Hormones and Behavior 107 (2019) 83–95

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Hormonal regulation of social ascent and temporal patterns of behavior in an African cichlid

Beau A. Alward*, Austin T. Hilliard, Ryan A. York, Russell D. Fernald

Department of Biology, Stanford University, Stanford, CA 94305, USA

ARTICLE INFO

Keywords:
Social behavior
Social status
Androgens
African cichlid

ABSTRACT

For many species, social rank determines which individuals perform certain social behaviors and when. Higher ranking or dominant (DOM) individuals maintain status through aggressive interactions and perform courtship behaviors while non-dominant (ND) individuals do not. In some species ND individuals ascend (ASC) in social rank when the opportunity arises. Many important questions related to the mechanistic basis of social ascent remain to be answered. We probed whether androgen signaling regulates social ascent in male *Astatotilapia burtoni*, an African cichlid whose social hierarchy can be readily controlled in the laboratory. As expected, androgen receptor (AR) antagonism abolished reproductive behavior during social ascent. However, we discovered multiple AR- and status-dependent temporal behavioral patterns that typify social ascent and dominance. AR antagonism in ASC males increased the time between successive behaviors compared to DOM males. Socially ascending males, independent of AR activation, were more likely than DOM males to follow aggressive displays with another aggressive display. Further analyses revealed differences in the sequencing of aggressive and courtship behaviors, wherein DOM males were more likely than ASC males to follow male-directed aggression with courtship displays. Strikingly, this difference was driven mostly by ASC males taking longer to transition from aggression to courtship, suggesting ASC males can perform certain DOM-typical temporal behavioral patterns. Our results indicate androgen signaling is necessary for social ascent and hormonal signaling and social experience may shape the full suite of DOM-typical behavioral patterns.

1. Introduction

Social animals often organize into hierarchies, where higher ranking members have access to key resources such as territory, food, and mates (Sapolsky, 2005; Wilson, 1975). Such hierarchies are established through agonistic interactions between conspecifics. Higher ranking individuals perform a variety of courtship behaviors that are part of the species-typical suite of acts that culminate in copulation and reproduction (Adkins-Regan, 2009). Studies in primates, birds, and fish have discovered key social and environmental factors that regulate social status (Fernald, 2012; Sapolsky, 2005). However, the mechanistic basis of social status is still unclear.

The African cichlid fish *Astatotilapia burtoni* has been used as a particularly popular model for these studies given the unique opportunities if offers for mechanistic analysis of status. In their native habitat, Lake Tanganyika, *A. burtoni* males exist as either dominant (DOM) or non-dominant (ND; Fig. 1A). ND males survey the social environment waiting for an opportunity to rise in social rank, a process that has been called social ascent, wherein males exhibit an increase in aggressive and reproductive behaviors typical of DOM males (Fig. 1A–B; Burmeister et al., 2005; Maruska, 2015; Maruska et al., 2013; Maruska and Fernald, 2013, Maruska and Fernald, 2010a, 2010b, 2010c). DOM males possess large gonads and high circulating levels of testosterone and its androgenic and estrogenic metabolites, while ND males have small gonads and low levels of circulating testosterone. Ascending (ASC) males experience a large increase in circulating testosterone to DOM-typical levels, even though their gonads remain small. Aggression in *A. burtoni* appears to be mediated by the actions of estrogenic metabolites of testosterone (Huffman et al., 2013; O'Connell and Hofmann, 2012a). However, the role of androgen signaling in mediating social behavior in *A. burtoni* is still unclear. For instance, blocking androgen receptors (AR) with the potent antagonist cyproterone acetate (CA) reduced two reproductive behaviors in stable DOM males (O'Connell and Hofmann, 2012a), yet injection of dihydrotestosterone, an androgen, failed to stimulate these two reproductive behaviors in ND males. Thus, while estrogen signaling regulates aggression regardless of

* Corresponding author.
E-mail addresses: balward@stanford.edu (B.A. Alward), ahilliar@stanford.edu (A.T. Hilliard), ryanyork@stanford.edu (R.A. York), rfernald@stanford.edu (R.D. Fernald).

https://doi.org/10.1016/j.yhbeh.2018.12.010
Received 30 August 2018; Received in revised form 7 December 2018; Accepted 17 December 2018
Available online 04 January 2019

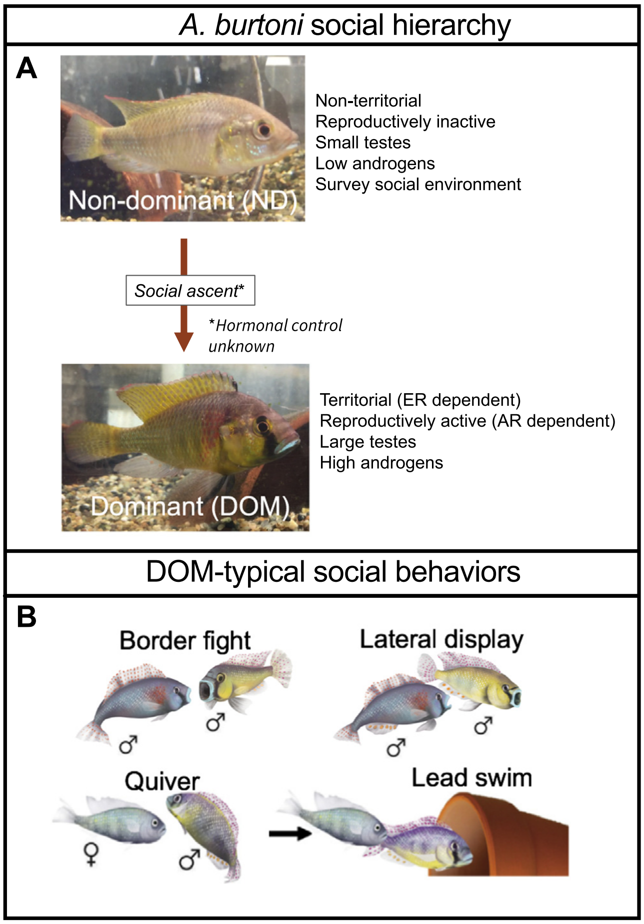

Fig. 1. Male *Astatotilapia burtoni* stratify along a social hierarchy. *A. burtoni* males exist in a social hierarchy, in which (A) ND males survey the social environment and attempt to rise to DOM male status when the opportunity arises (Fernald, 2017); (B) DOM males exhibit a complex suite of aggressive (e.g., border fight and lateral display) and reproductive (e.g., quiver and lead swim) behaviors that are readily quantifiable in naturalistic laboratory settings. ER = Estrogen receptor; AR = Androgen receptor.

social status, the role of androgen signaling is not straightforward. For example, how does an ND male, when given the opportunity, exhibit an increase in an AR-dependent behavior, courtship?

Seasonally-breeding songbirds, a model system in the hormonal control of behavior, may provide clues (Alward et al., 2018). Songbirds undergo a transformation in courtship behavior as they transition from non-breeding to breeding conditions, a process that could be viewed as similar to social ascent. For instance, when in non-breeding conditions (e.g., during the fall and winter), male canaries (*Serinus canaria)* and white crown sparrows (*Zonotrichea leucophrys*) possess small testes, low testosterone, and sing low levels of courtship song (Reviewed in Alward et al., 2018 and Schlinger and Brenowitz, 2009). However, when in breeding conditions (e.g., during the spring), males experience a dramatic increase in testosterone levels and courtship song, which is followed several days later by an increase in testes size. Laboratory studies have found the increase in courtship song is AR- and estrogen receptor (ER)-dependent (Reviewed in Ball et al., 2002 and Schlinger and Brenowitz, 2009). Moreover, global and localized manipulations using pharmacological techniques have found AR activation enhances courtship song (Sartor et al., 2005), but also controls multiple temporal aspects of song, such as the tempo of specific vocalizations and their sequential arrangement (Alward et al., 2017).

Do ascending *A. burtoni* males undergo similar AR-dependent shifts in the activation and temporal sequencing of specific behaviors during social ascent? Here, we address this question via an investigation of social ascent across multiple behavioral measures and temporal scales. To do so we augment standard measures of aggressive and courtship behaviors (Fernald and Maruska, 2012; Maruska and Fernald, 2013) with novel assays of behavioral sequence and interval variation across time scales relevant to ascent. We initially found blockade of AR abolished the rise in reproductive behavior normally seen in ASC fish, as expected given previous observations. However, upon factoring in temporal information, it was revealed that AR activation is also required for enhancing the speed of behavioral performance during ascent. Further investigation into these temporal patterns of behavior revealed that socially ascending fish—regardless of AR activation—significantly diverged from stable DOM males in the sequelae of behavior at multiple time-scales. For instance, socially ascending males were more likely than stable DOM males to perform aggressive displays twice in a row, while DOM males were significantly more likely than socially ascending males to follow aggressive behavior with courtship behavior. Strikingly, several of these differences disappeared when we used a longer interval for defining bouts of behavior, suggesting ASC males can perform DOM-like behavior sequencing patterns but take longer to do so. Our findings expand our knowledge of the hormonal control of social status and, to best of our knowledge, identify for the first time that temporal patterns of behavior over multiple time-scales are key factors in demarcating ASC and DOM males. Furthermore, our data suggest social behavior in *A. burtoni* is highly dissociable, with motivation and temporal aspects of behavior likely being mediated by independent mechanisms.

2. Materials and methods

2.1. Animals

Adult *A. burtoni* were derived from wild-caught stock from Lake Tanganyika, Africa, and laboratory-bred in aquaria under environmental conditions that mimic their natural equatorial habitat (28 °C; pH 8.0; 12:12 h light/dark cycle with full spectrum illumination; constant aeration). Aquaria contained gravel-covered bottoms with terra cotta pots cut in half to serve as shelters and spawning territories. Fish were fed cichlid pellets and flakes (AquaDine, Healdsburg, CA, USA) each morning. All experimental procedures were approved by the Stanford Administrative Panel for Laboratory Animal Care.

2.2. Social manipulation and CA injection

2.2.1. Rationale

A. burtoni, like many other teleosts, have two ARs (Harbott et al., 2007). While ND males have lower levels of circulating androgens compared to DOM males, they are not undetectable (Parikh et al., 2006). Androgens could promote social ascent in two ways: low levels of androgens in ND males promote ascent once the opportunity arises and/or androgens act rapidly on the day of ascent promoting a rise in social status. Thus, in our injection procedures we aimed to address these two issues. To block ARs, we injected the AR antagonist cyproterone acetate (CA), a potent steroidal anti-androgen that blocks androgen binding to the ligand binding domain of ARs through competitive binding (Fourcade and McLeod, 2004), approximately 36 h before the opportunity to ascend. Previous work in *A. burtoni* using CA showed that maximal behavioral effects were observed two days after injection, but significant behavioral effects occurred on the day of and day after injection (O'Connell and Hofmann, 2012a). According to clinical studies CA has a half-life of 30–40 h (Wirth et al., 2007). Thus, our injection procedure likely blocks ARs on the day of injection up until the day of ascent.

