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Androgens and dominance: Sex-specific patterns in a highly social fish (*Neolamprologus pulcher*)

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ABSTRACT

In most vertebrates, aggression and dominance are tightly linked to circulating testosterone. Fish, however, have two androgens (testosterone, T and 11-ketotestosterone, 11KT) that influence aggression and dominance. To date, few studies have compared the relationship between androgen levels and the outcome of aggressive contests in both females and males of the same species. To investigate sex differences in androgens we staged size-matched, limited-resource (territory) contests with 14 female–female and 10 male–male pairs of the highly social cichlid *Neolamprologus pulcher*. We then examined androgen levels in recently established dominants, who won the contest and subsequently acquired a territory (for 3 h), and subordinates, who lost and did not acquire a territory. Newly dominant females had higher plasma T but similar 11KT levels to newly subordinate females. In contrast, newly dominant males had higher 11KT but similar T levels to subordinate males. The ratio of 11KT to T, which demonstrates physiological importance of T conversion to 11KT, was positively correlated with submissive behavior in female winners, and correlated weakly with aggressive behavior in male winners ($p = 0.05$). These findings provide support for the hypothesis that different androgens play equivalent roles in female versus male dominance establishment, and suggest that relative levels of 11KT and T are implicated in female dominance behavior and perhaps behavior of both sexes.

1. Introduction

Androgen hormones have been linked to aggressive behavior across a broad spectrum of vertebrates (reviewed by Nelson (2000)). Manipulation experiments have demonstrated that androgen removal decreases aggression while androgen treatment rescues or increases aggression (Edwards, 1970; Arnold, 1975; Balthazart, 1983; Moore, 1988; Kindler et al., 1991; Francis et al., 1992; Hume and Wynne-Edwards, 2005). These observations, paired with repeated demonstration that individuals with elevated androgen levels exhibit high levels of aggression (Lincoln et al., 1972; Scott et al., 1980; Johnsen, 1998) have led scientists to accept circulating androgens as potent mediators of male aggressive behavior. More recent findings have shown that female aggression also varies with circulating androgen levels (reviewed in Bouissou (1983); Staub and De Beer (1997)). Both male and female aggression and androgen levels have been strongly associated with dominance over conspecifics (Bouissou, 1983; Schoech et al., 1991; Desjardins et al., 2008b). To our knowledge, however, no study has measured androgen levels corresponding with dominance establishment in both males and females, and sex-specific roles of androgens in dominance establishment remain poorly understood.

Neolamprologus pulcher, a group-living African cichlid, is an ideal model to test predictions on aggression and dominance establishment in both males and females. Highly territorial, this fish lives in groups composed of a dominant breeding pair and up to 20 subordinate helpers (Balshine et al., 2001; Heg et al., 2005). All individuals constantly reinforce their position within the group dominance hierarchy through a variety of aggressive, submissive and affiliative behaviors. As in all group-living species, predation events and natural deaths create vacancies in the dominance hierarchy. When these vacancies occur in top breeding positions, conspecifics from within or outside the group compete over the newly vacant position (Balshine-Earn et al., 1998; Stiver et al., 2006; Fitzpatrick et al., 2008). The victor of these competitions fills the dominant breeding position. Maintaining a position of dominance requires the strength and stamina for territory defense, subordinate policing and reproduction. These behaviors have been associated with circulating androgen levels in fish (Neat et al., 1998; Parikh et al., 2006), and androgen levels have been implicated in aggressive behavior during male dominance establishment across vertebrate

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taxa (Bouissou, 1983; Wingfield, 1985; Moore, 1988; Oliveria, 2004). Importantly, a previous experiment with *N. pulcher* found that winners and losers had equally elevated androgen levels immediately following a contest, although no changes in estradiol were seen in either sex (Desjardins et al., 2006). The androgen increases observed in both contestants may reflect short-term aggression-induced fluctuations rather than long-term patterns associated with status change, as established dominants in the field have higher plasma androgen levels than subordinates (Desjardins et al., 2008b).

The first aim of this experiment was to determine whether individuals successfully acquiring a territory for 3 h after completion of competition would exhibit higher levels of circulating androgen hormones than individuals who were unsuccessful in territory acquisition. The second aim was to explore whether females and males differ in androgen response to territory acquisition and establishment of dominance. To investigate endocrine and behavioral correlates of territory acquisition, we staged competitions for a shelter-containing territory between size-matched individuals of the cichlid fish, *N. pulcher*. As this fish species is highly philopatric and completely reliant on shelter in its territory for survival and reproduction (Balshine et al., 2001), we staged contests for shelter and then compared plasma androgens, relative gonad investment, and measures of aggression between competition winners and losers. Plasma T and 11-KT were assayed, as these are considered the most active androgens in females and males, respectively (Borg, 1994). Specifically, we predicted to find higher circulating androgen levels in both female and male individuals that successfully gained control of a territory (winners) compared to individuals that did not (losers).

