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# Androgenization of Dominant Males in a Cichlid Fish: Androgens Mediate the Social Modulation of Sexually Dimorphic Traits

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## Abstract

We present data on the relationship between social status and male secondary sex traits, both morphological and ethological, in *Oreochromis mossambicus* at the onset of sexual maturation. There is a differential expression of morphological (dorsal fin height, anal fin height, mandible width and premaxilla length) and behavioural (nuptial coloration, per cent time defending a territory, spawning pit volume, pit hovering and courtship rate) traits according to social status, with dominants presenting the highest expression of these dimorphic traits. Social status also affects the development of the genital papillae, which is considered to be a good predictor of androgen levels in cichlid fishes. The results support a causal model in which social status modulate androgen levels that in turn modulate the expression of behavioural and morphological male traits.

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## Introduction

Androgens may play a very important proximate causal role as mediators between social status, as an expression of the outcomes of male–male competition, and the expression of male secondary sex characters, both morphological and behavioural, that may serve as cues to both males and females. Indeed, there is an increasing amount of evidence that the levels of androgens are strongly affected by social factors, namely by the outcomes of the social interactions in which the individual participates (for fish: *Xiphophorus helleri*, see Hannes et al. 1984; Hannes 1986; *Sparisoma viride*, see Cardwell & Liley 1991; *Astatotilapia burtoni*, see Francis et al. 1993; *Chromis dispilus*, see Pankhurst & Barnett 1993; *Oreochromis mossambicus*, see Oliveira et al. 1996; for other vertebrates: reptiles, Greenberg & Crews 1990; birds, Harding & Follett 1979; Wingfield et al. 1987; mammals, Eberhart et al. 1980; Sachser & Pröve 1984; Creel et al. 1993). Conversely, the expression of many male secondary sex traits is under androgen control. In teleosts, androgens have been implicated in the differentiation of many morphological male secondary

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sex characters, such as nuptial colour patterns (e.g. *Astatotilapia burtoni*, see Fernald 1976; Reinboth 1978; *Gasterosteus aculeatus*, see Borg 1981, 1987; *Oncorhynchus nerka*, see Idler et al. 1961; *Trichogaster trichopterus*, see Johns & Liley 1970), development of median fins (e.g. *Betta splendens*, see Leitz 1987; *T. trichopterus*, see Johns & Liley 1970; *Xiphophorus helleri*, see Baldwin & Goldin 1939), development of a gonopodium in poecilids (Turner 1942, 1947, 1960), sonic muscles in sound producing fishes (e.g. *Opsanus tau*, see Fine & Pennypacker 1986; *Porichthys notatus*, see Brantley et al. 1993), and electrocytes in the electric organ of mormyrids and gymnotids (Zakon 1993). In relation to the role of androgens in the causation of male behaviour in teleost fishes, both castration and exogenous administration experiments have provided positive evidence, in many instances, of the effects of androgens in determining behaviour (for a review see Liley & Stacey 1983; Borg 1994). In cichlid fishes, androgens have been shown to influence the expression of male displaying characters, namely nuptial coloration, courtship, territorial behaviour and spawning pit building (Levy & Aronson 1955; Kramer et al. 1969; Reinboth & Rixner 1972; Wapler Leong & Reinboth 1974; Fernald 1976; Reinboth 1978; Billy & Liley 1985; Munro & Pitcher 1985).

It has been demonstrated in *Oreochromis mossambicus* that the social status of males modulates the androgen levels and that androgen levels were positively correlated with male displaying characters such as nest size, courtship rate and the dark coloration (Oliveira et al. 1996). In this species, there is also a marked sexual dimorphism involving the height of the dorsal and anal fins and the proportions of the jaw (both set of traits being positively allometric in the males; Oliveira & Almada 1995). Interestingly, these two sets of sexually dimorphic characters appear to be involved in male–male competition (the median fins in lateral displays and the jaw in mouthfighting; Oliveira & Almada 1995).

In the present paper we investigate the hypothesis that androgens mediate the effects of social status in the expression of: 1. morphological secondary sex characters (jaw structure and anal/dorsal fins); and 2. behavioural displaying characters (nuptial dark coloration, nest size, courtship rate). These traits may be important clues both in male–male competition and in female mate choice. We aimed specifically to evaluate to what extent the social status acquired by each fish after the formation of groups in adolescent animals led to interindividual differences in androgen levels that in turn affect the expression of the secondary sex characters, both morphological and behavioural.

## Methods

### Synopsis of the *Oreochromis mossambicus* Mating System

*O. mossambicus* is an African lek-breeding cichlid. Males form dense aggregations of territories in which they dig and defend nest pits. Territorial males adopt a conspicuous dark coloration. Ripe females visit the breeding arenas and when male courtship is successful they enter the pits to spawn, after which they pick up the eggs into the mouth, where fertilization takes place, and leave the arena. The eggs and fry are mouth-brooded by the female outside the spawning sites, and the fry are release after ≈20 d in nursery grounds (Bruton & Boltt 1975). Thus, males do not participate in parental care and their nests, whose size varies with male status (Oliveira et al. 1996), appear to serve an epigamic function and are important during the spawning process. Indeed, Nelson (1995) demonstrated, based on binary choice tests, that females prefer males with larger spawning pits. This appears to be a general trend in lekking cichlids, since McKaye et al. (1990) also demonstrated a female preference for males with larger and more centrally located nests in another arena breeding cichlid (*Cyrtocara eucinostomus*). Mating interference and egg cannibalism from other breeding males are common in cichlids including *O. mossambicus* (Fryer & Iles 1972; Trewavas 1983), thus spawning place inside a pit may reduce the likelihood these and so the female may gain a direct benefit from choosing to spawn with a male with a larger pit.

