Hormones and social behavior of cichlid fishes: a case study in the Mozambique tilapia

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ABSTRACT

The Mozambique tilapia, *Oreochromis mossambicus*, is used as a case study to illustrate different aspects of the relationship between androgens and social behavior in cichlid fish. First the mating system of *O. mossambicus* and its variations is described, namely the occurrence of alternative male mating tactics and male-male courtship, and the influence of social status on mating decisions and mating success. A discussion of a two-way causal relationship between androgens and social behavior follows, including the implications of social modulation of androgens for the expression of androgen-dependent male displaying characters (i.e. differential expression of secondary sex characters and male social behavior according to social status). The mechanisms regulating the action of androgens upon male behavior are also discussed with original data on the activity of brain aromatase. It is shown that male *O. mossambicus* have higher levels of brain aromatase activity than females, but aromatase activity is related neither to the gonadosomatic index nor to social status. In contrast, an androgen environment with predominance of the non-aromatizable androgen 11-ketotestosterone over the aromatizable testosterone is indicative of high social status and territorial possession. This suggests that 11-ketotestosterone may be playing a major role in the expression of male characters, both behavioral and morphological. The role of testosterone in female aggression is also discussed. An association between female aggression and plasma testosterone was found during the mouthbrooding cycle. Finally, the relevance of cichlids for comparative studies of vertebrate behavioral endocrinology is highlighted.

INTRODUCTION: ANDROGENS AND SOCIAL BEHAVIOUR IN MALE CICHLIDS

The relationship between sex steroids and behavior in teleosts has been mainly studied from the point of view of the effects of steroids upon behavior. Androgens in particular have been shown to play a major role in the expression of breeding behaviours (Liley and Stacey, 1983; Villars, 1983; Borg, 1994). Based on early ethological work (e.g.van Iersel, 1953; Wiepkema, 1961; Baggerman, 1966), which indicated that different motivational systems may be involved in different aspects of breeding behavior, Liley and Stacey (1983) have proposed that the study of the underlying mechanisms to different aspects of breeding behavior should be analyzed separately. The breeding behaviors include:

- Those that occur before spawning, i.e. the establishment of breeding territories, the preparation of the spawning site (which in some species can be also used as a nest) and the attraction of a mate to the spawning site;
- The spawning episode, that is male ejaculation and female egg laying behavior in external fertilizers and copulation in species with internal fertilization;
- The parental behavior, which may involve guarding larvae and/or eggs against potential

Table 1. Effect of exogenous administration of androgens and/or castration on male nuptial coloration in cichlid fishes with sexual dichromatism. KT, 11-ketotestosterone; 11-OHT, 11 ß-hydroxytestosterone; DHT, 5ß-dihydrotestosterone; T, testosterone; A, androstenedione.

Species	Treatment	Effect	Reference
Sarotherodon macrocephala	Castration	-	Levy and Aronson, 1955
	Testosterone after castration	+	Levy and Aronson, 1955
Pseudocrenilabrus multicolor	Castration	-	Reinboth and Rixner, 1972
	Testosterone after castration	+	Reinboth and Rixner, 1972
	Testosterone given to females	+ (male coloration)	Reinboth and Rixner, 1972
Astatotilapia burtoni	Testosterone propionate	+ ("eye bar")	Fernald, 1976
	Testosterone given to females	+ (male coloration with "anal spots")	Wapler-Leong and Reinboth, 1974
	11-KT>11OHT> DHT>A>T	+ (melanophores in pelvic fins and in "anal spots" in juveniles)	Reinboth, 1978

predators, fanning to oxygenate the eggs, together with the inhibition of feeding directed to its own eggs.

