

# The biomechanical basis of evolutionary change in a territorial display

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## Summary

1. Few studies have examined how the anatomy of an animal signal contributes to, or limits, the evolution of signal differentiation among closely related species.

2. In *Anolis* lizards, adult males extend a large, conspicuous dewlap as part of a territorial advertisement display. Males of species from the island of Jamaica rely on the rapid extension of the dewlap to facilitate display detection by territorial neighbours and conspecific females. Males of other species on the island of Puerto Rico extend the dewlap at considerably slower speeds and instead rely on other strategies to maintain an effective display. What initially prompted this divergence in display between the islands is unknown, but evidence suggests that it may have something to do with the way the dewlap is extended during display.

3. Our goal was to determine whether an innovation in the dewlap lever in Jamaican lineages, but absent in Puerto Rican lineages, accounted for the evolution of the high-speed dewlap on Jamaica. We began by modelling the performance of the *Anolis* dewlap as a first-order lever system. We then simulated changes to this system relating to its morphology, articulation and input force in biologically realistic ways to predict how such changes impact dewlap speed. Finally, we compared these predictions with data on the morphology of the dewlap lever system from museum specimens and actual dewlap speed recorded for lizards in the field.

4. This investigation revealed that changes to the dewlap lever have affected the performance of the dewlap display in at least two ways. Within islands, structural changes to the hyoid morphology seem to have led to differences in dewlap speed among species. Between islands, however, differences in dewlap speed were most likely the result of a major increase in muscle contraction velocity that powers the dewlap extension in Jamaican species, but has not originated in species on Puerto Rico.

5. Our study shows how the biomechanics of a social signal can have important implications for understanding why closely related species might differ in signal behaviour, despite apparent similarities in the selection pressures that act on the signal.

**Key-words:** *Anolis*, dewlap morphology, evolutionary differentiation, hyoid apparatus, island divergence, visual signal

## Introduction

What accounts for the remarkable diversity of animal signals has been a topic of long-standing interest for communication biologists and evolutionary ecologists alike. The way in which social signals differ among closely related taxa can provide valuable clues to the function and evolu-

tion of those signals, such as the key components that convey information to receivers (Campbell *et al.* 2010; Grant & Grant 2010; Scott *et al.* 2010; Cardoso & Hu 2011) or facilitate the reception of signals in different environments (Dingle, Halfwerk & Slabberkoorn 2008; Tobias *et al.* 2010; Ord & Gracia-Porta 2012). Of special interest to many researchers is the role of divergence in social communication among populations – especially signals that mediate access to mates – in driving reproductive isolation

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and the subsequent evolution of new species (Boughman 2002; Price 2008; Seehausen *et al.* 2008; Verzijden *et al.* 2012). We now have an impressive amount of information on why animal signals differ among populations and species (Bradbury & Vehrencamp 2011), and empirical evidence for the role of animal communication in speciation is mounting (e.g. Seehausen *et al.* 2008; Grant & Grant 2010). However, this research has focussed almost exclusively on the evolution of the outward phenotype of the signal, with little consideration of the structural anatomy that underlies it. Selection might favour specific types of changes in signals, but those changes must fall within the bounds of motor patterns and morphological traits that can be readily modified. This is especially relevant for dynamic signals, such as vocalizations or body movements making up visual displays, because the production of this class of signal is often constrained by morphology and physiology (Suthers & Margoliash 2002; Riede *et al.* 2006; indeed, conveying information on physiological limits can be an important function of so-called ‘honest’ signals: Zahavi & Zahavi 1997).

Mechanistic perspectives are certainly not lacking in communication research – quite the contrary (e.g. see Nava, Conway & Martins 2009; Henningsen & Irschick 2012; and reviews by Lailvaux & Irschick 2006 and Irschick *et al.* 2007) – but where this approach has been adopted, it generally focuses on model organisms and with limited consideration (if any) of how biomechanical constraints affect signal performance and differentiation among taxa. With the emergence of the ‘sensory drive’ concept in animal communication (*sensu* Endler 1992), many researchers turned their attention to the sensory physiology of receivers and its role in signal divergence (e.g. Ryan 1986; Wilczynski, Rand & Ryan 2001; Nava, Conway & Martins 2009; Tobias *et al.* 2010; Ord 2012). By contrast, similar acknowledgement that the physiology of signallers is also important has been more limited. For example, recent reviews of animal communication often discuss the role of receiver sensory systems in shaping signal design (Hebets & Papaj 2005; Brumm & Naguib 2009; Bro-Jørgensen 2010; Bradbury & Vehrencamp 2011; Miller & Bee 2012), but rarely mention any consequence that the physical anatomy of signallers might have on the evolution of signal diversity. This represents an important gap in our understanding of how the biomechanics of signal production contribute to, or limit, signal evolution.

In rare cases where studies have explicitly considered these evolutionary implications, the evolutionary dynamics of the communication system in question and the origin of its diversity have become much clearer. For example, the complex song produced by different species of Darwin’s finch depends on the morphology of the beak: big-beaked birds are mechanically constrained to produce simple song designs, while smaller-beaked birds are not (Podos 2001). The evolution of beak morphology has itself been shaped by diet: large-beaked birds specialize on cracking open large, hard seeds, while small-beaked birds feed on small,

soft seeds or insects that require precision in handling (reviewed in Grant & Grant 2008). That is, to a large extent, the origin of song diversity in Darwin’s finches has been an evolutionary by-product of adaptations in beak morphology to different food resources among species and not direct selection on song specifically. Nevertheless, the differentiation that has resulted in songs following changes in beak morphology has probably played a central role in speciation because song design is a key mechanism enforcing reproductive isolation among divergent finch populations (Grant & Grant 1996, 2009). Identifying the origin of song diversity in Darwin’s finches was only possible through an understanding of the biomechanics of song production (Podos *et al.* 1995; Podos 1997) and its study in relation to signal diversity and ecology across species (Podos 2001). Similar mechanistic divergences in beak morphology and correlated changes in song structure have now been documented in other birds (Badyaev *et al.* 2008; Derryberry 2009).

As the examples above would suggest, those few studies that have investigated the biomechanics of signal production in an evolutionary setting have generally been restricted to the acoustic domain. Our understanding of the biomechanics of movement-based displays is far more limited by comparison, despite the obvious dependency of animal movement on anatomy. In particular, there has been almost no study of how the biomechanics of movement might account for performance differences in display among closely related species (we know of only one example: Johnson & Wade 2010). We report such a study here on the elaborate visual display of territorial *Anolis* lizards. We investigated how the biomechanics underlying a conspicuous component of the territorial display – the extension of a large throat fan or dewlap – might account for striking differences in display performance observed among species.

The *Anolis* dewlap has been of special interest to evolutionary ecologists because of its putative role in speciation (Streelman & Danley 2003; Losos 2009; see below). The genus is exceptional in species richness, with nearly 400 species described throughout the Caribbean and Americas (Losos 2009). The *Anolis* mating system is centred on adult males establishing large territories that overlap the home ranges of females in an effort by males to monopolize mating opportunities (Stamps 1983; Jensen & Nunez 1998; Jenssen, Lovern & Congdon 2001). Males defend territories vigorously against neighbour intrusions using elaborate species-typical visual displays of headbobs and the extension of an often large and brightly coloured dewlap (Ord 2008). In other lizards, strenuous body movements such as headbobs have been shown to convey reliable information on physical condition and potential fighting ability (Brandt 2003; Ord & Evans 2003; Brandt & Allen 2004; Perry *et al.* 2004). The function of the dewlap is less clear. There are a variety of hypotheses suggesting that the colour or size of the dewlap conveys important cues on species identity (Losos

1985; Vanhooydonck *et al.* 2009) and potential fighting ability (Vanhooydonck *et al.* 2005), but experimental evidence has been conflicting (Ord & Stamps 2009; Henningsen & Irschick 2012; Lailvaux, Gilbert & Edwards 2012). What is clear, however, is that the dewlap is important for signal detection (Fleishman 2000; Leal & Fleishman 2004; Fleishman, Leal & Persons 2009), both for the detection of the dewlap itself and the headbobbing display that often accompanies it (Ord & Stamps 2008). Without reliable detection, the information that might be conveyed by a signal is lost. Difficulty in detecting territorial displays is an acute problem for *Anolis* lizards because seeing displays becomes increasingly difficult with decreases in ambient light (e.g. in shade) and increases in distracting motion or visual 'noise' from windblown vegetation (Ord *et al.* 2007; Ord & Stamps 2008; Ord, Stamps & Losos 2010). The rapid extension of a dewlap or dewlap like flag has been shown to enhance display detection in these situations (Persons *et al.* 1999; Fleishman & Persons 2001; Ord & Stamps 2008).

The speed at which the dewlap is extended varies among species. For example, *A. gundlachi* on Puerto Rico extends the dewlap slowly at the start of the headbob display and only retracts it after the headbobs have finished, whereas *A. lineatopus* on Jamaica extend the dewlap rapidly and frequently, with and without accompanying headbob movements. Both species have large, pale yellow dewlaps. That is, there is little relation between the size or colour of the dewlap and the speed at which it was used in display (Ord, Charles & Hoffer 2011). Different species do, however, communicate in different environments, and these environments vary in the adverse conditions that affect signal detection. This accounts for a portion of the variation in dewlap speed among closely related species (Ord, Stamps & Losos 2010). However, dramatic differences in dewlap speed remain between species from Jamaica and Puerto Rico: Jamaicans generally produce high-speed dewlap extensions, whereas Puerto Ricans do not (e.g. see Fig. 3).

