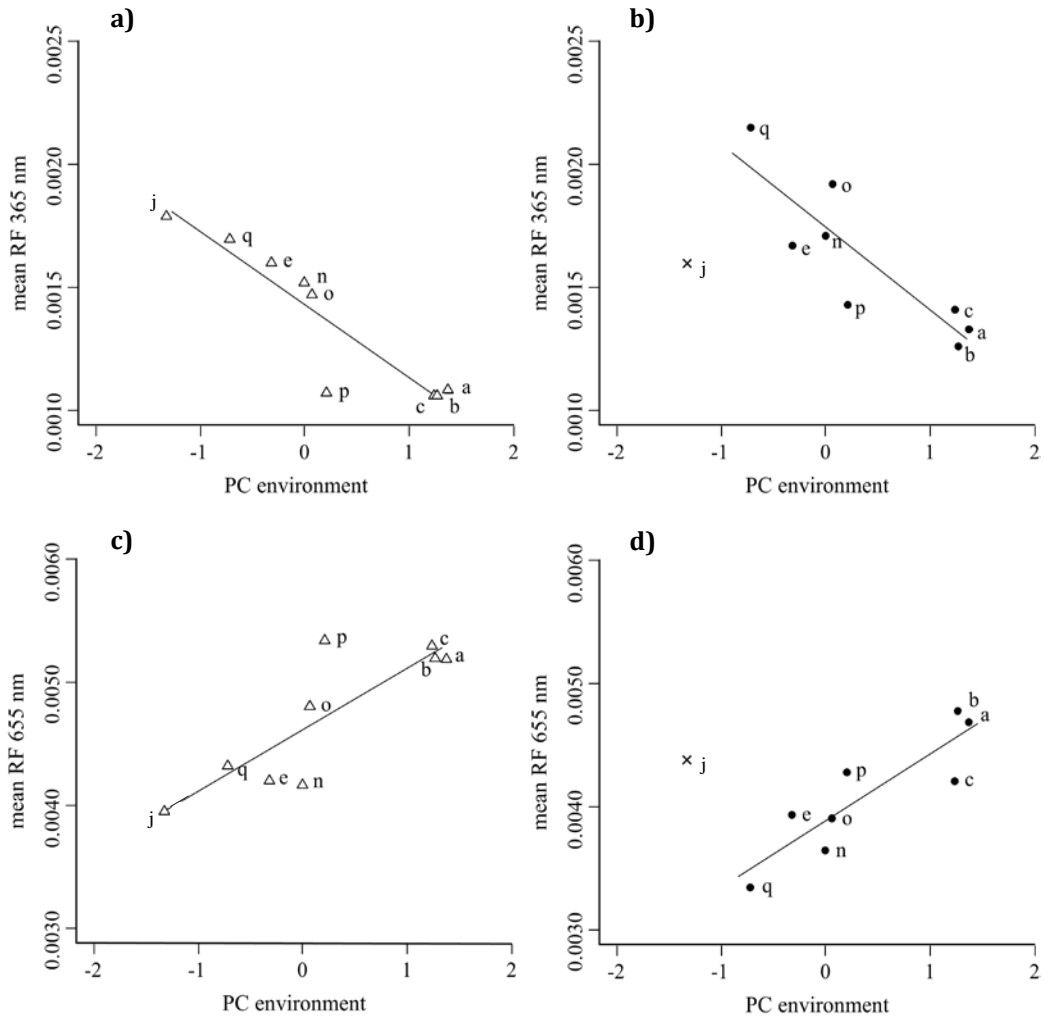


Fig. 6.4 Relationship between dewlap colour and climate-related environmental conditions. Pglm regressions revealed significant associations between PC environment and (a, b) mean relative reflectance in UV (RF 365 nm), and (c, d) mean relative reflectance in red (RF 655 nm). Data points for males (a, c) are represented by triangles and for females (b, d) by filled dots; letters denote corresponding study populations (see figure 6.3a for abbreviations). For females (b, d), *P*-values were only significant after excluding the San Salvador population and the regression lines were therefore fit discarding this population; the San Salvador data point (j) is represented by the crossed symbol.

6.4 Discussion

In a first step, we focused on documenting the interpopulational variation in dewlap design and display of *A. sagrei* males and females, distributed across the Caribbean. In accordance with previous findings (Vanhooydonck et al. 2009), we found that dewlap size and pattern differed significantly among populations of *A. sagrei*. Also dewlap colour, which was defined by brightness, hue and reflectance in UV and red, showed a high degree of interpopulational variation. These results were obtained for both sexes separately, which highlights the importance of also incorporating data on female dewlap design in studies addressing dewlap diversity. For dewlap display, however, we only found interpopulational differences in *A. sagrei* males. The low number of female individuals that actually exhibited dewlap extensions (DE) together with the low DE rates that were obtained, may largely explain this result. The observation that females use dewlap displays far less compared to males, is a commonly seen pattern that has already been reported for several anole species including *A. sagrei* (Partan et al. 2011; Driessens et al. 2014).

In a second step, we assessed whether variation in climate-related environmental conditions could explain this remarkable dewlap diversity observed among populations. Our results showed that *A. sagrei* lizards occurring in xeric environments had a higher proportion of solid dewlaps with relatively high reflectance at UV. In addition, we found that lizards inhabiting mesic environments had primarily marginal dewlaps showing relatively high reflectance in red. Excluding data sampled outside the breeding season gave the same results in males. For females, however, dewlap colour (i.e., RF 365 nm and RF 655 nm) correlated significantly to PC environment only after exclusion of the population sampled in San Salvador. Our results provide clear evidence for a relationship between signal design and prevailing environmental conditions in both sexes of *A. sagrei*, and additionally suggest that the interpopulational variation in signal design is not just the result of random genetic drift or haphazard changes in female preferences. Whether the observed variation in dewlap colour and pattern

among *A. sagrei* populations is truly adaptive in terms of optimal signal transmission under disparate environmental conditions, remains to be tested. Generally, colour signals should reflect the strongest regions of wavelengths in the ambient light, but at the same time stand out sufficiently against background vegetation in order to maximize their visibility within certain environments (Endler 1990). A thorough study of efficacy of the dewlap signal would thus require information on the spectral properties of the ambient light, the reflectance of the background and the visual system of the receiver. Following Ng et al. (2013a), we used global environmental parameters to estimate variation in prevailing environmental conditions and therefore, cannot present conclusive data on this matter here. Yet, we have reasons to believe that the observed variation in dewlap colour and design may aid signal efficacy. In his study on the colour of lights in tropical forests around the world, Endler (1993b) distinguished four major light environments when the sun is not blocked by clouds: forest shade, woodland shade, small gaps and large gaps. In forest shade, the greenish leaf radiance dominates the irradiance spectrum because most of the light is transmitted through or reflected from leaves. In woodland shade, bluish sky radiance dominates because much of the light comes from the sky, through canopy holes. Large gaps receive a mixture of yellow-red irradiation (from the sun) and blue radiation (from the sky), combining into a white light. Smaller gaps will see less light from the sun and sky, and more light transmitted through or reflected from leaves, and are therefore characterized by reddish light. Assuming that the 'xeric' environments sampled in our study have light conditions that resemble Endler's 'woodland' and 'large gap' light environments, the relatively high UV-reflectance of lizard dewlaps in these populations may enhance their detectability. Likewise, if 'mesic' environments sampled here have light conditions akin to Endler's 'forest shade' and 'small gaps', the reddish colours of the dewlaps would benefit visibility, because they will reflect the light that is most available there and will additionally appear highly conspicuous against the dense green vegetation backgrounds (Endler 1992). It should be noted that the evolution of red and UV-reflection in *Anolis* dewlaps might be correlated. In *A. sagrei*, the

orange-red colours of the dewlap are produced by drosoppterin, a pigment that is known to absorb UV (Macedonia et al. 2000; Steffen and McGraw 2007, 2009). Dewlaps of lizards with high drosoppterin concentrations will therefore show strong reflectance in red, but low reflectance in UV (Steffen and McGraw 2009). This trade-off is also evident in our measurements, both within and among populations. One could thus argue that low UV-reflectance in mesic environments is a pleiotropic effect for selection for red colours, or vice versa. The obtained differences in dewlap pattern among *A. sagrei* populations could be explained along similar lines. In our xeric environments characterized by relatively open sites with high incoming radiation, additional colour contrasts created by patterning may not be required to assure visibility, resulting in a higher proportion of solid dewlaps here. Whereas in our mesic environments characterized by dense green sites with high rainfall and less incoming radiation, additional colour contrasts, such as the adjacency of a yellow margin with a red dewlap centre, are likely essential to ensure signal visibility (especially in 'forest shade' and 'small gaps' light environments, Endler 1993b).

So far, two other studies have connected differences in *Anolis* dewlap colouration to variation in the physical environment. Ng et al. (2013a) showed that *A. distichus* males from environments with more rainfall and a higher percentage tree cover had dewlaps with higher reflectance in red, which corresponds nicely to our results. However, inconsistent to what we report here for *A. sagrei*, Ng et al. (2013a) found that precipitation level additionally predicted the brightness, cut-on wavelengths and size of *A. distichus* dewlaps. Also, Ng et al. (2013a) failed to explain variation in UV-reflectance among *A. distichus* populations, while we found a clear association between UV-reflectance and prevailing environmental conditions for *A. sagrei*. In the other study, on *A. cristatellus*, Leal and Fleishman (2004) also found habitat-related differences in dewlap colouration, but here the pattern was opposite: males from mesic habitats had bright and UV-reflective dewlaps, while males from xeric habitats had duller and redder dewlaps. It is unclear whether the disparity in results between these studies is due to differences in methodology, or reflects actual differences between the species.

Alternatively, the relationship between environmental conditions and dewlap design reported here, may come about by factors unrelated to light conditions and signal efficacy. For instance, climatic conditions and habitat structure are likely to affect the abundance and composition of prey species. Diet affects the colouration of many animals (Olson and Owens 1998), including some lizards (Merkling et al. 2016). However, a common garden breeding experiment in *A. distichus* (Ng et al. 2013b) and a two-factor experiment assessing effects of nutritional stress and carotenoid supplementation in *A. sagrei* (Steffen et al. 2010) do not support the idea that differences in dewlap colouration have a dietary origin. Climate and habitat structure may also affect the abundance and range of predators. In this regard, the observed relationship between climate-related environment and dewlap design among *A. sagrei* populations might be rather attributed to variation in predation pressure (Vanhooydonck et al. 2009). Yet, results from another study using data of the same *A. sagrei* populations in combination with estimates of relative predation pressure do not provide indications in that direction (chapter 5). Finally, the number of syntopic *Anolis* species may largely depend on climate-related environmental conditions and may therefore, impact dewlap design via reproductive character displacement (Vanhooydonck et al. 2009). Previously reported results (chapter 5) indeed showed that *A. sagrei* males (but not females) from populations with a higher number of syntopic *Anolis* species are more likely to have a ‘spotted’ dewlap pattern. Nevertheless, the number of syntopic species did not correlate with the proportion of solid and marginal dewlap patterns (chapter 5), nor did it correlate with climate-related environmental conditions (Spearman’s rho test: number of co-occurring *Anolis* species – PC environment, $\rho = 0.19$, $P = 0.467$, $N=17$). Based on these findings, we believe that the relationship between dewlap design and environmental conditions discussed in this paper (i.e. higher proportion of solid dewlaps and more UV-reflectance in xeric environments versus higher proportion of marginal dewlaps and more reflectance in red in mesic environments) is likely not the result of reproductive character displacement.

In addition to dewlap design, we linked display behaviour to variation in climate-related environmental conditions. We found that males use significantly more dewlap displays in mesic compared to xeric environments. For females, in contrast, no significant relation between signal diversity and prevailing environmental conditions was obtained. Previous research has noted that male anoles adjust their signalling behaviour in order to compete with distractions in their environment decreasing visibility, e.g. visual background noise and low light conditions (Fleishman 1988; Ord et al. 2011). For example, lizards inhabiting poorly lit or windy environments compensate by enhancing the speed of display movements or extending the duration of displays (Ord et al. 2007, 2010). Our finding that males increase dewlap use only in the mesic environments, characterized by a lower visibility due to poor light conditions and complex habitat structures, might thus be a simple and adaptive strategy to enhance signal transmission. Based on previous results (chapter 5), we believe that the contribution of predation and the number of syntopic species in explaining climate-related variation in dewlap use, is limited; see previous paragraph for this discussion on dewlap design.