Available evidence based on castration and replacement therapy with exogenous androgens and/or anti-androgens suggests a major role for androgens in the control of pre-spawning behavior, but not in the spawning reflex or in parental care. Studies on the mechanisms of spawning reflex suggest a direct neural control of sperm release in male teleosts. Direct innervation of the testis in male Nile tilapia (*Oreochromis niloticus*) has been described, with nerve bundles distributed in the walls of the efferent duct and thinner ones penetrating the interstices among lobules containing spermatogenic cells (Nakamura, 1995). Male bluehead wrasse (*Thalassoma bifasciatum*) control the amount of sperm ejaculated and adjust it to the size of the spawning female, supporting further a neural control of sperm release (Shapiro et al., 1994). Moreover, electrical stimulation of the pre-optic area in the hypothalamus elicits sperm release (Demski et al., 1975). Several aspects of parental care seem to be regulated by prolactin, which in some cases may be acting together with androgens, such as in parental aggression (Liley and Stacey, 1983; Wendelaar-Bonga et al., 1984; de Ruiter et al., 1986; Kindler et al., 1991). A potential trade-off between androgens and parental behaviors has been suggested, since androgens have been implicated in the expression of aggression, which should be reduced during the parental phase. In fact, species with marked parental phases during their breeding cycles usually show a decrease in androgen levels during parental care (Wingfield et al., 1990). However, in many species of teleost fishes a significant reduction of androgens is also associated with the initiation of spermiation when progestin levels increase. Laboratory manipulations of parental fishes would help to clarify a potential role of androgens.

For pre-spawning behaviors, castration experiments have yielded contradictory results. However, gonadectomy generally inhibits the expression of reproductive behaviors, as well as secondary sex characters, in most teleost species. The administration of exogenous androgens to castrates restores and promotes the development of these characters (for a review see Liley and Stacey, 1983 and Borg, 1994). Moreover, the administration of androgens to intact individuals promotes the expression of reproductive behaviors in most fish species (Liley and Stacey, 1983; Borg, 1994). In cichlid fishes, courtship and nest-building behaviors have been maintained in castrated *Hemichromis bimaculatus* (Noble and Kumpf, 1936), *Sarotherodon melanotheron* (Aronson, 1951), *Aequidens latifrons* (Aronson et al., 1960), *Sarotherodon melanotheron heudelotii* and *Oreochromis niloticus* (Heinrich, 1967). But castration also inhibited the expression of reproductive behaviors in *Pseudocrenilabrus multicolor*, namely territorial aggression, sexual behavior and nest building, which were fully re-established after testosterone administration (Reinboth and Rixner, 1972). These contradictory results could be at least partly explained by incomplete gonadectomies and/or to the regeneration of testicular tissue. Additional factors are the social context of the experiment, the social status and sexual condition of the castrated individuals.

Total castration of male *Astatotilapia burtoni* reduces circulating levels of 11-ketotestosterone and testosterone, followed by a reduction in aggression, while partially castrated individuals have intermediate levels of both androgens and aggressive behaviors (Francis et al. 1992). However, castrated territorial males maintain their dominant status in dyadic encounters with intact non-territorial males, although they show lower levels of both androgens and aggression. Testosterone-treated females acquire male nuptial coloration, establish territories in which they dig nests and to which they attract non-treated females (Reinboth and Rixner, 1972). Furthermore, female *A. burtoni* display the full male behavioral repertoire and coloration pattern 15 days after a single injection of testosterone (0.2 mg / g body weight) with these effects lasting for up to 6-8 months (Wapler Leong and Reinboth, 1974). Intact *A. burtoni* males injected intramuscularly with testosterone (0.15 mg / g body weight) increase the frequency of approaches and attacks directed towards stimuli fish, blind juvenile *Tilapia mariae* (Fernald, 1976), but courtship and nest-building behaviors were not affected. This has been attributed to a lack of appropriate stimuli in the experimental set-up to trigger an unambiguous response. Approaching can be viewed as a decision point in the behavioral continuum of the species (Fernald, 1976) and depending on the response of the approached opponent, the individual may initiate an attack or a courtship sequence (Fernald, 1976). Approaches more frequently preceded courtship sequences than agonistic ones and since stimuli fish were blind, they did not respond to approaches by male *A. burtoni*, which may thus be considered as courtship attempts (Fernald, 1976). testosterone may therefore be viewed as acting both on agonistic and sexual behaviors or in the general social arousal.