It seems that Jamaican *Anolis* rely on a high-speed dewlap to ensure the production of a conspicuous territorial display regardless of the viewing conditions of the habitat they occupy (Ord, Charles & Hoffer 2011). Puerto Rican *Anolis*, on the other hand, do not extend the dewlap as rapidly as the Jamaican species and have instead evolved a range of alternative strategies for enhancing display detection. These include tailoring the speed and duration of displays to match the conditions at the time of display (Ord *et al.* 2007; Ord, Stamps & Losos 2010) and selectively timing displays to avoid periods of high visual noise (Ord, Stamps & Losos 2010; Ord, Charles & Hoffer 2011). These contrasting communication strategies may not be limited to Jamaica and Puerto Rico, but reflect deeper divergences in the trajectory of display evolution in the *Anolis* phylogeny. The Jamaican clade is nested within the broader Western Caribbean radiation and shares elements of display design with other species

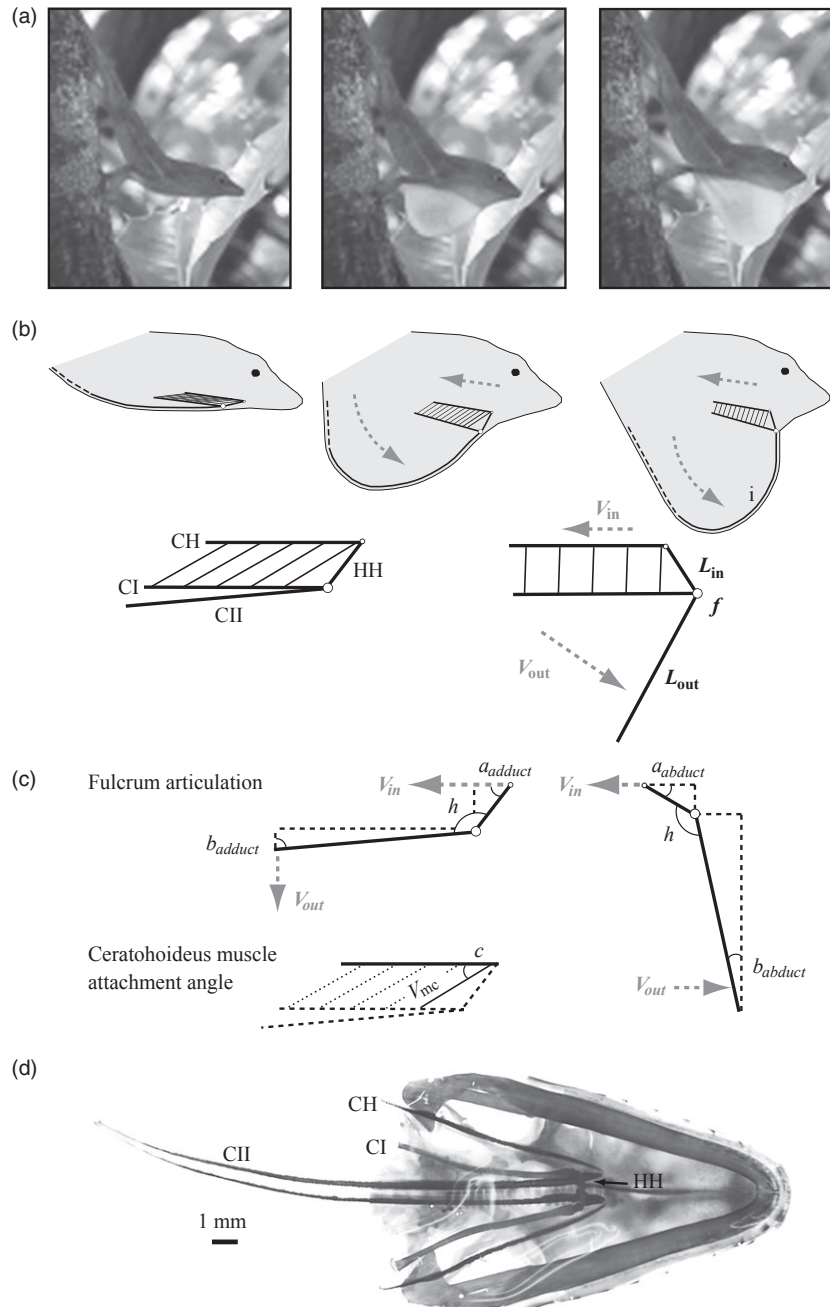
belonging to the same radiation (e.g. from Cuba and the Cayman Islands); the Puerto Rican clade belongs to the Eastern Caribbean radiation and shares elements of display design with other species belonging to that radiation (e.g. Hispaniola; Ord 2012). What initially prompted this divergence in the trajectory of display evolution deep in the anole phylogeny remains unknown, but it seems related to how the dewlap is extended in display (Ord, Charles & Hoffer 2011).

Inspired by pioneering studies on birds that have examined the biomechanics underpinning the origin of song differentiation (Podos 2001; Badyaev *et al.* 2008; Derryberry 2009), our goal was to determine whether evolutionary divergence in dewlap speed reflects an underlying structural change to the hyoid apparatus that controls the dewlap extension. Specifically, we tested the hypothesis that an adaptation in the hyoid that affects the performance of the dewlap display has allowed Jamaican species to evolve a territorial display centred on the rapid extension of the dewlap, which in effect has allowed these lizards to perform a display that is likely detectable in a range of environmental conditions (Ord & Stamps 2008; Ord, Charles & Hoffer 2011). By extension, this hypothesis also implies that Puerto Rican species are limited by the structure of the hyoid apparatus in their capacity to produce high-speed dewlap extensions. This has subsequently prompted the evolution of alternative behavioural adaptations for enhancing display detection in visually challenging environments (selective timing and plasticity in display).

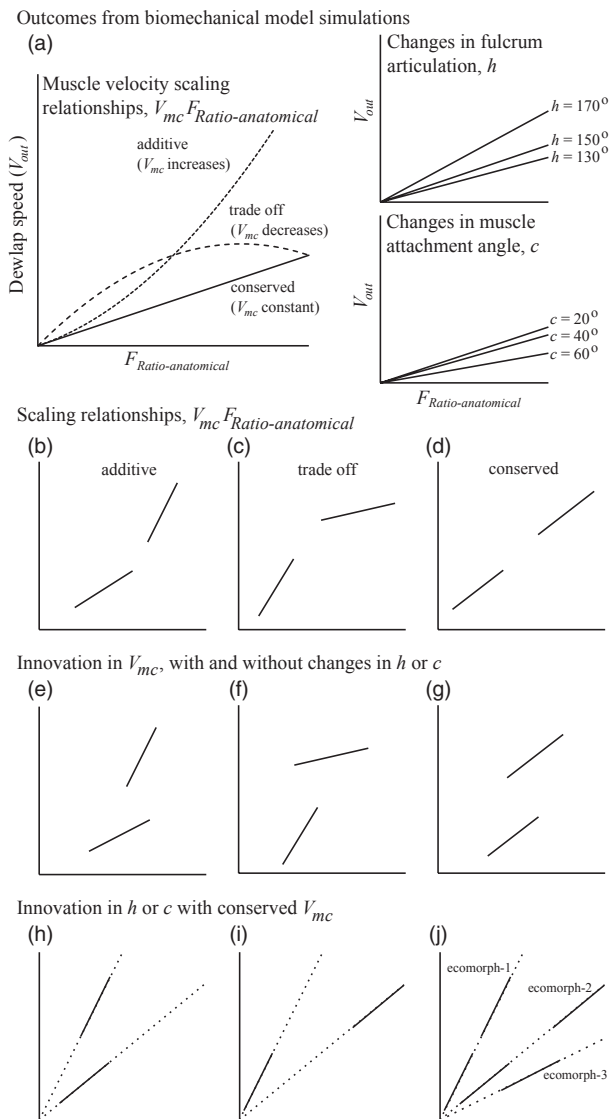
We began our study by first developing a biomechanical model that mimicked the mechanism of the dewlap extension. This model was built on a strong understanding of how the *Anolis* hyoid apparatus functioned in extending the dewlap (Bels 1990; Font & Rome 1990; O'Bryant & Wade 2002; Wade 2005; Johnson *et al.* 2011). The model identified several key components of the hyoid that dictate the maximum speed of the dewlap extension. We then simulated how evolutionary change to these components might lead to variation among species in dewlap speed and in particular the major differences in speed exhibited by lizards from Jamaica and Puerto Rico. The outcome of these simulations revealed several plausible scenarios. We subsequently formulated these scenarios into separate statistical functions and tested them against each other using a phylogenetic comparative analysis of data compiled from 11 *Anolis* species from the two islands.

## Materials and methods

Our study focussed on four Jamaican species (*A. grahami*, *A. valencienni*, *A. lineatopus* and *A. sagrei*) and 7 Puerto Rican species (*A. poncensis*, *A. gundlachi*, *A. cristatellus*, *A. pulchellus*, *A. krugi*, *A. stratulus* and *A. evermanni*). These species represent most of the *Anolis* diversity on both islands (4 of 7 and 7 of 11, respectively) and were selected to include a range of species with different dewlap speeds, body sizes and ecologies. The phylogenetic relationships among these species are presented in Fig. 3.



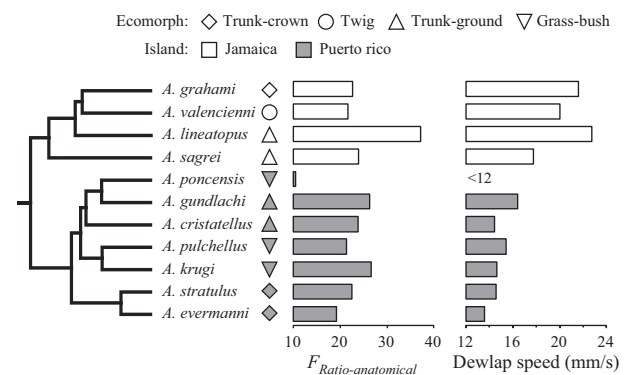
**Fig. 1.** The structural anatomy of the *Anolis dewlap* illustrated here by *A. lineatopus*. The top row (a) shows sequential video stills of an adult male extending his dewlap during a territorial advertisement display. The second row (b) provides a schematic of how the hyoid apparatus functions during the dewlap extension. Shown is the relationship of the ceratohyal (CH), the first ceratobranchial (CI), the hypohyal (HH) and the second ceratobranchial (CII) in the lever system. Dashed arrows illustrate the direction of movement or velocity.  $V_{in}$  is the amount of velocity used to move the lever system and is produced by the contraction of the muscles that bridge the ceratohyal and first ceratobranchial [NB: it is unclear whether the direction of movement is a posterior shift in the CH relative to the fixed position of the CI (as illustrated here and in Font & Rome 1990) or an anterior shift in the CI relative to the fixed position of CH (as suggested by Bels 1990)]. This contraction, combined with the rotation of the anatomical in- and out-levers ( $L_{in}$  and  $L_{out}$ ) around the fulcrum ( $f$ , the basihyal) results in  $V_{out}$  or the speed of the dewlap extension. We used the leading edge of the dewlap ( $i$ ) to measure  $L_{out}$  (see text for details). The third row (c) illustrates the angle of articulation of the HH relative to the CII,  $h$ , and its relationship with  $a$  and  $b$ , as well as the angle of muscle fibres relative to the CH,  $c$ . Both the articulation of the lever and attachment angle of the ceratohyoideus muscles affect the performance of the dewlap lever system (see Fig. 2). The bottom row (d) shows a ventral view of a cleared and stained specimen of an adult male, highlighting the structure of the hyoid apparatus *in situ*.