Dewlap colours of female *A. sagrei* followed a similar pattern of covariation with environmental conditions as seen in males. This may indicate genetic correlation (as expected if the same genes affect dewlap design in males and females). Yet, preliminary analyses on *A. sagrei* data provide no support for the genetic byproduct hypothesis in explaining female dewlap size. Alternatively, female dewlaps may experience the same selection regime as males (which would be expected if both are selected for efficacy). The interpretation of our results for the female dewlap is hampered by the fact that a relationship was found only after exclusion of the San Salvador data, which were obtained outside the breeding season. This opens the interesting possibility that female dewlap design changes considerably with the reproductive status of the female. In a previous publication, we already hinted at the possible significance of UV-reflection as an indicator of

receptivity in female *A. sagrei* (Driessens et al. 2015). Clearly, the female dewlap is understudied and deserves closer attention.

In conclusion, our observations reveal an association between climate-related environmental conditions and aspects of dewlap design and use in *Anolis sagrei*. We have presented some indications that this association may result from differential selection on signal efficacy. Yet, detailed measurements of local light conditions and backgrounds, as well as the visual perception of *A. sagrei* are required to further test this idea. Our results additionally invite a closer look at female dewlaps. Testing more species with inclusion of data on the female sex too, would thus further enrich our knowledge of the evolution of dewlap diversity and its role in speciation.

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The Ethical committee on animal experimentation approved protocols for the use of live animals in this study (ECD 2011-64).

The authors declare that they have no conflict of interest.

CHAPTER 7

General Discussion

Science is the acceptance of what works and the rejection of what does not. That needs more courage than we might think.

JACOB BRONOWSKI - MATHEMATICIAN, BIOLOGIST

In this PhD dissertation, I explored the function and the evolution of a visual signalling device, the dewlap in *Anolis sagrei*, by combining an experimental approach in the laboratory (Part I: chapters 2-3) with a comparative approach in the field (Part II: chapters 4-6). In contrast to most studies on this subject, I also looked at the female dewlap. Based on findings and personal observations acquired throughout my PhD trajectory, I here discuss some general insights into the signalling function of the brown anole dewlap and the evolutionary processes shaping geographic signal diversity.

7.1 Signalling functions of the dewlap

From chapters 2 and 3, it is clear that the both sexes of the brown anole differ remarkably in dewlap design and display rate. Notably, male dewlaps are larger and more conspicuous in colour, and are in addition, far more used than in females. Nevertheless, in both sexes dewlap displays are used in a social context rather than in a predator context (chapter 2).

7.1.1 Predator context - males and females

Neither *A. sagrei* males nor females increased dewlapping in response to a predatory curly-tailed lizard (chapter 2), challenging the hypothesis that the dewlap serves as a pursuit deterrent signal. This finding nicely corresponds to other behavioural studies showing that in the presence of predatory cues, *A. sagrei* males do not change their display behaviour (Elmasri et al. 2012) or even decrease their display intensity and/or amplitude (Simon 2007; Steinberg et al. 2014). Yet, in one study was suggested that males and females do use their dewlap to deter curly-tailed lizards, as relative dewlap size was larger on islands where predatory *Leiocephalus* lizards co-occurred with *A. sagrei* (Vanhooydonck et al. 2009). In an attempt to replicate these results using an extended dataset (chapter 5), I could not provide this indirect support for pursuit deterrence signalling to curly-tailed lizards or other predators. In summary, based on my findings (chapters 2 and 5) and the lack of ‘direct’ evidence for predator deterrence in *A. sagrei*, I suggest that the dewlap does not function as a pursuit

deterrent signal in either sex of the brown anole lizard.

7.1.2 Social context - males

When evaluating male dewlap displays in an intrasexual context (chapter 2), my results showed no significant role of the dewlap during agonistic male-male interactions. A few other studies on *A. sagrei* found similar results (Tokarz et al. 2003; Simon 2011), suggesting that the dewlap on its own is not essential for territorial defence. However, it should be noted that I studied dewlap display rates in isolation. In doing so, I may have underestimated the complexity of the signal. Perhaps, the way in which dewlap extensions are combined with other display types (head-nods, HN; and primarily push-ups, PU; chapter 2) and body postures can still contribute in the advertisement of male quality for access to territories and mates. The displays of the *A. sagrei* males show a high degree of within-individual variability along a number of dimensions (e.g. total number of HN and PU per display bout, use of the dewlap with and without erection of the crest and/or bobbing; Scott 1984; Partan et al. 2011), which further indicates that a specific combination of display types may direct the information content of the signal. Future behavioural studies should therefore focus on dewlap extensions as a coherent part of a multi-component display and preferably not, as a separate display type on itself. In addition, duration and amplitude of display types, as well as the duration of intervals between display bouts may convey information about the sender that is important, but was not tested for in my study.

Another idea that may explain why I did not observe an increase in dewlap frequency during the staged male-male interactions, may involve the actual distance between opponents. Earlier studies on *Anolis*, including my study species, have shown that a different set of display types may be used when male competitors are at short versus long distances (e.g. Decourcy and Jenssen 1994; McMann 2000). In most studies, including this one (chapter 2), staged male-male interactions are studied in a short-distance context, which is typically characterized by aggressive displays and/or fights. However, intrasexual competition can also involve long-distance territorial displays that act as signals

of male quality and resident status, thus discouraging seen and unseen rivals from intruding. For example, Henningson and Irschick (2012) showed that relative dewlap size signals bite force and thus male fighting capacity in *A. carolinensis* males. Yet, reducing dewlap size did not change the outcome of staged short-distance interactions between these males, leading Henningson and Irschick (2012) to suggest that dewlap size signals male fighting quality primarily during long-distance territorial displays. In chapters 3 and 4, I showed that absolute male dewlap size is an excellent predictor of bite force in *A. sagrei* and may as such, act as a redundant signal of body size in the advertisement of fighting capacity. Yet, in some *A. sagrei* populations, male dewlap size can also act as direct honest signal of bite force (independent of body size; chapter 4). Based on suggestions from Henningson and Irschick (2012) and the low dewlapping rates found during my short-distance male-male interactions (chapter 2), I believe that also in *A. sagrei* the advertisement of fighting capacity through dewlap and body size may occur especially during long-distance territorial displays. In addition to size, dewlap colour may signal male quality, specifically health state, during these agonistic interactions (chapter 3). On the other hand, during aggressive short-distance interactions, more direct features of assessing one another might be used (Henningson and Irschick 2012), i.e. head size (strongly correlated to bite force and predictor of male combat outcome in *A. cristatellus*; e.g. Perry et al. 2004; Lappin and Husak 2005), body condition, and/or behavioural traits like push-ups (honest signal of endurance and predictor of male combat outcome in *A. cristatellus* males; e.g. Perry et al. 2004). Indeed, in *A. sagrei*, primarily PU displays played a major role during the staged, short-distance male-male interactions (chapter 2). All the suggestions made here, need additional testing in the laboratory and in the field to shed more light on the importance of dewlap displays in signalling fighting success during short versus long-distance interactions.

When evaluating the use of the male dewlap in an intersexual context (chapter 2), I found dewlap displays to be important during the staged, short-distance male-

female interactions; as a separate display component, as well as in combination with HN and PU. Based on the literature and my results, I here briefly discuss four potential functions of the male dewlap during courtship in the brown anole (e.g. Andersson 1994; Simon 2011).

Firstly, male dewlap displays might increase the conspicuousness of the signalling male towards potential females and in this way facilitate mate attraction. In my experiment, males increased their display rates for all display types (HN, PU and DE; chapter 2) only during intersexual interactions, indicating that a high degree of conspicuousness can be an important aspect of courtship. Although I found support for this idea in a short-distance context, increased conspicuousness might be especially valuable over long-distances, at the initial stage of the male-female interactions.

Secondly, during courtship, male dewlap displays may signal sexual identity. In chapter 3, I explicitly tested this idea for *A. sagrei* and provided support. Notably, in this species, body size together with relative dewlap area and colour act as redundant messages in the advertisement of sexual identity.

Thirdly, dewlap use in males might stimulate or accelerate female receptivity. Direct evidence for this idea has so far, only been acquired for *A. carolinensis*. Crews (1974, 1975) suggested that male DE accelerate female receptivity at the beginning of the breeding season and that continued exposure is necessary for maintaining ovarian recrudescence. Future behavioural experiments where unreceptive *A. sagrei* females are exposed to *A. sagrei* males with intact versus disabled dewlaps, might offer a simple, though, fruitful approach to test this idea.

Lastly, male dewlap displays may also advertise individual quality to females. In chapter 3, I found that male dewlap colouration in both the centre and edge region conveys information on aspects of individual quality, specifically health state (i.e. multiple messages: body condition, immune response and hematocrit). Whether these messages conveyed by dewlap colouration actually increase male attractiveness to females, remains to be tested. Although evidence for female preference based on male dewlap colouration has been demonstrated in *Anolis carolinensis* (Sigmund 1983; but see MacDonald and Echternacht 1991), no such

evidence has been found for *A. sagrei* females. Typically, females settle on territories prior to the emergence of males in several seasonal lizard species, which precludes females from choosing between reproductively active males in the early breeding season (Jenssen et al. 2001). Tokarz (1998) previously observed that *A. sagrei* females do not tend to enter territories of other males and generally do not display to neighboring males. Moreover, after territorial displacements, the *A. sagrei* females just mated with the new resident males. In a recent study conducted in the laboratory, Flanagan and Bevier (2014) showed that *A. sagrei* females did not show any preferences for males with higher territory quality. Based on these findings and knowing that *A. sagrei* females can mate with multiple males and store sperm for more than two months (Calsbeek et al. 2007), I suggest that sperm competition and multiple paternity rather than female mate choice are important mechanisms through which females acquire 'good genes'. This idea is poorly explored in *Anolis* and the issue offers plenty of future research possibilities.

7.1.3 Social context - females

When studying female dewlap displays in an intrasexual context (chapter 2), my results demonstrated that the dewlap does not serve an important role during female-female interactions in *A. sagrei*. However, this finding can possibly be attributed to my experimental set-up; females were only confronted to their mirror image (no real adversaries), and a light source on top of a perch was used as the resource to compete for. Still, I did observe elevated PU and HN rates in this experimental set-up in comparison to the non-social contexts (predator, non-predator; chapter 2).

A growing number of studies have recently drawn specific attention to the role of female ornamentation during intraspecific competition for ecological resources, in a variety of species (see 'general introduction' for more details). In *Anolis*, several studies have demonstrated that females indeed defend exclusive territories, involving aggressive signalling to other females (*A. aeneus*: Stamps 1977; *A. bahorucoensis*: Orrell and Jenssen 1998; *A. carolinensis*: Nunez et al. 1997;

A. lineatopus: Rand 1967; *A. nebulosus*: Jenssen 1970a). None of these studies have, however, looked at the link between female dewlap components and individual quality or competitive success over resources. Although I could not find support for a role of the *A. sagrei* dewlap in signalling individual quality to female opponents (chapter 2 and 3), further research is required to support or reject this idea, using more appropriate methods (e.g. food and/or nest sites as ecological resources to compete for, staged female-female interactions in the field, short-distance versus long-distance interactions, other female quality indexes than the ones used in chapter 3). In particular, because I also found female dewlap size to show strong positive allometry in *A. sagrei*, suggesting that this trait is shaped by sexual or social selection (chapter 3, see also Harrison and Poe 2012).

When evaluating female dewlap use in an intersexual context, I found a strong increasing trend in DE rate compared to the control treatment. This suggests that also in females, the dewlap functions predominantly in a courtship context. As for the male dewlap, I discuss four possible functions of the female dewlap.

Firstly, dewlapping might increase the females' conspicuousness and as such facilitate mate attraction. As previously stated, the idea of increased conspicuousness might be especially important to initially attract mates at a distance; home ranges in *A. sagrei* can extend up to 25 m² (Schoener and Schoener 1982a; Sigmund 1983).

Secondly, the female dewlap may function in advertising sexual identity to males (chapter 3 and paragraph on male dewlap) and as such, may help avoiding costly mating errors.

Thirdly, the female dewlap may serve a role in the advertisement of sexual receptivity to males. This would be highly beneficial for the female, because sexual coercion imposed by a male can be costly to both individuals and can additionally reduce female fecundity and survival (Clutton-Brock and Parker 1995; Le Galliard et al. 2005). In chapter 6, I already suggested that dewlap colouration (i.e. UV reflectance) in *A. sagrei* females may vary in function of the breeding season and reproductive state. Yet, additional behavioural experiments

are required to provide direct evidence for the idea that female dewlap colouration (UV) functions in the advertisement of sexual receptivity to males.