Injection of the anti-androgen cyproterone acetate (0.3-1.0 mg/g body weight) to male *O. mossambicus* inhibits both agonistic and nest-build behavior, but not courtship, 3 to 5 days after the administration, with the inhibitory effects lasting for 1 to 7 days (Kramer et al., 1969). Injection of methyltestosterone also had a negative effect on aggression, nest building and courtship, while luteinizing hormone (LH) or gonadotropin releasing factor (GnRH) had positive effects on all these three behaviors. A negative feedback mechanism of testosterone acting on reproductive behavior in *O. mossambicus* has been suggested to explain the results (Kramer et al., 1969). A positive effect of the mammalian gonadotropins follicle stimulating hormone (FSH) and LH on the expression of nuptial coloration has been demonstrated in the cichlids *Symphysodon aequifasciata axelrodi* and *Pterophyllum scalare* (Blüm and Fiedler, 1965). LH also induced the expression of agonistic behaviors and the development of the genital papilla. The direct role of gonadotropins and their interaction with androgens on the control of social behavior in cichlids is still an open question.

As well as an activational role, androgens may also play an organizational role in the social behavior of cichlids. Clemens and Inslee (1968) described for the first time sex reversal of female *O. mossambicus* following the administration of testosterone during the first two months of life (the most effective period for sex reversal were the first 21 days after hatching). The sex-reversed females developed as males and when they became adults, they displayed male dark nuptial coloration and dug nests when in the presence of receptive non-treated females (Clemens and Inslee, 1968). These results have been confirmed by Billy

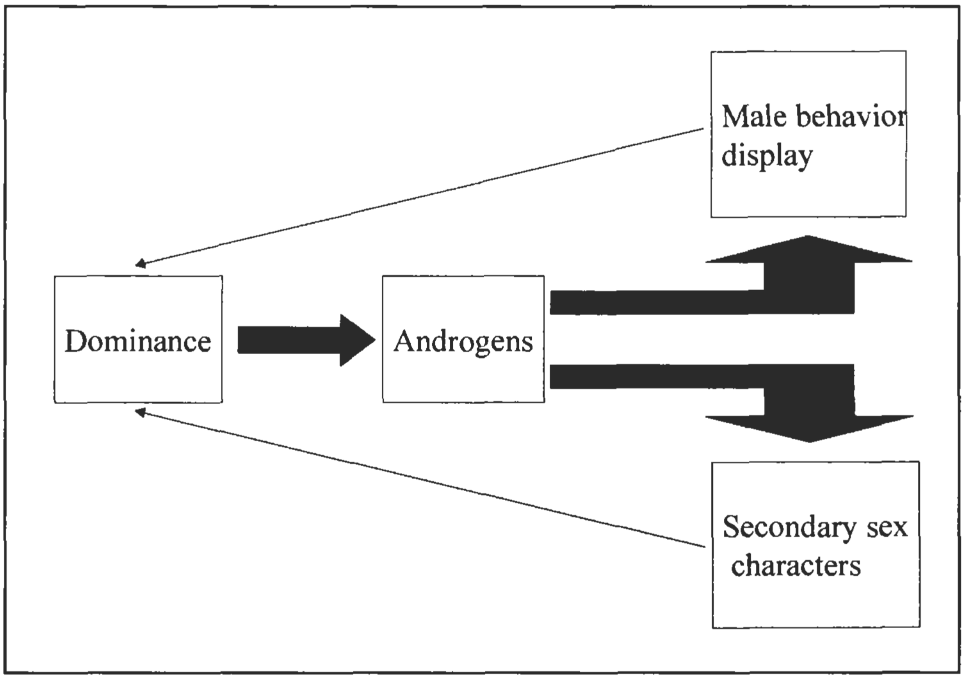

Figure 1. Causal model for the androgen mediation in the social control of the expression of male displaying behaviors and secondary sex characters in male *O. mossambicus*.