### Subjects and Maintenance

The fish used in the present study came from two broods from a stock bred at our laboratory (for details on this stock see Oliveira 1995) In this work, males ranged from 3.6 to 8.1 cm standard length, and females ranged from 3.1 to 7.1 cm standard length. Fish were fed daily, except for the observation days, with commercial food flakes, and were kept at 24 ± 2°C with a photoperiod of 12 h light and 12 h dark.

### Procedures

Sixteen groups of immature juveniles (c. 20–30 d post-buccal incubation) of six individuals each were formed, in standard aquaria ($62 \times 34 \times 39\,\mathrm{cm}^3$) and the groups were reared until the onset of sexual maturity, as revealed by the first appearance of dark individuals and nest beginnings in the substrate (c. 8–9 mo old fish). At this time behavioural observations were carried out (see details below). The individuals were then killed with an overdose of the anaesthetic quinaldine (Sigma), morphometric measurements (see Table 1) were taken with a calliper to the nearest 0.1 mm, and the individuals were dissected for gonad extraction. The following measurements were also taken: body weight, gonad weight and size of genital papillae (to the nearest 0.1 mm using a calibrated micrometer on a stereomicroscope). The length of the caudal fin (CFL) was obtained as a derivative variable as: $\mathrm{CFL} = \mathrm{TL} - \mathrm{SL}$. We included this variable in the present study as a control one since it does not show any sexual dimorphism (Oliveira 1995).

Sexing was achieved by direct inspection of the gonads and the aceto-carmine method proposed by Guerrero & Sheldon (1974) was used when necessary. The stage of maturation of the gonads was assessed following a scale for macroscopic inspection proposed by Dadzie (1969, 1974) and by Arthington & Milton (1986) — gonad maturation index (GMI: 1 = juvenile; 2 = inactive; 3 = maturing; 4 = mature; 5 = spent) — and by using the gonado-somatic index (GSI = gonad weight/body weight × 100).

*Table 1:* Morphometric measurements used in the present study (adapted from Oliveira & Almada 1995)

| Abbreviations | Term | Method of measurement |
|---|---|---|
| AFH | Anal fin height | Length of the largest fin ray of the second anal fin |
| DFH | Dorsal fin height | Length of the largest fin ray of the second dorsal fin |
| MW | Mandible width | Maximum width of the lower lip |
| PL | Premaxilla length | Anterior edge of the upper lip to the posterior edge of the jaw |
| PW | Premaxilla width | Maximum width of the upper lip |
| TL | Total length | Anterior edge of the upper lip to the tip of middle rays of caudal fin |
| SL | Standard length | Anterior edge of the upper lip to the base of the caudal fin |
| SNL | Snout length | Anterior edge of the upper lip to the anterior edge of the orbit |

### Behavioural Observations

All observations were carried out between 1100 and 1800 h. Because of to the large number of groups (n = 16) to be followed we subdivided the groups into two subsets (of eight groups each) which were observed under different protocols.

The first set of groups (n = 28 males) was followed for eight consecutive weeks and observations were carried out following two sampling procedures. 1. Behavioural sampling procedure with continuous recording (see Martin & Bateson 1993) for both agonistic and sexual interactions, in periods of 5 min each; each group was observed for six such periods each week, giving an observation effort of 30 min per group each week. 2. Scanning observations with instantaneous sampling (see Martin & Bateson 1993) for the behavioural activities pit digging and pit hovering (i.e. hovering near the bottom above the spawning pit), in periods of 5 min each; each group was observed for six such periods each week, giving an observation effort of 30 min per group each week. Based on this procedure the percentage of time spent on each behavioural activity was computed. In these groups we also recorded the following variables each week: presence of nests and the identity of the nesting male; nest volumes; and the dark coloration intensity for each individual.

In the second set of groups (n = 18 males) we used animal focal observations with continuous recording (Martin & Bateson 1993) for both agonistic and sexual interactions, based on which we could assess dominance ranks and mating success. Each group was observed on two consecutive days, and on each day six focal observations of 5 min each were performed, totalling 1 h of observation per fish, and 6 h per group. In these groups we also recorded the following variables:

presence of nests and the identity of the nesting male; nest volumes; and the dark coloration intensity for each individual.

In each group, individuals were recognized by natural markings and by relative size differences.

We considered that an agonistic interaction had occurred when one or more of the following behaviour patterns was seen: charge, butting/biting, chasing, lateral display, frontal display, carouseling, tail beating, mouth-fighting, pendeling, fleeing, and submission. We also recorded the identity of the participants and the outcome of the interactions. An individual was considered to be a loser in an agonistic interaction when he retreated, adopted a submissive posture or fled from the opponent. We considered that a courtship episode had occurred when one or more of the following acts were detected: tilting, leading, circling, tail-wagging and quivering. A detailed description of these agonistic and sexual behaviour acts may be found in Baerends & Baerends van Roon (1950) and in Neil (1964) (see Table 2 for a summary).