Fourthly, in contrast to Batesman's principle where females are expected to be the 'choosy' sex (Bateman 1948), females may signal individual quality to males in order to be chosen as mating partner (e.g. Amundsen 2000, Amundsen and Forsgren 2001). Studies on *A. sagrei* have demonstrated that males can discriminate among individual females and use this ability to control mating decisions (Tokarz 1998, 2006). Yet, it remains unclear what factors males use to decide to mate with specific females. In chapter 3, I found correlations between female dewlap design (size, brightness) and DE rates during intersexual interactions, which may suggest that females want to impress males by signalling reproductive quality ('good genes') via dewlap traits. Although I could not find a significant association between female dewlap design and several estimates of individual quality (chapter 3), additional experiments in the laboratory and in the field are required to further explore this idea. In particular, associations between female dewlap traits and qualities related to reproductive performance (e.g. egg size and quality, number of successive eggs produced, the quality of yolk involving nutritive content) rather than to health state and physical performance (chapter 3), should clarify whether females indeed use their dewlap to signal reproductive quality to males.

7.2 Dewlap diversity and underlying evolutionary processes

The dewlap is a 'dynamic' signalling system when considering aspects of design, use and information content. Seasonality (breeding versus non-breeding season), in particular, is known to substantially affect dewlap size and display intensity (e.g. Tokarz et al. 2002; Irschick et al. 2006; Lailvaux et al. 2015); hormone levels can even vary on a monthly base (testosterone and corticosterone levels in *A. sagrei* males, Tokarz et al. 1998). For the comparative approach (Part II), I have tried to minimize noise due to seasonal differences by sampling *A. sagrei* populations consistently during the first half of the breeding season (March - May;

with exception of one population); seasonal year-to-year fluctuations could not be taken into consideration, given the limited timeframe of the study.

7.2.1 Interpopulational variation in dewlap characteristics

In line with previous findings (Kolbe et al. 2004; Vanhooydonck et al. 2009), I found extensive variation among *A. sagrei* populations for nearly all male and female dewlap characteristics. This interpopulational variation in dewlap design and use is considerably greater than the variation observed within populations or within other *Anolis* species (chapters 4 to 6; Losos 2009), making the brown anole exceptional in this regard.

7.2.2 Selective processes

When reconsidering the same selective pressures as in Vanhooydonck et al. (2009), i.e. predation pressure, sexual selection and species recognition (chapter 5), I could only find support for the species recognition hypothesis in explaining interpopulational dewlap diversity. Notably, my results showed that the more congeneric species occur on a given island, the more complex the dewlap pattern in *A. sagrei* males becomes (i.e. a higher proportion of males with ‘spotted’ dewlaps). Previous studies have already provided evidence for species recognition in *A. sagrei* (Vanhooydonck et al. 2009) and various other *Anolis* species (e.g. Rand and Williams 1970; Webster and Burns 1973; Losos 1985; Macedonia et al. 2013). Surprisingly, I could not obtain support for species recognition in *A. sagrei* females, which challenges the findings reported by Vanhooydonck et al. (2009). Future research seems required to investigate whether brown anole females potentially use other signalling traits (perhaps HN display patterns or dorsal colour patterning) to communicate species identity.

Rather unexpectedly, I could not find a significant contribution of predation pressure or sexual selection in shaping dewlap design and displays in *A. sagrei* (but see significant correlation between island size and male dewlap displays in chapter 5). Nor could sexual selection explain interpopulational differences in the

dewlap size – bite force relationship in *A. sagrei* males (chapter 4). These results may be due to the fact that both predation pressure and sexual selection are notoriously difficult to quantify – my crude estimates may simply have failed to capture these processes in an adequate way. In chapters 4 and 5, I already pointed out some perils of using sexual size dimorphism (SSD) as a metric for quantifying sexual selection (e.g. Calsbeek and Sinervo 2004; Cox et al. 2007). The use of operational sex ratios, as well as the amount and duration of agonistic interactions might be a better approach for future research. Likewise, I highlighted the difficulties of accurately quantifying predation pressure (chapter 5; e.g. Endler 1986; Itescu et al. 2016). Combining the total number of predatory species with measures of predator abundance and diet composition might be most appropriate to estimate predation rates imposed by a large spectrum of predators (e.g. birds, snakes, lizards, cats). Furthermore, although logistically challenging to do, a capture-mark-release-recapture program (Calsbeek and Cox 2010) provides one of the best methods to accurately estimate the strength of ecological selection, including predation pressure. Despite the pitfalls of wielding proxy measurements for quantifying selective processes (i.e. SSD, island size, TBF, model attack rate, presence/absence of predatory *Leiocephalus* lizards), several studies on dewlaps were already fruitful in showing a significant contribution of sexual selection and predation pressure using these crude estimates (e.g. Vanhooydonck et al. 2009; Harrison and Poe 2012; Klomp et al. 2016).

Another reason to explain my results may involve the adopted statistical approach. In chapters 4 and 5, only the separate contributions of predation pressure and sexual selection were tested, whereas in real-world scenarios signalling traits are subjected to a mixture of selective agents (opposing, as well as reinforcing). This idea probably explains the partial discordance between my results (chapter 5) and findings of Vanhooydonck et al. (2009); these authors adopted an information theoretic approach to compare models of selection incorporating a combination of multiple factors (see chapter 5 for my statistical approach and motivation). Nevertheless, an array of studies on dewlap evolution does support sexual selection and predation pressure hypotheses using both

crude estimates and univariate statistical analyses treating various selective factors independently (e.g. Harrison and Poe 2012; Klomp et al. 2016). My findings might thus, also be explained by that fact that other selective factors than sexual selection and predation pressure are far more important in explaining interpopulational dewlap diversity in *A. sagrei*.

I therefore extended my scope to the role of climate and habitat conditions in driving dewlap design and display behaviour (chapter 6); a selective factor that was so far, largely unexplored in *A. sagrei*. It turned out that variation in climate-related environmental conditions could explain interpopulational differences in dewlap colour and pattern, in both sexes of *A. sagrei*. Notably, I showed that males and females occurring in ‘xeric’ environments had a higher proportion of solid dewlaps with higher UV-reflectance. On the other hand, lizards inhabiting ‘mesic’ environments had primarily marginal dewlaps showing high reflectance in red. Whether the divergence in dewlap design is truly adaptive in terms of optimal signal efficacy under disparate environmental conditions (sensory drive; Leal and Fleishman 2004), needs further testing with incorporating of ambient light conditions and backgrounds. Nonetheless, in chapter 6, I presented some indications (based on qualitative predictions only; Endler 1993a, b) that the observed differences in dewlap colour and pattern may indeed result from differential selection for signal visibility. In addition to changes in dewlap design, I found an increase in male dewlap use in ‘mesic’ environments. This finding may further support the idea of sensory drive, as increased display rates in males can greatly enhance signal transmission in environments characterized by complex habitat structures, relatively low light levels and lots of ambient noise (Ord et al. 2007, 2010).

To my knowledge, this is the first study providing evidence for a role of climate-related environmental conditions in shaping female dewlap design, on an intraspecific level (see Harrison and Poe 2012, for evidence on the interspecific level). Dewlap design in *A. sagrei* females may have experienced the same selection regime as in *A. sagrei* males, in order to optimize signal visibility

(sensory drive). Yet, I cannot rule out the genetic byproduct hypothesis with respect to dewlap colouration and patterning (Lande 1980); preliminary results provide, however, no genetic correlation for dewlap size in *A. sagrei* females (Driessens et al. in prep; see also Harrison and Poe 2012). Furthermore, the interpretation of my results for the female dewlap remains hampered by one ‘outlier’ population that was sampled outside the breeding season (chapter 6). Future studies on the female dewlap should therefore further address the importance of the sensory drive, as well as the other described selective processes.

7.2.2 *Stochastic processes*

In addition to the discussed selective processes, stochastic processes like random genetic drift or mutation order (M-O) may also have a contribution in explaining dewlap divergence (Lande 1976; Mani and Clarke 1990). In several *Anolis* species, phylogeographic studies have found significant genetic differentiation among populations, even in the absence of phenotypic differentiation (e.g. Jackman et al. 2002; Glor et al. 2003, 2005). These observations can be described to genetic drift. Furthermore, by using a replicated experiment in nature, Kolbe et al. 2012 showed that founder effects can play an important role in the divergence of *A. sagrei* island populations, even when adaptation occurs. Notably, founding events generated significant among-island genetic and morphological differences that persisted throughout the course of their experiment, despite all *A. sagrei* populations adapting in response to narrow vegetation and perches (i.e. evolution of shorter hindlimbs). Despite the evidence for drift and founder events in *Anolis*, it remains unclear whether these stochastic processes can also explain phenotypic dewlap divergence.

Under M-O, dewlap divergence can occur among populations adapting to similar selection pressures because of the stochastic order in which beneficial traits arise. Although it is believed that M-O may have a significant contribution in shaping signal divergence, especially in large populations under strong selection (Mani and Clarke 1990), no study has so far looked to this process in *Anolis*. More

studies assessing the importance of M-O would be useful, and also the interaction between genetic drift and M-O processes merits further investigation.

7.2.3 Phenotypic plasticity

Besides genetic processes, phenotypic plasticity can be important in explaining interpopulational variation in a variety of traits (Price et al. 2003). The potential significance of phenotypic plasticity in *A. sagrei* was demonstrated years ago in a study on variation in limb length, in response to narrow versus broad perches (Losos 2011). In several anole species, dewlap characteristics, including male dewlap size appear to be highly plastic according to season and resource availability. For example, Irschick et al. (2006) showed that dewlap size is an honest signal of bite force in *A. carolinensis* males, only during the breeding season. Accordingly, Lailvaux et al. (2012) showed that the honest dewlap size - bite force relationship in *A. carolinensis* males got disrupted under limited resource availability. In chapter 4, my results showed that interpopulational variation in the relative dewlap size - bite force relationship in *A. sagrei* males may also depend on resource availability and may therefore, rather be explained by phenotypic plasticity than by genetic change. Furthermore, in *A. sagrei* females, I observed a substantially lower UV-reflectance for dewlap colouration in one population that was sampled outside the breeding season (chapter 6), which may hint towards phenotypic plasticity. Generally, the role of phenotypic plasticity remains largely understudied in a context of dewlap divergence and therefore deserves special attention for future research, in males and especially, in females.

7.3 General concluding remarks

Overall, I showed that the dewlap in *A. sagrei* functions primarily in social contexts and, in particular, during courtship. It is beyond doubt that the *A. sagrei* dewlap is more conspicuous and more often used in males than in females. However, my results showed that, despite being less elaborate, the female dewlap also has a signalling function. The information conveyed by dewlap components can vary among populations (e.g. relative male dewlap size is an honest signal of

biting capacity only in some *A. sagrei* populations) and according to season (e.g. potentially dewlap colour in females), and remains therefore idiosyncratic. Identifying the evolutionary processes that underlie the observed intraspecific dewlap diversity is challenging. This is mainly due to the interplay of evolutionary processes in real-world scenarios, as well as due to the methodological difficulties to accurately quantify the strength of selective processes. Nevertheless, I could show indirect support for species recognition in shaping complex dewlap colour patterns in *A. sagrei* males. Furthermore, I could demonstrate that climate-related environmental conditions help in explaining intraspecific diversity in male and female dewlap colouration and patterning. Future studies focusing on the interplay of processes, including stochastic events, selective mechanisms and phenotypic plasticity would be fruitful to further unravel what determines dewlap diversity. To end, with this PhD dissertation, I hope to provide groundwork for future research on the function and evolution of the *Anolis* dewlap, and to stimulate an increased interest in the so far ‘scarcely studied’ female ornaments.