and Liley (1985) who also found that reproductively mature sex-reversed females have functional testis and display the full male behavioral repertoire. If individuals (both males and females) are precociously exposed to androgens out of the critical period (i.e. without the induction of gonadal sex reversal), they will be more aggressive as adults when compared to control individuals which have not been exposed to androgens as young. Females exposed to androgens as young, will also respond more effectively to androgen treatments when adult, showing a higher expression of male typical behaviors such as the dark nuptial coloration and courtship and agonistic behaviors (Billy and Liley, 1985). Thus, androgens seem to play a role not only in the activation of social behavior but also at an organizational level during the ontogeny in cichlids.

Hormones may also affect behavior by acting upon somatic structures with an epigamic function. Secondary sex characters play an important role in intraspecific communication, allowing sex recognition and potentially conveying information about the social status or the sexual state of the individual. It is also widely accepted that most secondary sexual characters may have evolved through sexual selection, through either male to male competition or female mate choice. Most male secondary sex characters in teleost fishes have been shown to be androgen-dependent (for reviews see Liley and Stacey, 1983; Villars, 1983; Borg, 1994). Castration inhibits the expression of male secondary sex characters while ovariectomy has no effect, which indicates that ovaries are not inhibiting the expression of male secondary sex characters in females. The negative effects of castration on secondary sex characters can be reversed by exogenous androgen administration. In studies in which different androgens have been tested 11-ketotestosterone has been shown to be the most effective androgen on eliciting the expression of secondary sex characters in male teleosts (e.g. Borg, 1994).

In cichlids two common secondary sex characters are the genital papilla and nuptial coloration (Fryer and Iles, 1972). In tilapine fishes there is a sexual dimorphism in the shape and size of genital papilla, which is accentuated during the breeding season and that can be used to sex individuals (Trewavas, 1983;

Table 2. Spearman rank correlation coefficients (r_s) between urinary aondrogen levels and social status in male *O. mossambicus* (N=13). Significance levels are: p<0.01 (**) and p<0.001 (***). Social status was estimated as the number of victories over the total number of interactions in which the individual participated (modifed from Oliveira et al., 1996).

	Social Status at the 1st Day After Group Formation	Social Status at the 5th Day After Group Formation
Baseline testosterone level	0.23	0.003
Testosterone level after group formation	0.71**	0.80**
Baseline 11-ketotestosterone level	0.18	0.27
11-ketotestosterone level after group formation	0.70**	0.80**

Oliveira and Almada, 1995). Gonadectomy had a negative effect on the size of genital papilla in male and female *Sarotherodon macrocephala*, but administration of testosterone and 17ß-estradiol were effective in promoting papilla development in ovariectomized females (Aronson and Holz-Tucker, 1947). Moreover, *Tilapia mariae* fry subjected to a long term oestradiol treatment developed as normal males without any vestiges of ovarian tissue in their gonads but developed female-like genital papilla (Jensen and Shelton, 1979). Thus, genital papilla development in females seems to be largely estrogen dependent. On the other hand, androgens seem to control male the development of the genital papilla, since administration of androgens to castrated males restores it in *Sarotherodon macrocephala* (Levy and Aronson, 1955). Furthermore, the size of the genital papilla is positively correlated with androgen levels measured in the urine of male *O. mossambicus* (r = 0,67, p<0.01, Oliveira and Almada, 1998a), which allows its use as a bioassay of androgen levels in cichlid species (Schwanck, 1980; Oliveira and Almada, 1998a). Taken together these results suggest that after the period of gonadal differentiation sex steroids are effective in the modulation of the differentiation of cichlid genitalia. Available data also suggests that male nuptial coloration in cichlids is another character under androgen control (see Table 1).

