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Variation in steroid hormone levels among Caribbean *Anolis* lizards: Endocrine system convergence?

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ABSTRACT

Variation in aggression among species can be due to a number of proximate and ultimate factors, leading to patterns of divergent and convergent evolution of behavior among even closely related species. Caribbean *Anolis* lizards are well known for their convergence in microhabitat use and morphology, but they also display marked convergence in social behavior and patterns of aggression. We studied 18 *Anolis* species across six ecomorphs on four different Caribbean islands to test four main hypotheses. We hypothesized that species differences in aggression would be due to species differences in circulating testosterone (T), a steroid hormone implicated in numerous studies across vertebrate taxa as a primary determinant of social behavior; more aggressive species were expected to have higher baseline concentrations of T and corticosterone. We further hypothesized that low-T species would increase T and corticosterone levels during a social challenge. Within three of the four island assemblages studied we found differences in T levels among species within an island that differ in aggression, but in the opposite pattern than predicted: more aggressive species had lower baseline T than the least aggressive species. The fourth island, Puerto Rico, showed the pattern of baseline T levels among species we predicted. There were no patterns of corticosterone levels among species or ecomorphs. One of the two species tested increased T in response to a social challenge, but neither species elevated corticosterone. Our results suggest that it is possible for similarities in aggression among closely related species to evolve via different proximate mechanisms.

Introduction

Variation in social behavior among species can be studied by considering the evolutionary forces driving the observed variation (Westneat and Fox, 2010) and by examining mechanistic differences in neuroendocrine and neuromuscular pathways among species that differ in behavior (Adkins-Regan, 2005; Johnson and Wade, 2010). Each approach offers powerful insights into how behavior, and the physiological substrates underlying it, can evolve over evolutionary time, but combining these approaches to simultaneously study proximate and ultimate determinants of inter-species behavioral differences can help us better understand how evolution has shaped the diversity of behavioral strategies that exist in the present. Convergent evolution of social behavior in particular, where similar behavior has evolved due to similar selective pressures and not phylogenetic relatedness, can provide replication of behavior so that mechanisms of behavioral variation can be tested.

Although convergent evolution is traditionally studied in terms of morphological traits (McGhee, 2011; Rosenblum, 2006; Taylor and McPhail, 2000), there are numerous examples of behavioral convergence, including maternal care strategies in mammals (Fisher et al., 2002), web making in Hawaiian spiders (Blackledge and Gillespie, 2004), echolocation call structure in bats (Jones and Holderied, 2007), and display behavior in Caribbean *Anolis* lizards (Johnson et al., 2010; Ord et al., 2013). Although studying convergent evolution of a trait as potentially complex and dynamic as 'behavior' is challenging, the replication of such an integrated trait in fact makes it ideal for tests of how proximate mechanisms that regulate behavior evolve. If we are to understand how behavior evolves, then we must explore not only selective pressures that favor certain behavioral strategies, but also what is changing at lower levels of biological organization to cause the behavioral changes. It is here that studying examples of convergence in behavior can be so useful. Behavior, whether it be social behavior, foraging behavior, or anti-predator behavior, is the result of numerous physiological and neuroendocrine pathways functioning together to produce an outcome. Thus, just as species differences in behavior can result from divergence in any one or more of these pathways or connections, convergence between species can be the result of the evolution of a multitude of pathways or combinations of pathways (Adkins-Regan, 2005). Such multiple possible mechanistic solutions to 'solving' a functional problem (i.e., the 'behavior') is akin to many-to-one mapping of morphological traits, where multiple morphological combinations can

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result in identical functional outcomes (Losos, 2011; Wainwright, 2007; Wainwright et al., 2005). We focus on social behavior, specifically aggression, and ask whether species convergence in aggression is due to similar underlying mechanisms among species and divergence is due to differences in mechanisms.

Of the myriad pathways that could underlie variation in aggression, testosterone (T) and other androgens have received perhaps the most attention (Hirschenhauser and Oliveira, 2006; Oliveira, 2009; Simon and Lu, 2006; Wingfield, 2005; Wingfield et al., 1990, 2006). There are numerous examples of baseline circulating T levels being associated with variation among species in mating system, courtship behavior, and aggression, especially in interspecific studies (Wingfield, 2005), as well as large-scale environmental factors as latitude, elevation, and breeding season length (Bókony et al., 2009; Eikenaar et al., 2012; Garamszegi et al., 2008; Goymann et al., 2004; Hau et al., 2010; Moore and Jessop, 2003). In the case of aggression, Goymann et al. (2004) found significant differences in baseline T levels among tropical bird species that differ in the degree and duration of territoriality, and two species of *Sceloporus* lizards that differ in aggression have different circulating T levels (Hews et al., 2012). Peripheral, instead of central, androgen actions have been shown in manakins to be important in regulating male display behavior (Fuxjager et al., 2013). In each of these cases, higher T levels were associated with increased aggression or display behavior and are in accordance with the plethora of experimental studies in a variety of vertebrate taxa showing that removal of T decreases aggression, and supplemental T increases aggression (e.g., Marler and Moore, 1988; Tokarz et al., 2002). Nevertheless, individual variation in behavior within a species is not always associated with circulating T levels (Johnson et al., 2011; Kabelik et al., 2008a, 2008b), but is instead likely due to individual variation in other aspects of androgen signaling (Ball and Balthazart, 2004; Hews et al., 2012; Rosvall et al., 2012). Further, empirical work to date suggests that polygynous species with no parental care should maintain maximum baseline T levels throughout the breeding season (Goymann et al., 2007; Wingfield et al., 1990), but the extent to which such species do this remains somewhat unclear. It is possible that some polygynous species without parental care could maintain low baseline T, such as with increased breeding season length in the tropics (Eikenaar et al., 2012), and increase T levels during social interactions (Goymann et al., 2004), but this scenario has been little explored. Thus, an ideal system to test the role of T in determining inter-specific differences in aggression would be an assemblage of relatively closely related species that do not vary much in mating system or degree of parental care and live at similar latitude, but differ markedly in aggression.