References

*If I have seen further, it is by standing on the
shoulders of giants.*

ISAAC NEWTON - PHYSICIST

Alonso-Alvarez C, Doutrelant C, Sorci G (2004) Ultraviolet reflectance affects male-male interactions in the blue tit (*Parus caeruleus ultramarinus*). *Behav Ecol* 15:805-809

Amézquita A, Lima AP, Jehle R, Castellanos L, Ramos Ó, Crawford AJ, Gasser H et al. (2009) Calls, colours, shape, and genes: a multi-trait approach to the study of geographic variation in the Amazonian frog *Allobates femoralis*. *Biol J Linn Soc* 98:826-838

Amundsen T (2000) Why are female birds ornamented? *Trends Ecol Evol* 15: 149-155

Amundsen T, Forsgren E (2001) Male mate choice selects for female coloration in a fish. *P Natl Acad Sci USA* 98:13155-13160

Anderson R, McBrayer LD, Herrel A (2008) Bite force in vertebrates: opportunities and caveats for use of a nonpareil whole-animal performance measure. *Biol J Linn Soc* 93:709-720

Andersson M (1994) *Sexual selection*. Princeton University Press, Princeton

Andersson SJ, Örnborg J, Andersson M (1998) Ultraviolet sexual dimorphism and assortative mating in blue tits. *Proc R Soc B Biol Sci* 265:445-450

Bartlett MS (1950) Tests of significance in factor analysis. *Brit J Math Stat Psy* 3:77-85

Bartlett MS (1954) A note on multiplying factors for various chi-squared approximations. *J R Stat Soc B* 16:296-298

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. *Heredity* 2:349-368

Bean D, Cook JM (2001) Male mating tactics and lethal combat in the nonpollinating fig wasp *Sycoscapter australis*. *Anim Behav* 62:535-542

Bels VL (1990) The mechanism of dewlap extension in *Anolis carolinensis* Reptilia: Iguanidae with histological analysis of the hyoid apparatus. *J Morphol* 206:225-244

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 57:289-300

Bennett AF (1985) Temperature and muscle. *J Exp Biol* 155:333-344

Bernal XE, Rand AS, Ryan MJ (2006) Acoustic preferences and localization performance of blood-sucking flies (*Corethrella coquillett*) to tungara frog calls. *Behav Ecol* 17:709-715

Billen J, Morgan ED (1998) Pheromone communication in social insects – sources and secretions. In: Vander Meer RK, Breed MD, Winston ML, Espelie KE (eds) *Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites*. Westview Press, Boulder, Oxford, pp 3–33

Blanco G, De La Puente J (2002) Multiple elements of the black-billed magpie's tail correlate with variable honest information on quality in different age/sex classes. *Anim Behav* 63:217–225

Blomberg SP, Garland T Jr, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717-745

Blumstein DT, Evans CS, Daniel JC (2000) JWatcher V1.0. An introductory user's guide. <http://www.jwatcher.ucla.edu>. Accessed March 2012

Bonneaud C, Marnocha E, Herrel A, Vanhooydonck B, Irschick DJ, Smith TB (2016) Developmental plasticity affects sexual size dimorphism in an anole lizard. *Funct Ecol* 30:235-243

Boughman JW (2002) How sensory drive can promote speciation. *Trends Ecol Evol* 17:517–577

Boul KE, Funk WC, Darst CR, Cannatella DC, Ryan MJ (2007) Sexual selection drives speciation in an Amazonian frog. *Proc R Soc B Biol Sci* 274:399-406

Bradbury JW, Vehrencamp SL (1998) *The principles of animal communication*. Sinauer Associates, Sunderland

Bradley BR, Rey-Millet Y (2013) *A Photographic Guide to the Birds of the Cayman Islands*. Bloomsbury Publishing Plc, London

Brandley NC, Speiser DI, Johnsen S (2013) Eavesdropping on visual secrets. *Evol Ecol* 27:1045–1068

Brodie III ED (1993) Differential avoidance of coral snake banded patterns by free ranging avian predators in Costa Rica. *Evolution* 47:227-235

Bussiere LF, Gwynne DT, Brooks R (2008) Contrasting sexual selection on males and females in a role-reversed swarming dance fly, *Rhamphomyia longicauda* Loew (Diptera: Empididae). *J Evol Biol* 21:683–1691

- Butcher GS, Rohwer S (1989) The evolution of conspicuous and distinctive coloration for communication in birds. *Curr Ornithol* 6:51–107
- Calsbeek, R, Cox RM (2010) Experimentally assessing the relative importance of predation and competition as agents of selection. *Nature* 465:613-616
- Calsbeek R, Sinervo B (2004) Progeny sex is determined by relative male body size within polyandrous females' clutches: cryptic mate choice in the wild. *J Evol Biol* 17:464-470
- Calsbeek R, Bonneaud C, Prahbu S, Manoukis N, Smith TB (2007) Multiple paternity and sperm storage lead to increased genetic diversity in *Anolis* lizards. *Evol Ecol Res* 9:495–503
- Campbell P, Pasch B, Pino JL, Crino OL, Phillips M, Phelps SM (2010) Geographic variation in the songs of Neotropical singing mice: testing the relative importance of drift and local adaptation. *Evolution* 64:1955–72
- Campbell TS (1996) Northern range expansion of the brown anole *Anolis sagrei* in Florida and Georgia. *Herpetol Rev* 27:155–157
- Campbell TS, Hammontree JT (1995) Geographic distribution: *Anolis sagrei*. *Herpetol Rev* 26:107
- Candolin U (2003) The use of multiple cues in mate choice. *Biol Rev* 78:575-595
- Cantwell LR, Forrest TG (2013) Response of *Anolis sagrei* to acoustic calls from predatory and nonpredatory birds. *J Herpetol* 47:293-298
- Carpenter CC (1978) Ritualistic social behaviors in lizards. In: Greenberg N, MacLean PD (eds) *Behavior and neurology of lizards*. NIMH, Rockville, pp 253–267
- Caspari E (1952) Pleiotropic gene action. *Evolution* 1-18
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17:540-552
- Clark RW (2004) Timber rattlesnakes (*Crotalus horridus*) use chemical cues to select ambush sites. *J Chem Ecol* 30:607-617
- Clark DL, Macedonia JM, Rowe JW, Stuart MA, Kemp DJ, Ord TJ (2015) Evolution of displays in Galapagos lava lizards: comparative analyses of signallers and robot playbacks to receivers. *Anim Behav* 109:33-44

Clutton-Brock TH (2007) Sexual selection in males and females. *Science* 318:1882–1885

Clutton-Brock TH (2009) Sexual selection in females. *Anim Behav* 77:3–11

Clutton-Brock TH, Albon SD, Gibson RM, Guinness FE (1979) The logical stag: adaptive aspects of fighting in red deer (*Cervus elaphus* L.). *Anim Behav* 27:211–225

Clutton-Brock TH, Parker GA (1995) Sexual coercion in animal societies. *Anim Behav* 49:1345–1365

Cochran PA (1989) *Anolis sagrei* (Brown Anole): behavior. *Herpetol Rev* 20:70

Cook EG, Murphy TG, Johnson MA (2013) Colorful displays signal male quality in a tropical anole lizard. *Naturwissenschaften* 100:993–996

Cooper WE (2001) Multiple roles of tail display by the curly-tailed lizard *Leiocephalus carinatus*: Pursuit deterrent and deflective roles of a social signal. *Ethology* 107:1137–1149

Cooper WE (2007) Escape and its relationship to pursuit-deterrent signalling in the Cuban curly-tailed lizard *Leiocephalus carinatus*. *Herpetologica* 63:144–150

Cooper WE (2010) Timing during predator-prey encounters, duration and directedness of a putative pursuit-deterrent signal by the zebra-tailed lizard, *Callisaurus draconoides*. *Behaviour* 147:13–14

Cooper WE Jr (2011) Pursuit deterrence, predation risk, and escape in the lizard *Callisaurus draconoides*. *Behav Ecol Sociobiol* 65:1833–1841

Cooper WE Jr, Caldwell JP, Vitt LJ. 2008. Conspicuousness and vestigial escape behavior by two dendrobatid frogs, *Dendrobates auratus* and *Oophaga pumilio*. *Behaviour* 146:325–349

Cox RM, Butler MA, John-Alder HB (2007) The evolution of sexual size dimorphism in reptiles. In: Fairbairn DJ, Blanckenhorn WU, Szekely T (eds) *Sex, Size & Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. Oxford University Press, Oxford, pp 38–49

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

References

- 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
- Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Sunderland
- Crews D (1974) Effects of group stability, male–male aggression and male courtship behavior on environmentally-induced ovarian recrudescence in the lizard, *Anolis carolinensis*. *J Zool* 172:419–441
- 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
- Cummings ME (2007) Sensory trade-offs predict signal divergence in surfperch. *Evolution* 61:530–545
- Cuthill IC, Bennett ATD, Partridge JC, Maier EJ (1999) Plumage reflectance and the objective assessment of avian sexual dichromatism. *Am Nat* 160:183–200
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772
- Darwin C (1871) *The descent of man, and selection in relation to sex*. John Murray, London
- Dean CL, Smith HT, Engeman RM (2004) Geographic distribution: *Leiocephalus carinatus armouri* (Little Bahama curly-tailed lizard). *Herpetol Rev* 35:82
- Decourcy KR, Jenssen TA (1994) Structure and use of male territorial headbob signals by the lizard *Anolis carolinensis*. *Anim Behav* 47:251–262
- Dial BE (1986) Tail display in 2 species of iguanid lizards - a test of the predator signal hypothesis. *Am Nat* 127:103–111
- Dobzhansky T (1951) *Genetics and the Origin of Species*. Columbia University Press, New York
- Driessens T, Huyghe K, Vanhooydonck B, Van Damme R (2015) Messages conveyed by assorted facets of the dewlap, in both sexes of *Anolis sagrei*. *Behav Ecol Sociobiol* 69:1251–1264

References

- Driessens T, Vanhooydonck B, Van Damme R (2014) Deterring predators, daunting opponents or drawing partners? Signaling rates across diverse contexts in the lizard *Anolis sagrei*. *Behav Ecol Sociobiol* 68:173–184
- Duellman WE, Schwartz A (1958) Amphibians and reptiles of southern Florida. *Bull Florida State Mus* 3:181-324
- 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
- Dziuban CD, Shirkey EC (1974) When is a correlation matrix appropriate for factor analysis? *Psychol Bull* 81:358–361
- 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
- Edward DA, Chapman T (2011) The evolution and significance of male mate choice. *Trends Ecol Evol* 26:647-654
- Edwards JR, Lailvaux SP (2012) Display behavior and habitat use in single and mixed populations of *Anolis carolinensis* and *Anolis sagrei* lizards. *Ethology* 118:494-502
- Elmasri OL, Moreno MS, Neumann CA, Blumstein DT (2012) Response of brown anoles (*Anolis sagrei*) to multimodal signals from a native and novel predator. *Curr Zool* 58:791–796
- 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 (1980) Natural selection on color patterns. *Evolution* 34:76–91
- Endler JA (1982) Convergent and divergent effects of natural selection on color patterns in two fish faunas. *Evolution* 36:178–188
- Endler JA (1983) Natural and sexual selection on color patterns in poeciliid fishes. *Environ Biol Fishes* 9:173–190
- Endler JA (1986) *Natural Selection in the Wild*. Princeton University Press, Princeton

Endler JA (1990) On the measurement and classification of color in studies of animal color patterns. *Biol J Linn Soc* 41:315–352

Endler JA (1992) Signals, signal conditions, and the direction of evolution. *Am Nat* 139:S125–S153

Endler JA (1993a) Some general comments on the evolution and design of animal communication systems. *Phil Trans R Soc B* 340:215–225

Endler JA (1993b) The color of light in forests and its implications. *Ecol Monogr* 63:1–27

Endler JA (2012) A framework for analysing colour pattern geometry: adjacent colours. *Biol J Linn Soc* 107:233–253

Farr, James A (1977) Male rarity or novelty, female choice behavior, and sexual selection in the guppy *Poecilia reticulata* Peters (Pisces: Poeciliidae). *Evolution* 31:162–168

Felsenstein J (1985) Phylogenies and the comparative method. *Am Nat* 125:1–15

Finkbeiner SD, Briscoe AD, Reed RD (2014) Warning signals are seductive: relative contributions of color and pattern to predator avoidance and mate attraction in *Heliconius* butterflies. *Evolution* 68:3410–3420

Fisher R A (1930) *The genetical theory of natural selection*. The Clarendon Press, Oxford

Fisher RA (1958) *The genetical theory of natural selection*. 2nd edn. Dover, New York

FitzGibbon CD, Fanshawe JH (1988) Stotting in Thomson's gazelles: an honest signal of condition. *Behav Ecol Sociobiol* 23:69–74

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 (1988) Sensory and environmental influences on display form in *Anolis aeneus*, a grass anole from Panama. *Behav Ecol Sociobiol* 22:309–316