### THE DEWLAP MECHANISM

Bels (1990) and Font & Rome (1990) described in detail the anatomy of the *Anolis* hyoid apparatus as it related to the extension of the dewlap, including the structural elements involved and the muscles that power the extension. These studies revealed that the hyoid operates as a first-order lever, the biomechanical properties of which are well studied (Smith & Savage 1956; Barel 1983; Wainwright & Shaw 1999; Westneat 2004). In descriptive terms, from a resting position, the contraction of the ceratohyoideus muscles between the first ceratobranchials (CI) and ceratohyals (CH) rotates the hypohyals (HH) away from the nose of the lizard, which in turn protracts and depresses the second ceratobranchials (CII), causing extension of the dewlap (Fig. 1; Bels 1990; Font & Rome 1990). In mechanical terms, the speed of dewlap extension (i.e. the output speed,  $V_{out}$ ) is a product of the following: (i) the fulcrum ratio ( $F_{ratio}$ ) which is a function of the lengths of the CII and HH and their orientations to output and input movements; and (ii) the input speed ( $V_{in}$ ) generated by ceratohyoideus muscle contraction. To create our biomechanical model, we first separated the effects of these variables on dewlap speed ( $V_{out}$ ) and then combined them into a single functional model. A glossary of terms used is provided in Table 1.

**Fig. 2.** Simulation outcomes from the biomechanical model of the dewlap lever system and corresponding predictions of how changes to the hyoid could produce island divergences in dewlap speed ( $V_{out}$ ) as a function of the anatomical fulcrum ratio ( $F_{ratio-anatomical}$ ) under different scenarios of how contraction velocity of the ceratohyoideus muscles ( $V_{mc}$ ) relates to the fulcrum, the orientation of  $L_{in}$  to  $L_{out}$  ( $h$ ) and the attachment angle of the ceratohyoideus muscles between the CH and CI ( $c$ ). The second row (b–d) illustrates how dewlap speed and  $F_{ratio-anatomical}$  should be related among species from Jamaica (upper line) and Puerto Rico (lower line) assuming additive, trade-off or conserved effects of  $V_{mc}$ . The third row (e–g) illustrates how dewlap speed and  $F_{ratio-anatomical}$  should be related if there has been an innovation in base level  $V_{mc}$  (leading to a difference in intercepts) with (e, f) or without (g) an accompanying innovation in  $h$  or  $c$  (difference in slope). The first two panels of the bottom row (h–i) illustrate how dewlap speed and  $F_{ratio-anatomical}$  should be related if there has been an innovation in the Jamaican clade relating to  $h$  or  $c$  only. The last panel of the bottom row (j) illustrates how dewlap speed and  $F_{ratio-anatomical}$  should be related if species within ecomorphs have converged in  $h$  or  $c$ .



**Fig. 3.** The phylogeny of the dewlap lever system ( $F_{ratio-anatomical}$ ) compared with the actual velocity of the dewlap extension ( $V_{out}$ : dewlap speed) measured from free-living territorial males. *A. poncensis* had a tiny dewlap making it difficult to reliably quantify the speed of the extension. Visual inspect of videos confirmed that the speed of the dewlap extension was extremely slow and certainly vastly lower than the minimum speed recorded for any other species (<12 mm s<sup>-1</sup>).

**Fulcrum ratio ( $F_{ratio}$ ):** the hyoid lever amplifies speed from the ceratohyoideus by a factor equal to the  $F_{ratio}$ , which is partly determined by the lengths of the HH and CII. Given a constant input velocity from the ceratohyoideus muscle, a decrease in the length of the HH shifts the fulcrum,  $f$ , closer to where effort is applied and allows the CII to be moved over a longer arc, resulting in the dewlap being extended at greater speed. Conversely, an increase in length of the HH shifts  $f$  away from the point where effort is applied and decreases the output speed. We refer to the lengths of HH and CII as the anatomical in- and out-levers ( $L_{in}$  and  $L_{out}$ ), respectively. When the directions of input and output movement are perpendicular to  $L_{in}$  and  $L_{out}$ , the performance of the anatomical lever can be summarized as follows:

$$F_{ratio-anatomical} = \frac{L_{out}}{L_{in}} \approx \frac{\text{CII length}}{\text{HH length}} \quad \text{eqn 1}$$

This special case does not often hold, however, and transmission of speed through the lever also depends on the angle,  $\alpha$ , between the HH and the vector of input movement,  $V_{in}$ , and the

**Table 1.** Glossary of Terms

| Term                   | Definition  |
|------------------------|---|
| <i>a</i>               | The angle of the hypohal (HH) relative to the direction of $V_{in}$ (see eqn 2).  |
| <i>b</i>               | The angle of the second ceratobranchial (CII) relative to the direction of $V_{out}$ (see eqn 2).   |
| <i>c</i>               | Angle of orientation of the ceratohyoideus muscle fibres relative to CH (see eqn 3)   |
| CH                     | Ceratohyal  |
| CI                     | First ceratobranchial   |
| CII                    | Second ceratobranchial, the leading edge of which corresponds to the out-lever of the dewlap fulcrum  |
| <i>f</i>               | The fulcrum point where the in-lever (HH) and out-lever (CII) articulate  |
| $F_{ratio-anatomical}$ | Anatomical fulcrum ratio: the length of the out-lever (CII) relative to the length of the in-lever (HH; see eqn 1)  |
| $F_{ratio-functional}$ | Functional fulcrum ratio: an elaboration of the fulcrum that incorporates the effects of <i>a</i> and <i>b</i> on $V_{out}$ (see eqns 2 and 3)  |
| <i>h</i>               | The angle of articulation between the in-lever (HH) and out-lever (CII) at the fulcrum point ( <i>f</i> ). This angle influences the relationship of <i>a</i> to <i>b</i> and <i>vice versa</i> . |
| HH                     | Hypohal, which corresponds to the in-lever of the dewlap fulcrum  |
| $L_{in}$               | The in-lever, which corresponds to the HH   |
| $L_{out}$              | The out-lever, which corresponds to the leading edge of the CII   |
| $V_{in}$               | The input velocity applied to the $L_{in}$ and powers the dewlap extension (see eqn 3)  |
| $V_{mc}$               | Contraction velocity of the ceratohyoideus muscle (see eqn 3)   |
| $V_{out}$              | The output speed of the lever system, which corresponds to the speed of the dewlap extension (see eqn 4)  |

angle, *b*, between the CII and the vector of output movement,  $V_{out}$  (Fig. 1c). Because the direction of  $V_{in}$  corresponds to the path of movement of the CH, it remains parallel to the long axis of the head during rotation of the lever (Fig. 1b). Conversely, movement of the distal portion of the CII,  $V_{out}$ , is consistently perpendicular to the CH as the CII rotates out from the throat. Assuming constancy in the starting orientation of the hyoid relative to the direction of  $V_{in}$  (i.e. a constant angle between CII and CH when the hyoid is at rest), *a* and *b* can be determined by the angle formed by the HH and CII, *h* (Fig. 1c). As the dewlap is extended, the values of *a* and *b* will continually change as HH and CII rotate around *f* for a fixed value of *h*. The functional outcome of this is that as *h* decreases for a given anatomical  $F_{ratio}$ , the proportion of  $V_{in}$  applied to  $L_{in}$  and subsequently translated to the  $L_{out}$  is progressively reduced as the dewlap is extended. We can compute the functional  $F_{ratio}$  and approximate its dynamic relationship by averaging the functional  $F_{ratio}$  for the hyoid in its adducted position (dewlap retracted) and in its abducted position (dewlap fully extended), such that:

$$\bar{F}_{ratio-functional} = \left( \frac{CII \text{ length} \times \sin b_{adduct}}{HH \text{ length} \times \sin a_{adduct}} + \frac{CII \text{ length} \times \sin b_{abduct}}{HH \text{ length} \times \sin a_{abduct}} \right) \div 2 \quad \text{eqn 2}$$

Input Velocity ( $V_{in}$ ): the relationship between skeletal gearing and output speed depends on properties of the muscle powering

the movement. We modelled input speed generated by the ceratohyoideus muscle as a function of (i) contraction velocity of the ceratohyoideus muscles,  $V_{mc}$ , and (ii) the orientation of the ceratohyoideus muscle fibres relative to the  $L_{in}$ , *c* (Fig. 1c). We modelled a constant  $V_{mc}$ , which requires that the ceratohyoideus muscle can maintain shortening velocity as leverage and resistance vary over the course of hyoid rotation (McHenry 2012a,b), such as when the load is negligible or the ceratohyoideus contracts at a rate that maximizes power. The amount of  $V_{mc}$  translated to the  $L_{in}$  depends on the orientation of the muscle relative to  $L_{in}$ : as *c* or the angle of  $V_{mc}$  relative to the CH decreases,  $V_{mc}$  becomes increasingly perpendicular to the  $L_{in}$ , and a higher proportion of  $V_{mc}$  is applied to the  $L_{in}$ , resulting in an increase in dewlap speed. This relationship is modelled as follows:

$$V_{in} = V_{mc} \cos c \quad \text{eqn 3}$$

Finally, the combined effects of the lengths of HH and CII, *h*,  $V_{mc}$  and *c* can be integrated into a single model to compute  $V_{out}$  by multiplying the functional  $F_{ratio}$  by the input velocity (i.e. eqn 2 by eqn 3):

$$\text{Dewlap display speed} = V_{out} = V_{in} \bar{F}_{ratio-functional} \quad \text{eqn 4}$$