The dark coloration intensity was estimated on a scale from 0 to 4 (0 = neutral; 1 = dark 1; 2 = dark 2; 3 = dark 3; 4 = black) according to the coloration patterns described by Neil (1964) (see Table 2 for a summary). An average coloration intensity was computed for each fish based on 12 measurements of the coloration intensity taken at the beginning of each observation period.

Nest volume was estimated from measurements of the diameter and depth at the centre of the pits, assuming that the nests have the approximate shape of an inverted cone. In order to reduce inter-group variability in pit depths, each aquarium was provided with approximately the same amount of sand (7 cm layer).

To estimate the social status of each individual two methods were used. 1. A dominance index (DI) according to the formula $DIi = Vi/Vi + Di$, where Vi is the number of victories and Di is the number of defeats of each individual, i. This index expresses the success of each fish in the set of agonistic interactions on which it participated. 2. Individuals were classified into three categories of dominance (dominant, intermediate, subordinate) according to a classification procedure based on the analysis of a $n \times 2$ (individuals × victories/defeats) contingency table using a simulation procedure (ACTUS, Estabrook & Estabrook 1989). According to this procedure individuals were classified as dominants or subordinates when they presented a number of victories or defeats, respectively, larger than would be expected by chance ($\alpha = 5\%$). Individuals that failed to show significant asymmetry in the number of observed victories or defeats vs. the number of simulated ones were classified as being of intermediate rank. Based on this method, from the first subset nine males were classified as dominants, seven as of intermediate rank and 12 as subordinates. From the second subset of males, six were classified as dominants, six as of intermediate rank and six as subordinates. The use of these two methods was necessary for using different statistical procedures, and is justified by the fact that they are mutually consistent, i.e. individuals of the three categories differ significantly in their DIs (males of subset 2 one-way ANOVA, $F_{2,25} = 41.7$, $p > 0.001$; multiple comparisons of subordinates vs. intermediate rank $p = 0.036$, intermediate rank vs. dominants $p = 0.0002$, and subordinates vs. dominants $p = 0.0001$).

*Table 2:* Brief description of the social behavioural patterns & colour patterns according to Baerends & Baerends van Roon (1950) and Neil (1964)

| Behavioural pattern | Description |
|---|---|
| Agonistic interactions | |
| Butting | The fish swims rapidly towards the opponent and rams it in the head or in the flanks usually with open mouth |
| Lateral display | With the opponents in a parallel or antiparallel position they fully erect the dorsal and anal fins and fully spread the caudal and pelvic fin; at its maximum intensity it can be combined with erecting the branchiostegal membrane |
| Tail beating | While displaying laterally the fish beats the tail sideways |
| Carouseling | The opponents circle each other in lateral display |
| Frontal display | When the opponents approach each other frontally they erect the gill covers and the branchiostegal membrane. Usually the mouth is open |
| Mouth fighting | Both opponents try to grip the jaws of the other one, and having seized each other firmly by the mouth, they push and pull with tail beats |
| Submission posture | The fish hangs in the water column at an angle of 20° relative to the horizontal with the head upwards |
| Pendeling | Two nestholder males in a head to head position rush at each other with the dorsal and anal fins closed against the body. Just before contact with the opponent the fish avoid colliding with the other fish |
| Courting | |
| Tilting | Body is held at an angle of about 30° with the horizontal with the unpaired fins against the body |
| Leading | In a tilting posture the males swims in front of the female towards the nest |
| Circling | When the female is close to the nest the male circles around the nest; when the female joins the male they start to circle the nest with the male behind |
| Tail-wagging | Inside the nest the male wags his caudal fin in front of the female |
| Quivering | The male vibrates the body and presumably ejects sperm |
| Coloration patterns | |
| Neutral | body and fins silver dull |
| Dark 1 | body begins to darken in the region above the pelvic fins |
| Dark 2 | Darkening of the body progresses with scattered darker areas on the belly and on the caudal fin; the sub-opercular region lightens |
| Dark 3 | Dark dotted body with black dorsal and caudal fins and white sub-opercular region |
| Black | the body presents a velvet texture with lower jaw and opercle white; the tips of the dorsal, anal and caudal fins and the pelvic fins are bright red |

Because both group sets showed no differences in the trends presented we merged the compatible data in the proceeding analysis. Thus, for the morphological traits, data from both subsets was used. From the 18 males of the second subset, only eight contributed to this data set ($n = 36$), except for GSI ($n = 46$), since 10 of them were processed for aromatase assays and morphometric data were not collected. For the behavioural traits only males of the second subset were used ($n = 28$).

