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Divergent hormonal responses to social competition in closely related species of haplochromine cichlid fish

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ABSTRACT

The diverse cichlid species flocks of the East African lakes provide a classical example of adaptive radiation. Territorial aggression is thought to influence the evolution of phenotypic diversity in this system. Most vertebrates mount hormonal (androgen, glucocorticoid) responses to a territorial challenge. These hormones, in turn, influence behavior and multiple aspects of physiology and morphology. Examining variation in competition-induced hormone secretion patterns is thus fundamental to an understanding of the mechanisms of phenotypic diversification. We test here the hypothesis that diversification in male aggression has been accompanied by differentiation in steroid hormone levels. We studied two pairs of sibling species from Lake Victoria belonging to the genera *Pundamilia* and *Mbipia*. The two genera are ecologically differentiated, while sibling species pairs differ mainly in male color patterns. We found that aggression directed toward conspecific males varied between species and across genera: *Pundamilia nyererei* males were more aggressive than *Pundamilia pundamilia* males, and *Mbipia mbipi* males were more aggressive than *Mbipia lutea* males. Males of both genera exhibited comparable attack rates during acute exposure to a novel conspecific intruder, while *Mbipia* males were more aggressive than *Pundamilia* males during continuous exposure to a conspecific rival, consistent with the genus difference in feeding ecology. Variation in aggressiveness between genera, but not between sibling species, was reflected in androgen levels. We further found that *M. mbipi* displayed lower levels of cortisol than *M. lutea*. Our results suggest that concerted divergence in hormones and behavior might play an important role in the rapid speciation of cichlid fishes.

Introduction

Identifying the mechanisms that drive population differentiation and speciation has proven to be one of the most challenging problems in evolutionary biology (Fisher, 1930; Lande, 1981; Van Doorn et al., 2009). The adaptive radiations of haplochromine cichlid fishes in the East African Great Lakes provide textbook examples of rapid diversification through natural and sexual selection (Kocher, 2004; Salzburger and Meyer, 2004; Schluter, 2000). The rock-dwelling communities of these lakes comprise several species complexes or genera that are strongly differentiated in ecology. By contrast, within genera, sibling species tend to be ecologically more similar, yet strikingly different in male nuptial coloration (Seehausen, 2000). This color variation is a target of sexual selection by female mate choice and plays a central role in the evolution and maintenance of haplochromine species richness (e.g., Genner and Turner, 2005; Kocher, 2004; Maan et al., 2004; Seehausen et al., 1997). Since haplochromine males can be highly territorial, it has been proposed that interference competition among males for mating and/or foraging territories can be a source of selection (Genner et al., 1999; Seehausen and Schluter, 2004). Indeed, several studies have indicated that male–male competition can generate negative frequency-dependent selection between competing species (Dijkstra et al., 2010; Seehausen and Schluter, 2004).

Across cichlid species there is striking variation in the (intrinsic) rate of territorial aggression (Genner et al., 1999; Ribbink et al., 1983). This behavioral variation influences outcomes of competition for both mates and ecological resources, and therefore has implications for selection, patterns of gene flow and the evolution and maintenance of phenotypic diversity (Dijkstra et al., 2010; Genner et al., 1999; see also: Owen-Ashley and Butler, 2004; West-Eberhard, 1983). A clear understanding of the evolutionary consequences of aggressive behavior requires understanding the physiological causes and consequences of agonistic interactions. Variation in hormones could underlie differences in aggression between species (e.g., O'Connell and Hofmann, 2011; Oliveira, 2009). However, hormones are not only a causal factor for male social behavior, but also their excretion rates are influenced in

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turn by the social environment, in particular by interactions between conspecifics, suggesting a complex two-way relationship between hormones and behavior (Wingfield et al., 1990; reviewed in Oliveira, 2004).

In addition to their role in behavior, hormones also regulate multiple aspects of physiology and morphology. Consequently, hormones are thought to mediate trade-offs among life history traits that are important for survival and reproduction (McGlothlin and Ketterson, 2008). It follows then that ecological or social factors may select for higher rates of aggressiveness via increases in competition-induced circulating levels of androgens. Competitive challenges may also induce a stress response by activating the hypothalamic–pituitary–adrenal (HPA) axis, resulting in a rapid glucocorticoid release that helps the animal to respond appropriately to stressful stimuli. However, increased androgen and glucocorticoid secretion rates can exert negative effects on the immune system and other physiological variables (Folstad and Karter, 1992; Wendelaar Bonga, 1997). As hormones exert (antagonistic) pleiotropy over behavior and other aspects of an animal's phenotype, selection on hormone-mediated behaviors could play an important role in creating and maintaining polymorphic phenotypes (e.g. in a frequency-dependent manner) (Kitano et al., 2010; Pryke et al., 2007; for review see Zera et al., 2007). We therefore propose that studying competition-induced shifts in hormone levels may advance our understanding of the rapid evolution of the haplochromine cichlid radiation. Specifically, we ask in the present study whether interspecific variation in aggression is reflected in parallel patterns of steroid hormones in four closely related sympatric haplochromine species.

Steroid hormones, such as androgens and glucocorticoids, affect a variety of morphological, physiological and behavioral traits (reviewed by Nelson, 2005). As noted above, androgen release is modulated by the social environment, in particular through interactions with conspecifics (e.g. Cardwell and Liley, 1991, reviewed in Oliveira, 2004). Circulating androgen levels are increased in periods of social instability that constitute a challenge to the animal (Wingfield et al., 1990), preparing the animal for future competitive situations (reviewed in Oliveira, 2004). In a comparative context, the challenge hypothesis has been useful in predicting competition-induced shifts in hormone levels according to several social and life history variables, such as length of breeding season and mating system (for recent reviews see Gleason et al., 2009; Goymann, 2009; Hirschenhauser and Oliveira, 2006).