Findings in other species suggest CA can enter the *A. burtoni* brain and exert an effect on ARs. Administration of CA in mice causes a reduction in GABA concentrations in the brain (Earley and Leonard,

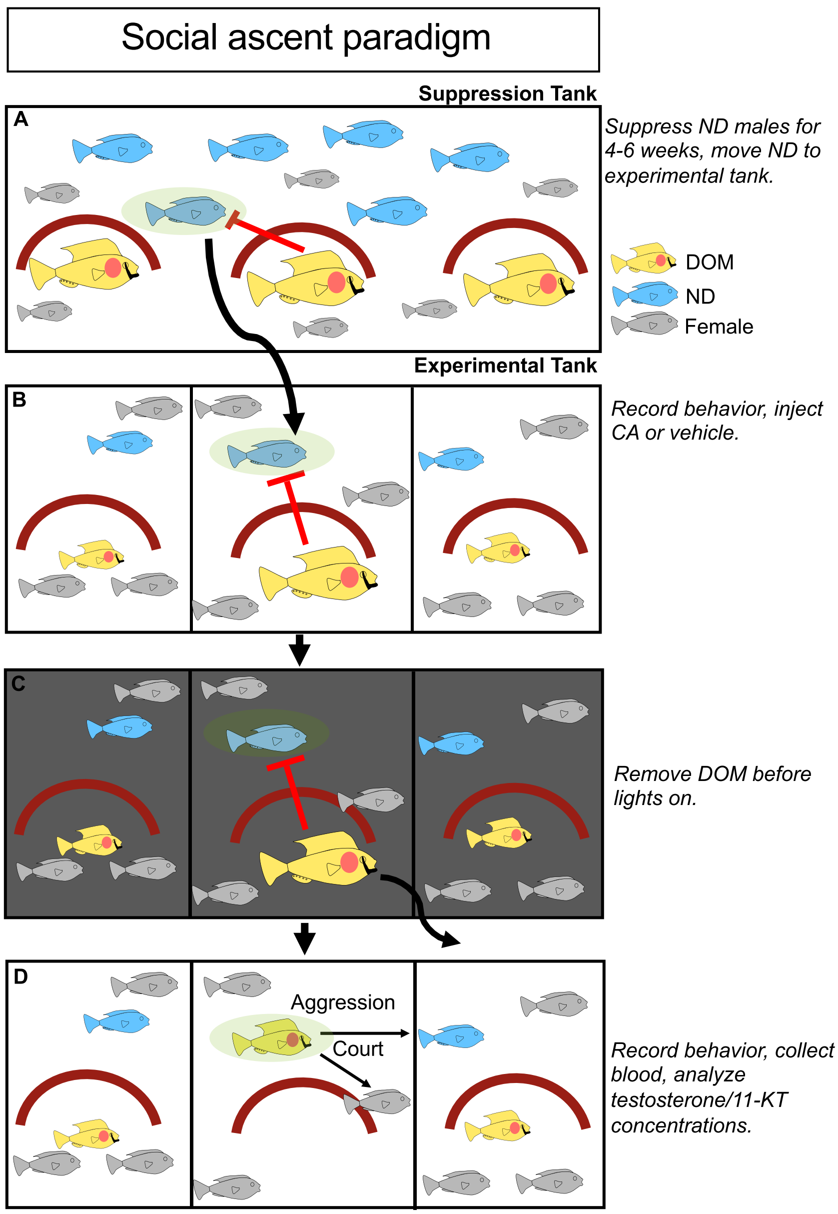

Fig. 2. Social manipulations are used to generate non-dominant *A. burtoni* males that are allowed to ascend. Schematic showing the social ascent paradigm combined with the injection of CA or vehicle. (A) Males were placed into community suppression tanks for 4–5 weeks that contained three large dominant males (i.e., suppressor males) and females. (B) Suppressed males were then transferred to a central compartment of an experimental tank for 2 days prior to social opportunity. The central compartment contained a larger resident dominant male and 3 females and was separated with transparent acrylic barriers on either side from community tanks containing females, subordinate males, and dominant males that were smaller in size than the subject fish. On the day of transfer to the experimental tank, fish were allowed to acclimate for 1 h; then, behavior was recorded for 30 min and fish were injected with either vehicle (sesame oil) or CA, an AR antagonist. Fish were filmed again the following day. (C) Then, on the third day (i.e. the day of social ascent) the resident dominant male was removed from the central compartment 1 h before the lights turned on in the morning. (D) At light onset, behavior was filmed for five hours and fish were removed and blood was collected for analyses of concentrations of the androgens testosterone and 11-KT. Upside down red "U"s indicate the halved terra cotta pots that functioned as the males' spawning sites. DOM = dominant; ND = non-dominant. KT = ketotestosterone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1977), and in rats reduces the uptake of testosterone in the brain (Sar and Stumpf, 1973) and reduces the binding of DHT to AR in the brain (Lieberburg et al., 1977), suggesting CA can cross the blood-brain barrier (Earley and Leonard, 1977). Previous work has shown the zebrafish (*Danio rerio*) blood-brain barrier is similar to that of mammals (Li et al., 2017). Therefore, it is likely that our CA injections are able to exert effects throughout the *A. burtoni* brain and periphery.

2.2.2. Experimental design

We used a previously described "social ascent" paradigm (Maruska et al., 2011; Maruska and Fernald, 2011; Maruska and Fernald, 2010a, 2010b, 2010c) that is shown in Fig. 2. Briefly, to establish socially-suppressed ND fish, males were placed into aquaria for 4–5 weeks with several larger dominant suppressor males, females, and ND males. At the end of the suppression period, subjects were moved into the central compartment of an experimental tank that contained one larger resident dominant male and three females. This central compartment was separated with transparent acrylic barriers from flanking compartments that contained two smaller males and three females so that fish could interact visually but not physically with community animals. After an hour acclimation, we recorded fish from 1:30 pm to 2:00 pm (see Recording and scoring of behavior below). Then, the focal male was removed, injected intraperitoneally with CA (for a subset of ND males that would be allowed to socially ascend, hereon called ASC + CA; concentration of CA = 0.83 μg/gbw) or vehicle (sesame oil; given to DOM, ASC, and ND fish) and immediately placed back into the central compartment of the experimental tank. The concentration of CA was determined based on previous work showing that injecting CA at this concentration results in significant behavioral effects two days after injection (O'Connell and Hofmann, 2012a, 2012b). Two days later—the day of ascent—the larger resident suppressor male was removed with a net 30 min before light onset using infrared night vision goggles (Model 26-1020; Bushnell, Overland Park, KS, USA), providing an opportunity for the ND male to ascend at light onset. Stable DOM and stable ND males were used as controls and compared to males ascending in social status. Stable ND males were suppressed in community tanks for 4–5 weeks and transferred to the same experimental tank as described above. On the day of ascent, however, removal of the suppressor DOM male was only simulated by dipping a net into the tank before light onset without removal of the dominant resident. Stable DOM were

DOM males that maintained their status in community tanks for 4–5 weeks and were then placed in the experimental tank with females but no larger resident male, which maintains their dominance status. On the day of ascent, a net was dipped into the water before light onset to simulate resident male removal.

We included females in this study that were visually identified as gravid (Maruska and Fernald, 2010a). Two assays were always ran in parallel and after each assay, all females from both tanks were removed and placed in large buckets, then the males were removed and placed into a bucket separate from females. Then, the tanks were siphoned and cleaned. At the beginning each new assay, females were randomly assigned to an experimental tank and compartment. If any females were brooding after an assay, they were replaced by another female that appeared to be gravid based on visual inspection. Males were also assigned randomly to flanking compartments and experimental tanks. If a male appeared injured based on damaged fins, it was replaced by an uninjured male. At all times, the males and females were smaller than the focal males included within the central compartment.

2.3. Recording and scoring of behavior

Behavior was quantified during essential 30-minute intervals: after an hour of acclimation on day 1, but immediately before the injection ("pre-injection") from 1:30 pm to 2:00 pm; on day 2 from 1:30 to 2:00 pm (baseline (BL)); and on day 3 (day of ascent) from 9:00 am to 9:30 am and 1:30 to 2:00 pm. Focal fish were removed from the experimental compartment at 2:00 pm on the day of ascent, when tissue was harvested for physiological measurements and blood collection (see Morphological and steroid hormone analyses). Behavior was recorded using a digital video camera (Sony, HDR). Videos were scored by an observer without knowledge of the fish identification number. Based on previous work, multiple types of behavior were quantified (Fernald and Hirata, 1977): subordinate behavior (flee); territorial or agonistic behaviors (lateral display, border fight, and attack female); and reproductive behaviors (chase female, quiver, lead swim, pot entry, and dig). Fleeing was defined as a rapid retreat swim from an approaching dominant male or female. Lateral displays were classified as presentations of the side of the body to another fish with erect fins, flared opercula, and trembling of the body. Border fights were interactions with neighboring dominant males across the acrylic barrier typified by head-on lunges and rams against the barrier with an open mouth. Attack female was defined as the male lunging a short distance towards a female, biting her on her side, and floating backwards a short distance. Chase female was defined as a rapid swim directed towards a female and was grouped with reproductive behaviors here because they were only directed towards females within the same compartment, and chases are a normal component of the courtship repertoire. Quiver was defined as a rapid vibration of the body by the male with presentation of the anal fin egg spots to a female, and lead swim was defined as swimming towards the shelter accompanied by back-and-forth motions of the tail (waggles) as the male attempted to lead females towards the pot. We defined pot entry as any time the focal male entered the half terra cotta pot at the center of his territory and digging as any time the male scooped gravel from inside its pot or around its pot into its mouth and subsequently released it around its pot. We additionally classified chase female, quiver, and lead swim as courtship behavior as they are a part of the normal courtship repertoire and involve the active pursuit of a female (Fernald and Hirata, 1977). For ASC, ASC + CA, and ND fish across all behaviors, there were no statistical differences between the pre-injection period and baseline (Table S2; Wilcoxon test, $p \geq 0.22$ for all comparisons; $N = 6$–8 per group). DOM males exhibited changes in behavior from the pre-injection and BL period, but no changes between BL and the day of ascent (Supplementary Results and Fig. S1). We took this as evidence of stable DOM males needing about a day to acclimate to the experimental tank to re-establish a territory in the new environment. Therefore, we only analyzed statistically behavior during the following periods: BL, morning of social ascent, and afternoon of social ascent. Videos were scored in Scorevideo (Matlab). The results of scoring videos were saved into log files that were subjected to a variety of analyses using custom R software (log files and code are available at: available at https://github.com/FernaldLab).

2.4. Analyses of temporal patterns of behavior

2.4.1. Interbehavior interval

The log files containing the results from Scorevideo were analyzed for interbehavior intervals (IBI) using custom R software (available at https://github.com/FernaldLab). IBI is determined by calculating the time between successive behaviors and averaging across all IBIs within a given log file. We determined average IBI during the morning and afternoon for DOM, ASC, and ASC + CA fish. ND fish performed almost no behavior so were not included in analyses of temporal patterns of behavior.

2.4.2. Behavior sequence analysis

We conducted behavior sequence analysis using custom R software (available at https://github.com/FernaldLab). Behavior sequences were determined by quantifying the number of times different behaviors followed a given behavior. We counted the number of times a given sequence occurred and called this behavior count. We also determined the probability of different behaviors following a given behavior and called this behavior transition probability (TP). For example, if a fish performed a lateral display eight separate times and the first four times it performed a lateral display and followed it by attack male, and the next four times it followed it by chase female, the behavior count of lateral display → attack male is 2, the lateral display → chase female is 6. The TP for lateral display → attack male is 0.33 (2/6) and the TP for lateral display → chase female is 0.67 (4/6).