### Androgen Levels

The size of the genital papillae was used as a measure of androgen levels in males. In fact, Levy & Aronson (1955) demonstrated, through castration and exogenous administration of testosterone, that the genital papillae of tilapias is an androgen dependent character and that its development may be used as a bioassay to evaluate the levels of circulating androgens. This procedure was subsequently used by Schwanck (1980) to assess the effect of androgen levels on the establishment of dominance relationships in *Tilapia mariae* males. To validate the use of the genital papillae size as an indicator of relative androgen levels, we measured the genital papillae (GPAP) and collected urine samples from 14 adult males. Urine samples were processed for radio-immunoassays, and testosterone (T) and 11-ketotestosterone (KT) concentrations in the urine were assessed as in Oliveira et al. (1996). The correlations obtained between these measures (GPAP vs. T, $r = 0.54$, $p < 0.05$; GPAP vs. KT, $r = 0.54$, $p < 0.05$; and GPAP vs. T + KT, $r = 0.67$, $p < 0.01$) confirmed that the size of the genital papillae is a good indicator of androgen concentrations, especially if the total amount of both androgens (i.e. T plus KT) is considered, and so we decided to use it as a relative measure of androgen levels.

### Data Analysis

The comparison of the different variables among the three classes of dominance (dominants, intermediates, and subordinates) was performed with a one-way ANOVA followed by multiple comparison tests (Spjotvoll–Stoline test for different sample sizes; Spjotvoll & Stoline 1973), except for data that violated the ANOVA assumptions. For these days the non-parametric Kruskal–Wallis test was used followed by the non-parametric multiple comparison test of Dunn (Zar 1984).

To evaluate the androgen role in the social control of the expression of sexually dimorphic characters (anal fin height (AFH), dorsal fin height (DFH), mandible width (MW), premaxilla length (PL), premaxilla width (PW) and snout length (SNL)) and reproductive behaviour (dark coloration, percentage of time defending a territory, courtship rate, pit volume, pit digging, pit hovering) we used two criteria. 1. Comparison of the adjusted determination coefficients (R2) between two multiple regression models in which the secondary sex character was the dependent variable; in one of the models the independent variables were the dominance index ($V/V + D$) and body size (SL); in the second model these independent variables were maintained but the androgen level (GPAP) was added as a third independent variable. In this way one can test, using F statistics, if the inclusion of the androgen levels in the model increases its determination (Pedhazur 1982). 2. Using the path analysis method we decomposed the correlations between the secondary sex characters and the dominance index into the three possible paths expressed in the regression model (see Fig. 1): direct effect (DE), indirect effect (IE) through the androgens (GPAP), and spurious effect (SE) resulting from a covariation of the dependent variable with body size (SL) (Pedhazur 1982). That is:

$$\mathrm{Rdy} = \mathrm{Pdy} + \mathrm{PdaPay} + \mathrm{RdsPsy} \Leftrightarrow \mathrm{Rdy} = \mathrm{DE} + \mathrm{IE} + \mathrm{SE}$$

We then compared the relative importance of the indirect effects of dominance on each dependent variable via the androgens with the direct effect.

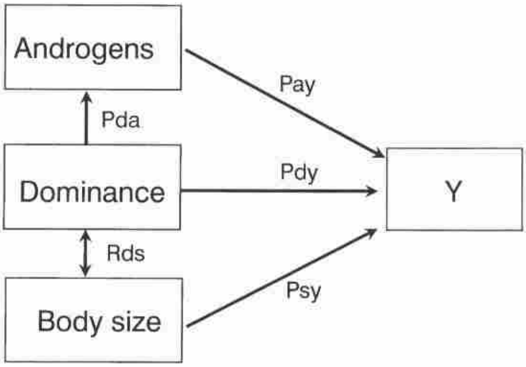

*Fig. 1:* Path diagram to illustrate the decomposition of the correlation coefficient between dominance and a sexually dimorphic variable Y (Rdy) into its three components: direct effect of dominance on Y expressed by the path coefficient, Pdy; the indirect effect of dominance on Y via the androgens expressed by the path coefficients Pda and Pay (i.e. Pda × Pay); and the spurious effect of dominance on Y due to a correlation between dominance and body size (Rds) and the covariation of Y with size (Psy), expressed by the path Rds × Psy

## Results

### Dominance and Sexual Maturation

Since the objectives of this paper concern the effects of dominance on the development of male secondary sex traits, both morphological and behavioural, it was important to have a prior assessment of the relationship between the social status and the level of gonadal maturation. Indeed, as the fishes in this study were reaching sexual maturity, one possible effect of dominance could be an acceleration or delay of the maturation process. Although fishes of different social status did not differ in their level of gonadal maturation (GMI: Kruskal–Wallis, $H_{2,33} = 3.63$, ns) they differed in GSIs (one-way ANOVA, $F_{2,43} = 3.21$, $p = 0.05$), with dominant males showing higher values (see Fig. 2).

### Dominance and Male Morphological Sexual Characters

Of the six variables previously diagnosed as sexually dimorphic in *O. mossambicus* (Oliveira & Almada 1995), only two did not present a variation with social status (PW and SNL) (see Table 3). The other four (DFH, AFH, MW and PL) tended to be larger in dominant males and to decrease with the social status of the individuals (see Table 3 and Fig. 3).