Fleishman LJ (1992) The influence of the sensory system and the environment on motion patterns in the visual displays of anoline lizards and other vertebrates. *Am Nat* 139:S36–S61

Fleishman LJ (2000) Signal function, signal efficiency and the evolution of anoline lizard dewlap color. In: Rosenqvist G, Amundsen T, Espmark Y (eds) *Animal signals: signaling and signal design in animal communication*, Tapir Academic Press, Trondheim, pp 209-236

Fleishman LJ, Leal M, Persons MH (2009) Habitat light and dewlap color diversity in four species of Puerto Rican anoline lizards. *J Comp Physiol A* 195:1043-1060

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

Fleishman LJ, Loew ER, Leal M (1993) Ultraviolet vision in lizards. *Nature* 365:397

Forsman A, Lindell LE (1996) Resource dependent growth and body condition dynamics in juvenile snakes: an experiment. *Oecologia* 108:669–675

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

Foster SA, Endler JA (1999) *Geographic variation in behavior: perspectives on evolutionary mechanisms*. Oxford University Press, New York

Foster SA, Scott RJ, Cresko WA (1998) Nested biological variation and speciation. *Phil Trans R Soc B* 353:207–218

Fox SF, Perea-Fox S, Franco RC (1994) Development of the tail autotomy adaptation in lizards under disparate levels of predation at high and low elevations in Mexico. *Southwest Nat* 39:311–322

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

Garland T Jr, Ives AR (2000) Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *Am Nat* 155:346–364

- Gerhardt H, Huber F (2002) Acoustic communication in insects and anurans. University of Chicago Press, Chicago
- Glor RE, Kolbe JJ, Powell R, Larson A, Losos JB (2003) Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk-ground anoles (*Anolis cybotes* group). *Evolution* 57:2383-2397
- Glor RE, Losos JB, Larson A (2005) Out of Cuba: overwater dispersal and speciation among lizards in the *Anolis carolinensis* subgroup. *Molec Ecol* 14:2419-2432
- Gordon RE (1956) The Biology and Biodemography of *Anolis carolinensis* Voigt. PhD Dissertation, Tulane University, New Orleans
- Gorman GC (1968) The relationships of *Anolis* of the Roquet species group (Sauria: Iguanidae) – III Comparative study of display behavior. *Breviora* 284:1-31
- Graeb BD, Dettmers JM, Wahl DH, Cáceres CE (2004) Fish size and prey availability affect growth, survival, prey selection, and foraging behavior of larval yellow perch. *T Am Fish Soc* 133:504-514
- Grafen A (1990) Biological signals as handicaps. *J Theor Biol* 144:246-517
- Greenberg G, Noble GK (1944) Social behavior of the American chameleon (*Anolis carolinensis* Voigt). *Physiol Zool* 17:392-439
- Greene HW (1988) Antipredator mechanisms in reptiles. In: Gans C, Huey RB (eds) *Biology of the Reptilia*, Vol. 16. John Wiley & Sons, New York, pp 1-152
- Grether GF, Kolluru GR, Nersissian K (2004) Individual color patches as multicomponent signals. *Biol Rev* 79:583-610
- Griffin DR (1958) *Listening in the Dark*. Yale University Press, New Haven
- 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
- Grose J (2011) Modelling and the fall and rise of the handicap principle. *Biol Philos* 26:677-696
- Guilford T, Dawkins MS (1991) Receiver psychology and the evolution of animal signals. *Anim Behav* 42:1-14

- Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696-704
- Hack MA (1997) Assessment strategies in the contests of male crickets, *Acheta domesticus* (L.). *Anim Behav* 53:733-747
- Hardy ICW, Briffa M (2013) *Animal Contests*. Cambridge University Press, Cambridge
- 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
- Harvey PH, Pagel MD (1991) *The comparative method in evolutionary biology*. Oxford University Press, Oxford
- Hasson O (1991) Pursuit-deterrent signals: communication between prey and predator. *Trends Ecol Evol* 6:325-329
- Hasson O, Hibbard R, Ceballos G (1989) The pursuit deterrent function of tail-wagging in the zebra-tailed lizard (*Callisaurus draconoides*). *Can J Zool* 67:1205-1209
- Hebets EA, Papaj DR (2005) Complex signal function: developing a framework of testable hypotheses. *Behav Ecol Sociobiol* 57:197-214
- Heinen-Kay JL, Morris KE, Ryan NA, Byerley SL, Venezia RE, Peterson MN, Langerhans RB (2015) A trade-off between natural and sexual selection underlies diversification of a sexual signal. *Behav Ecol* 26:533-542
- Henderson RW, Crother B (1989) Biogeographic patterns of predation in West Indian colubrid snakes. In: Woods CA (eds) *Biogeography of the West Indies Past, Present, and Future*. Sandhill Crane Press, Inc., Gainesville, pp 479-518
- Henningsen JP, Irschick DJ (2012) An experimental test of the effect of signal size and performance capacity on dominance in the green anole lizard. *Funct Ecol* 26: 3-10
- Herrel A, O'Reilly JC (2006) Ontogenetic scaling of bite force in lizards and turtles. *Physiol Biochem Zool* 79:31-42
- Herrel A, Aerts P, Fret J, De Vree F (1999b) Morphology of the feeding system in agamid lizards: ecological correlates. *Anat Rec* 254:496-507

References

- Herrel A, Spithoven L, Van Damme R, De Vree F (1999a) Sexual dimorphism of head size in *Gallotia galloti*: testing the niche divergence hypothesis by functional analysis. *Funct Ecol* 13:289–297
- Herrel A, Van Damme R, Vanhooydonck B, De Vree F (2001) The implications of bite force for diet in two species of lacertid lizards. *Can J Zoolog* 79:662-670
- Hertz PE, Huey RB, Stevenson RD (1993) Evaluating temperature regulation by field-active ectotherms – the fallacy of the inappropriate question. *Am Nat* 142:796-818
- Hicks RA, Trivers RL (1983) The social behavior of *Anolis valencienni*. In: Rhodin AGJ, Miyata K (eds) *Advances in Herpetology and Evolutionary Biology: Essays in Honor of Ernest E. Williams*. Museum of comparative Zoology, Harvard University, Cambridge, pp 570-595
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978
- Hobel G, Gerhardt HC (2003) Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). *Evolution* 57:894–904
- Huey RB (1982) Temperature, physiology, and the ecology of reptiles. In: Gans C, Pough FH (eds) *Biology of the Reptilia*. Academic Press, New York, pp 25–91
- Hughes M (1996) Size assessment via a visual signal in snapping shrimp. *Behav Ecol and Sociobiol* 38:51-57
- Hurd PL, Enquist M (2005) A strategic taxonomy of biological communication. *Anim Behav* 7:1155-1170
- Husak JF, Macedonia JM, Fox SF, Saucedo RC (2006) Predation cost of conspicuous male coloration in collared lizards (*Crotaphytus collaris*): an experimental test using clay-covered model lizards. *Ethology* 112:572-580
- Huyghe K, Vanhooydonck B, Scheers H, Molina-Borja M, Van Damme R (2005) Morphology, performance, and fighting capacity in male lizards, *Gallotia galloti*. *Funct Ecol* 19:800-807
- Irschick DJ, Briffa M, Podos J (2015) *Animal signaling and function: an integrative approach*. Wiley-Blackwell, Hoboken, New Jersey

References

- Irschick DJ, Ramos M, Buckley C, Elstrott J, Carlisle E, Lailvaux SP, Bloch N, Herrel A, Vanhooydonck B (2006) Are morphology-performance relationships invariant across different seasons? A test with the green anole lizard (*Anolis carolinensis*). *Oikos* 114:49-59
- Irschick DJ, Vitt LJ, Zani PA, Losos JB (1997) A comparison of evolutionary radiations in mainland and Caribbean *Anolis* lizards. *Ecology* 78:2191-2203
- Irwin DE, Thimgan MP, Irwin JH (2008) Call divergence is correlated with geographic and genetic distance in greenish warblers *Phylloscopus trochiloides*: A strong role for stochasticity in signal evolution? *J Evol Biol* 21:435-48
- Itescu Y, Schwarz R, Meiri S, Pafilis P (2016) Intra-specific competition, not predation, drives lizard tail loss on islands. *J Anim Ecol* (Accepted Author Manuscript)
- Jackman TR, Irschick DJ, de Queroz K, Losos JB, Larson A (2002) Molecular phylogenetic perspective on evolution of the lizards of the *Anolis grahami* series. *J Exp Zool B Mol Dev Evol* 294:1-16
- Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: a comparison of body condition indices. *Oikos* 77:61-67
- Jaksic FM, Greene ER (1984) Correlates of tail losses in twelve species of *Liolaemus* lizards. *J Herpetol* 14:137-141
- Jennions MD, Backwell PRY (1996) Residency and size affect fight duration and outcome in the fiddler crab *Uca annulipes*. *Biol J Linn Soc* 57:293-306
- Jenssen TA (1970a) The ethoecology of *Anolis nebulosus*. *J Herpetol* 4:1-38
- Jenssen TA (1970b) Female response to filmed displays of *Anolis nebulosus* (Sauria, Iguanidae). *Anim Behav* 18:640-647
- Jenssen TA (1977) Evolution of anoline lizard display behavior. *Am Zool* 17:203-215
- Jenssen TA (1978) Display diversity in anoline lizards and problems in interpretation. In: Greenberg N, MacLean PD (eds) *Behavior and neurology of lizards*. NIMH, Rockville, pp 26
- 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, Lovern M, Congdon JD (2001) Fieldtesting the protandry-based mating system for the lizard *Anolis carolinensis*: does the model organism have the right model? *Behav Ecol Sociobiol* 50:162–172

Jenssen TA, Orrell KS, Lovern MB (2000) Sexual dimorphisms in aggressive signal structure and use by a polygynous lizard. *Anolis carolinensis*. *Copeia* 2000:140–149

Johansson BG, Jones TM (2007) The role of chemical communication in mate choice. *Biol Rev* 82:265–289

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: “backup signals” and “multiple messages.” *Phil Trans R Soc B* 35:329–338

Johnstone RA (1997) The evolution of animal signals. In: Krebs JR, Davies NB (eds) *Behavioural Ecology: an evolutionary approach*. Blackwell Publishing, Oxford, pp 155–178

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

Jones G (1997) Acoustic signals and speciation: the roles of natural and sexual selection in the evolution of cryptic species. *Adv Study Behav* 26:317–354

Jones IL, Hunter FM (1993) Mutual sexual selection in a monogamous seabird. *Nature* 362:238–239

Jones IL, Hunter FM (1999) Experimental evidence for mutual inter- and intrasexual selection favouring a crested auklet ornament. *Anim Behav* 57:521–528

Kaiser HF (1970) A second generation Little Jiffy. *Psychometrika* 35:401–415

Kamilar JM, Cooper N (2013) Phylogenetic signal in primate behavior, ecology and life history. *Phil Trans R Soc B* 368:20120341

References

- Karsten KB, Andriamandimbiarisoa LN, Fox SF, Raxworthy CJ (2009) Sexual selection on body size and secondary sexual characters in 2 closely related, sympatric chameleons in Madagascar. *Behav Ecol* 20:1079-1088
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol Biol Evol* 30:772-780
- Kennedy MW, Nager RG (2006) The perils and prospects of using phytohaemagglutinin in evolutionary ecology. *Trends Ecol Evol* 21:653-655
- Keyser AJ, Hill G (2000) Structurally based plumage coloration is an honest signal of quality in male blue grosbeaks. *Behav Ecol* 11:202-209
- Klomp DA, Ord TJ, Das I, Diesmos A, Ahmad N, Stuart-Fox D (2016) Ornament size and colour as alternative strategies for effective communication in gliding lizards. *J Evol Biol* 29:1689-1700
- 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. *P Natl Acad Sci USA* 103:8733-8738
- Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature* 431:177-181
- Kolbe JJ, Leal M, Schoener TW, Spiller DA, Losos JB (2012). Founder effects persist despite adaptive differentiation: a field experiment with lizards. *Science* 335:1086-1089
- Kraaijeveld K, Kraaijeveld-Smit FJL, Komdeur J (2007) The evolution of mutual ornamentation. *Anim Behav* 74:657-677
- Kriner E, Schwabl H (1991) Control of winter song and territorial aggression of female robins (*Erithacus rubecula*) by testosterone. *Ethology* 87:37-44
- Kwiatkowski MA (2003) Variation in conspicuousness among populations of an Iguanid lizard, *Sauromalus obesus* (= *ater*). *Copeia* 2003:481-492
- Lachmann M, Számadó SZ, Bergstrom CT (2001) Cost and conflict in animal signals and human language. *P Natl Acad Sci USA* 98:13189-13194