We determined the IBI needed to most accurately group successive behaviors into bouts for behavior sequence analysis. Behaviors that occurred outside of this value, which we will call "bout IBI", were not included in a given behavior sequence analysis. We first determined the average IBI across DOM, ASC, and ASC + CA log files, regardless of treatment or time of day. This value (10 s; see Results) was used as bout IBI for behavior sequence analysis across DOM, ASC, and ASC + CA fish. Since ASC + CA fish had significantly longer IBIs compared to DOM and ASC fish (see Results), we conducted a second round of behavior sequence analysis for DOM, ASC, and ASC + CA fish using a bout IBI of 5.5 s, the average IBI across DOM and ASC files regardless of treatment or time of day, to determine if observed behavior sequence differences (see Results) were due to differences in the ordering of behaviors or ASC + CA males taking longer to transition from behavior to behavior. In DOM, ASC, and ASC + CA males, we quantified behavior sequences containing male-directed aggressive behavior (lateral displays and border fights) that were two behaviors in length, since ASC + CA males essentially performed no reproductive behavior (see Results). For DOM and ASC males, we conducted additional analyses on behavior sequences containing all behavior types. For this round of behavior sequence analysis we used bout IBIs of 5.5 s and 9 s. The bout IBI of 9 s was used to determine if observed differences in behavior sequencing between DOM and ASC fish were due to ASC males taking longer to transition from one behavior to the next. After finding differences between DOM and ASC males in sequencing of some behavior sequences that were two behaviors in length (see Results), we wondered if this pattern persisted for successively longer behavior lengths. Specifically, in addition to analyzing sequences that were two behaviors in length, we analyzed sequences that were three, four, and five behaviors in length (sequences containing six or more behaviors were too infrequent for meaningful analysis and were thus not analyzed). Once we determined that differences between DOM and ASC males in the sequencing of two and three behaviors followed the same pattern (e.g., starting with a male-directed aggressive behavior and ending with a reproductive behavior; see Results for detailed description of findings), we conducted additional analyses over categories of behavior to determine if this pattern persisted for successively longer behavior sequence lengths. Categories were defined as such: Male-directed aggressive behavior (lateral display and border fight), reproductive behavior (dig, pot entry, chase female, quiver, and lead swim), and courtship behavior (chase female, quiver, and lead swim). We used the courtship category as these behaviors involve the active pursuit of a female for mating. As each successive sequence analysis was aimed at identifying if the general pattern of starting with a particular type of behavior and ending with the same or another type of behavior persisted at successively longer lengths, the types of behaviors in between the starting and ending behavior were not considered and thus replaced with an "N" in the figures of results, where N = any behavior.

For behavior sequences that were subjected to sequence analysis, sometimes a fish did not perform a component behavior. For instance, when analyzing the occurrence of the behavior sequence "lateral display → chase female" a fish may not have performed chase female. Because quantifying the occurrence of a behavior sequence in a fish that did not perform a component behavior may be confounded, fish that did not perform a component behavior of a behavior sequence were excluded from the analysis of that particular behavior sequence. Excluded fish for this reason occurred very infrequently and for any analysis when it did a maximum of two fish had to be excluded. The sample sizes for each test are shown in the Results section.

2.5. Morphological and steroid hormone analyses

Focal fish were assessed for standard length (SL), body mass (BM), testes mass, and gonadosomatic index [GSI = (gonad mass/body mass) ∗ 100]. Blood samples were also collected. With capillary tubes from the caudal vein, centrifuged for 10 min at 5200*g*, and the plasma was removed and stored at −80 °C until assayed. Immediately after blood collection fish were killed by cervical transection. Testes were removed and weighed to calculate GSI. For one ASC + CA fish, data for SL, BM, and testes mass could not be recorded (ASC + CA SL, BM, testes mass, and GSI final $N = 8$).

Plasma testosterone and 11-ketotestosterone (11-KT) levels were measured using commercially available enzyme immunoassay (EIA) kits (Cayman Chemical Company, Ann Arbor, MI, USA) as previously described and validated for this species (Maruska and Fernald, 2010b). Briefly, for testosterone and 11-KT assays, a 5-μl sample of plasma from each subject was extracted two times using 200 μl of ethyl ether and evaporated under a fume hood before re-constitution in EIA assay buffer (dilution 1:40 to 1:50). EIA kit protocols were then strictly followed, plates were read at 405 nm using a microplate reader (UVmax Microplate Reader; Molecular Devices, Sunnyvale, CA, USA) and steroid concentrations were determined based on standard curves. All samples were assayed in duplicate and intra-assay coefficients of variation (CV) were: testosterone (15.2%); 11-KT (8.9%). One DOM sample could not be assayed for testosterone due to possible contamination (DOM testosterone concentration final $N = 6$), one ASC + CA plasma sample was not collected after the experiment, and 11-KT concentrations could not be determined for an ASC + CA fish due to limited sample (ASC + CA testosterone concentration final N = 8; ASC + CA 11-KT concentration final $N = 7$).

2.6. Statistical analysis

A majority of the data did not meet the assumptions of normality and/or homogeneity of variance. Moreover, for some dependent variables, data points within one or more groups were highly variable. Therefore, we used non-parametric statistical approaches that use the median as the measure of central tendency. This approach avoids the requirement of meeting assumptions of parametric statistics and also minimizes the influence of potential outliers. We determined for all variables if there were outliers using a standard approach (i.e., outlier ≥ 3rd quartile + 2 ∗ Interquartile Range ∗ 1.5 or outlier ≤ 3rd quartile − 2 ∗ Interquartile Range ∗ 1.5). For some variables, an outlier was identified. However, each time an outlier was identified, it was never the same fish, suggesting outliers did not systematically affect the overall pattern of results. Moreover, when we re-ran tests upon removing an outlier, it did not affect the overall pattern of results in our study. Therefore, outliers were not removed. For reference, in Table S1 demonstrate the minimal effects outliers had on our results.

All statistical tests were performed in the R statistical computing environment or Prism 7.0. We used Kruskal-Wallis ANOVAs followed by Dunn's post-hoc tests for comparisons of physiological and behavioral measures across groups. To perform within-subject comparisons, we used Friedman's Tests followed by Dunn's post-hoc tests. When comparing only two groups, we used Mann-Whitney tests (between-subjects) or Wilcoxon tests (within-subjects). Raster plots were generated using custom software packages in R (available at https://github.com/FernaldLab). IBI analysis and behavior sequence analysis were also conducted using custom R software (available at https://github.com/FernaldLab). Correlational analyses were conducted using Spearmen's rho. Effects were considered significant at $p \leq 0.05$.

2.7. Results

2.7.1. DOM, ASC, and ASC + CA males have similar levels of circulating sex steroid hormones

We found that the DOM, ASC, ASC + CA, and ND males used in this study were similar in measures of body size (Fig. S2A; standard length, Kruskal-Wallis ANOVA, $p = 0.57$; Fig. S2B; body mass, Kruskal-Wallis ANOVA, $p = 0.74$; $N = 6$–8 per group). As expected, DOM males had significantly larger gonads (Fig. S2C; Kruskal-Wallis ANOVA, $p = 0.0018$; N = 6–8 per group) and higher gonadosomatic indices [(gonad mass/body weight) × 100] (Fig. S2D; Kruskal-Wallis ANOVA, $p = 0.0015$; N = 6–8 per group) compared to the other groups. DOM, ASC, and ASC + CA males had higher levels of testosterone than ND males and did not differ from one another (Fig. S2E; Kruskal-Wallis ANOVA, $p = 0.0041$; N = 6–8 per group). Circulating levels of 11-KT (Fig. S2F; Kruskal-Wallis ANOVA, $p = 0.0006$; N = 6–7 per group) were significantly higher in DOM and ASC males compared to ND males (Fig. S2F). DOM males also had higher levels of 11-KT compared to ASC + CA fish, which did not differ significantly from ASC or ND males. These results are in line with previous observations (Maruska et al., 2013; Maruska and Fernald, 2010a, 2010b, 2010c; Parikh et al., 2006).

2.7.2. Androgen signaling is required for enhancing reproductive—but not aggressive—behavior during social ascent

We analyzed a panel of behaviors encompassing multiple categories (Fernald and Hirata, 1977; examples are in Fig. 1B), including subordinate behavior, fleeing, where the male flees from a stimulus male; male-directed aggressive behavior, such as border fights and lateral displays; female-directed aggressive behavior, where the male attacks a stimulus female; and reproductive behavior, including chase female, quiver at female, lead swim, pot entry, and digging. These behaviors are described in detail in the Methods section. Behaviors during the BL, morning, and afternoon period were analyzed.

Behavior across groups on the day of ascent was visualized in raster plots to assess qualitative differences as a function of group (Fig. 3). Representative plots from each group show DOM, ASC, and ASC + CA males performed similarly high levels of aggressive behavior in the morning and afternoon, while ND performed almost no aggressive behavior. DOM and ASC males performed similarly high levels of reproductive behavior in the morning and afternoon, while ASC + CA and ND males performed virtually no reproductive behavior.

Given these observations, we next queried the extent to which there are quantitative differences between ASC and ASC + CA during ascent by analyzing changes in behavior from BL to the morning and afternoon

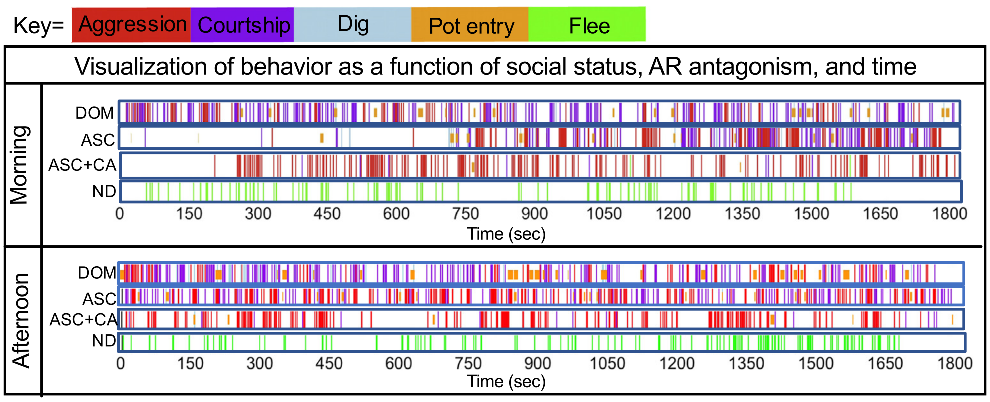

Fig. 3. Qualitative visualization of behavior. Raster plots showing behavior from individual fish from each group in the morning and afternoon. Each colored line represents a particular type of behavior. The x-axis represents time. Pot entry here is designated as durational; once the bar denoting pot entry is over the fish has exited the pot.