### Dominance and Male Reproductive Behaviour

For the analysis of the relationship between dominance and reproductive behaviour only fishes of the first subset mentioned in the Methods were used

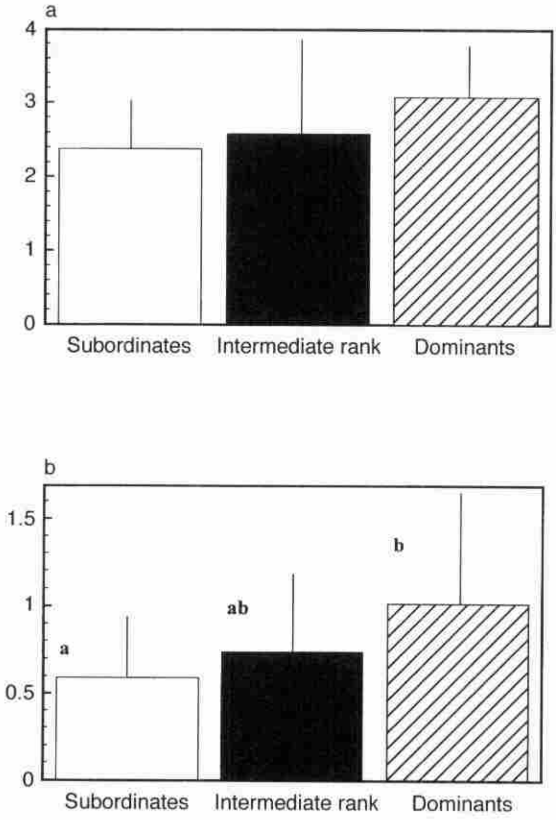

*Fig. 2:* Variation of gonadal development with social status: a. gonadal maturation index; b. gonado-somatic index. Groups (i.e. dominants vs. intermediate rank vs. subordinates) that differ significantly from each other are noted with different letters

*Table 3:* Comparison of morphological sexually dimorphic traits among males of different social status: dominants (D), intermediate rank (I) and subordinates (S). To control for allometric effects of body size the residuals of a linear regression between each variable and standard length were used. The following data are presented: sample size (n), F-value and associated p-value(one-way ANOVA), and the results of multiple comparisons among the three dominance groups (D, I, and S; homogeneous groups are denoted with the same letter)

| Variable | n | $F_{2,n-3}$ | p | D | I | S | Multiple comparisons ($\alpha$) |
|---|---|---|---|---|---|---|---|
| AFL | 36 | 3.62 | $<0.05$ | a | ab | b | $<0.05$ |
| DFL | 36 | 7.75 | $<0.01$ | a | a | b | $<0.05$ |
| MW | 36 | 2.82 | 0.074 | a | ab | b | $<0.10$ |
| PL | 36 | 4.49 | $<0.05$ | a | ab | b | $<0.05$ |
| PW | 36 | 2.31 | ns | – | – | – | – |
| SNL | 34 | 1.54 | ns | – | – | – | – |

($n = 28$ males). All the behavioural traits investigated, except pit digging, differed according to social status, with dominants differing significantly from subordinates and intermediate rank fishes exhibiting an intermediate position (see Fig. 4).

### Androgens as Mediators Between Social Status and Male Secondary Sexual Characters

There is a positive correlation between the androgen level, as measured by the size of the genital papillae, and the dominance index after controlling for body size (i.e. SL) (0.351, $n = 36$, $p < 0.05$). In order to try to assess to what extent one of the two correlated variables is more likely to be causal to the other we compared the coefficients of determination (R2) of the regression equations when size of the genital papilla is taken as the independent variable and the dominance index as the dependent one, and vice-versa, in both cases controlling for body size (i.e. SL). Since the explained percentage of variance in the dependent variable is greater when the size of the genital papilla is the dependent variable ($R^2 = 0.655$) than in the reverse situation ($R^2 = 0.244$), it is more likely that the social status may modulate the androgen level than the opposite. This result is in agreement with previous findings that social status modulates the levels of testosterone and 11-ketotestosterone after the formation of groups in this species (Oliveira et al. 1996). Thus, we hypothesized that the relationship between dominance status and the degree of expression of the secondary sexual characters in males of *O. mossambicus* is mediated by androgens. To test this hypothesis we compared the following two regression models:

$$y = b1 \text{ dominance} + b2 \text{ androgens} + b3 \text{ body size} + e \qquad (1)$$

vs.

$$y = b1 \text{ dominance} + b2 \text{ body size} + e$$

If the effects were mediated by androgens it would be predicted that inclusion of the size of the genital papilla would significantly increase the determination of