Lailvaux SP, Irschick DJ (2007) The evolution of performance-based male fighting ability in Caribbean *Anolis* lizards. *Am Nat* 170:573-586

Lailvaux SP, Gilbert RL, Edwards JR (2012) A performance-based cost to honest signalling in male green anole lizards (*Anolis carolinensis*). *Proc R Soc B Biol Sci* 279:2841-2848

Lailvaux SP, Hathway J, Pomfret J, Knell RJ (2005). Horn size predicts physical performance in the dung beetle *Euoniticellus intermedius*. *Funct Ecol* 19:32-639

Lailvaux SP, Herrel A, Vanhooydonck B, Meyers JJ, Irschick DJ (2004) Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). *Proc R Soc B Biol Sci* 271:2501-2508

Lailvaux SP, Leifer J, Kircher BK, Johnson MA (2015) The incredible shrinking dewlap: signal size, skin elasticity, and mechanical design in the green anole lizard (*Anolis carolinensis*). *Ecol Evol* 5:4400-4409

Lande R (1976) Natural selection and random genetic drift in phenotypic evolution. *Evolution* 30:314-334

Lande R (1980) Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292-305

Lappin AK, Husak JF (2005) Weapon performance, not size, determines mating success and potential reproductive output in the collared lizard (*Crotaphytus collaris*). *Am Nat* 166:426-436

Le Galliard JF, Fitze PS, Ferriere R, Clobert J (2005) Sex ratio bias, male aggression, and population collapse in lizards. *P Natl Acad Sci USA* 102:18231-18236

Leal M (1999) Honest signalling during predator-prey interactions in the lizard *Anolis cristatellus*. *Anim Behav* 58:521-526

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 B Biol Sci* 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, Rodriguez-Robles JA (1995) Antipredator responses of *Anolis cristatellus* (Sauria: Polychrotidae). *Copeia* 1995:155-161

References

- Leal M, Rodríguez-Robles JA (1997a) Signaling displays during predator-prey interactions in a Puerto Rican anole, *Anolis cristatellus*. *Anim Behav* 54:1147–1154
- LeBas NR (2006) Female finery is not for males. *Trends Ecol Evol* 21:170–173
- Lee JC, Clayton D, Eisenstein S, Perez I (1989) The reproductive cycle of *Anolis sagrei* in southern Florida. *Copeia* 1989:930–937
- Lengagne T, Slater PJB (2002) The effects of rain on acoustic communication: tawny owls have good reason for calling less in wet weather. *Proc R Soc B Biol Sci* 269:2121–2125
- Leonard ML, Horn AG (2005) Ambient noise and the design of begging signals. *Proc R Soc B Biol Sci* 272:651–656
- Leuck BE (1995) Territorial defense by male green anoles: an experimental test of the roles of residency and resource quality. *Herpetol Monogr* 9:63–74
- Liang K, Zeger SL (1986) Longitudinal data analysis using generalized linear models. *Biometrika* 73:13–22
- Licht P, Gorman GC (1970) Reproductive and fat cycles in Caribbean *Anolis* lizards. *Univ Calif Publ Zool* 95:1–52
- Lissmann HW (1958) On the function and evolution of electric organs in fish. *J Exp Biol* 35:156–191
- Lister BC, Aguayo AG (1992) Seasonality, predation and the behaviour of a tropical mainland anole. *J Anim Ecol* 61:717–733
- 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 *Anolis dewlap* color. *Copeia* 4:905–910
- Losos JB (1990) Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol Monogr* 60:369–388
- Losos JB (2009) *Lizards in an evolutionary tree: Ecology and Adaptive Radiation of Anoles*, University of California Press, Berkeley, Los Angeles, London

References

- Losos JB (2011) Detective work in the West Indies: integrating historical and experimental approaches to study the evolutionary diversification of island lizards. In: Losos JB (eds) In the light of evolution: essays from the laboratory and field, Roberts and Company, Greenwood village, Colorado, pp 73-92
- Losos JB, Chu LR (1998) Examination of factors potentially affecting dewlap size in Caribbean anoles. *Copeia* 1998:430-438
- 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
- Losos JB, Schluter D (2000) Analysis of an evolutionary species-area relationship. *Nature* 408:847-850
- Losos JB, Irschick DJ, Schoener TW (1994) Adaptation and constraint in the evolution of specialization of Bahamian *Anolis* lizards. *Evolution* 48:1786-1798
- Loyau A, Jalme MS, Sorci G (2005) Intra- and intersexual selection for multiple traits in the peacock (*Pavo cristatus*). *Ethology* 111:810-820
- Lozano GA (1994) Carotenoids, parasites, and sexual selection. *Oikos* 70:309-311
- MacArthur RH, Wilson EO (2015) *Theory of Island Biogeography*. Princeton University Press, Princeton
- MacDonald D, Echternacht AC (1991) Red-throated and gray-throated *Anolis carolinensis*: do females know the difference. *Anolis Newsletter* 4:92-100
- Mace TR (2000) Time budget and pair-bond dynamics in the Comb-crested Jacana *Irediparra gallinacea*: a test of hypotheses. *Emu* 100:31-41
- Macedonia JM, Stamps JA (1994) Species recognition in *Anolis grahami* (Sauria, Iguanidae): evidence from responses to video playbacks of conspecific and heterospecific displays. *Ethology* 98:246-264
- Macedonia JM, Clark DL, Riley RG, Kemp DJ (2013) Species recognition of color and motion signals in *Anolis grahami*: evidence from responses to lizard robots. *Behav Ecol* 24:846-852
- 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

Mahler DL, Ingram T, Revell LJ, Losos JB (2013) Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science* 341:292–295

Maia R, Eliason CM, Bitton PP, Doucet SM, Shawkey MD (2013) Pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol Evol* 4:906–913

Mani GS, Clarke BC (1990) Mutational order: a major stochastic process in evolution. *Proc R Soc B Biol Sci* 240:29–37

Marshall DC, Cooley JR (2000) Reproductive character displacement and speciation in periodical cicadas, with description of new species, 13-year *Magicicada neotredecim*. *Evolution* 54:1313–1325

Martín J, López P (2013) Effects of global warming on sensory ecology of rock lizards: increased temperatures alter the efficacy of sexual chemical signals. *Funct Ecol* 27:1332–1340

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

Martin MD, Mendelson TC (2012) Signal Divergence is Correlated with Genetic Distance and not Environmental Differences in Darters (Percidae: *Etheostoma*). *Evol Biol* 39:231–241

Mayer CM, Wahl DH (1997) The relationship between prey selectivity and growth and survival in a larval fish. *Can J Fish Aquat Sci* 54:1504–1512

Maynard-Smith J (1982) *Evolution and the Theory of Games*. Cambridge University Press, Cambridge

Maynard-Smith J (1991) Honest signalling: the Philip Sidney Game. *Anim Behav* 42: 1034–1035

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

McMann S (1998) Display behavior and territoriality in the lizard *Anolis sagrei*. Dissertation, University of Miami

- McMann S (2000) Effects of residence time on displays during territory establishment in a lizard. *Anim Behav* 59:513–522
- McMann S, Patterson AV (2012) Display behavior of resident brown anoles (*Anolis sagrei*) during close encounters with neighbors and non-neighbors. *Herpetol Conserv Biol* 7:27–37
- Means DB (1990) Geographic distribution: *Anolis sagrei*. *Herpetol Rev* 21:96
- Merkling T, Hamilton DG, Cser B, Svedin N, Pryke SR (2016) Proximate mechanisms of color variation in the frillneck lizard: geographical differences in pigment contents of an ornament. *Biol J Linn Soc* 117:503–515
- Molina-Borja M, Padron-Fumero M, Alfonso-Martin T (1998) Morphological and behavioral traits affecting the intensity and outcome of male contests in *Gallotia galloti galloti* (family Lacertidae). *Ethology* 104:314–322
- Møller AP (2011) When climate change affects where birds sing. *Behav Ecol* 22:212–217
- 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* (Volume 1). Harvard University Press, Cambridge, pp 90–147
- Moore RP, Robinson WD (2004) Artificial bird nests, external validity, and bias in ecological field studies. *Ecology* 85:1562–1567
- Murphy TG (2006) Predator-elicited visual signal: why the turquoise-browed motmot wag-displays its racketed tail. *Behav Ecol* 17:547–553
- Murphy TG, Hernández-Mucino D, Osorio-Beristain M, Montgomerie R, Omland KE (2009) Carotenoid-based status signaling by females in the tropical streak-backed oriole. *Behav Ecol* 20:1000–1006
- Nakagawa S (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav Ecol* 15:1044–1045
- Nevo E, Capranica RR (1985) Evolutionary origin of ethological reproductive isolation in cricket frogs, *Acris*. *Evol Biol* 19:147–214

Ng J, Glor RE (2011) Genetic differentiation among populations of a Hispaniolan trunk anole that exhibit geographical variation in dewlap color. *Mol Ecol* 20:4302-4317

Ng J, Kelly AL, MacGuigan DJ, Glor RE (2013b) The Role of Heritable and Dietary Factors in the Sexual Signal of a Hispaniolan *Anolis* Lizard, *Anolis distichus*. *J Hered* 104:862–873

Ng J, Landeen EL, Logsdon RM, Glor RE (2013a) Correlation between *Anolis* lizard dewlap phenotype and environmental variation indicates adaptive divergence of a signal important to sexual selection and species recognition. *Evolution* 67:573–582

Nicholson KE, Harmon LJ, Losos JB (2007) Evolution of *Anolis* lizard dewlap diversity. *PLoS ONE* 2:e274

Nicholson KE, Paterson AV, Richards PM (2000) *Anolis sagrei* (brown anole) cannibalism. *Herpetol Rev* 31:173-174

Nunez SC, Jenssen TA, Ersland K (1997) Female activity profile of a polygynous lizard (*Anolis carolinensis*): evidence of intersexual asymmetry. *Behaviour* 134:205–223

O'Neill EM, Beard KH (2011) Clinal variation in calls of native and introduced populations of *Eleutherodactylus coqui*. *Copeia* 2011:18–28

Olson VA, Owens IPF (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol Evol* 13:510–514

Ord TJ, Martins EP (2006) Tracing the origins of signal diversity in anole lizards: phylogenetic approaches to inferring the evolution of complex behaviour. *Anim Behav* 71:1411–1429

Ord TJ, Stamps JA (2009) Species identity cues in animal communication. *Am Nat* 174:585–593

Ord TJ, Charles GK, Hofer RK (2011) The evolution of alternative adaptive strategies for effective communication in noisy environments. *Am Nat* 177:54–64

Ord TJ, Peters RA, Clucas B, Stamps JA (2007) Lizards speed up visual displays in noisy motion habitats. *Proc R Soc B Biol Sci* 274:1057–1062

Ord TJ, Stamps JA, Losos JB (2010) Adaptation and plasticity of animal communication in fluctuating environments. *Evolution* 64:3134–3148

- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W (2013) Caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.2. Available at: <https://cran.r-project.org/web/packages/caper/>. Accessed April 2016
- Orrell KS, Jenssen TA (1998) Display behavior of *Anolis bahorucoensis*: an anole with a diminutive dewlap. *Caribb J Sci* 34:113–125
- Orrell KS, Jenssen TA (2002) Male mate choice by the lizard *Anolis carolinensis*: a preference for novel females. *Anim Behav* 63:1091–1102
- Orrell KS, Jenssen TA (2003) Heterosexual signaling by the lizard *Anolis carolinensis*, with intersexual comparisons across contexts. *Behaviour* 140:603–634
- 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
- Paluh DJ, Hantak MM, Saporito RA (2014) A test of aposematism in the dendrobatid poison frog *Oophaga pumilio*: The importance of movement in clay model experiments. *J Herpetol* 48:249–254
- Pan W (2002) Goodness-of-fit tests for GEE with correlated binary data. *Scand J Stat* 29:101–110
- Panhuis TM, Wilkinson GS (1999) Exaggerated eye span influences male contest outcome in stalk-eyed flies. *Behav Ecol Sociobiol* 46:221–227
- 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
- Paterson AV (2002) Effects of an individual's removal on space use and behavior in territorial neighborhoods of brown anoles *Anolis sagrei*. *Herpetologica* 58:382–393
- Paterson AV, McMann S (2004) Differential headbob displays towards neighbors and nonneighbors in the territorial lizard *Anolis sagrei*. *J Herpetol* 38:288–291