2. Materials and methods

2.1. Study animals and holding conditions

Territory competitions were performed between 24 September and 26 November 2004 using *N. pulcher* held at McMaster University in Hamilton, Ont., Canada. Fish used in this study were derived from a wild-caught stock of fish originating from the southern shore of Lake Tanganyika, received in 2001 and 2002. To mimic natural lake conditions water temperature was kept between 25 and 28 °C and a 13:11 light:dark cycle was maintained. Fish were fed flake cichlid food daily, ad libitum. Tanks contained 2.0 cm of fine coral sand as substrate, an AquaClear Mini mechanical filter and a thermometer. Twenty male and 28 female subordinates were selected from stable 190-l group tanks (92 × 41 × 50 cm) for this study. Subordinate status was determined by repeated observations of submissive behaviors by the focal fish toward a dominant individual of the same sex. Fish were measured and given a unique fin clip for individual identification. The fish used had a mean standard length (±standard error) of 5.92 ± 0.11 cm (range 4.50–7.98 cm) and mean body mass of 5.76 ± 0.34 g (range 2.316–13.697 g).

Individuals were grouped into sex- and size-matched pairs, and then each member of a pair was randomly placed into one end compartment of a 30-l experimental tank (48 × 26 × 30 cm) partitioned into three equal 16 × 26 × 30 cm compartments. Each compartment was separated by one opaque and one transparent barrier. The central compartment (not containing any fish) contained a flowerpot half that served as a shelter and as the resource over which the animals competed. Paired, sex-matched fish always originated from different tanks to avoid effects of familiarity and previous interactions, and each fish was only used once. The mean size difference (±one standard deviation) between paired individuals was 0.20 ± 0.03 cm (0.19 ± 0.05 cm was the mean difference between paired females and 0.22 ± 0.04 cm was the mean difference between paired males).

2.2. Territory competition

Fish were placed in pairs in the experimental tank compartments. Tanks were completely covered with opaque plastic sheeting to occlude all light, and fish were left in the dark for 60 min to acclimate to their new environment. After 60 min, the plastic sheeting and opaque barriers were removed. The remaining transparent barriers allowed both fish to see each other and the shelter in the middle enclosure. This stage lasted for 10 min. Following this light acclimation period, transparent tank partitions were removed so that both fish were able to swim freely throughout the entire tank. All interactions were videotaped and aggressive behaviors were later scored from the videotapes by observers blind to the identities of the fish. Trials lasted for 20 min and were always conducted between 9:00 and 13:00 to control for daily variation in androgens and behaviors (Oliveira et al., 2001; Werner et al., 2003). All aggressive and submissive interactions were recorded (see Table 1 for an updated ethogram for this species). Time spent within the shelter was also recorded. At the end of each 20 min competition, winner status was assigned to the fish guarding the shelter and loser status was assigned to the fish furthest away from the shelter, often this individual was near the top of tank. One transparent partition was then replaced to sequester the loser into a compartment at one end of the tank (as before the competition). The winner retained access to the other two-thirds of the tank including the shelter. The two fish remained in this arrangement for 3 h, after which blood samples were collected. Studies have shown that both winners and losers can have similarly elevated androgen levels immediately after an aggressive interaction (Neat and Mayer, 1999; Desjardins et al., 2006); by waiting 3 h our samples reflected longer-term hormone patterns following social status establishment and territory acquisition.

2.3. Hormone assays

Blood collection and hormone assay protocols in this study were based on Desjardins et al., 2006, 2008b). Three hours after the competition was completed, both fish were caught, weighed, measured (standard length and total length), and anesthetized with a lethal dose of benzocaine (ethyl-*p*-aminobenzoate, 1.0 μg/ml). Blood was collected by caudal severance with heparinized microcapillary tubes. The time from researchers approaching the tank after the experiment to completion of blood sampling was less than 3 min. Blood was spun at 8000 rpm for 10 min after which separated plasma was removed and frozen at −20 °C for later measurement of T and 11-KT. A mean of 29 ± 12 μl of plasma was obtained from blood samples. Gonads, livers, and brains were removed and weighed. Fish were sexed by examination of gonads.

Steroid extraction from plasma was performed by shaking aliquots with 5 ml of diethyl ether, twice. The aqueous phase was frozen in a bath of acetone and dry ice, and the organic phase was decanted and dried. Dry extracts were resuspended in assay buffer and frozen at −20 °C. Extraction losses were independently monitored by extracting five samples hot spiked with a known volume of radioactivity, and recoveries after performing a double extraction were 88 ± 4%. Antibodies originated from Cayman Chemical. T and 11KT were measured by radioimmunoassay according to plasma steroid measurement techniques described by McMaster et al. (1995). Stated values are mean concentrations of duplicate aliquots whose values were within the 5–95% binding limits of the appropriate standard curve after linearization with a log–logit