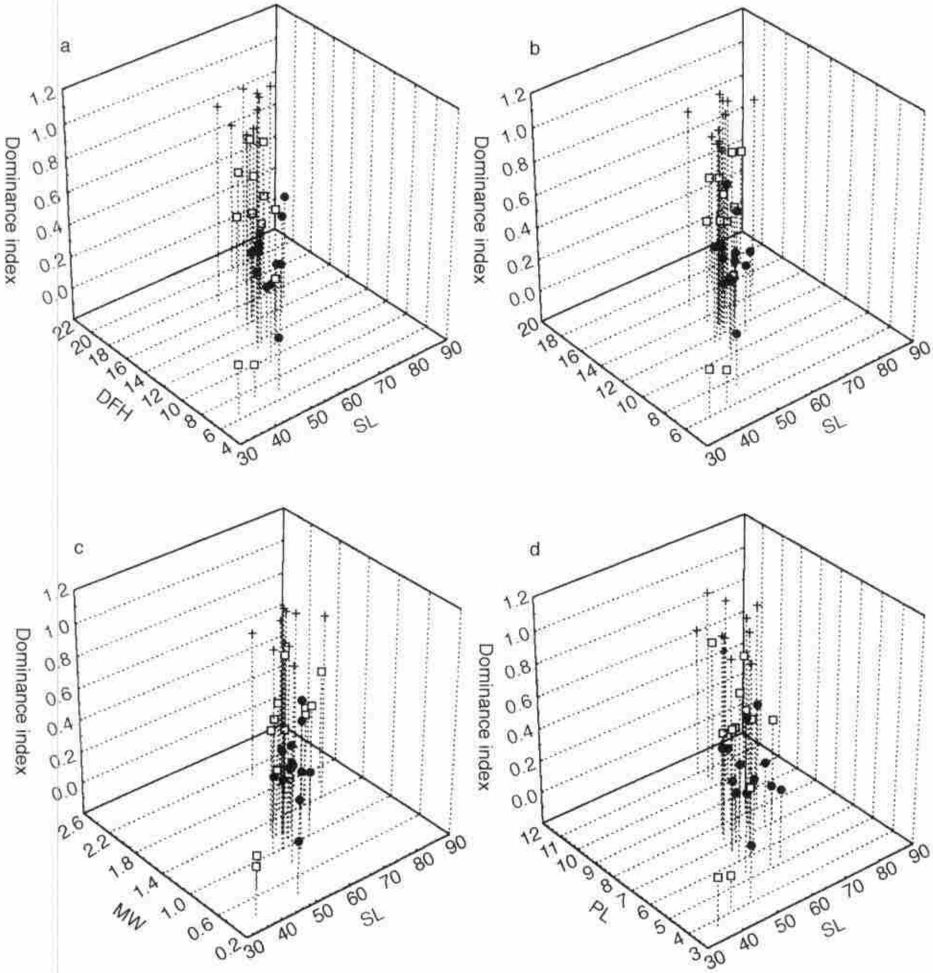

*Fig. 3:* Variation of sexually dimorphic morphological traits in males according to social status (i.e. dominance index = V/V + D) and to body size (SL): a. dorsal fin height (DFH); b. anal fin height (AFH); c. mandible width (MW); d. premaxilla length (PL). Crosses = dominants; open squares = intermediate rank; dark circles = subordinates

the model (i.e. the adjusted R2). This hypothesis would also predict that after the decomposition of the correlation between the sexually dimorphic traits and the social dominance into the three possible paths expressed in the regression model (1) (i.e. direct effect, indirect effect via the androgens and spurious effect due to the body size) the indirect effects of social dominance on the expression of the sexually dimorphic traits via the androgen level would be greater than the direct effects. From Table 4 it can be seen that both predictions are confirmed for all but one sexually dimorphic character (MW), and that CFL used as a control variable is