#### SIMULATING DEWLAP PERFORMANCE

There are a number of possible scenarios for how evolutionary change might have occurred in the *Anolis* hyoid apparatus to produce higher dewlap speeds. Generally, any change in the lengths of the CII or HH ( $F_{ratio-anatomical}$ ), the angle between the HH and the CII (*h*, which influences the relationship of *a* to *b* and *vice versa*), the contraction velocity of the ceratohyoideus muscles ( $V_{mc}$ ), or the alignment of muscle fibre pennation relative to the CH (*c*), would all lead to changes in dewlap speed ( $V_{out}$ ). However, the relationship of these parameters to dewlap speed differs in important ways and some variables have the potential to exert greater influence on dewlap speed than others. To clarify these relationships, we simulated changes in  $F_{ratio-anatomical}$ , *h*,  $V_{mc}$  and *c* with the ultimate goal of identifying the conditions that might lead to prominent divergences in dewlap display speed (i.e. those documented for Jamaican and Puerto Rican *Anolis* species).

First, selection for increased dewlap speed could result in increases in both  $F_{ratio-anatomical}$  and  $V_{mc}$  or increases in  $F_{ratio-anatomical}$  at the expense of  $V_{mc}$  (and *vice versa*) because of some physiological/developmental trade-off or increases primarily in  $F_{ratio-anatomical}$  with  $V_{mc}$  held largely constant over time (or *vice versa*). We plotted these scenarios as a function of  $F_{ratio-anatomical}$  (Fig. 2a; *h* and *c* were held constant): a scenario of ‘additive’ effect in which increases in both  $F_{ratio-anatomical}$  and  $V_{mc}$  were possible was visualized by increasing  $V_{mc}$  by 0.2 units for every unit of increase in  $F_{ratio-anatomical}$  (starting values were 1 and 1, respectively); a scenario reflecting a trade-off between  $F_{ratio-anatomical}$  and  $V_{mc}$  was visualized by decreasing  $V_{mc}$  by 0.2 units for every unit of increase in  $F_{ratio-anatomical}$  (starting values were 3 and 1, respectively); and a scenario in which  $V_{mc}$  has been evolutionarily conserved was visualized by setting  $V_{mc}$  to 1 (starting values were 1-fixed and 1, respectively). NB: the specific parameter values were not especially important here (e.g. increasing the magnitude of change in  $V_{mc}$  relative to  $F_{ratio-anatomical}$  simply results in accentuating the steepness of slopes generated).

Next, we assessed the effect of evolutionary changes in *h* on dewlap speed across the same range of  $F_{ratio-anatomical}$  values (Fig. 2a, top right panel;  $V_{mc}$  and *c* were held constant). To provide a biologically relevant benchmark of *h*, we used an X-ray image of *A. carolinensis* that depicted the morphology of the hyoid apparatus with the dewlap extended (this image was presented in Losos (2009) and credited to B. Brainerd and D.

Irschick). We measured the angle of the HH to the CII using ImageJ ver 1.42q (W. Rasband 1997–2009, NIH) and obtained a value of  $150^\circ$ . We then varied  $h$  around this benchmark by  $\pm 20^\circ$  and computed the corresponding values of  $a$  and  $b$  based on the approximated position of the HH and CII relative to  $V_{in}$  and  $V_{out}$  when the hyoid is adducted and abducted (see Fig. 1b,c) and plotted the effect of changes to  $h$  on dewlap speed (Fig. 2a; simulations for additive and trade-off scenarios of  $V_{mc}$  showed the same trend of increasing slope with increasing values of  $h$ ).

Finally, we varied the angle of fibre pennation for the ceratohyoideus muscles,  $c$ , and examined the combined effects of it and  $F_{ratio-anatomical}$  on  $V_{out}$  (Fig. 2a, bottom right panel;  $V_{mc}$  and  $h$  were held constant). The only information available on ceratohyoideus fibre pennation was a descriptive note in Font & Rome (1990) stating that the fibres of the ceratohyoideus muscle in *A. equestris* were roughly parallel to the CII when the hyoid was adducted. This would correspond to a low value of  $c$ . We arbitrarily set  $c$  to  $20^\circ$  and increased its value by  $20^\circ$  increments and plotted its effect on dewlap speed (Fig. 2a; again, simulations assuming additive and trade-off scenarios of  $V_{mc}$  showed the same trend of increasing slope with decreasing values of  $c$ ).

The predictions summarized in Fig. 2(a) not only illustrate the effects of  $F_{ratio-anatomical}$ ,  $h$ ,  $V_{mc}$  and  $c$  on  $V_{out}$ , but also the distinctive patterns in the way  $V_{out}$  should change with  $F_{ratio-anatomical}$  depending on changes in  $V_{mc}$ ,  $h$  and  $c$  (Fig. 2b–j). That is, given only information on anatomical  $F_{ratio}$ , it was possible to assess whether divergences in dewlap speed between species from Jamaica and Puerto Rico reflected changes in  $F_{ratio-anatomical}$  exclusively or have been accentuated by divergences in  $V_{mc}$ ,  $h$  or  $c$  as well. This was a critical outcome from our simulations because obtaining reliable estimates of  $V_{mc}$ ,  $h$  or  $c$  was difficult (see next section).

#### QUANTIFYING $F_{RATIO-ANATOMICAL}$ , $V_{MC}$ , $H$ AND $C$

To compute  $F_{ratio-anatomical}$ , a measure of the HH was obtained using cleared and stained preserved specimens (Fig. 1d; see ‘Measurements from Preserved Specimens’). Estimating the appropriate length of the CII was not as straightforward. Videos of dewlapping adult males (see ‘Measurements of Specimens in Life’) revealed the full length of the CII was not functioning equally in the lever system; only part of the CII physically extended the dewlap (Fig. 1a,b). The histology of the CII explains why: there is a gradual reduction in the mineralization of the CII along its length (Bels 1990). This causes the CII to lose rigidity and bow under the stress of the extended dewlap to produce its characteristic curvature in *Anolis* (Fig. 1a). This means that the calcified portion of the CII, and not the entire length of the CII, functions as the  $L_{out}$ . Identifying the point at which calcium levels in the CII drop below a certain value might approximate the functional length of  $L_{out}$  (e.g. see Bels 1990). However, a more accurate and direct measure is obtained by observing where the maximum bowing of the CII actually occurs during the extension of the dewlap. Specifically,  $L_{out}$  corresponds to the ‘leading edge’ of the dewlap, from the external position of  $f$  below the throat to the point where the CII loses rigidity and can no longer be seen to push the dewlap membrane outward (this length corresponds to the solid line labelled ‘i’ in Fig. 1b). We measured this length using high-resolution video stills that depicted adult males extending the dewlap during territorial display (see ‘Measurements of Specimens in Life’ below).

Obtaining an appropriate measure of  $V_{mc}$  was a problem. Past work has confirmed that the ceratohyoideus muscles are responsible for powering the dewlap lever mechanism (Bels 1990; Font & Rome 1990), but the specific attributes of the muscles that contribute to variation in  $V_{mc}$  remain unclear. Several studies have found seasonal effects and sex differences in the overall size and histology of the muscles (O’Bryant & Wade 1999, 2002; Rosen *et al.* 2004;

Johnson *et al.* 2011), but there appears to be little association between these characteristics and the frequency of dewlap use (Johnson & Wade 2010; Johnson *et al.* 2011). We could find no information on whether variation in muscle size, fibre length or other physiological characteristics (or a combination of these characteristics) might determine interspecific differences in maximal contraction velocity. This meant that any measure we might take of the ceratohyoideus muscle would be a putative measure of  $V_{mc}$  and one potentially unrelated to maximal contraction velocity.

We faced similar difficulties with estimating  $h$  and  $c$ . The only way to measure  $h$  was through X-rays of live lizards extending their dewlap (see Losos 2009). Obtaining such images for the sample sizes and number of species needed in our study was impractical. Estimating the angle of muscle fibre pennation,  $c$ , was more tractable, but we encountered challenges relating to the accuracy of measuring  $c$  from preserved museum specimens. Generally, though, there seemed to be little variation among *Anolis* species in fibre pennation (M. A. Johnson, pers. comm.), and pennation seemed to conform to the Font & Rome (1990) description of being roughly parallel to the CII in *A. equestris* ( $c \leq 20^\circ$ ).

To circumvent these data limitations, we investigated the effects of  $V_{mc}$ ,  $h$  and  $c$  through simulations of our biomechanical model. These revealed distinct patterns in the way dewlap speed changes as a function of  $F_{ratio-anatomical}$  depending on how  $V_{mc}$ ,  $h$  and  $c$  varied among species (Fig. 2a). We converted these predicted relationships (Fig. 2b–j) into quadratic or linear functions of  $F_{ratio-anatomical}$  (Table 2) and used an analytical framework to evaluate the fit of each of these functions to observed interspecific variation in dewlap speed and  $F_{ratio-anatomical}$ . The fit of predictions to species data informed how  $F_{ratio-anatomical}$  and corresponding changes in  $V_{mc}$ ,  $h$  or  $c$  have impacted the performance of the dewlap display among species. The only scenario that this approach would fail to identify would be one in which change only occurs in  $V_{mc}$ ,  $h$  or  $c$  with  $F_{ratio-anatomical}$  largely conserved among species. However, we relied on estimates of the range of  $F_{ratio-anatomical}$  values recorded across species to investigate this particular scenario.