Perry G, Levering K, Girard I, Garland T Jr (2004) Locomotor performance and dominance in male *Anolis cristatellus*. *Anim Behav* 67:37–47

Peters RA, Evans CS (2003) Design of the jacky dragon visual display: signal and noise characteristics in a complex moving environment. *J Comp Physiol A* 189:447–459

Picq S, Alda F, Bermingham E, Krahe R (2016) Drift-driven evolution of electric signals in a Neotropical knifefish. *Evolution* 70:2134–44

Platt SG, Fontenot LW (1994) Geographic distribution. *Anolis sagrei*. *Herpetol Rev* 25:33

Podos J (2001) Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* 409:185–188

Pough FH, Andrews RM, Cadle JE, Savitsky AH, Wells KD (2001) *Herpetology*. Prentice Hall, Upper Saddle River

Price JJ, Yunes-Jiménez L, Osorio-Beristain M, Omland KE, Murphy TG (2008) Sex-role reversal in song? Females sing more frequently than males in the Streak-backed Oriole. *Condor* 110:387–392

Price T (1998) Sexual selection and natural selection in bird speciation. *Phil Trans R Soc B* 353:251–260

Price TD, Qvarnström A, Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. *Proc R Soc B Biol Sci* 270:1433–40

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. Available at: <http://www.R-project.org/>. Accessed April 2016

Rand AS (1967) Ecology and social organization in the iguanid lizard *Anolis lineatopus*. *Proc US Nat Mus* 122:1–79

Rand AS, Williams EE (1969). The anoles of La Palma: aspects of their ecological relationships. *Breviora* 327:1–19

Rand AS, Williams EE (1970) An estimation of redundancy and information content of anole dewlaps. *Am Nat* 104:99–103

Rangen SA, Clark RG, Hobson KA (2000) Visual and olfactory attributes of artificial nests. *Auk* 117:136–146

- Reby D, McComb K (2003) Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Anim Behav* 65:519–530
- Rendall D, Owren MJ, Ryan MJ (2009) What do animal signals mean? *Anim Behav* 78:233–240
- Rensch B (1959) *Evolution Above the Species Level*. Columbia University Press, New York
- Revell LJ (2010) Phylogenetic signal and linear regression on species data. *Methods Ecol Evol* 1:319–329
- Revell LJ (2012) Phytools: an R package for phylogenetic comparative biology (and other things). *Meth Ecol Evol* 3:217–223
- Richards-Zawacki CL, Yeager J, Bart HPS (2013) No evidence for differential survival or predation between sympatric color morphs of an aposematic poison frog. *Evol Ecol* 27:783–795
- Ricklefs RE, Bermingham E (2004) History and the species-area relationship in Lesser Antillean birds. *Am Nat* 163:227–239
- Ricklefs RE, Lovette IJ (1999) The roles of island area per se and habitat diversity in the species–area relationships of four Lesser Antillean faunal groups. *J Anim Ecol* 68:1142–1160
- Riechert S (1987) Games spiders play: Behavioural variability in territorial diputes. *Behav Ecol Sociobiol* 3:135–162
- Roberts SC, Gosling LM, Thornton EA, McClung J (2001) Scent-marking by male mice under the risk of predation. *Behav Ecol* 12:698–705
- Robinson MR, Kruuk LEB (2007) Function of weaponry in females: the use of horns in intrasexual competition for resources in female Soay sheep. *Biol Lett* 3:651–654
- Rodríguez-Robles JA (1992) Notes on the feeding behavior of the Puerto Rican Racer, *Alsophis portoricensis* (Serpentes: Colubridae). *J Herpetol* 26:100–102
- Rodríguez Schettino L (1999) *The Iguanid Lizards of Cuba*. University Press of Florida, Gainesville
- Rohwer S (1975) The social significance of avian winter plumage variability. *Evolution* 29:593–610

- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systems Biol* 61:539–542
- Rouse JW, Haas RH, Schell JA, Deering DW (1974) Monitoring vegetation systems in the Great Plains with ERTS. Third Earth Resources Technology Satellite-1 Symposium (NASA SP-351), Washington, pp 309–317
- Rowe C (1999) Receiver psychology and the evolution of multicomponent signals. *Anim Behav* 58:921–931
- Rubenstein DR, Lovette IJ (2009) Reproductive skew and selection on female ornamentation. *Nature* 462:786–789
- Rundle HD, Nosil P (2005) Ecological speciation. *Ecol Lett* 8:336–352
- Ryan MJ, Rand AS (1993) Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* 47:647–57
- Saks L, Mcgraw K, Horak P (2003) How feather color reflects its carotenoid content. *Funct Ecol* 17:555–561
- 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
- Santos JC, Cannatella DC (2011) Phenotypic integration emerges from aposematism and scale in poison frogs. *Proc Natl Acad Sci USA* 108:6175–6180
- Schleich HH, Kästle W, Kabisch K (1996) Amphibians and Reptiles of North Africa. Koeltz Scientific Books, Königstein
- Schoener TW, Schoener A (1980a) Densities sex ratios and population structure in four species of Bahamian *Anolis* lizards. *J Anim Ecol* 49:19–54
- Schoener TW, Schoener A (1980b) Ecological and demographic correlates of injury rates in some Bahamian *Anolis* lizards. *Copeia* 1980:839–850
- Schoener TW, Schoener A (1982a) Interspecific variation in home-range size in some *Anolis* lizards. *Ecology* 63:809–823
- Schoener TW, Schoener A (1982b) The ecological correlates of survival in some Bahamian *Anolis* lizards. *Oikos* 39:1–16

Schoener TW, Slade JB, Stinson CH (1982) Diet and sexual dimorphism in the very catholic lizard genus, *Leiocephalus* of the Bahamas. *Oecologia* 53:160–169

Schoener TW, Spiller DA, Losos JB (2002) Predation on a common *Anolis* lizard: can the food-web effects of a devastating predator be reversed? *Ecol Monogr* 72:383–407

Schwartz A, Henderson RW (1991) Amphibians and reptiles of the West Indies: descriptions, distributions, and natural history. University Press, Florida, Gainesville

Schwartz AK, Hendry AP (2007) A test for the parallel co-evolution of male colour and female preference in Trinidadian guppies *Poecilia reticulata*. *Evol Ecol Res* 9:71–90

Scott MP (1984) Agonistic and courtship displays of male *Anolis sagrei*. *Breviora* 479:1–22

Scott-Phillips TC (2008) Defining biological communication. *J Evol Biol* 21:387–395

Searcy WA, Nowicki S (2005) *The Evolution of Animal Communication*. Princeton University Press, New Jersey, Princeton

Seddon N (2005) Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution* 59:200–215

Seehausen O, Terai Y, Magalhaes IS, Carleton KL, Mrosso HDJ, Ryutaro M, van der Sluijs I, Schneider MV, Maan ME, Tachida H, Imai H, Okada N (2008) Speciation through sensory drive in cichlid fish. *Nature* 455:620–626

Setchell JM, Wickings J (2005) Dominance, status signals and coloration in male mandrills (*Mandrillus sphinx*). *Ethology* 111:25–30

Sexton OJ, Brown KM (1977) The reproductive cycle of an iguanid lizard *Anolis sagrei*, from Belize. *J Nat Hist* 11:241–250

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 (2007) Not all signals are equal: Male brown anole lizards *Anolis sagrei* selectively decrease pushup frequency following a simulated predatory attack. *Ethology* 113:793–801

- Simon V (2011) Communication signal rates predict interaction outcome in the brown anole lizard, *Anolis sagrei*. *Copeia* 2011:38-45
- Skutch AF (1949) Life history of the Yellow-thighed Manakin Auk 66:1-24
- Smiseth PT, Örnborg J, Andersson S, Amundsen T (2001) Is male plumage reflectance correlated with paternal care in bluethroats? *Behav Ecol* 12:164–170
- Smith JM, Harper D (2003) *Animal signals*. Oxford University Press, Oxford
- Snell-Rood EC (2012) The effect of climate on acoustic signals: does atmospheric sound absorption matter for bird song and bat echolocation? *J Acoust Soc Am* 131: 1650–1658
- Sokal RR, Rohlf FJ (1995) *Biometry: The Principles and Practices of Statistics in Biological Research*. 3rd edn. Freeman WH, New York
- Stamps JA (1977) The relationship between resource competition, risk, and aggression in a tropical territorial lizard. *Ecology* 58:349–358
- Stamps JA, Losos JB, Andrews RM (1997) A comparative study of population density and sexual size dimorphism in lizards. *Am Nat* 149:64–90
- Stapley J, Wordley C, Slate J (2011) No evidence of genetic differentiation between anoles with different dewlap color patterns. *J Hered* 102:118-124
- Steffen JE (2009) Perch-height specific predation on tropical lizard clay models: implications for habitat selection in mainland neotropical lizards. *Rev Biol Trop* 57:859-864
- 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* 2:239–246

Steinberg DS, Losos JB, Schoener TW, Spiller DA, Kolbe JJ, Leal M (2014) Predation-associated modulation of movement-based signals by a Bahamian lizard. *Proc Natl Acad Sci* 25:9026-9027

Stevens M (2013) Signalling and Communication. In: Stevens M (eds) *Sensory ecology, behaviour, and evolution*. Oxford University Press, Oxford, pp 73–88

Stuart-Fox DM, Ord TJ (2004) Sexual selection, natural selection and the evolution of dimorphic coloration and ornamentation in agamid lizards. *Proc R Soc B Biol Sci* 271:2249–2255

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

Stuart-Fox DM, Moussalli A, Whiting MJ (2007) Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. *Am Nat* 170:916-930

Számadó S (1999) The validity of the handicap principle in discrete action-response games. *J Theor Biol* 198:593-602

Számadó S (2003) Threat displays are not handicaps. *J Theor Biol* 221:327–348

Számadó S (2011) The cost of honesty and the fallacy of the handicap principle. *Anim Behav* 81:3-10

Takahashi M, Arita H, Hiraiwa-Hasegawa M, Hasegawa T (2008) Peahens do not prefer peacocks with more elaborate trains. *Anim Behav* 75:1209–1219

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst Biol* 56:564–577

Telemeco RS, Baird TA, Shine R (2011) Tail waving in a lizard (*Bassiana duperreyi*) functions to deflect attacks rather than as a pursuit-deterrent signal. *Anim Behav* 82:369-375

Thompson FR, Burhans DE (2004) Differences in predators of artificial and real songbird nests: Evidence of bias in artificial nest studies. *Conserv Biol* 18:373-380

- Tobias JA, Montgomerie R, Lyon BE (2012) The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. *Phil Trans R Soc B* 367:2274-2293
- Tokarz RR (1985) Body size as a factor determining dominance in staged agonistic encounters between Male Brown Anoles (*Anolis sagrei*). *Anim Behav* 33:746-753
- Tokarz RR (1987) Effects of cortisone treatment on male aggressive behavior in a lizard (*Anolis sagrei*). *Horm Behav* 21:358-370
- Tokarz RR (1998) Mating pattern in the lizard, *Anolis sagrei*: implications for mate choice and sperm competition. *Herpetologica* 54:388-394
- Tokarz RR (2002) An experimental test of the importance of the dewlap in male mating success in the lizard *Anolis sagrei*. *Herpetologica* 58:87-94
- 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
- Tokarz RR, Paterson AV, McMann S (2003) Laboratory and field test of the functional significance of the male's dewlap in the lizard *Anolis sagrei*. *Copeia* 3:502-511
- Tokarz RR, Paterson AV, McMann S (2005) Importance of dewlap display in male mating success in free-ranging Brown Anoles (*Anolis sagrei*). *J Herpetol* 39:174-177
- Tokarz RR, McMann S, Seitz L, John-Alder H (1998) Plasma corticosterone and testosterone levels during the annual reproductive cycle of male brown anoles (*Anolis sagrei*). *Physiol Biochem Zool* 71:139-146
- Tokarz RR, McMann S, Smith LC, John-Alder H (2002) Effects of testosterone treatment and season on the frequency of dewlap extensions during male-male interactions in the lizard *Anolis sagrei*. *Horm Behav* 41:70-79
- Townsend DS (1989) Sexual selection, natural selection, and a fitness trade-off in a tropical frog with male parental care. *Am Nat* 133:266-272
- Trivers RL (1976) Sexual selection and resource-accurring abilities in *Anolis garmani*. *Evolution* 30:253-269