Anolis lizards of the Greater Antilles in the Caribbean represent such an ideal model system with which to study convergent behavior, as well as the proximate mechanisms that underlie it. Although the details of mating system are poorly known for many species, they appear to be mostly polygynous and do not display parental care as seen in birds and mammals (Losos, 2009). In addition, these lizards display remarkable, and well-studied, convergent evolution in habitat use and body shape (Losos, 2009). Species on different Caribbean islands have independently evolved into assemblages of coexisting species that partition the vertical strata of the forest and have accompanying morphological adaptations associated with their differential habitat use (Losos, 2009; Williams, 1983). Such species that utilize similar habitats and show convergence in behavior and morphology related to that habitat use are referred to as ecomorphs. For example, species of the same ecomorph on different islands that have converged on similar limb morphology have apparently done so in response to selection that has optimized locomotor performance on their preferred substrates (Irschick and Losos, 1999; reviewed in Losos, 2009). Further, it appears that the differences in habitat use among ecomorphs, and the accompanying differences in habitat complexity and signal efficacy, have resulted in convergence in social behavior (Johnson et al., 2010; Losos, 1990a; Ord et al., 2013). Although behavioral convergence is not perfect, species within an ecomorph appear to have independently evolved similarities in display rate (Losos, 1990a), degree of overlap among male territories (Johnson et al., 2010), and predominant type of signal used during social interactions (Ord et al., 2013).

These findings allowed us to make predictions about which ecomorphs/species might have higher or lower T levels. Trunk-ground anoles have high sexual size dimorphism (Butler et al., 2000), high territory overlap, and spend more time displaying compared to twig anoles at the other end of the behavioral continuum, while other ecomorphs fall somewhere in between these extremes (Johnson et al., 2010; Losos, 1990a). We expected T levels to parallel this continuum of social behavior. We focus on T for the reasons articulated above, but also because experimental studies have shown that T manipulation directly influences aggression and display behavior of male anoles (e.g., Cox et al., 2009; Lovern et al., 2001; Neal and Wade, 2007; Tokarz et al., 2002) and it is T, not conversion to estradiol or 5α-dihydrotestosterone, that is important in regulating male anole social behavior (reviewed in Wade, 2011).

Our goal in this study was to take an important first step in understanding the underlying mechanisms of social behavior divergence and convergence in *Anolis* lizards. Thus, we studied the potential role of circulating T in 18 *Anolis* species across six ecomorphs on four different Caribbean islands. Our first hypothesis was that ecomorphs on an island would differ in T levels consistent with their social behavior differences. We predicted that within each island, more 'aggressive' species (i.e., those that perform more aggressive displays) would have higher circulating levels of T than those that are less 'aggressive' (i.e., those that perform less aggressive displays). For example, on Jamaica, 'trunk-ground' anoles would have high T levels, whereas 'twig' anoles would have low levels. Other ecomorphs would have intermediate levels. Our second hypothesis was that the same ecomorph on different islands would have the same rank order of T levels, and that patterns of ecomorph differences in T levels within an island would be repeated across islands. That is, 'trunk-ground' anoles in Jamaica, the Dominican Republic, the Bahamas, and Puerto Rico would all have the highest T levels, whereas 'twig' anoles on all those islands would have the lowest levels, and other ecomorphs would be intermediate. This hypothesis predicts that even if T levels within an ecomorph across islands are not of the same magnitude, the same pattern of relative ecomorph differences seen within an island would be replicated across islands. Given the energetic demands of high activity levels involved with increased display frequency (perhaps due to T levels), and the observation that baseline corticosterone is important for energy mobilization during such energetically expensive activity (Hau et al., 2010; Sapolsky et al., 2000), our third hypothesis was that corticosterone levels would positively relate to T levels across species. Our fourth hypothesis, based on tenets of the Challenge Hypothesis and the fact that Caribbean anoles have an extended breeding season, was that two species with low T levels would elevate them during a simulated social challenge.

Methods

Data collection

We sampled species (see Table 1) on Jamaica in and around Discovery Bay Marine Laboratory, Jamaica in late April of 2011. We sampled Bahamian species in and around the 'Fountain of Youth' and the Bimini Nature Trail on South Bimini, Bahamas in early May of 2011. We sampled species on Hispaniola in and around Coralsol Resort near Barahona, Dominican Republic in late April of 2012. We sampled Puerto Rican species in El Yunque National Forest in and around El Verde Field Station, Puerto Rico in early May of 2012. Although we sampled in two different years, we were consistent in time of year and sampled in as small a window of time as possible within a year (2 weeks per site) to maximize sample size but minimize any potential seasonal effects. We chose this time of year, because it is definitely within the breeding season of all