#### MEASUREMENTS FROM PRESERVED SPECIMENS

The HH was measured from close-up digital photographs of the hyoid apparatus of cleared and stained specimens. All specimens were of adult males. Sample sizes for each species varied depending on the availability of specimens and ranged from 2 to 14 males

**Table 2.** Predictions generated from simulations of the biomechanical model (Fig. 2) converted into statistical functions tested in species comparative analyses (Table 3). Functions were also tested with a covariate for body size (SVL)

| Simulation predictions  | Statistical function tested                       |
|---|---|
| (i) Additive or trade-off changes in anatomical $F_{ratio}$ and $V_{mc}$ : Fig. 2(b, c)                   | $F_{ratio-anatomical} \pm F_{ratio-anatomical}^2$ |
| (ii) Conserved $V_{mc}$ with changes only in anatomical $F_{ratio}$ : Fig. 2(d)                           | $F_{ratio-anatomical}$                            |
| (iii) Changes in anatomical $F_{ratio}$ with island innovations in $V_{mc}$ and $h$ or $c$ : Fig. 1(e, f) | $F_{ratio-anatomical} + island \pm island^*$      |
| (iv) Changes in anatomical $F_{ratio}$ with an island innovation in $V_{mc}$ only: Fig. 1(g)              | $F_{ratio-anatomical} + island$                   |
| (v) Changes in anatomical $F_{ratio}$ with an island innovation in $h$ or $c$ only: Fig. 1(h, i)          | $F_{ratio-anatomical} \pm island^*$               |
| (vi) Changes in anatomical $F_{ratio}$ with convergences within ecomorphs in $h$ or $c$ only: Fig. 1(j)   | $F_{ratio-anatomical}$<br>Not formally tested     |

**Table 3.** Evolutionary predictors of species variation in the fulcrum ratio ( $F_{\text{ratio-anatomical}}$ ) among 11 *Anolis* species (see Fig. 2). Models were implemented in a phylogenetic framework using PGLS; the ‘phylogeny’ model was an intercept only model that effectively assumed phenotypic variation primarily reflected the phylogenetic relationships among species. The  $\alpha$  parameter provides a measure of the extent the phenotype has been free to vary (potentially adaptively) among species (high values) or was correlated to phylogeny (low values)

| Evolutionary model | AIC <sub>c</sub> | $\Delta$ AIC <sub>c</sub> | AIC <sub>w</sub> | $r_{\text{phylogenetic}}$ | $\alpha$ |
|--------------------|------------------|---------------------------|------------------|---------------------------|----------|
| Allometry          | 54.0             | 0.0                       | 0.56             | 0.43                      | 15.5+    |
| Island             | 54.5             | 0.5                       | 0.43             | 0.39                      | 15.5+    |
| Ecomorph           | 61.6             | 7.6                       | 0.01             |                           |          |
| Phylogeny          | 66.2             | 12.2                      | 0.00             |                           |          |

with an average of six males per species. Cleared and stained specimens were prepared as described in Sanger *et al.* (2012). Briefly, specimens were preserved in 10% neutral buffered formalin, skinned and eviscerated, and dehydrated in a graded ethanol series. The specimens were cleared using a combination of trypsin (Fisher Scientific, Pittsburgh, PA 15275, USA) and potassium hydroxide. Cartilage was stained using alcian blue 8GX (Sigma-Aldrich Corp. St. Louis, MO, USA) and bone with alizarin red (Sigma). All specimens were stored in 100% glycerol. Scaled digital photographs were taken using a Zeiss Discovery V8 microscope and associated Zeiss Axiovision software.

We then used ImageJ to measure the length of the HH on the left side. We used the straight-line tool to trace the length of the HH from its articulation with the CII to its articulation with the CH (see Fig. 1d). The recorded distance in pixels was calibrated to mm after tracing a set distance on a ruler. HH length for each specimen was the mean of two nonsequential measurements. Species values for HH length were the means among specimens, and these were used to compute  $F_{\text{ratio-anatomical}}$ .

One person took the majority of measurements (45/64 specimens), but a second measurer contributed HH measurements for some specimens of four species (*A. valencienni*, *A. gundlachi*, *A. cristatellus* and *A. evermanni*). To evaluate consistency in measurements, HH length was measured from the same 10 specimens (these specimens belonged to three different species: *A. gundlachi*, *A. cristatellus* and *A. evermanni*). There was some variability in HH length between measurers ( $r = 0.75$ ), but importantly a regression confirmed that the intercept was not significantly different from zero (95% confidence intervals, CI:  $-0.40, 0.84$ ; i.e. the ‘on average’ measured length was similar), and the slope was not significantly different from 1 (95% CIs:  $0.43, 1.75$ ; i.e. measurements were taken in a consistent manner). We also note that based on the two nonsequential HH length measurements taken from each specimen, intra-observer repeatability was high ( $r = 0.98$  and  $0.99$ ).

#### MEASUREMENTS FROM SPECIMENS IN LIFE

We measured the  $L_{\text{out}}$  length of the CII as the leading edge of the dewlap at maximum extension (shown in Fig 1b as the solid line highlighted by ‘i’). This measurement was taken for 10 adult males for each species using stills extracted from digital video of free-living males performing territorial advertisement displays (see Ord, Stamps & Losos 2010 for details on locations and recording protocols). These videos were a subset of those used to quantify dewlap speed (next paragraph). Three estimates of the leading edge were obtained from different display clips for each adult male. These measurements were made using ImageJ and converted from pixels to mm using a calibration value obtained at the time of

video recording (an object of known size was placed in-frame at the site a lizard had displayed; see Ord *et al.* 2007; Ord, Stamps & Losos 2010). These three measurements were averaged for each male and then averaged again across all adult males for a given species to obtain a species mean value. These species mean values were used to compute  $F_{\text{ratio-anatomical}}$ .

Estimates of maximum dewlap speed were taken from Ord, Stamps & Losos (2010) and its corresponding archive in the Dryad Digital Repository (doi: 10.5061/dryad.1619). Details on methods used to quantify speed are presented in Ord *et al.* (2007) and Ord, Stamps & Losos (2010). Briefly, digital videos (30 frames per second) were taken of free-living adult males on their territories for a period of 25–30 minutes. Instances of display behaviour were exported from this video record as separate clips and the maximum speed of movements, measured in millimetres moved per second ( $\text{mm s}^{-1}$ ), were quantified using the ‘Analysis of Image Motion’ program (AIM; Peters, Clifford & Evans 2002) run in MATLAB. These analyses focussed only on video clips in which the speed of movements could be unambiguously assigned to the extension of the dewlap. In almost all cases, a clip depicted a series or bout of dewlap extensions, and maximum dewlap speed was measured as the fastest instantaneous extension speed recorded for that bout. Our level of precision for measuring display speed was dependent on the NTSC video format and was  $\pm 0.033$  s. This maximum dewlap speed was averaged across separate display bouts performed by individuals and averaged again across individuals to obtain a species mean value. This approach was preferred over simply taking the fastest dewlap extension recorded for a given lizard or taxa because estimates can be sensitive to imprecision in image calibrations for camcorder distance. This earlier study also surveyed two separate populations for *A. lineatopus*, *A. gundlachi* and *A. pulchellus*. These population estimates were in turn averaged to provide a single mean for the species. Sample sizes for each species ranged from 4 to 27 males with an average of 19 males per species.

Dewlap speed data could not be obtained for *A. poncensis*. This Puerto Rican species has an unusually small dewlap that made quantifying the speed of its extension virtually impossible. Careful inspect of videos for this species showed that the dewlap extension was extremely slow and certainly much lower than the minimum speed recorded for other species on Puerto Rico ( $<12 \text{ mm s}^{-1}$ ). Unfortunately, without a direct estimate of the dewlap speed for this species, it was not possible to include *A. poncensis* in some of our analyses. When this was the case, we instead provide a qualitative comparison of *A. poncensis* by illustrating the approximated position of the species in figures.

We also collected data on ambient air temperature at the site of first display for each individual lizard. These measurements were taken with a digital thermometer immediately following video recordings in the field. Species values for ambient temperature during display were evaluated as the means among individuals. For the three species in which two separate populations were surveyed (see above), we averaged temperature estimates across populations.

#### STATISTICAL ANALYSES

Our objective was to determine whether modifications to the hyoid apparatus have allowed Jamaican lizards to move their dewlap more rapidly than Puerto Rican species. Based on simulations from our biomechanical model (Fig. 2a), our general predictions were the following: (i) species from Jamaica have higher values of  $F_{\text{ratio-anatomical}}$  than Puerto Rico (e.g. Fig. 2b–d); and (ii) variance in  $F_{\text{ratio-anatomical}}$  would be positively correlated with maximum dewlap speed across species, with the magnitude of the relationship dependent on how  $V_{\text{mc}}$ ,  $h$  or  $c$  varied among species (Fig. 2b–j). Our analyses were subsequently conducted in two parts.