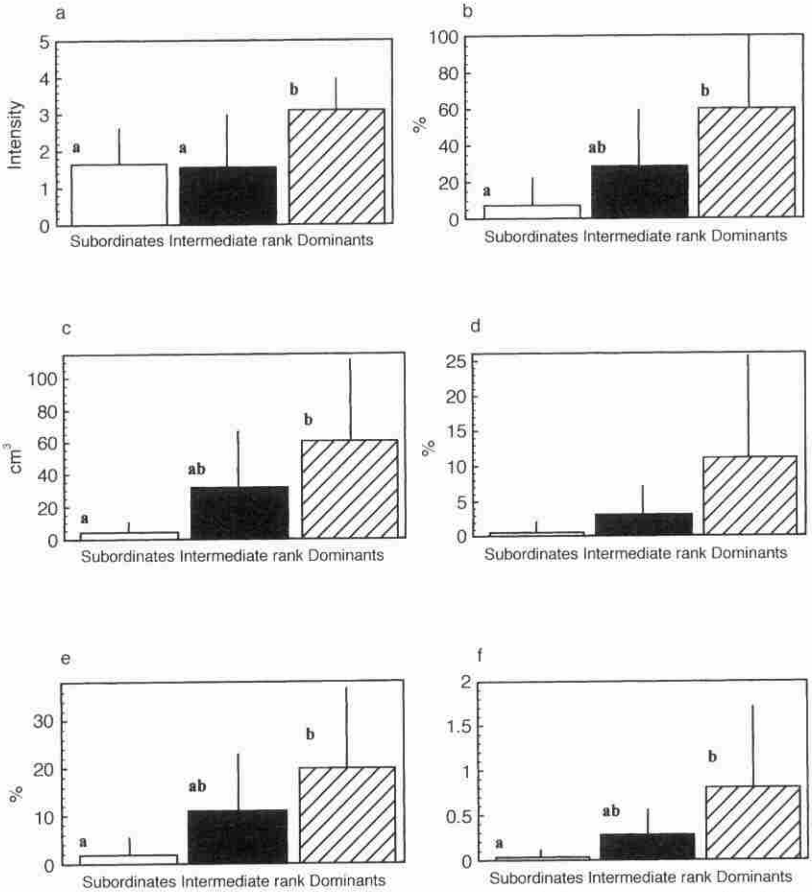

*Fig. 4:* Variation of male reproductive behaviour with social status: a. nuptial dark coloration ($H_{2,25} = 7.88$, $p < 0.05$); b. percentage of time defending a territory ($H_{2,25} = 9.92$, $p < 0.01$); c. nest volume ($H_{2,25} = 8.68$, $p < 0.05$); d. percentage of time spent digging the spawning pit ($H_{2,25} = 4.98$, $p = 0.08$); e. percentage of time hovering in the spawning pit ($H_{2,25} = 7.75$, $p < 0.05$); f. courtship rate in acts/5 min ($H_{2,25} = 7.83$, $p < 0.05$). Groups (i.e. dominants vs. intermediate rank vs. subordinates) that differ significantly (multiple comparisons Dunn's test) from each other are denoted by different letters

indeed the only character that does not present IE > DE. A similar procedure was applied to test the hypothesis that the relationships between social status and the behavioural traits involved in reproduction are also mediated by androgens (see Table 5). From Table 5 one can conclude that both criteria are again fulfilled in most cases. The inclusion of androgen levels in the model increases significantly its determination in all cases except for courtship rate, for which the increase in Adj R2 is not significant. The indirect effect via the androgens is higher than the direct effects except for courtship rate and nest digging.

*Table 4:* Comparison of the two linear regression models to evaluate the role of androgens on the expression of morphological sexually dimorphic traits in males; DE, direct effect of dominance on Y; IE, indirect effect of dominance on Y via the androgens; SE, spurious correlation between dominance and Y due to a covariation with body size (SL); (Adj $R^2$) adjusted coefficients of determination for each model; F (p), F and associated p-values to test the increment in proportion of variance accounted for

| | Y = b1 dom + b2 androgens + b3 size + e | | | | | Y = b1 dom + b2 size + e | |
|---|---|---|---|---|---|---|---|
| Y | r | DE | IE | S | Adj $R^2$ | Adj $R^2$ | F (p) |
| DFL | 0.52 | 0.14 | 0.16 | 0.22 | 0.869 | 0.835 | 8.6 (<0.01) |
| AFL | 0.50 | 0.11 | 0.16 | 0.23 | 0.901 | 0.865 | 12.0 (<0.005) |
| CFL | 0.45 | 0.12 | 0.10 | 0.23 | 0.691 | 0.685 | 0.6 (ns) |
| PW | 0.40 | −0.04 | 0.26 | 0.17 | 0.824 | 0.725 | 18.6 (<0.005) |
| MW | 0.42 | 0.05 | 0.14 | 0.22 | 0.741 | 0.719 | 2.8 (ns) |
| PL | 0.48 | 0.03 | 0.33 | 0.12 | 0.897 | 0.736 | 51.6 (<0.005) |
| SNL | 0.43 | −0.02 | 0.24 | 0.18 | 0.821 | 0.745 | 13.2 (<0.005) |

*Table 5:* Comparison of the two linear regression models to evaluate the role of androgens on the expression of male reproductive behavioural traits; DE, direct effect of dominance on Y; IE, indirect effect of dominance on Y via the androgens; SE, spurious correlation between dominance and Y due to a covariation with body size (SL); Adj $R^2$ adjusted coefficients of determination for each model; F (p), F and associated p-values to test the increment in proportion of variance accounted for

| | Y = b1 dom + b2 androgens + b3 size + e | | | | | Y = b1 dom + b2 size + e | |
|---|---|---|---|---|---|---|---|
| Y | r | DE | IE | SE | Adj $R^2$ | Adj $R^2$ | F (p) |
| Dark colour | 0.53 | 0.17 | 0.51 | −0.16 | 0.598 | 0.387 | 17.3 (<0.005) |
| Territory (% time) | 0.64 | 0.35 | 0.62 | −0.33 | 0.801 | 0.474 | 24.7 (<0.005) |
| Courtship rate | 0.54 | 0.51 | 0.31 | −0.28 | 0.282 | 0.222 | 2.76 (ns) |
| Pit volume | 0.69 | 0.43 | 0.56 | −0.30 | 0.765 | 0.401 | 51.1 (<0.005) |
| Pit digging | 0.69 | 0.60 | 0.46 | −0.37 | 0.636 | 0.197 | 18.1 (<0.005) |
| Pit hovering | 0.54 | 0.22 | 0.52 | −0.20 | 0.566 | 0.375 | 6.6 (<0.05) |

## Discussion

Because the present study is based on correlational evidence the results must be interpreted as preliminary. They raise a number of questions that require further experimental work.

The data presented above support the following causal model: social status modulates androgen levels that in turn affect both morphological secondary sex characters and behaviour patterns involved in reproduction. (see Fig. 5).