- Tso IM, Liao CP, Huang RP, Yang EC (2006) Function of being colorful in web spiders: attracting prey or camouflaging oneself? *Behav Ecol* 17:606-613
- Turnbow N (2006) Geographic distribution *Anolis sagrei*. *Herpetol Rev* 37:361
- Turner FB, Medica PA, Jennrich RI, Maza BG (1982) Frequencies of broken tails among *Uta stansburiana* in Southern Nevada and a test of the predation hypothesis. *Copeia* 1982:835-840
- 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, Meyers JJ, Irschick DJ (2009) What determines dewlap diversity in *Anolis* lizards? An among-island comparison. *J Evol Biol* 22:293-305
- 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, Irschick DJ (2006) The quick and the fast: the evolution of acceleration capacity in *Anolis* lizards. *Evolution* 60:2137-2147
- 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, Huyghe K, Holáňová V, Van Dongen S, Herrel A (2015) Differential growth of naturally and sexually selected traits in an *Anolis* lizard. *J Zool* 296:231-238
- Vehrencamp S (2000) Handicap, index, and conventional signal elements of bird song. In: Espmark Y, Amundsen T, Rosenquist G (eds) *Signalling and Signal Design in Animal Communication*. Tapir Academic Press, Trondheim, pp 277-300

- 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
- Watson NL, Simmons LW (2010a) Reproductive competition promotes the evolution of female weaponry. *Proc R Soc B Biol Sci* 277:2035–2040
- Watson NL, Simmons LW (2010b) Mate choice in the dung beetle *Onthophagus sagittarius*: are female horns ornaments? *Behav Ecol* 21:424–430
- Webster TP, Burns JM (1973) Dewlap color variation and electrophoretically detected sibling species in a Haitian lizard, *Anolis brevirostris*. *Evolution* 27:368–377
- Weiss SL (2002) Reproductive signals of female lizards: pattern of trait expression and male response. *Ethology* 108:793–813
- West-Eberhard MJ (1983) Sexual selection, social competition and speciation. *Q Rev Biol* 58:155–183
- West-Eberhard MJ (1984) Sexual selection, competitive communication and species-specific signals in insects. *Insect communication* 283–324
- Whittaker RJ, Fernández-Palacios JM (2007) *Island biogeography: ecology, evolution, and conservation*. Oxford University Press, Oxford
- Wilczynski W, Ryan MJ (1999) Geographic variation in animal communication systems. In: Foster SA, Endler J (eds) *Geographic Diversification of Behavior: An Evolutionary Perspective*. Oxford University Press, Oxford, pp 234–261
- Wilkins MR, Seddon N, Safran RJ (2013) Evolutionary divergence in acoustic signals: causes and consequences. *Trends Ecol Evol* 28:156–166
- Williams EE (1972) The origin of faunas. Evolution of lizard congeners in a complex island fauna: a trial analysis. *Evol Biol* 6:47–89
- Williams EE, Rand AS (1977) Species recognition, dewlap function and faunal size. *Am Zool* 17:261–270
- Yee J, Lee J, Desowitz A, Blumstein DT (2013) The Costs of Conspecifics: Are Social Distractions or Environmental Distractions More Salient? *Ethology* 19:480–488
- York JR, Bairds TA (2016) Juvenile collared lizards adjust tail display frequency in response to variable predatory threat. *Ethology* 122:37–44

References

Zahavi A (1975) Mate selection: a selection for handicap. *J Theor Biol* 53:205–214

Zahavi A (1977) The cost of honesty: further remarks on the handicap principle. *J Theor Biol* 67:603–605

Zuk M, Ligon JD, Thornhill R (1992) Effects of experimental manipulation of male secondary sex characters on female mate preference in Red Junglefowl. *Anim Behav* 44:999–1006

Zuk M, Tinghitella RM (2008) Rapid evolution and sexual signals. In: d'Ettorre P, Hughes DP (eds) *Sociobiology of Communication*. Oxford University Press, Oxford, pp 139-156

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until being strong is the only option you have.*

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SCIENTIFIC CURRICULUM VITAE

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- 2014 Driessens T., Vanhooydonck B. and Van Damme R. (2014) Detering predators, daunting opponents or drawing partners? Signaling rates across diverse contexts in the lizards *Anolis sagrei*. *Behavioural ecology and social biology*, 68:173-184.
- 2011 Robert E. M. R., Schmitz N., Boeren I., Driessens T., Herremans K., De Mey J., Van de Castele E., Beeckman H. and Koedam N. (2011) Successive *Cambia*: a developmental oddity or an adaptive structure? *Plos One*, 6(1):e16558.
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APPENDIX

Supporting information (chapters 4 - 6)

Table A.1 Genetic populations with corresponding GenBank accession numbers of the specimens used to assess phylogenetic relationships among our 17 *A. sagrei* study populations. Coordinates of sampling locality are additionally provided for each study population.

Fig. A.1 Representation of the phylogenetic relationships among our *A. sagrei* study populations. The obtained Bayesian tree is based on mtDNA (ND2 and tRNA's) haplotypes from Kolbe et al. (2004). These authors sampled *A. sagrei* on Andros, Crooked, Grand Bahama, Grand Cayman, Little Cayman, San Salvador, Cuba, South Abaco, South Bimini and Staniel Cay and we assume that our study populations occurring on these islands had the same genetic identity. No specific information was listed for Acklins, Chub Cay and Pidgeon Cay, and we therefore assumed that lizards of these study populations are closest related to specimens collected from the nearest islands, i.e. Crooked Island, Berry Islands and Staniel Cay, respectively (R.G. Reynolds, personal communication). Notably, for Acklins, we incorporated the genetic sequence of a specimen from Crooked Island and this specimen was different from the one we used to represent our Crooked Island study population; the same working strategy was adopted for Chub Cay and Pidgeon Cay. For Jamaica, Kolbe et al. (2004) described the co-occurrence of two haplotypes and possible hybridization. We performed separate analyses with either of the haplotypes, which resulted in the same phylogenetic tree. Consequently, we selected randomly one haplotype, as a representative for our Jamaican study population. All details of the used *A. sagrei* specimens from Kolbe et al. (2004) with corresponding GenBank accession numbers and sampling localities are provided in Table A.1.

Table A.1

Species	Study pop.	Coordinates (study pop.)	Genetic pop. (Kolbe et al. 2004)	Accession nr. (Kolbe et al. 2004)
Outgroup				
<i>Anolis bremeri</i>	-	-	Las Canas, Cuba	AY655165
<i>Anolis homolechis</i>	-	-	La Habana, Cuba	AY655164
<i>Anolis quadriocellifer</i>	-	-	El Cayuco, Cuba	AY655168
Ingroup				
<i>Anolis sagrei</i>	Acklins	22°41'22.61"N 73°58'55.49"W	Crooked Island, Bahamas	AY655373
<i>Anolis sagrei</i>	Andros	24°42'16.71"N 77°47'14.51"W	South Andros, Bahamas	AY655390
<i>Anolis sagrei</i>	Cayman Brac	19°41'11.37"N 79°52'27.18"W	Cayman Brac	AY655410
<i>Anolis sagrei</i>	Chub Cay	25°24'59.56"N 77°53'35.84"W	Berry Islands, Bahamas	AY655386
<i>Anolis sagrei</i>	Crooked Island	22°45'18.88"N 74°11'48.19"W	Crooked Island, Bahamas	AY655372
<i>Anolis sagrei</i>	Grand Bahama	26°31'50.77"N 78°39'41.62"W	Grand Bahama, Bahamas	AY655400
<i>Anolis sagrei</i>	Grand Cayman	19°17'2.03"N 81°22'24.33"W	George Town, Grand Cayman	AY655478
<i>Anolis sagrei</i>	Jamaica	18°30'24.81"N 77°42'58.25"W	Portland, Jamaica	AY655430
<i>Anolis sagrei</i>	Little Cayman	19°39'50.58"N 80° 5'0.39"W	Little Cayman	AY655419
<i>Anolis sagrei</i>	Pidgeon Cay	24° 9'35.17"N 76°26'6.71"W	Staniel Cay, Bahamas	AY655381
<i>Anolis sagrei</i>	San Salvador	24° 7'2.60"N 74°27'52.23"W	San Salvador, Bahamas	AY655376
<i>Anolis sagrei</i>	Santa Clara	22°24'8.81"N 80° 0'6.95"W	Santa Clara, Cuba	AY655354
<i>Anolis sagrei</i>	Soroa 1	22°48'26.60"N 83° 1'6.60"W	Soroa, Cuba	AY655178
<i>Anolis sagrei</i>	Soroa 2	22°46'40.00"N 83° 0'7.70"W	Soroa, Cuba	AY655181
<i>Anolis sagrei</i>	South Abaco	26°6'12.8" N 77°11'2" W	Great Abaco, Bahamas	AY655394
<i>Anolis sagrei</i>	South Bimini	25°42'11.8" N 79°18'0.5" W	South Bimini, Bahamas	AY655394
<i>Anolis sagrei</i>	Staniel Cay	24°10'22.40"N 76°26'40.98"W	Staniel Cay, Bahamas	AY655378

Abbreviations: pop. = population, nr. = number

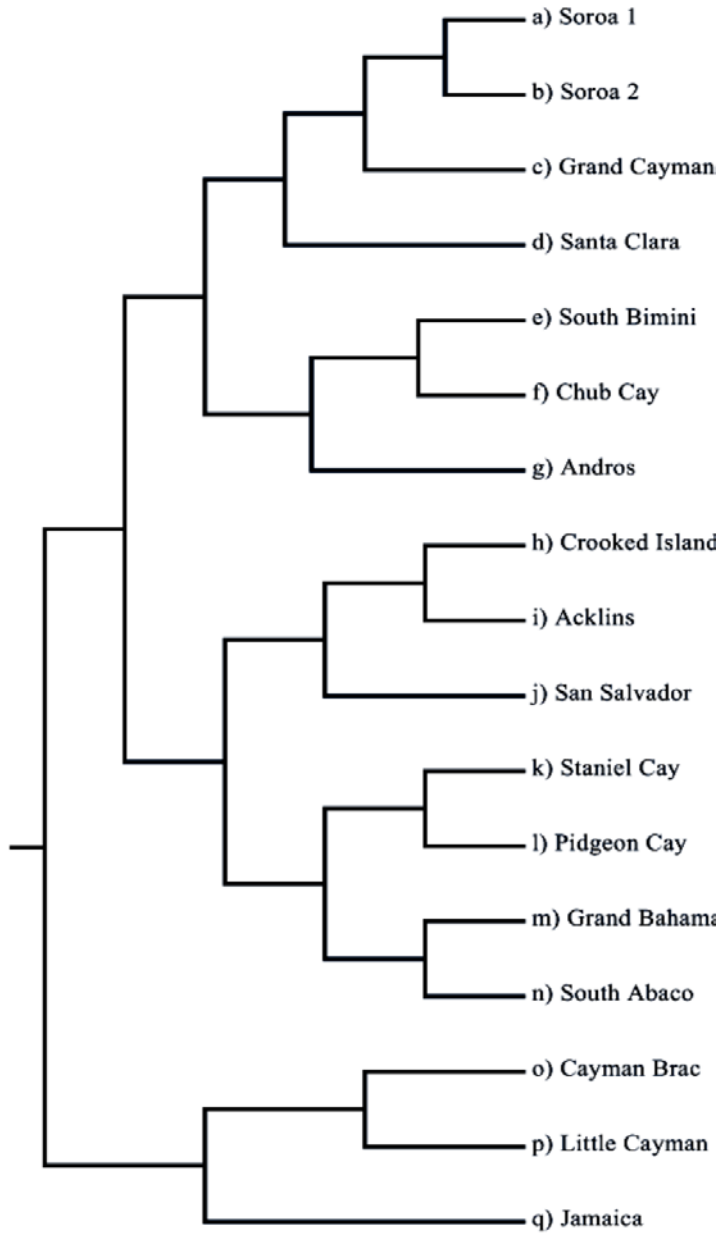


Fig. A.1



*Anolis homolechis on Cuba,
photo taken by S. De Decker*

*Although Nature needs thousands or millions of
years to create a new species, man needs only a few
dozen years to destroy one.*

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