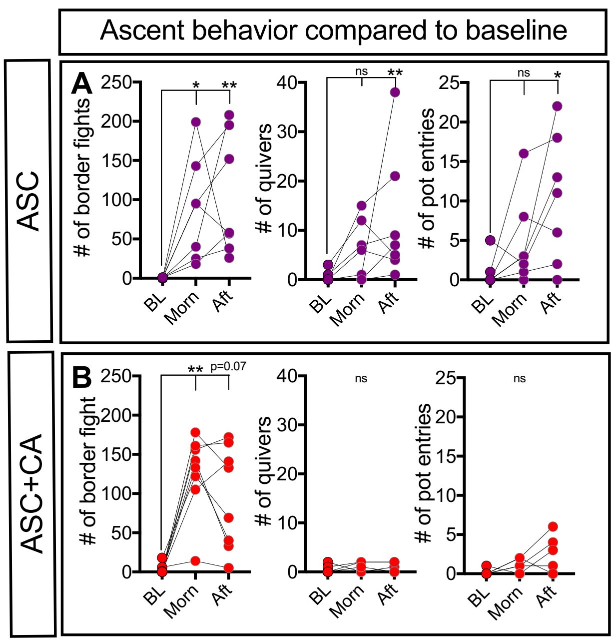

Fig. 4. Androgen signaling during social ascent is required for a rise reproductive but not aggressive behavior. Plots showing change in male-directed aggressive (border fight) and reproductive (quiver, pot entry) behaviors from BL to the morning and afternoon on the day of social ascent for (A) ASC and (B) ASC + CA fish. Each time-point along the x-axis refers to the 30-min period during which behaviors were scored. Each circle is an individual fish; connected circles represent the same fish. Brackets over time-points indicate the comparison over which a difference or lack of difference was observed based on Friedman's tests followed by post-hoc Dunn's tests. ns = non-significant; *$p < 0.05$, **$p < 0.01$. ASC = ascender; CA = cyproterone acetate; BL = baseline; morn = Morning; Aft = Afternoon.

on the day of ascent. ASC males performed more border fights in the morning and afternoon on the day of ascent compared to baseline (Fig. 4A; Friedman's ANOVA, $p = 0.02$; $N = 7$) and more lateral displays in the afternoon on the day of ascent compared to baseline (Fig. S3A; Friedman's ANOVA, p = 0.02; N = 7). ASC + CA males performed more border fights in the morning on the day of ascent compared to baseline (Fig. 4A; Friedman's ANOVA, $p = 0.005$, $N = 8$), while the increase in border fights in the afternoon compared to baseline was only a statistical trend (Fig. 4A). ASC + CA males performed more lateral displays in the morning and afternoon on the day of ascent compared to baseline (Fig. S3A; Friedman's ANOVA, $p = 0.001$). Neither group showed a change in attacks directed towards females (Fig. S3; $p \geq 0.41$ for both tests; N = 7–8 per group). ASC males also significantly reduced fleeing behavior (Fig. S3A; $p = 0.0014$; N = 7). For ASC + CA males, there was a non-significant decrease in fleeing behavior from baseline to day of ascent (Fig. S3B; $p = 0.08$; N = 8). However, while ASC males showed a significant increase in reproductive behaviors in the afternoon compared to baseline (Fig. 4A and Fig. S3A; $p < 0.05$, N = 7), ASC + CA males did not (Fig. 4B and Fig. S3B; $p \geq 0.12$ for all tests; N = 8), suggesting androgen signaling is required for a key aspect of social ascent, an increase in the performance of reproductive behavior.

We next determined whether ASC or ASC + CA male behaviors differed on the day of social ascent compared to stable DOM and ND males in the morning and afternoon. In the morning ASC and ASC + CA males performed levels of aggressive behavior that were not different than DOM males (Fig. 5A and Fig. S4A; Kruskal Wallis ANOVAs, $p < 0.05$ for all main effects; $N = 6$–8 per group). There was a main effect of group in the morning on the number of attacks directed towards females, where ASC + CA males attacked females more than ND males (Fig. S4A; Kruskal Wallis ANOVA, $p = 0.04$; N = 6–8 per group). There was a main effect of group in the morning on the levels of reproductive behavior (Fig. 5A and Fig. S4A; Kruskal Wallis ANOVAs, $p < 0.05$ for all main effects; N = 6–8 per group). Specifically, in the morning DOM males performed significantly more quivers and pot entries than ND males. Moreover, in the morning ASC males performed levels of reproductive behavior that were not different than levels seen in DOM males; however, they also did not differ statistically from ASC + CA or ND males. ASC + CA males performed virtually no reproductive behavior and did not differ from ND males (Fig. 5A and Fig. S4A), further supporting the notion that androgen signaling is required for the performance of DOM-typical levels of reproductive behavior.

Overall, in the afternoon the pattern of behavioral differences was similar to the morning (Fig. 5B and Fig. S4B, and Supplementary Results), but some differences emerged. For instance, while ASC and ASC + CA males differed statistically from ND males in the number of lateral displays performed, DOM males did not differ statistically from ASC, ASC + CA, or ND males (Kruskal Wallis ANOVA, $p = 0.0013$; Fig. S4B). This surprising lack of a difference between DOM males and all of the other groups in the afternoon prompted us to analyze temporal changes in the performance of lateral displays in DOM males. We found that DOM males performed significantly fewer lateral displays over time (Median difference in lateral displays between morning and afternoon: −18; morning lateral display range: 78–13 = 65; afternoon lateral display range: 46–13 = 33; morning-afternoon difference range: 65–1 = 64; Wilcoxon test, $p = 0.01$; $N = 7$; data not graphed). Overall then, our findings suggest that while androgen signaling in ASC males is required for the performance of DOM-typical levels of reproductive behavior, it is not sufficient for the expression of DOM-typical temporal behavioral patterns.

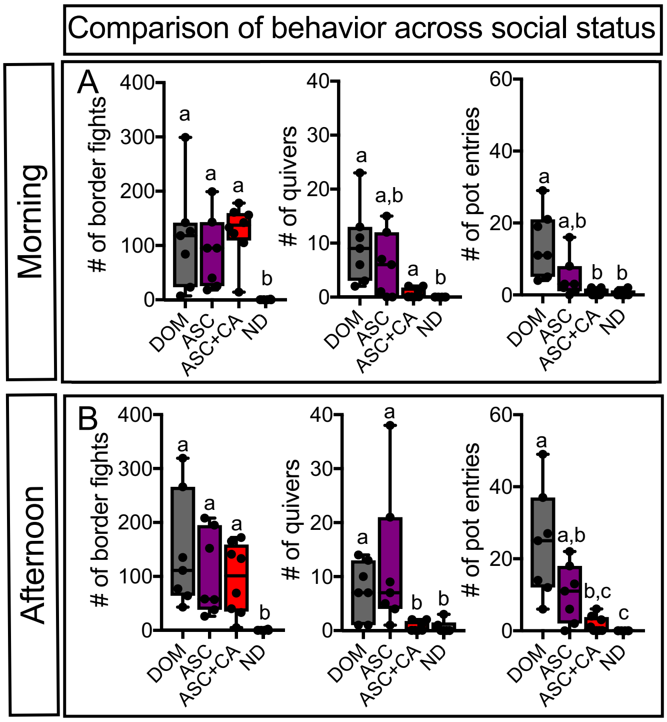

Fig. 5. Androgen receptor antagonism virtually abolishes reproductive but not aggressive behavior in socially ascending fish. Box and whisker plots showing numbers of male-directed aggressive (border fight) and reproductive (quiver, pot entry) behavior in the (A) morning (scored during the first 30 min following lights-on) and (B) afternoon (scored during the final 30 min of recording) performed by DOM, ASC, ASC + CA, and ND fish. Each circle represents an individual fish. Top and bottom whiskers represent maximum and minimum, respectively; top and lower boxes represent third and first quartiles, respectively; line within box represents the median. Letters indicate if a group was statistically different (different letters) or statistically indistinguishable (same letters) from the other groups. DOM = dominant; ASC = ascending; ND = non-dominant; CA = cyproterone acetate.

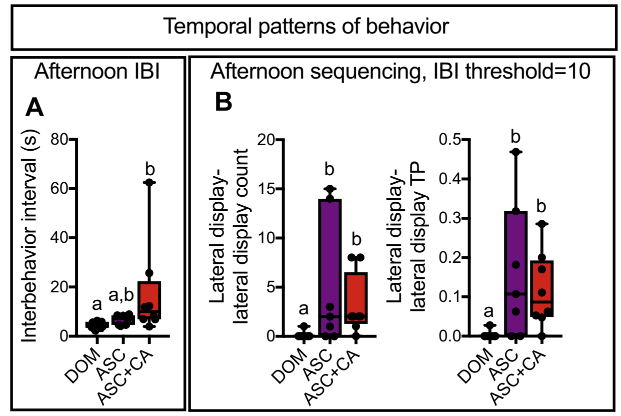

Fig. 6. Androgen signaling influences temporal patterns of behavior. Box and whisker plots showing (A) IBI and (B) behavior sequencing across DOM, ASC, and ASC + CA fish. Each circle represents an individual fish. Top and bottom whiskers represent maximum and minimum, respectively; top and lower boxes represent third and first quartiles, respectively; line within box represents the median. Letters indicate if a group was statistically different (different letters) or statistically indistinguishable (same letters) from the other groups. DOM = dominant; ASC = ascender; IBI = interbehavior interval; Count = number of times sequence occurred; TP = transition probability, the probability a given behavior followed an initial behavior.

2.8. Social ascent is characterized by AR-dependent and status-dependent changes in temporal patterns of behavior

2.8.1. Time between successive behaviors is AR-dependent

After finding that differences between the groups changed over the course of the day, we next asked if blocking androgen signaling in socially ascending fish disrupts the speed at which behaviors are performed, which can be measured using inter-behavior interval (IBI). We measured the IBI in the morning and afternoon by measuring the interval between successive behaviors, and computed the average of this measure for DOM, ASC, and ASC + CA fish. ND males were not included in this analysis because they performed virtually no behavior. ASC + CA fish performed behavior at significantly slower speed in the afternoon (i.e., with significantly longer IBI) than DOM fish (Kruskal Wallis ANOVA, $p = 0.0097$; Fig. 6A), while ASC fish were not different from either DOM or ASC + CA fish ($p = 0.19$ and 0.91, respectively). There were no effects of group on IBI in the morning (Fig. S5A). These data imply androgen signaling in socially ascending fish is required for enhancing the speed of behavior performance to DOM levels, not just the occurrence of individual reproductive behaviors.

2.8.1.1. Lateral displays are repeated more often by ASC regardless of AR activation. We also wondered whether androgen signaling in socially ascending fish controls the sequencing of behavior. Since ASC + CA fish performed virtually no reproductive behavior, we only quantified the sequencing of aggression and compared it to DOM and ASC fish. We analyzed behavioral sequences within bouts of behavior, which were defined as two or more behaviors separated by 10 s or less, the average IBI across all DOM, ASC, and ASC + CA fish regardless of time of day (See Methods). In the afternoon we found a significant effect of group—but not CA treatment—on the sequencing of male-directed aggressive behavior (Kruskal Wallis ANOVAs, $N = 7–8$, $p < 0.05$). Specifically, ASC and ASC + CA fish performed lateral displays twice in a row more often than DOM fish (Fig. 6B; Dunn's test, $p < 0.05$ for both comparisons). ASC and ASC + CA males were also more likely to follow a lateral display with another lateral display compared to DOM males (Fig. 6B; Dunn's test, $p < 0.05$ for both comparisons). There were no significant differences in the sequencing of aggression in the morning (Kruskal Wallis ANOVAs, $N = 7–8$, $p \geq 0.09$ for all comparisons).