The first step of the model is the modulation of the androgen level by the outcomes of social interactions. In the present study the androgen levels, as expressed by the size of the genital papilla, were positively correlated with male social status, and the determination coefficients, after controlling for body size, indicate that social status explains more of the variance of androgen levels than the reverse. It is also possible that androgens affect directly the social status by acting on motivational mechanisms. However, we have demonstrated previously that when all-male groups are formed, the dominance scores measured after 1 d of group formation are good predictors of androgen levels measured 4 d later, while the androgen levels measured prior to group formation could not predict the dominance score achieved by each fish (Oliveira et al. 1996). In another mouth-brooding cichlid (*Astatotilapia burtoni*) Francis et al. (1992) showed that while castration of males decreased their aggressive behaviour it had no effect on their social status. Thus, the available evidence supports the first step of the model. Moreover, this conclusion makes sense if the breeding ecology of *O. mossambicus* is considered, since when males arrive at the arenas they have to compete among themselves for the acquisition and maintenance of reproductive territories.

The second step of the model involves the action of androgens as modulators both of morphological secondary sex traits and of behavioural displaying characters (i.e. nest size, dark coloration, and courting). Both effects are supported by the data presented in this paper. There is a large body of evidence implicating androgens in the control of both morphological secondary sex traits and of behavioural displaying characters in teleosts (see introduction for references). The oper-

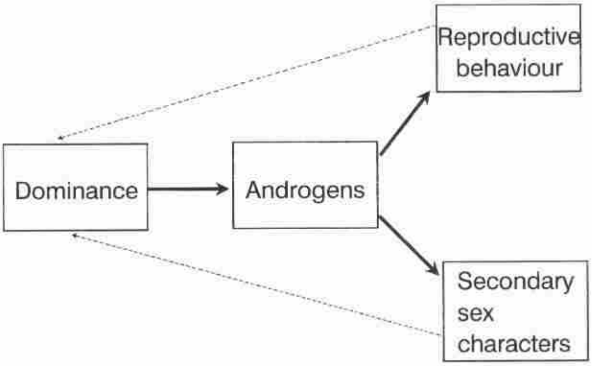

*Fig. 5:* Causal model for the role of androgens as mediators on the social modulation of the expression of sexually dimorphic traits, both morphological and ethological, in males of *Oreochromis mossambicus*. Arrows indicate causal relationships supported by linear regression models; dashed lines represent possible positive feedback mechanisms not investigated in the present study, but suggested by the available literature (see text for details)

ation of this model would have, at least, the following four evolutionary implications.

1. Honest signalling: males regulate the expression of secondary sex characters, both morphological and behavioural, according to their resource-holding potential (RHP). In a system in which male–male agonistic interactions are frequent, males displaying characters that do not correspond to their RHP may incur in heavy costs by provoking agonistic confrontations that they will not be able to win.

2. Amplifier effect on dominance: dominant males, by signalling their status both morphologically and behaviourally, may reinforce their social status by a positive feedback mechanism (dashed arrow on Fig. 5 going from morphological secondary characters and behavioural displaying characters towards dominance). Thus, small initial differences in RHP may result in increasingly larger differences in status.

3. Compartmentalization of deleterious effects: it is interesting to note that from the three median fins only the dorsal and the anal fins show a response to androgens. The fact that the caudal fin plays a crucial role in swimming in this species may have restrictive effects on its responsiveness to sexual selection. This may be an example of evolutionary compartmentalization (sensu Ketterson & Nolan 1994) of the response of median fins to androgens in a way that separates beneficial effects of androgens on dorsal and anal fins from hydrodynamic detrimental effects on the caudal fin. The caudal fin size may be important in swimming performance both in subordinate and dominant fish, namely when escaping from predators, precluding its response to sexual selection. The disassociation of the response of the different fins to androgens may be achieved by a local modification of the number or the affinity of steroid receptors present.

4. Androgens and life-histories: if hormones do play a mediating role between the outcomes of social interactions and the expression of male traits, this opens the way for the evolution of a number of alternative life history patterns. Indeed, the social status of a fish may affect its maturation (e.g. *Xiphophorus variatus*, see Borowsky 1973, 1978; *A. burtoni*, see Fraley & Fernald 1982), the adoption of alternative male tactics (for a review see Taborsky 1994), or even sex change (*Anthias squamipinnis*, see Fishelson 1970; *Labroides dimidiatus*, see Robertson 1972; *Amphiprion* spp., Fricke & Fricke 1977; *Thalassoma bifasciatum*, Warner & Swearer 1991; *Trimma okinawae*, see Sunobe & Nakazono 1993; for a general review see Shapiro 1979).

All these possibilities can, in principle, allow compensatory responses to evolve so that subordinate fishes minimize the disadvantages of their status by adopting the life history pattern that makes the best of their bad situation. In many species, social status is related to body size. Thus, if a subordinate and smaller individual delays or suspends its sexual maturation it may divert more resources to growth, subsequently overcoming its relative size disadvantage. The same compensatory principle as also been proposed by other authors both for alternative mating tactics (Taborsky 1994; Gonçalves et al. 1996) and for socially controlled mechanisms of sex-change (Warner 1975; Warner et al. 1975). In our case, because we worked with young adults, and the gonadal maturation state (i.e. GSI) was correlated with the degree of androgenization it is likely that the subordinates were delaying their sexual maturation. This phenomena, first studied in *Xiphophorus* (Borowsky 1973, 1978), may indeed be much more widespread among teleosts, as it is much less conspicuous than sex change or the adoption of sneaking, and it requires specific ontogenetic studies to be revealed.

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