First, we determined whether  $F_{\text{ratio-anatomical}}$  varied among species and then evaluated whether island origin (Prediction 1) or some other factor accounted for species differences in  $F_{\text{ratio-anatomical}}$ . In this analysis, we compared the likelihood of an 'island' model relative to three other probable models of  $F_{\text{ratio-anatomical}}$  evolution. The island model assumed divergence in  $F_{\text{ratio-anatomical}}$  has primarily occurred among species from Jamaica and Puerto Rico (e.g. Fig. 2b–d; Prediction 1). The predictor variable in this model was island origin scored as '1' for species from Jamaica and '0' for species from Puerto Rico. The 'allometry' model assumed  $F_{\text{ratio-anatomical}}$  has been constrained by allometry. For example, small species might have low values of  $F_{\text{ratio-anatomical}}$  because of developmental or morphological limits to the elongation of the CII associated with their small body size. In this instance, any divergence observed in  $F_{\text{ratio-anatomical}}$  between islands reflects a difference in the mean body size of species from the two islands, not an innovation in hyoid morphology. In the allometry model, the predictor variable was species mean snout-vent length (SVL). SVL was measured from distance-calibrated video stills and was the length of the body from the tip of the nose to the base of the tail, averaged across three separate video stills for a given individual, which were in turn averaged across individuals for a given species. We chose to use body size measured from video stills rather than preserved specimens because our sample sizes were larger for the former (NB: the correlation between SVL estimates from video stills and preserved specimens was high,  $r = 0.71$ ). The 'ecomorph' model considered that the *Anolis* of the Greater Antillean islands, which includes Jamaica and Puerto Rico, exhibit a common set of ecomorphs through convergent evolution (ecomorphs are defined as species that share ecological and morphological characteristics: see Losos *et al.* 1998; Losos 2009). These ecomorphs are named depending on where in the environment species typically prefer to perch. Of the species included in our study, there were three ecomorphs on Puerto Rico – grass–bush, trunk–ground and trunk–crown – and three ecomorphs on Jamaica, two of which were shared with Puerto Rico – twig, trunk–ground and trunk–crown (Fig. 3). The ecomorph model assumed that species within a given ecomorph category (regardless of island origin) shared a common  $F_{\text{ratio-anatomical}}$  and that this common  $F_{\text{ratio-anatomical}}$  differed among ecomorphs. This model was opposite to the island model as it assumed convergence in  $F_{\text{ratio-anatomical}}$  among species on each island, whereas the island model assumed divergence in  $F_{\text{ratio-anatomical}}$  among species on each island. Ecomorphs were coded as separate dummy variables (i.e. species were, '1', or were not, '0', a particular ecomorph). Finally, the 'phylogeny' model was a null model that assumed variance among species in  $F_{\text{ratio-anatomical}}$  was simply the outcome of evolutionary differentiation by Brownian motion. This model was equivalent to an intercept only ANOVA with no predictor variable. It assumed that variation among species in  $F_{\text{ratio-anatomical}}$  reflected phylogenetic relationships without respect to island origin, body size or the ecomorph of species.

Next, we tested the prediction that differences in the speed of dewlap extensions among species reflected differences in  $F_{\text{ratio-anatomical}}$  among species and that the nature of this relationship depended on the extent species also differed in  $V_{\text{mc}}$ ,  $h$  and  $c$  (Prediction 2). Each of the simulated relationships between dewlap speed and  $F_{\text{ratio-anatomical}}$  from our biomechanical model (Fig 2b–i, but not j; see next paragraph) was translated into statistical functions (see Table 2) that included various combinations of  $F_{\text{ratio-anatomical}}$ , island origin and body size (SVL) as independent variables [SVL was included as a covariate in some models because allometry was found to be potentially important in the evolution of  $F_{\text{ratio-anatomical}}$  (see 'Results')]. These statistical functions were then fit to species data. The first of these functions was formulated to test the influence of changes in  $F_{\text{ratio-anatomical}}$  and  $V_{\text{mc}}$ : a quadratic function of  $F_{\text{ratio-anatomical}}$  to simulate a situation in which  $V_{\text{mc}}$  changed incrementally (positively or negatively) with changes in

$F_{\text{ratio-anatomical}}$  among species (Table 2; Fig. 2b,c); or a linear function of  $F_{\text{ratio-anatomical}}$  in which  $V_{\text{mc}}$  was assumed to be conserved among species (Table 2; Fig. 2d). A second set of functions assessed the extent dewlap speed varied as a function of  $F_{\text{ratio-anatomical}}$ , and the extent islands differed in this relationship because of a fundamental divergence in  $V_{\text{mc}}$ . That is, the nature of the relationship between dewlap speed and  $F_{\text{ratio-anatomical}}$  differed among species on each island because of a change in the base value of  $V_{\text{mc}}$  on one island and not the other. Several of these functions also assumed an evolutionary divergence in either the orientation of  $L_{\text{in}}$  to  $L_{\text{out}}$  ( $h$ ) or pennation of the ceratohyoideus muscle fibres ( $c$ ). Each of these scenarios was modelled using a linear function of  $F_{\text{ratio-anatomical}}$  with a covariate for island (Table 2; Fig. 2e–g), as well as interaction term between  $F_{\text{ratio-anatomical}}$  and island for the models that assumed an additional effect of an evolutionary divergence in  $h$  or  $c$  (Table 2; Fig. 2e,f). In a third set of functions, we tested the influence of changes in  $F_{\text{ratio-anatomical}}$  coupled with only a base level change in  $h$  or  $c$  on one of the islands. Here, a linear function of  $F_{\text{ratio-anatomical}}$  included an interaction term between  $F_{\text{ratio-anatomical}}$  and island, but assumed no difference in intercept value between islands (Table 2; Fig. 2h,i).

We also examined the possibility that species within ecomorphs on either island have converged on similar values of  $h$  or  $c$ . This might produce island divergences in dewlap speed if  $F_{\text{ratio-anatomical}}$  also tended to differ between islands (Fig. 2j; Prediction 1). With the sample sizes of our current study, we were unable to assess this particular scenario statistically. Instead, we relied on a qualitative comparison of plots with the expected patterns shown in Fig. 2j.

Finally, we tested the effects of temperature on dewlap speed. Because ambient temperature is a key variable affecting biomechanical performance in lizards (Gunderson & Leal 2012), species variation in dewlap speed may be unrelated to variation in the hyoid apparatus (either in  $F_{\text{ratio-anatomical}}$ ,  $V_{\text{mc}}$ ,  $h$  or  $c$ ) and instead may simply reflect temperature effects on display performance. If so, an island difference in dewlap speed would occur if species on each island occupied habitats that were broadly different in temperature; specifically, Jamaican species have faster dewlaps because they display in hotter environments than species on Puerto Rico. To consider this scenario, we included a linear model in which the only predictor of dewlap speed was mean temperature at the time of display.

The level of support for models was computed using the Phylogenetic Generalized Least Squares (PGLS) analysis in the program COMPARE 4.6b (Martins 2004). We used the phylogeny of Nicholson *et al.* (2005) presented with branch length information by Losos (2009). The phylogeny was trimmed to the species of interest using MESQUITE version 2.6 (Maddison & Maddison 2010). The log-likelihood value for each model calculated by PGLS was converted into an  $AIC_c$  score (Akaike Information Criterion with a correction for small sample size) using the equation presented in Burnham & Anderson (2002). The model with the lowest  $AIC_c$  value has the highest support and was interpreted to account for more of the variance among species in  $F_{\text{ratio-anatomical}}$  or dewlap speed compared to other models tested. However, any model within two  $AIC_c$  units of this best fitting model ( $\Delta AIC_c \leq 2.0$ ) was considered equally well supported (Burnham & Anderson 2002). We also calculated  $AIC_w$  for each model to provide an indication of the weight of evidence in favour of a given model relative to others considered in a given model set. Values of  $AIC_w$  range from 0 to 1 (effectively no support to exclusive support for a given model).

We report the phylogenetic effect size ( $r_{\text{phylogenetic}}$ ) and estimates of PGLS  $\alpha$  for the best fitting model and all equally supported models (i.e.  $\Delta AIC_c \leq 2.0$ ). PGLS  $\alpha$  is a parameter computed using maximum likelihood and can be interpreted in a variety of ways. For example, it is often considered to reflect how freely a trait had undergone adaptive change. When  $\alpha$  approaches zero, traits are tightly correlated with phylogeny and much of the

interspecific variation observed in phenotypes today can be attributed to evolutionary relationships among species. Very large  $\alpha$  values (15.5+), on the other hand, indicate little phylogenetic signal in comparative data suggesting a trait is potentially the product of adaptation to some stationary phenotypic optimum (Hansen & Martins 1996; Hansen 1997). However, parameters like  $\alpha$  should be interpreted with caution because estimates of phylogenetic signal can reflect a number of quite different biological scenarios and are sensitive to taxon sampling, phylogenetic uncertainty and measurement error in phenotypic traits (e.g. see Revell, Harmon & Collar 2008).

## Results

### EVOLUTION OF ANATOMICAL $F_{\text{RATIO}}$

Our estimates of  $F_{\text{ratio-anatomical}}$  were variable among species, with a wide range between the species with the lowest  $F_{\text{ratio-anatomical}}$  (*A. poncensis*: 10.46) and the species with the highest  $F_{\text{ratio-anatomical}}$  (*A. lineatopus*: 37.18). Model fitting inferred these differences in  $F_{\text{ratio-anatomical}}$  were best explained by the overall size of species or island origin (Table 3). However, effect sizes were small, with body size and island origin accounting for only minimal variance in  $F_{\text{ratio-anatomical}}$  among species (18.5% and 15.2%, respectively). The small effect of island origin was made even more obvious when  $F_{\text{ratio-anatomical}}$  was mapped across the anole phylogeny, which revealed extensive overlap in  $F_{\text{ratio-anatomical}}$  between the islands (Fig. 3). Ecomorphology could not be considered a compelling explanatory model of  $F_{\text{ratio-anatomical}}$  (Table 3).

Based on the small effect sizes and the distribution of  $F_{\text{ratio-anatomical}}$  among species, we concluded that none of our evolutionary models provided a compelling explanation for species differences in  $F_{\text{ratio-anatomical}}$ . This included the phylogenetic null model in which the high-estimated  $\alpha$  implies little correlation between  $F_{\text{ratio-anatomical}}$  and phylogeny. It also follows from these results that the observed differences in dewlap speed among species from Jamaica and Puerto Rico (Fig. 3) do not reflect an evolutionary divergence in  $F_{\text{ratio-anatomical}}$  between the islands (Prediction 1). However,  $F_{\text{ratio-anatomical}}$  did vary among species within islands and beyond what might be reasonably expected for biological noise or measurement error (e.g. the standard error of  $F_{\text{ratio-anatomical}}$  across species was 3.5 times larger than the mean standard error of the CII and the HH within species). This excluded the possibility that differences among species in the performance of the dewlap lever were solely the result of evolutionary changes in  $V_{\text{mc}}$ ,  $h$  or  $c$ .