To determine if the IBI used influenced our results, we conducted the above comparisons again, except we defined bouts of behavior as two or more behaviors separated by 5.5 s or less, the average IBI across DOM and ASC fish regardless of time of day (see Methods for detailed reasoning for using multiple IBIs). This did not alter the general pattern of results (Fig. S5B), indicating differences in behavioral sequencing are not due to differences in the tempo of behavioral performance. Lastly, since DOM, ASC, and ASC + CA fish were statistically indistinguishable in their performance of lateral displays in the afternoon, our findings cannot be attributed to differences in the occurrence of behavior (See Fig. S4). These results as a whole suggest another temporal dimension of behavior, the sequencing of behavior, demarcates socially ascending fish from stable DOM fish.

2.8.1.2. Ordering of successive behaviors differs between DOM and ASC in the afternoon. Given our observation that sequencing patterns of aggressive behavior demarcate socially ascending versus stable DOM male *A. burtoni*—regardless of AR activation—we wondered how the full suite of DOM-typical behaviors were sequenced differently in ASC and DOM males. ASC + CA males were excluded from this analysis because they performed virtually no reproductive behavior. We set the acceptable time interval between behaviors to be analyzed as 5.5 s, the average IBI across DOM and ASC fish regardless of time of day (see Methods for detailed reasoning for using a bout IBI of 5.5 s), and measured behavioral sequences comprising two, three, four, and five behaviors to determine if identified patterns existed for successively longer behavior sequences (See Methods for detailed reasoning).

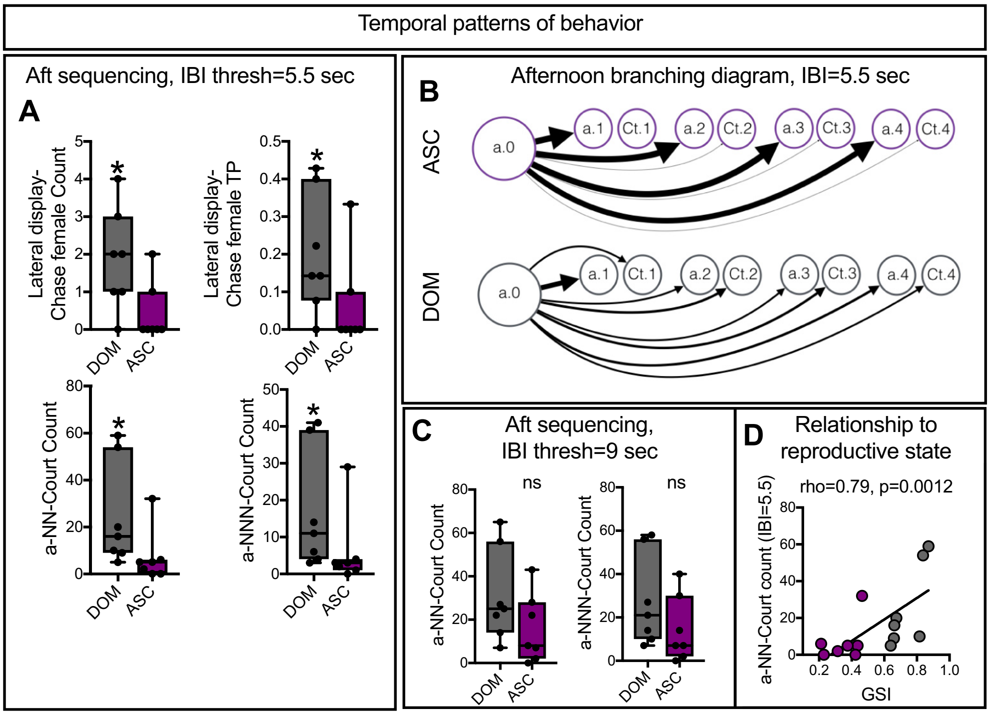

Fig. 7. Socially ascending and DOM males are demarcated based on behavior sequencing patterns. Behavior sequencing patterns in the afternoon using a (A) 5.5 s bout IBI. (B) Behavioral sequencing patterns for male-directed aggression (male-directed aggression (= a) represented in a behavior branching diagram. Letters in this diagram refer to a type of behavior, the size of arrows indicate the number of times male-directed aggression "a" was followed by "a" or courtship (= Ct) at different parts of a behavior sequence. The largest arrow = 53 occurrences; the smallest arrow = 1 occurrence. For example, for the ASC fish, there were no occurrences from a.0 to Ct.1 (hence, there is no arrow connecting a.0 to Ct.1), there was one instance where "a" was followed by "Ct" two behaviors later (represented by the a.0 branching to Ct.2), one instance of "a" being followed by "Ct" three behaviors later (represented by the a.0 branching to Ct.3), and one instance of "a" being followed by "Ct" four behaviors later (represented by the a.0 branching to Ct.4). (C) Behavior sequencing patterns in the afternoon using a 9 s bout IBI. (D) Relationship between gonadosomatic index (GSI) and a behavior sequence of four behaviors in length. For (A) and (C), each circle represents an individual fish. Box and whisker plots were used, where top and bottom whiskers represent maximum and minimum, respectively; top and lower boxes represent third and first quartiles, respectively; and the line within the box represents the median. DOM = dominant; ASC = ascender; N = any behavior; IBI = inter-behavior interval; *sec* = seconds; Count = number of times sequence occurred; TP = transition probability (i.e., the probability a behavior followed an initial behavior). *$p < 0.05$; ns = non-significant.

There were striking differences in behavior sequences between DOM and ASC males in the afternoon on the day of ascent. DOM males were more likely to follow lateral displays with female chases and performed this sequence more often than ASC males (Fig. 7A and Fig. S5; Mann-Whitney test, $p < 0.05$ for both comparisons; $N = 7$ for both groups). DOM males were more likely to perform a lead swim after exiting their pot and performed this sequence more often than ASC males (Fig. 7A and Fig. S6; $p < 0.05$ for both comparisons; $N = 5$–7). We also asked how different categories (i.e., male-directed aggression, reproductive, and courtship behavior) of behavior were sequenced and found that ASC males were more likely to follow male-directed aggression with male-directed aggression than DOM males (Fig. S6). Similarly, there was a statistical trend wherein DOM males were more likely to follow male-directed aggression with a reproductive behavior than ASC males (Fig. S6; $p = 0.07$ for both; $N = 7$ for both groups). DOM and ASC male behavioral sequences did not differ in the morning (Mann-Whitney test, $p \geq 0.09$ for all comparisons of behavior sequences; N = 7 for both groups). Thus, although ASC males have intact androgen signaling, they do not perform DOM-typical sequencing patterns of aggressive and reproductive behavior, suggesting another temporal aspect of behavior demarcates socially ascending males from DOM males.

2.8.2. Longer behavioral sequences distinguish DOM from ASC males

Beyond the ordering of two successive behaviors, we next asked whether longer behavioral sequences differed between groups (i.e. sequences > 2 behaviors) and found that, for multiple sequence lengths, many differences associated with male-directed aggressive behaviors emerged in the afternoon. In three-behavior sequences, the same general pattern as for two-behavior sequences remained, but some new differences emerged (Fig. 7 and Fig. S6). DOM males, after performing two border fights successively, were more likely to perform a female chase compared to ASC males (Mann-Whitney test, $p = 0.03$; N = 7 for both groups). This pattern persisted when behavioral categories were analyzed (Fig. 7A and Fig. S6). For instance, after performing two male-directed aggressive behaviors, DOM males were more likely to perform a reproductive or courtship behavior compared to ASC males (Mann-Whitney test, $p < 0.05$ for both comparisons; N = 7 for both groups). Strikingly, this pattern of differences persisted for sequences that included four and five behaviors: DOM males performed more behavior sequences that started with male-directed aggression and culminated in reproductive or courtship behavior three and four behaviors later (Fig. 7A–B and Fig. S6; Mann-Whitney test, $p < 0.05$ for both comparisons; N = 7 for both groups).

Thus, ASC- and DOM-typical behavior sequencing patterns are detectable in short and long behavior sequences. Specifically, ASC males typically follow aggressive behavior with more aggressive behavior, while DOM males typically follow aggressive behavior with reproductive or courtship behavior. Therefore, androgen signaling in ASC males is not sufficient for activating DOM-typical sequencing patterns of aggressive and reproductive behavior, suggesting yet another complex distinction between ASC and DOM social status. Our findings on behavioral sequences could not be attributed to overall behavioral frequency, which we addressed in several ways (see Supplemental Results and Fig. S7–S10).

2.8.3. A longer IBI eliminates certain transition differences

The differences in behavioral sequencing between DOM and ASC males might depend, in part, on the time used for defining a bout. For instance, it could be that ASC and DOM males perform courtship behavior after male-directed aggression at similar rates, but ASC males take longer than DOM males to transition from male-directed aggression to courtship. Half of the ASC males had an IBI approximately 2 or more seconds longer than the bout IBI used of 5.5 s and the highest IBI in the ASC group was ~9 s. Thus, we conducted the behavioral sequence analysis again but used an IBI of 9 s instead of 5.5. Using a 9-second instead of a 5.5-second interval did not alter the general pattern of differences observed between the groups when behavior chains that

Table 1
Correlations to gonadal measures. $N = 14$.

Behavior	GSI		Testes Mass	
	rho	*P*	rho	*P*
dd Count	−0.53	0.054	−0.41	0.14
dd TP	−0.6	0.025*	−0.43	0.12
dC Count	0.76	0.0025**	0.56	0.04*
dC TP	0.63	0.01*	0.48	0.08
xs Count	0.65	0.014*	0.54	0.04
xs TP	0.62	0.02*	0.55	0.04*
aaRepro TP	0.51	0.06	0.63	0.02*
aaCourt TP	0.6	0.02*	0.65	0.01*
bbC TP	0.67	0.01*	0.56	0.04
a-NN-Repro Count	0.68	0.0093**	0.49	0.07
a-NN-Court Count	0.79	0.0012**	0.49	0.07
a-NNN-Repro Count	0.69	0.0084**	0.49	0.07
a-NNN-Court Count	0.75	0.003**	0.53	0.05*

* $p \leq 0.05$;
** $p < 0.01$.

were two and three behaviors long were considered (Table S3). However, differences between DOM and ASC were no longer present for behavior sequences that were four and five behaviors long (Fig. 7C and Table S3). Therefore, the differences in behavioral sequences between DOM and ASC males are in general stable for short sequences, but differences in longer sequences as a function of status are eliminated when longer functional bout intervals are considered. These results suggest ASC males can perform some DOM-typical behavior sequences, but may take more time between successive behaviors.

2.9. Behavioral sequencing correlates with reproductive state

Finally, we tested whether the differences in behavioral sequencing in ASC and DOM males in the afternoon were related to reproductive state. Hence, we correlated the significantly different behavioral sequences to GSI and testes mass. Numerous significant linear correlations were observed (Table 1). The strongest positive correlation was between GSI and the occurrence of a chain of four behaviors, in which male-directed aggression was followed by courtship behavior three behaviors later (rho = 0.79, $p = 0.0012$; Fig. 7D). These data may suggest reproductive state is related to the sequencing of behavior. However, many of these correlations disappeared when we analyzed the two groups separately. For example, for GSI, 10/12 correlational tests that were significant were not significant within either ASC or DOM males. There was a significant correlation between GSI and the number of times DOM males performed male-directed aggression and courtship three behaviors later, but this correlation did not hold for ASC males. GSI did, however, correlate significantly to the number of times ASC males performed lateral display → chase female. For testes mass, no correlational tests were significant when they were analyzed within each group. It should be noted that some significant correlations may have disappeared within status merely due to halving the sample sizes (e.g., 14 data points versus 7). However, it could be that GSI and testes mass are not linearly related to behavior sequencing and another variable may better explain DOM- and ASC-typical behavior sequencing patterns.