Glucocorticoids coordinate behavioral and physiological responses to acute and chronic stressors (Sapolsky et al., 2000). For example, glucocorticoids mobilize energy resources and coordinate other physiological aspects of the stress response, aiding the animal in surviving stressful situations (Romero, 2002). At least indirectly, glucocorticoids are important modulators of aggression as well (Soma et al., 2008) and, correspondingly, glucocorticoid secretion rates increase in periods of social instability (Goymann and Wingfield, 2004). Although glucocorticoid responses are essential to survival, glucocorticoid can suppress the gonadal axis (Moberg, 1985) and long term exposure of glucocorticoids can lead to a multitude of deleterious effects, including neuron death (Sapolsky, 1993). Animals must therefore strike a balance between glucocorticoid levels that help survive stressful situations while limiting (long-term) glucocorticoid secretion to prevent deleterious effects.

Within an evolutionary context, studying hormonal responses to social challenges may contribute to our understanding of the mechanism of diversification in cichlids, since steroid hormones not only regulate behavior (and vice versa), but also affect a variety of key life history traits such as sexual signaling and immune function. We test here the hypothesis that diversification in male aggression has been accompanied by differentiation in steroid hormone levels across several Lake Victoria cichlid species. We focused on two sympatric sibling species pairs of haplochromine cichlids from two different genera that have the same mating system but vary in male color, the rate of aggressiveness and foraging ecology (Fig. 1): (1) *Pundamilia pundamilia* and *Pundamilia nyererei* and (2) *Mbipia lutea* and *Mbipia mbipi* (Seehausen, 1996). Sibling species within each genus are morphologically very similar but differ markedly in male nuptial coloration and aggression (Fig. 1) with *P. nyererei* being more aggressive than *P. pundamilia* and *M. mbipi* being more aggressive than *M. lutea* (Dijkstra et al., 2010; Verzijden et al., 2008, 2009; Verzijden unpublished). Although the two genera have not been previously compared, we hypothesized that they would likewise differ in average aggressiveness (Fig. 1). *Mbipia* and *Pundamilia* spp. occupy different trophic niches and accordingly display divergent ecomorphology (Seehausen et al., 1998): *Pundamilia* prefer zooplankton and benthic insects, which are more or less uniformly distributed within the lake. *Mbipia* are more dependent on *Aufwuchs* (i.e., spatially clustered epilithic algae and associated organisms, Bouton et al., 1997; Seehausen et al., 1998), which constitutes a more defendable resource (Bouton et al., 1997; Seehausen et al., 1998). Thus, in *Mbipia* aggressive behavior has a dual function in that it enables males to compete for and attract potential mates and to defend a feeding territory. As a consequence, we predicted that *Mbipia* would exhibit higher levels of territorial aggression than *Pundamilia* (Fig. 1).

In the present study, we investigated how interspecific variation in two types of territorial challenges from a conspecific rival is reflected in variation in circulating androgen and glucocorticoid levels. To this end, we analyzed agonistic behavior patterns and subsequent hormonal responses across three experimental contexts: continuous territory defense against a familiar male; a simulated territorial intrusion challenge by an unfamiliar male; and a social isolation control. We quantified aggressive displays and attacks and measured circulating levels of testosterone (T), the teleost-specific androgen 11-ketotestosterone (11-KT) (Kime, 1993) and the glucocorticoid hormone cortisol (CORT).

We expected that the behavioral and hormonal responses toward an unfamiliar intruding rival would be stronger than toward a familiar neighbor. Further, we predicted that *Mbipia* males would exhibit higher levels of aggression, and have higher levels of circulating steroids than males of *Pundamilia* (Fig. 1). In a previous study (Dijkstra et al., 2011), we found that red and blue *Pundamilia* phenotypes differed in aggression levels, yet this phenotype difference was not reflected in circulating steroid hormone levels. Importantly, in that study red and blue males were from a location in Lake Victoria where they hybridize and behave like incipient species or color morphs (Seehausen, 2009). In the current study, in contrast, we focused on reproductively isolated

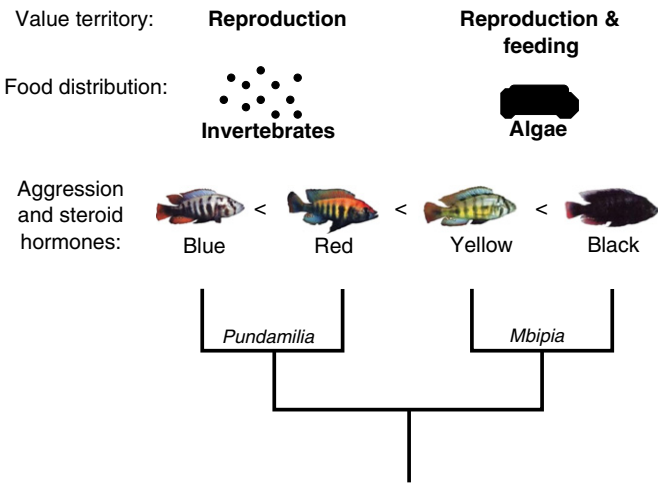

Fig. 1. Summary description of trophic ecology as well as the expected relative aggression and steroid hormone levels for the four species used in the current study, *Pundamilia pundamilia* (males are blue), *P. nyererei* (red), *Mbipia lutea* (yellow) and *M. mbipi* (black). Photos by Ole Seehausen.