### EVOLUTION OF DEWLAP SPEED AS A FUNCTION OF ANATOMICAL $F_{\text{RATIO}}$

Models that included island origin and a linear function of  $F_{\text{ratio-anatomical}}$  or  $F_{\text{ratio-anatomical}}$  and its interaction with island origin received the highest support ( $F_{\text{ratio-anatomical}} + \text{island}$  and  $F_{\text{ratio-anatomical}} + \text{island} * F_{\text{ratio-anatomical}}$ ;

Table 4). Effect sizes for these models were also extremely large, with 84.6–88.4% of the variance in dewlap speed among species being accounted for by the combined effect of island origin and  $F_{\text{ratio-anatomical}}$ . Both models showed that within islands, increases in  $F_{\text{ratio-anatomical}}$  have led to increases in dewlap speed, but divergences in  $F_{\text{ratio-anatomical}}$  among species alone cannot account for island differences in dewlap speed (see also 'Evolution of Anatomical  $F_{\text{ratio}}$ ' above). Rather, each model assumes a different evolutionary scenario for what has caused the islands to diverge in dewlap speed. The scenario receiving the most support was the one inferring a marked increase in base level  $V_{\text{mc}}$  within the Jamaican lineage (or a major decrease within the Puerto Rican lineage; for example, compare the simulated outcome in Fig. 2(g) with actual distribution of data in Fig. 4). Alternatively, rather than an increase in base level  $V_{\text{mc}}$ , a less supported but still plausible scenario was an evolutionary innovation in Jamaican lineages relating to the orientation of  $L_{\text{in}}$  to  $L_{\text{out}}$  ( $h$ ) or the angle of muscle pennation ( $c$ ; Table 4; see Fig. 2h). This has potentially enabled Jamaican lizards to produce faster dewlap extensions for a given  $F_{\text{ratio-anatomical}}$  and  $V_{\text{mc}}$ . The probability of these two scenarios can be inferred from their AIC<sub>w</sub> values in Table 4 (57% support for an innovation in base level  $V_{\text{mc}}$  vs. 30% support for an innovation in  $h$  or  $c$ ).

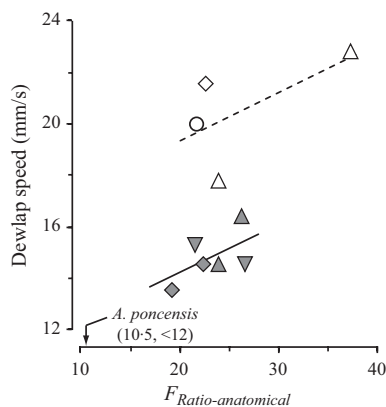
Visual inspect of the data in Fig. 4 provided little qualitative support for a third evolutionary scenario in which species within ecomorphs have converged on common  $L_{\text{in}}$  to  $L_{\text{out}}$  angles,  $h$ , or angles of muscle pennation,  $c$  (see Fig 2j). In addition, temperature differences experienced by species received little support as an alternative explanatory model for variation in dewlap speed among species and subsequently could not account for differences between islands (Table 4).

## Discussion

Morphological modifications to the fulcrum ratio of the *Anolis* hyoid apparatus (anatomical  $F_{\text{ratio}}$  or  $F_{\text{ratio-anatomical}}$ ) have been associated with changes among species in how fast the dewlap was extended in territorial display (especially those on Puerto Rico; Fig. 4). Furthermore, the nature of this relationship matched outcomes from a biomechanical model of how changes to the fulcrum ratio would impact dewlap performance (e.g. Fig. 2g). We conclude then that differences among species in the morphology of the fulcrum ratio and dewlap speed are functionally linked. Past work on the communication of these lizards also implies that within island divergence among species in fulcrum ratio and subsequently dewlap speed reflects selection for enhanced signal detection when species communicate in habitats that differ in visibility. This is because faster dewlap extensions are expected to increase the efficiency of advertising territory ownership in low light or visually noisy environments (Ord, Stamps & Losos 2010). The variance among species in the components determining the morphology of the fulcrum further suggest this

**Table 4.** Evolutionary predictors of species variation in the speed of dewlap extensions among 10 *Anolis* species (*A. poncensis* was excluded from these analyses because its small dewlap made quantifying speed difficult). NB: the sign of the predictor variables were computed as part of fitting functions to the data (e.g.  $F_{\text{ratio-anatomical}} \pm \text{island} * F_{\text{ratio-anatomical}}$  in Table 2 was subsequently computed to be  $F_{\text{ratio-anatomical}} + \text{island} * F_{\text{ratio-anatomical}}$  when fitted to the data). See Table 3 for other details

| Evolutionary model   | AIC <sub>c</sub> | ΔAIC <sub>c</sub> | AIC <sub>w</sub> | $r_{\text{phylogenetic}}$ | $\alpha$ |
|--|------------------|-------------------|------------------|---------------------------|----------|
| $F_{\text{ratio-anatomical}} + \text{island}$  | 114.3            | 0.0               | 0.57             | 0.94                      | 9.0      |
| $F_{\text{ratio-anatomical}} + \text{island} * F_{\text{ratio-anatomical}}$                              | 115.5            | 1.2               | 0.30             | 0.92                      | 3.73     |
| $F_{\text{ratio-anatomical}} + \text{island} + \text{SVL}$   | 118.9            | 4.6               | 0.06             |                           |          |
| $F_{\text{ratio-anatomical}} + \text{island} * F_{\text{ratio-anatomical}} + \text{SVL}$                 | 119.2            | 4.9               | 0.05             |                           |          |
| $F_{\text{ratio-anatomical}} + \text{island} + \text{island} * F_{\text{ratio-anatomical}}$              | 120.8            | 6.5               | 0.02             |                           |          |
| $F_{\text{ratio-anatomical}}$  | 122.7            | 8.4               | 0.01             |                           |          |
| Temperature  | 123.0            | 8.7               | 0.01             |                           |          |
| $F_{\text{ratio-anatomical}} + \text{SVL}$   | 126.8            | 12.5              | 0.00             |                           |          |
| $F_{\text{ratio-anatomical}} - F_{\text{ratio-anatomical}}^2$  | 127.0            | 12.7              | 0.00             |                           |          |
| $F_{\text{ratio-anatomical}} + \text{island} + \text{island} * F_{\text{ratio-anatomical}} + \text{SVL}$ | 128.5            | 14.2              | 0.00             |                           |          |
| $F_{\text{ratio-anatomical}} - F_{\text{ratio-anatomical}}^2 + \text{SVL}$                               | 133.1            | 18.8              | 0.00             |                           |          |



**Fig. 4.** The relation of the dewlap lever system ( $F_{\text{ratio-anatomical}}$ ) and the speed of dewlap extensions performed by free-living adult males during territorial advertisement displays. Open symbols are species from Jamaica, while filled symbols are species from Puerto Rico. The shape of symbols corresponds to the ecomorph categories shown in Fig. 3. Also shown is the relative position of the Puerto Rican lizard, *A. poncensis*. This species was not included in analyses because of the poor precision in estimating dewlap speed (which was most likely well below the range of speeds estimated for other species; i.e.  $<12 \text{ mm s}^{-1}$ ). Divergence in intercept values between islands probably reflects an innovation in  $V_{\text{mc}}$  at some point in the history of the Jamaican clade (see Fig 2g). An alternative scenario assumes island divergences in dewlap speed have been caused by an innovation in the articulation of the HH and CII,  $h$ , or attachment angle,  $c$ , of the muscle powering the dewlap lever system. This latter scenario predicts a difference in slope but not intercept between islands (see Fig 2h and i). Regardless, within islands, there appears to be less variation in  $V_{\text{mc}}$ ,  $h$  and  $c$  among species (indicated by low scatter around regression lines). NB: the Jamaican species with the slowest estimated dewlap speed (compared to other Jamaicans) was *A. sagrei*, an invasive species from Cuba that was closely related to other species on Jamaica (see Fig. 3).

adaptive response has largely been concentrated in the elongation of the second ceratobranchials (CII, the out-lever), rather than the shortening of the hypohals (HH, the in-lever; coefficient of variation across species: CII = 0.27; HH = 0.18). Why structural change has been more concentrated in the CI is unclear, but could reflect a constraint

on evolutionary change in the HH, which must articulate with more bones within the hyoid apparatus than the CI.

Although dewlap speed varies with the morphology of the fulcrum within islands, modifications to the second ceratobranchials or hypohals cannot explain the dramatic divergence in dewlap speed among island clades. Based solely on the morphology of the fulcrum, species from Puerto Rico should have been capable of extending the dewlap just as fast as those species on Jamaica. If our biomechanical model provides an accurate representation of how the hyoid operates during the dewlap extension, and the available evidence suggests that it does (Bels 1990; Font & Rome 1990; Wade 2005), then the input speed must differ – and substantially so – between species from the two islands. Specifically, Jamaican *Anolis* have evolved some adaptation that has increased muscle contraction velocity ( $V_{\text{mc}}$ ), or perhaps the efficiency of its transfer to the second ceratobranchials via an innovation in muscle attachment angle ( $c$ ) or the orientation of the hypohals to the second ceratobranchials ( $h$ ). The most likely scenario seems to have been an evolutionary innovation in base level contraction velocity (Figs 2g and 4). Parameterizing our biomechanical model with values from the statistical function that corresponds to this scenario ( $F_{\text{ratio-anatomical}} + \text{island}$ ; Table 4) indicates that an increase in dewlap speed of  $c. 5 \text{ mm s}^{-1}$ , which matches the observed difference in dewlap speed between Jamaican and Puerto Rican species would require a 49% increase in contraction velocity. Such an increase in Jamaican anoles has presumably resulted from physiological changes to the ceratohyoideus muscle (e.g. fibre length or types). Alternatively, parameterization that assumes an increase in the efficiency of velocity transfer to the lever ( $F_{\text{ratio-anatomical}} + F_{\text{ratio-anatomical}} * \text{island}$ ; Table 4) suggests that Jamaicans could produce faster dewlaps if the orientation of the hypohals to the second ceratobranchials was  $20^\circ$  wider than in Puerto Ricans (e.g.  $h_{\text{Jamaica}} = 170^\circ$ ;  $h_{\text{Puerto Rico}} = 150^\circ$ ) or if the attachment angle of the ceratohyoideus muscle was virtually parallel to the second ceratobranchials in Jamaicans while

substantially offset in Puerto Ricans (e.g.  $c_{\text{Jamaica}} = 1^\circ$ ;  $c_{\text{Puerto Rico}} = 39^\circ$ ). Yet another possible explanation is that Jamaican and Puerto Rican anoles are capable of the same dewlap speeds for their given fulcrum ratios, but Jamaican species simply employ faster muscle contractions (i.e. they differ in muscle activation, a behavioural response). We consider each of these possibilities below but note that data on muscle properties or motor pattern are necessary to determine what exactly accounts for island differences in dewlap speed.