3. Discussion

We used a social ascent paradigm in *A. burtoni* combined with AR antagonism to dissect the suite of behavioral traits that characterize social ascent and dominance. We demonstrate that androgen signaling is required for the performance of reproductive behavior during social ascent. Moreover, we showed androgen signaling as well as social status influences the temporal patterning of behavior. Our pharmacological dissection of social ascent revealed androgen signaling and experience may interact to regulate social behavior in *A. burtoni*, and social status may be dissociable.

3.1. Androgen signaling may regulate multiple aspects of the social calculus used by male A. burtoni

The roles played by sex steroid hormone signaling in regulating aggressive and reproductive behavior is widely appreciated (Eisenegger et al., 2011) and our findings are consistent with other studies on the regulation of courtship behavior by androgen signaling in teleost fish (Almeida et al., 2014; O'Connell and Hofmann, 2012a). However, the hormonal mechanisms underlying the likelihood of an animal rising in social rank are less clear.

Using *A. burtoni*, we showed that blocking androgen signaling during social ascent virtually abolished all forms of reproductive behavior. Previous work (O'Connell and Hofmann, 2012a) showed that in *A. burtoni*, blocking ARs in DOM males reduced two reproductive behaviors, quivers and lead swims, but did not affect aggressive behavior. This study, however, did not quantify other reproductive behaviors outside of quivers and lead swims. Therefore, our results indicate androgen signaling is necessary for the performance of all forms of reproductive behavior in socially ascending *A. burtoni* males, suggesting it may also be required for all reproductive behavior in DOM males. Interestingly, ND males given exogenous androgens do not increase reproductive behavior (O'Connell and Hofmann, 2012a). Administration of an AR agonist to *A. nigrofasciata* males enhances digging behavior only during social opportunity (Sessa et al., 2013). Therefore, our results provide support for the notion that the social environment influences the sensitivity or responsiveness to steroid hormones (O'Connell and Hofmann, 2012a). Our data expand on this hypothesis in suggesting that AR has distinct regulatory effects during the rapid behavioral shift that occurs after perceiving social opportunity than it does during the behavior of ND males.

Male *A. burtoni* survey the environment to guide their behavior (Desjardins et al., 2012; Grosenick et al., 2007), a process that has been called "social calculus" (Fernald, 2017). Our results suggest that this social calculus may require androgen signaling. Studies in humans (Dreher et al., 2016; Eisenegger et al., 2011), non-human primates (Lacreuse et al., 2010), rodents (Aikey et al., 2002; Frye et al., 2008; Jacome et al., 2016), and birds (Alward et al., 2016, 2017; Bottjer and Hewer, 1992) that show androgens affect complex cognitive processes (McCall and Singer, 2012). For instance, testosterone shifts player strategy to enhance social status in an experimental gambling paradigm (Dreher et al., 2016). Hence, AR antagonism may have led to deficits in the recognition of or the ability to act on social opportunity (Fernald, 2017).

Multiple sensory modalities that are likely involved in an ND male's ability to recognize and act on social opportunity could have been affected by AR antagonism (Maruska and Fernald, 2012). In *A. burtoni*, ARs are expressed in the inner ear (Maruska and Fernald, 2010b) and in the cyprinid fish *Puntius schwanenfeldi*, androgen implants increased in the olfactory epithelium the magnitude and sensitivity of electro-olfactogram responses to pheromones (Cardwell et al., 1995). While it is unknown if ARs are expressed in the *A. burtoni retina*, AR mRNA is expressed in the zebrafish (*Danio rerio*) retina (Gorelick et al., 2008) and AR protein is present in the goldfish retina (Gelinas and Callard, 1997). In *A. burtoni*, AR mRNA is expressed in the central nucleus of the dorsal telencephalon (Dc; Harbott et al., 2007). Based on work in catfish and goldfish, Dc receives visual input. Androgen signaling may modulate olfactory systems as well since ARs are expressed in the *A. burtoni* olfactory bulb (Maruska and Fernald, 2010c). Moreover, the ventral nucleus of the ventral telencephalon (Vv) also expresses AR mRNA and Vv and Dc receive olfactory projections based on work in cod (Rooney et al., 1992), zebrafish (Rink and Wullimann, 2004), and rainbow trout (Folgueira et al., 2004). ARs are also expressed in the POA of *A. burtoni*, a well-conserved brain region that integrates cues from a variety of sensory modalities and controls sexual behavior across vertebrates (Goodson, 2005; Harbott et al., 2007; O'Connell and Hofmann, 2012b). Within 30 min of social opportunity expression of ARα and ARβ is enhanced in the supracommissural nucleus of the ventral telencephalon (Vs), a putative homolog of the extended amygdala (Maruska et al., 2013). ARβ expression is also enhanced in the medial zone of the dorsal telencephalon (Dm), a putative homolog of the amygdala. Combining the above observations with the current results suggests that androgen signaling may modulate the processing of multiple types of sensory signals, such as auditory, visual, and chemical, that may be involved in the ability to recognize and act on social opportunity. Moreover, given the enhancement in the expression of ARs in Vs and Dm during social opportunity (Maruska et al., 2013) and the lack of an effect by exogenous androgens on reproductive behavior in ND males (O'Connell and Hofmann, 2012a, 2012b), we hypothesize that a rapid increase in the sensitivity to androgen signaling in these brain regions may contribute to the recognition of social opportunity and/or the subsequent increase in reproductive behavior during social ascent.

3.2. AR-dependent and status-dependent temporal patterns of behavior delineate ASC from DOM males

While we were not surprised that AR blockade reduced courtship behavior during social ascent, we discovered that androgen signaling and social status influence temporal patterns of behavior in *A. burtoni*. Socially ascending males with blocked AR performed behavior at a slower speed (i.e., took more time between each successive behavior) than stable DOM males. Previous research has shown testosterone affects the timing of behavior in response to different social cues. For instance, in rhesus monkeys (*Macaca mulatta*) testosterone treatment sped up reaction times to social cues during a task testing recognition memory, which may have reflected an enhancing effect of testosterone on reward sensitivity or general arousal (King et al., 2012). Testosterone treatment in rhesus monkeys also increased attention to videos depicting fights between conspecifics (Lacreuse et al., 2010). Given the well-established role of testosterone in mediating arousal and sexual motivation (Adkins-Regan, 2009; Eisenegger et al., 2011), it is intriguing to consider the possibility that blockade of androgen signaling in socially ascending male *A. burtoni* reduced reaction times to certain social cues, which is reflected as a state of reduced arousal and social motivation. It could also be that the reduced speed of behavioral performance due to AR blockage is caused by a reduced ability to recognize social opportunity due to perturbed androgen signaling in one or more of the sensory structures mentioned above (e.g., ear, olfactory epithelium). In mice, winning a fight enhances AR immunoreactivity within the extended amygdala (Fuxjager et al., 2010), findings that are in line with the enhancement of ARα and ARβ expression during social opportunity (Maruska et al., 2013). Hence, it could be that blockade of androgen signaling reduces the ability to recognize and act on social opportunity, a form of "winning", thus not activating reward pathways, leading to reduced arousal that manifests as a slower speed of behavioral performance. In line with this hypothesis, recent work in *A. burtoni* has shown the social context influences sensitivity of the dopamine D2 receptor (Weitekamp et al., 2017), which is involved in the control of motivation and arousal (Wise, 2004). Future work is needed to determine the possible interaction between androgen signaling, sensory processing, arousal, and motivation as they relate to temporal aspects of social behavior in *A. burtoni*.

However, socially ascending fish, regardless of AR activation during social ascent, sequenced aggressive behavior differently than stable DOM males. We further showed that DOM males are more likely than ASC males to follow aggressive behavior with courtship behavior, a difference that persisted for behavioral sequences of successively longer lengths. Some of these differences disappeared when we used a longer bout IBI, implying ASC males can perform some of the DOM-typical behavioral sequences, but they take longer to switch from male-directed aggression to female-directed courtship. This difference among ASC and DOM males in the temporal patterning of behavior, regardless of AR activation in socially ascending fish, may reflect an important dimension of social status that to the best of our knowledge hitherto was not known. This does not necessarily mean androgen signaling is not involved in the regulation of DOM-typical behavior sequences. Indeed, it could be that long-term dominance, as a function of long-term androgen signaling, may be required to produce DOM-typical sequences. This prediction is based on findings in songbirds, where long-term exposure to steroid hormones is required to shape the neural substrates that ultimately generate birdsong that is used to attract females (Reviewed in Alward et al., 2018). It is also possible that other molecular systems are involved in DOM-typical temporal behavior patterns. For instance, it was recently shown that melatonin in plainfin midshipman (*Porichthys notatus*) lengthens the duration of courtship calls produced by males (Feng and Bass, 2016). Therefore, a complex interaction between different hormones and social experience in *A. burtoni* males may be required to shape the temporal patterns of behavior in DOM *A. burtoni*.

3.3. Differences in behavioral transitions may reflect AR- and status-dependent social task switching: a working model

The rise in aggressive and reproductive behavior to DOM-like levels in socially ascending fish is likely mediated by neurons activated by sensory signals from neighboring males and females, respectively. One explanation for the disconnect between the performance of *individual* aggressive and reproductive behavior, the *speed* of behavior, and the *transitions* from aggression to aggression, or aggression to courtship, may be differences in neural responsiveness or selectivity for sensory signals from males and females in socially ascending males versus DOM males. This could manifest as a difference in how fast males behave in general and in how fast they transition from male-directed aggression to female-directed courtship behavior, if the decision or speed to transition is coordinated differently based on sensitivity to sensory signals and/or social status. In other words, socially ascending and DOM males may differ in "social task switching".

Sex steroid hormones and experience may interact to control an animal's responsiveness to different sensory signals, a phenomenon that may explain the differences in the temporal patterning of behavior between socially ascending and DOM males. Indeed, multiple observations in different taxa support the idea that physiological or maturational state influences cognitive processes like task switching. In humans, performance on tests measuring task switching improves from childhood to puberty and adulthood, suggesting sex steroid hormones and/or experience are involved (Davidson et al., 2006). Pre-pubertal rats perform more perseverative behavior compared to post-pubertal and adult rats during the water maze task (Juraska and Willing, 2017). Critically, gonadectomized male and female rats performed poorly at a go/no-go task measuring urinary odor sex discrimination, but after receiving testosterone implants, both sexes performed significantly better (Kunkhyen et al., 2018).

Social experience and long-term exposure to steroid hormones in *A. burtoni* may contribute to differences in neural responsiveness to sensory signals from males and females in socially ascending and DOM males. Support for this hypothesis stems from work done in songbirds. For instance, in zebra finches a combination of experience, social cues, and hormones shape the selectivity of neurons that regulate song, a courtship behavior. Specifically, juvenile zebra finches during early stages of song learning possess neurons that respond to their own specie's song (Solis and Doupe, 1997). However, these neurons do not discriminate between their own song and their tutor's song until song is crystallized (Solis and Doupe, 1997), when birds experience an increase in circulating testosterone (Marler et al., 1987, 1988), the action of which has been shown to regulate aspects of song crystallization, including the temporal structure of song (Alward et al., 2017; Bottjer and

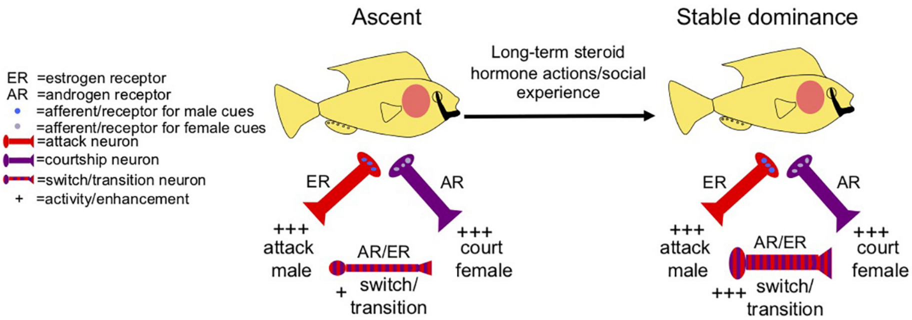

Fig. 8. Working model on the regulation of social behavior in *A. burtoni*. When given the opportunity, ASC males increase aggression directed towards males through the activation of estrogen receptor (ER) positive neurons through afferent input about sensory signals from neighboring males. ASC males will also increase courtship directed towards females as a result of activation of androgen receptor (AR) positive neurons through afferent input about sensory signals from females. However, the neurons that modulate how males switch or transition from male-directed aggression to female-directed courtship are not as active or sensitive compared to DOM males, so these transitions occur less frequently (as is the case for lateral display-chase female) and take more time to take place (as is the case for long behavior chains that start with male-directed aggression and end with a reproductive or courtship behavior). These "switch" neurons may undergo a transformation in function after an ASC male is exposed to higher levels of sex steroid hormones and social experience for several days, wherein now males more rapidly transition from male-directed aggression to female-directed reproductive or courtship behavior. As discussed in the main text, one status is not merely more efficient at these transitions; it could be that for ASC males the decision to transition from male-directed aggression to female-directed courtship is weighted by factors such as the state of territory establishment and fight outcome more strongly than DOM males, which have established their territory. While this simplified schematic denotes each behavior as being controlled by specific neurons, it could be networks of neurons control these distinct behaviors. Our model presents tractable hypotheses that once tested may reveal fundamental aspects of the regulation of social behavior across species. Key: neuron size positively corresponds to hypothesized activity/sensitivity; the striped switch/transition neurons indicate it could integrate cues about attacking male and when to switch to courting females.

Hewer, 1992; Korsia and Bottjer, 1991). Additionally, estradiol concentrations increase in the auditory cortex of male zebra finches when interacting with females or hearing another male's song (Remage-Healey et al., 2008). The estradiol produced in the auditory cortex of zebra finches due to these interactions may underlie the enhancement in the selectivity to the bird's own song of neurons in a downstream sensorimotor nucleus that controls song production (Remage-Healey and Joshi, 2012). Thus, a complex interplay between experience, social cues, and hormones like the one that shapes zebra finch vocal behavior may also shape temporal patterns of behavior in *A. burtoni*.

Taken together, we propose a working model in which socially ascending males undergo piecemeal changes in neural responsiveness to sensory signals from males and females that is controlled by sex steroid hormones and experience that eventually lead to the production of the full suite of DOM-like behavioral temporal patterns. In our working model (Fig. 8), male-directed aggression requires the activation of ER+ neurons stimulated by sensory signals from neighboring males, a prediction based on studies by O'Connell and colleagues showing estrogen signaling activates aggression in *A. burtoni* (Huffman et al., 2013; O'Connell and Hofmann, 2012a). Our model also posits the performance of courtship behavior depends on the activation of AR+ neurons by sensory signals from females (current findings, O'Connell and Hofmann, 2012a; van Breukelen, 2013). It is unclear if the extent of the control by androgen signaling of reproductive behavior in *A. burtoni* is the same for socially ascending and DOM males because the only study to the best of our knowledge that has tested the necessity of AR activation in the regulation of reproductive behavior measured only quivers and lead swims (O'Connell and Hofmann, 2012a). Also included in our model is that activation of AR+ neurons may contribute to a faster tempo of behavioral performance (Fig. 6A and Fig. S5A), reflecting general arousal or motivation.

However, socially ascending males, regardless of AR blockade, are more likely than stable DOM males to follow male-directed aggression with male-directed aggression. Stable DOM males, on the other hand, follow male-directed aggression more often with courtship behavior. These differences in behavioral sequencing patterns could be mediated by differential actions of sex steroid hormones acting over different time scales. For instance, activation of AR+ neurons before/during social ascent is required to enhance the tempo of behavior overall to DOM-typical levels. However, given our results that ASC males—regardless of AR activation—do not perform the same behavioral sequence patterns as stable DOM males, it could be that the long-term exposure to sex steroid hormones is required in ASC males to enhance the rapid transitions from male-directed aggression to courtship in a DOM-typical manner. Transitions from male-directed aggression to courtship may be controlled by AR/ER+ neurons or neural networks that we will call "switch neurons". In ASC males, these switch neurons do not discriminate as well or perform different computations between male and female sensory signals leading to fewer or slower transitions from male-directed aggression to courtship. The switch from male-directed aggression to courtship may be controlled by interactions between attack-male neurons and courtship neurons or neurons that specifically control behavior switching (Fig. 8). It is important to note that one status may not merely be more efficient at the different behavior transitions. For instance, it could be that for ASC males the decision to transition from male-directed aggression to female-directed courtship is weighted differently by factors such as the state of territory establishment and fight outcome compared to DOM males, which have established their territory and may be performing male-directed aggression only to maintain a territory so it can achieve more mating opportunities.

Work in *Drosophila* may shed light on the neural functions that underlie the switching between male-directed aggression and courtship. In *Drosophila melanogaster*, neurons integrate chemosensory cues differentially from males and females through a combination of excitatory and inhibitory neural responses (Clowney et al., 2015). A balance between excitation and inhibition pathways also allows males of the closely related Drosophila species *D. melanogaster* and *D. simulans* to rapidly discriminate between females from either species (Seeholzer et al., 2018). Therefore, it is possible a mechanism similar to the one used in *Drosophila* to discriminate between males and females is used by male *A. burtoni* to detect sensory signals from either sex and coordinate an appropriate response.

Some steroid hormones such as progestins and cortisol that may be relevant to *A. burtoni* social behavior (Maruska, 2015) were not discussed. While O'Connell and Hofmann (2012a, 2012b) showed activation or inhibition of the progesterone receptor (PR) enhances or reduces quivers and lead swims in DOM *A. burtoni*, very little is known about the role of PR in the regulation of male cichlid fish social behavior, so it was not included in our model. To the best of our knowledge, no functional studies on cortisol and *A. burtoni* male social behavior have been done. Future work into the role of these steroid hormones and other molecular systems will provide important insights, leading to a more comprehensive model of *A. burtoni* social behavior. Our working model nonetheless presents a foundation of testable hypotheses that may be applicable to a wide-range of species and social behaviors, especially those that rely on experience.

4. Conclusion: Pharmacological and behavioral dissection reveals social status is dissociable

Steroid hormones are known to regulate physiology, morphology, and behavior at different time scales to coordinate an animal's response to the environment (Adkins-Regan, 2009; Alward et al., 2017; Lee and Pfaff, 2008; Pfaff et al., 2008). Our results show that androgens may affect the complex social calculus used by animals as they determine their rank along a social hierarchy. Moreover, we provide evidence that gonadal steroids and social experience may also be involved in coordinating the temporal patterning of behavior, perhaps over distinct time scales or in integrating current behavior with experience. Our pharmacological and behavioral analyses revealed that the suite of behaviors that characterize social status are highly dissociable, suggesting non-redundant neural and hormonal mechanisms underlie an animal's social decision making, which is reflected in the performance of individual behaviors and their temporal patterning. This type of control by steroid hormones may be important in regulating complex social behaviors in other vertebrates.

Competing interests

We have no competing interests.

Author's contributions

Conceptualization, B.A.A. and R.D.F.; Methodology, B.A.A and R.D.F.; Investigation, B.A.A., A.T.H., and R.A.Y; Writing – Original Draft, B.A.A.; Writing – Review & Editing, B.A.A., R.D.F, A.T.H, and R.A.Y; Supervision, B.A.A. and R.D.F.

Acknowledgements

We thank Danielle Blakkan for help with the experimental set-up and injections and Paul Tran for scoring videos of the behavior.

Funding

This work was supported by an Arnold O. Beckman Foundation Postdoctoral Fellowship to BAA and National Institutes of Neurological Disorders and Stroke Grant (NS034950) and National Institutes of Mental Health Grants (MH101373 and MH096220) to RDF.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2018.12.010.