#### BIOMECHANICAL DIVERGENCE IN DEWLAP SPEED

In terms of differences in the ceratohyoideus muscles between species from the two islands, published data for a subset of the species examined in this study provide little evidence that Jamaican species possess muscle architectures that would substantially increase maximum contraction velocity. Johnson & Wade (2010) have reported some differences in muscle length and cross-sectional area of ceratohyoideus muscles among *Anolis* species from Jamaica, Hispaniola and mainland America. However, the Jamaicans were not especially unusual in either of these muscle attributes. For example, we reanalysed data presented in that paper and found no difference in the mean muscle length or cross-sectional area between Jamaican and non-Jamaican species (95% CIs computed using the Hansen Adaptive Model in COMPARE 4.6b (Martins 2004) for the size-free residuals of muscle length:  $\theta_{\text{Jamaican}} = -0.084, 0.064$ ,  $\theta_{\text{non-Jamaican}} = -0.091, 0.171$ ; and size-free residuals of cross-sectional area:  $\theta_{\text{Jamaican}} = -154.2, 74.9$ ,  $\theta_{\text{non-Jamaican}} = -46.9, 349.9$ ).

An alternative solution for increasing  $V_{\text{in}}$  is to change the orientation of the muscle fibres ( $c$ ; eqn 3) to increase the efficiency of energy transfer to the lever. While there is no published data on muscle fibre pennation for the *Anolis* ceratohyoideus muscle, informal inspection of the muscle suggests it is largely parallel to the second ceratobranchials (e.g.  $c < 20^\circ$ ; Font & Rome 1990) and varies little among *Anolis* species (M.J. Johnson, pers. comm.). Future work will have to clarify what other aspects of the ceratohyoideus muscles contributes to variation in input velocity and whether these attributes, or the orientation of the HH to the second ceratobranchials ( $h$ ), vary among species from Jamaica and Puerto Rico in the predicted manner.

#### BEHAVIOURAL DIVERGENCE IN DEWLAP SPEED

If the observed differences in dewlap speed between the islands do not reflect changes to the hyoid, then the observed performance differences have a behavioural rather than biomechanical basis; Puerto Rican lizards essentially 'choose' not to extend the dewlap as fast as lizards on Jamaica (a behavioural mechanism that results in lower muscle activation and slower dewlap speeds). Puerto Rican lizards communicate in a comparable range of visually challenging habitats with lizards on Jamaica and must

still perform an effective display in those environments. Puerto Rican lizards do have other adaptations for enhancing signal efficiency, which are absent on Jamaica. However, for these adaptations to be alternative evolutionary strategies for maintaining an effective display in the absence of a biomechanical constraint on the hyoid (see previous section 'Biomechanical Divergence in Dewlap Speed'), they would have to be equivalent in effect and cost of production (e.g. energy expenditure, time away from other activities or risk of predation risk). Rapidly extending the dewlap – the strategy employed by Jamaican species – and the incorporation of high-speed body movements into the display – one of the strategies employed by Puerto Rican species – have been confirmed experimentally as comparable means of enhancing display detection in low light and possibly visually noisy environments as well (Ord & Stamps 2008). It follows that the risk of predation should also be similar: if both types of display are equally conspicuous to conspecifics, then they will most likely be equally conspicuous to predators. In terms of energetic cost, however, rapidly extending the dewlap several times should be cheaper than performing the same number of exaggerated body movements simply because of the difference in mass displaced in each case (the hyoid and dewlap membrane vs. most of the body). This cost imbalance might have been resolved by Puerto Rican lizards through the selective timing of headbob displays to avoid periods when environmental conditions are most adverse for display detection (Ord, Charles & Hoffer 2011). By waiting until environmental visual noise is low, Puerto Ricans can perform headbob displays at more relaxed speeds (Ord, Charles & Hoffer 2011) and presumably at lower energetic cost (see Brandt 2003). In contrast, Jamaican lizards do not time headbob displays to exploit lulls in visual noise and instead apparently minimize production costs by incorporating rapid dewlap extensions into territorial displays rather than escalating headbob speeds (Ord, Stamps & Losos 2010; Ord, Charles & Hoffer 2011). Although the costs associated with these alternative behaviours remain to be tested, it seems likely that selective timing of displays in Puerto Rican anoles has lessened the demand for fast ceratohyoideus contraction (or more specifically high  $V_{\text{in}}$ ) and fast dewlapping (high  $V_{\text{out}}$ ) as a means for enhancing signal efficacy.

#### ORIGIN OF ISLAND-SPECIFIC DEWLAP SPEEDS

Even though they use their dewlaps differently, we suspect that Puerto Rican lizards have still been limited by biomechanical constraints on the extension of their dewlaps for two reasons:

1. The clear upward trend in dewlap speed as a function of the morphology of the fulcrum among species on Puerto Rico (Fig. 4) strongly implies that Puerto Rican species have responded to selection for increased signal efficiency by increasing dewlap speeds through the evolution of higher fulcrum ratios. That is, Puerto Rican

lizards have not simply relied on performing conspicuous headbob movements to maintain an efficient display in visually difficult environments (e.g. there is a strong, positive correlation between dewlap speed and visual noise among Puerto Rican species; Ord, Stamps & Losos 2010). Given this, it seems odd that Puerto Ricans have not evolved the same dewlap speeds as Jamaican lizards if not for some underlying constraint on muscle contraction velocity or its transfer to the dewlap lever. That is, if not for constraint, some innovation in muscle contraction velocity ( $V_{mc}$ ), pennation angle ( $c$ ) or the orientation of the hypohals to the second ceratobranchials ( $h$ ) in Puerto Rican anoles would likely evolve to alleviate the physiological costs associated with increasing dewlap speeds in noisy environments. This would in turn obviate the need to evolve other display strategies for enhancing signal efficiency (e.g. high-speed headbob movements or timing displays to overlap with lulls in visual noise).

2. Puerto Rican species are known to be contextually plastic in their dewlap extensions; lizards actively tailor the speed of dewlap extensions depending on the level of visual noise occurring at the time of display (Ord, Stamps & Losos 2010). This plasticity was reduced or absent in Jamaican species (Ord, Stamps & Losos 2010). The linear relationship between dewlap speed and the morphology of the fulcrum ratio implies that muscle contraction velocity ( $V_{mc}$ ) was very similar among Puerto Rican species (a linear correlation was only predicted to occur if  $V_{mc}$  did not vary greatly among species; Fig. 2a,d). This means that to track environmental changes in visual noise, those Puerto Rican species with low fulcrum ratios must expend more energy to plastically increase dewlap speeds as visual noise increases in their environment and may also have a reduced capacity to maintain the production of rapid dewlap movements, than species with high fulcrum ratios (e.g. see Brandt 2003 and references therein). Furthermore, plasticity is presumably physiologically costly, otherwise there would be little need for Puerto Rican lizards to evolve increased fulcrum ratios in environments with higher average levels of visual noise.

Taken together, our findings suggest that an innovation in muscle contraction velocity ( $V_{mc}$ , or possibly its transfer to the lever,  $c$  or  $h$ ) at some point in the history of the Jamaican radiation (or deeper in the phylogeny of the Western Caribbean radiation more broadly; see Introduction and Ord 2012) placed signal evolution on an alternative trajectory to that of the Puerto Rican (or Eastern Caribbean) radiation. The downstream effects of this innovation have been large, resulting in major differences in how species from the two islands cope with communication in visually difficult environments. On one hand, improvements in signal performance resulting from an underlying change in the biomechanics of dewlap

extension have apparently provided Jamaican lizards with a conspicuous territorial display for a range of environmental conditions (see Ord, Charles & Hoffer 2011). On the other hand, a constraint on the performance of the dewlap extension in Puerto Rican lizards has resulted in the evolution of other tactics for maintaining an effective signal (e.g. high-speed headbob movements, contextual plasticity and display timing: Ord *et al.* 2007; Ord, Stamps & Losos 2010; Ord, Charles & Hoffer 2011). In the latter instance, a biomechanical constraint affecting one component of the display has subsequently led to innovations in other aspects of display. It is also possible, however, that the innovation of selectively timing displays to avoid visual noise on Puerto Rico (Ord, Charles & Hoffer 2011) mitigated the need to evolve greater dewlap speeds in the first place. Distinguishing the probable sequence of evolutionary events will be difficult. It is clear, however, that display evolution and its underlying biomechanical basis have proceeded quite differently between Jamaican and Puerto Rican anoles.

## Conclusion

Identifying the adaptive significance of differences in signal behaviour among closely related species is obviously vital for understanding the selection pressures that act on animal signals. However, our study and others (e.g. Podos 2001; Gillooly & Ophir 2010) show that there are intrinsic factors related to the biomechanics of signal production that can also differ among closely related species and can have important effects on how animal signals respond to common selection pressures. Any discussion of the origin and maintenance of signal complexity and novelty in animal communication must consider the consequences of such intrinsic factors (see also related arguments by Lailvaux & Irschick 2006 and Irschick *et al.* 2007). These are not limited to biomechanical constraints, but include genetic correlations among phenotypic traits and other factors that affect the evolvability of the phenotype (Klingenberg 2008; Gosden *et al.* 2012; Sanger *et al.* 2012). Such intrinsic factors are a common focus of evolutionary biologists, but rarely discussed by communication biologists attempting to explain the evolution of signal diversity (outside the influence of receiver sensory systems: Hebets & Papaj 2005; Bradbury & Vehrencamp 2011; Miller & Bee 2012).

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