References

Adkins-Regan, E., 2009. Neuroendocrinology of social behavior. ILAR J. 50, 5–14.
Aikey, J.L., Nyby, J.G., Anmuth, D.M., James, P.J., 2002. Testosterone rapidly reduces anxiety in male house mice (*Mus musculus*). Horm. Behav. 460, 448–460.
Almeida, O., Canário, A.V.M., Oliveira, R.F., 2014. Castration affects reproductive but not aggressive behavior in a cichlid fish. Gen. Comp. Endocrinol. 207, 34–40.
Alward, B.A., Madison, F.N., Parker, S.E., Balthazart, J., Ball, G.F., 2016. Pleiotropic control by testosterone of a learned vocal behavior and its underlying neuroplasticity. eNeuro 3 (ENEURO.0145-15.2016).
Alward, B.A., Balthazart, J., Ball, G.F., 2017. Dissociable effects on birdsong of androgen signaling in cortex-like brain regions of canaries. J. Neurosci. 37, 8612–8624.
Alward, B.A., Cornil, C.A., Balthazart, J., Ball, G.F., 2018. The regulation of birdsong by testosterone: multiple time-scales and multiple sites of action. Horm. Behav. 104, 32–40.
Ball, G.F., Riters, L.V., Balthazart, J., 2002. Neuroendocrinology of song behavior and avian brain plasticity: multiple sites of action of sex steroid hormones. Front. Neuroendocrinol. 23, 137–178.
Bottjer, S.W., Hewer, S.J., 1992. Castration and antisteroid treatment impair vocal learning in male zebra finches. J. Neurobiol. 23, 337–353.
Burmeister, S.S., Jarvis, E.D., Fernald, R.D., 2005. Rapid behavioral and genomic responses to social opportunity. PLoS Biol. 3, e363.
Cardwell, J.R., Stacey, N.E., Lang, S.L.C., Tan, E.S.P., McAdam, D.S.O., 1995. Androgen increases olfactory receptor response to a vertebrate sex pheromone. J. Comp. Physiol. A. 176, 55–61.
Clowney, E.J., Iguchi, S., Bussell, J.J., Scheer, E., Ruta, V., 2015. Multimodal chemosensory circuits controlling male courtship in Drosophila. Neuron 87, 1036–1049.
Davidson, M.C., Amso, D., Anderson, L.C., Diamond, A., 2006. Development of cognitive control and executive functions from 4 to 13 years: evidence from manipulations of memory, inhibition, and task switching. Neuropsychologia 44, 2037–2078.
Desjardins, J.K., Hofmann, H.A., Fernald, R.D., 2012. Social context influences aggressive and courtship behavior in a cichlid fish. PLoS One 7, e32781.
Dreher, J., Dunne, S., Pazderska, A., Frodl, T., Nolan, J.J., 2016. Testosterone causes both prosocial and antisocial status-enhancing behaviors in human males. Proc. Natl. Acad. Sci. 113, 11633–11638.
Earley, C.J., Leonard, B.E., 1977. The effect of testosterone and cyproterone acetate on the concentration of γ-aminobutyric acid in brain areas of aggressive and non-aggressive mice. Pharmacol. Biochem. Behav. 6, 409–413.
Eisenegger, C., Haushofer, J., Fehr, E., 2011. The role of testosterone in social interaction. Trends Cogn. Sci. 15, 263–271.
Feng, N.Y., Bass, A.H., 2016. "Singing" fish rely on circadian rhythm and melatonin for the timing of nocturnal courtship vocalization. Curr. Biol. 26, 2681–2689.
Fernald, R.D., 2012. Social control of the brain. Annu. Rev. Neurosci. 35, 135–151.
Fernald, R.D., 2017. Cognitive skills and the evolution of social systems. J. Exp. Biol. 220, 103–113.
Fernald, R.D., Hirata, N.R., 1977. Field study of *Haplochromis burtoni*: quantitative behavioural observations. Anim. Behav. 25, 964–975.
Fernald, R.D., Maruska, K.P., 2012. Social information changes the brain. Proc. Natl. Acad. Sci. 109, 17194–17199.
Folgueira, M., Anadón, R., Yáñez, J., 2004. An experimental study of the connections of the telencephalon in the rainbow trout (*Oncorhynchus mykiss*). I: olfactory bulb and ventral area. J. Comp. Neurol. 480, 180–203.
Fourcade, R.O., McLeod, D., 2004. Tolerability of antiandrogens in the treatment of prostate cancer. UroOncology 4, 5–13.
Frye, C.A., Edinger, K., Sumida, K., 2008. Androgen administration to aged male mice increases anti-anxiety behavior and enhances cognitive performance. Neuropsychopharmacology 33, 1049–1061.
Fuxjager, M.J., Forbes-Lorman, R.M., Coss, D.J., Auger, C.J., Auger, A.P., Marler, C.A., 2010. Winning territorial disputes selectively enhances androgen sensitivity in neural pathways related to motivation and social aggression. Proc. Natl. Acad. Sci. 107, 12393–12398.
Gelinas, D., Callard, G.V., 1997. Immunolocalization of aromatase-and androgen receptor-positive neurons in the goldfish brain. Gen. Comp. Endocrinol. 106, 155–168.
Goodson, J.L., 2005. The vertebrate social behavior network: evolutionary themes and variations. Horm. Behav. 48, 11–22.
Gorelick, D.A., Watson, W., Halpern, M.E., Gene, Z., 2008. Androgen receptor gene expression in the developing and adult zebrafish brain. Dev. Dyn. 237, 2987–2995.
Grosenick, L., Clement, T.S., Fernald, R.D., 2007. Fish can infer social rank by observation alone. Nature 445, 429–432.
Harbott, L.K., Burmeister, S.S., White, R.B., Vagell, M., Fernald, R.D., 2007. Androgen receptors in a cichlid fish, *Astatotilapia burtoni*: structure, localization, and expression levels. J. Comp. Neurol. 73, 57–73.
Huffman, L.S., O'Connell, L.A., Hofmann, H.A., 2013. Aromatase regulates aggression in the African cichlid fish *Astatotilapia burtoni*. Physiol. Behav. 112, 77–83.
Jacome, L.F., Barateli, K., Buitrago, D., Lema, F., Frankfurt, M., Luine, V.N., 2016. Gonadal hormones rapidly enhance spatial memory and increase hippocampal spine density in male rats. Endocrinology 157, 1357–1362.
Juraska, J.M., Willing, J., 2017. Pubertal onset as a critical transition for neural development and cognition. Brain Res. 1654, 87–94.
King, H.M., Kurdziel, L.B., Meyer, J.S., Lacreuse, A., 2012. Effects of testosterone on attention and memory for emotional stimuli in male rhesus monkeys. Psychoneuroendocrinology 37, 396–409.
Korsia, S., Bottjer, S.W., 1991. Chronic testosterone treatment impairs vocal learning in male zebra finches during a restricted period of development. J. Neurosci. 11, 2362–2371.
Kunkhyen, T., Perez, E., Bass, M., Coyne, A., Baum, M.J., Cherry, J.A., 2018. Gonadal hormones, but not sex, affect the acquisition and maintenance of a Go/No-Go odor discrimination task in mice. Horm. Behav. 100, 12–19.
Lacreuse, A., King, H.M., Kurdziel, L.B., Partan, S.R., Caldwell, K.M., Chiavetta, M.R., Millette, M.M., Meyer, J.S., Grow, D.R., 2010. Testosterone may increase selective attention to threat in young male macaques. Horm. Behav. 58, 854–863.
Lee, A.W., Pfaff, D.W., 2008. Hormone effects on specific and global brain functions. J. Physiol. Sci. 58, 213–220.
Li, Y., Chen, T., Miao, X., Yi, X., Wang, X., Zhao, H., Lee, S.M.Y., Zheng, Y., 2017. Zebrafish: a promising in vivo model for assessing the delivery of natural products, fluorescence dyes and drugs across the blood-brain barrier. Pharmacol. Res. 125, 246–257.
Lieberburg, I., Maclusky, N.J., McEwen, B.S., 1977. 5α-dihydrotestosterone (DHT) receptors in rat brain and pituitary cell nuclei. Endocrinology 100, 598–607.
Marler, P., Peters, S., Wingfield, J., 1987. Correlations between song acquisition, song production, and plasma levels of testosterone and estradiol in sparrows. J. Neurobiol. 18, 531–548.
Marler, P., Peters, S., Ball, G.F., Dufty, A.M., Wingfield, J.C., 1988. The role of sex steroids in the acquisition and production of birdsong. Nature 336, 770–772.
Maruska, K.P., 2015. Social transitions cause rapid behavioral and neuroendocrine changes. Integr. Comp. Biol. 55, 1–13.
Maruska, K.P., Fernald, R.D., 2010a. Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish. Horm. Behav. 58, 230–240.

Maruska, K.P., Fernald, R.D., 2010b. Steroid receptor expression in the fish inner ear-varies with sex, social status, and reproductive state. BMC Neurosci. 11, 58.
Maruska, K.P., Fernald, R.D., 2010c. Reproductive status regulates expression of sex steroid and GnRH receptors in the olfactory bulb. Behav. Brain Res. 213, 208–217.
Maruska, K.P., Fernald, R.D., 2011. Plasticity of the reproductive axis caused by social status change in an African cichlid fish: II. Testicular gene expression and spermatogenesis. Endocrinology 152, 291–302.
Maruska, K.P., Fernald, R.D., 2012. Contextual chemosensory urine signaling in an African cichlid fish. J. Exp. Biol. 215, 68–74.
Maruska, K.P., Fernald, R.D., 2013. Social regulation of male reproductive plasticity in an African cichlid fish. Integr. Comp. Biol. 53, 938–950.
Maruska, K.P., Levavi-Sivan, B., Biran, J., Fernald, R.D., 2011. Plasticity of the reproductive axis caused by social status change in an African cichlid fish: I. Pituitary gonadotropins. Endocrinology 152, 281–290.
Maruska, K.P., Zhang, A., Neboori, A., Fernald, R.D., 2013. Social opportunity causes rapid transcriptional changes in the social behaviour network of the brain in an African cichlid fish. J. Neuroendocrinol. 25, 145–157.
McCall, C., Singer, T., 2012. The animal and human neuroendocrinology of social cognition, motivation and behavior. Nat. Neurosci. 15, 681–688.
O'Connell, L.A., Hofmann, H.A., 2012a. Social status predicts how sex steroid receptors regulate complex behavior across levels of biological organization. Endocrinology 153, 1341–1351.
O'Connell, L.A., Hofmann, H.A., 2012b. Evolution of a vertebrate social decision-making network. Science 336, 1154–1157.
Parikh, V.N., Clement, T.S., Fernald, R.D., 2006. Androgen level and male social status in the African cichlid, *Astatotilapia burtoni*. Behav. Brain Res. 166, 291–295.
Pfaff, D.W., Kow, L.M., Loose, M.D., Flanagan-Cato, L.M., 2008. Reverse engineering the lordosis behavior circuit. Horm. Behav. 54, 347–354.
Remage-Healey, L., Joshi, N.R., 2012. Changing neuroestrogens within the auditory forebrain rapidly transform stimulus selectivity in a downstream sensorimotor nucleus. J. Neurosci. 32, 8231–8241.
Remage-Healey, L., Maidment, N.T., Schlinger, B.A., 2008. Forebrain steroid levels fluctuate rapidly during social interactions. Nat. Neurosci. 11, 1327–1334.
Rink, E., Wullimann, M.F., 2004. Connections of the ventral telencephalon (subpallium) in the zebrafish (*Danio rerio*). Brain Res. 1011, 206–220.
Rooney, D., Doving, K.B., Ravaille-Veron, M., T., S., 1992. The central connections of the olfactory bulbs in cod, *Gadus morhua*. J. Hirnforsch. 33, 63–75.
Sapolsky, R.M., 2005. The influence of social hierarchy on primate health. Science 308, 648–653.
Sar, M., Stumpf, W.E., 1973. Effects of progesterone or Cyproterone acetate on androgen uptake in brain, pituitary and peripheral tissues. Proc. Soc. Exp. Biol. Med. 144, 26–29.
Sartor, J.J., Balthazart, J., Ball, G.F., 2005. Coordinated and dissociated effects of testosterone on singing behavior and song control nuclei in canaries (*Serinus canaria*). Horm. Behav. 47, 467–476.
Schlinger, B.A., Brenowitz, E.A., 2009. Neural and hormonal control of birdsong. In: Pfaff (Ed.), Hormones, Brain and Behavior. Elsevier B.V, Amsterdam, pp. 897–942.
Seeholzer, L.F., Seppo, M., Stern, D.L., Ruta, V., 2018. Evolution of a central neural circuit underlies *Drosophila* mate preferences. Nature 559, 564–569.
Sessa, A.K., Harris, R.M., Hofmann, H.A., 2013. Sex steroid hormones modulate responses to social challenge and opportunity in males of the monogamous convict cichlid, Amatitliana nigrofasciata. Gen. Comp. Endocrinol. 189, 59–65.
Solis, M.M., Doupe, A.J., 1997. Anterior forebrain neurons develop selectivity by an intermediate stage of birdsong learning. J. Neurosci. 17, 6447–6462.
van Breukelen, N.A., 2013. Androgen receptor antagonist impairs courtship but not aggressive behavior in the monogamous cichlid, *Amatitlania nigrofasciata*. Horm. Behav. 63, 527–532.
Weitekamp, C.A., Nguyen, J., Hofmann, H.A., 2017. Social context affects behavior, preoptic area gene expression, and response to D2 receptor manipulation during territorial defense in a cichlid fish. Genes Brain Behav. 16 (6), 601–611.
Wilson, E., 1975. Sociobiology: The New Synthesis. Harvard University Press, Cambridge, MA.
Wirth, M.P., Hakenberg, O.W., Froehner, M., 2007. Antiandrogens in the treatment of prostate cancer. Eur. Urol. 51, 306–314.
Wise, R.A., 2004. Dopamine, learning and motivation. Nat. Rev. Neurosci. 5, 483–494.