Further to the direct and indirect actions of androgens on behavior, it has emerged in recent years that the relationship between hormones and behavior is a two-way relationship, and that the behavior may itself modulate endocrine profiles (Wingfield et al., 1987; Cardwell and Liley, 1991; Oliveira et al., 1996; Borges et al., 1998). Social modulation of hormonal levels has also been demonstrated for cichlids. In *A. burtoni*, androgen levels reflect the individual social status and vary according to the social context. If, by experimental manipulation, the social status of an individual is reversed from dominant to subordinate its androgen levels will decrease. Conversely, a subordinate individual that becomes dominant will show an increase of androgen levels (Francis and Fernald, 1993). The social modulation of androgen levels appears to be under control of the pre-optic area of the hypothalamus where it has been shown that variations in the size of GnRH-immunoreactive cells in the POA respond to variations in social status in the same way (Francis et al., 1993). In contrast, no differences in androgen levels were found in male *Tilapia zillii* as consequence of winning or losing fights (Neat and Mayer, 1999), suggesting that these steroids may not mediate aggressive behaviors in this species. These contrasting results may reflect the fact that *T. zillii* and *A. burtoni* have different mating systems, monogamous vs. polygamous respectively.

The present paper will review available evidence on the interaction between androgens and social behavior in the Mozambique tilapia, *Oreochromis mossambicus*, a species that has been used as a study model in our laboratories.

SYNOPSIS OF *O. MOSSAMBICUS* MATING SYSTEM

O. mossambicus is an African lek-breeding cichlid (Fryer and Iles 1972). The social behavior of this species has received considerable attention and the basic social behavior patterns are well described (Seitz, 1949; Baerends and Baerends von Roon, 1950; Neil, 1964; N'Gokaka, 1984). During the breeding season males adopt a conspicuous dark nuptial coloration, that fades away in a few seconds if the fish is disturbed or defeated (Neil, 1964). They form dense aggregations of territories in which they dig and defend nest pits (Fryer and Iles 1972; Bruton and Boltt 1975; De Silva and Sirisena, 1988). Ripe females visit the breeding arenas and, when male courtship is successful, enter the pits to spawn. After which, females scoop the eggs into the mouth, where fertilization takes place, and then leave the arena (Baerends and Baerends von Roon, 1950; Bruton and Boltt 1975). Only females participate in parental care, mouthbrooding eggs and fry outside the spawning sites. The fry are released after approximately 20-22 days in nursery grounds (Fryer and Iles, 1972; Bruton and Boltt, 1975). Male spawning pits, whose size is positively correlated with male status (Oliveira et al., 1996), seem to serve an epigamic function and are important during the spawning process. Nelson (1995) demonstrated, with binary choice tests that females prefer males with larger spawning pits. This seems to be a general trend in lekking cichlids, since McKaye et al. (1990) had also shown a female preference for males with larger and more centrally located nests in *Copadichromis eucinostomus*. Mating interference and egg cannibalism from other breeding males is common in cichlids, including *O. mossambicus* (Fryer and Iles 1972; Trewavas 1983). The fact that spawning takes place inside a pit may reduce the likelihood of interference and cannibalism, and so the female may gain a direct benefit from choosing to spawn with a male with a larger pit.

Studies on the breeding behavior of *O. mossambicus* under captive conditions have revealed other interesting aspects of male breeding strategies, namely the synchronization of breeding behavior among males within the same tank and the occurrence of alternative mating tactics and male-male courtship (Oliveira and Almada, 1998b). In a tank that was checked daily for dark males and nests during 123 consecutive days, a trend was found for an alternation between periods when males dug nests and held territories (duration 13.2 ± 8.5 days, mean ± SD, n=6), with periods when no breeding activities were observed (8.8 ± 3.3 days, n=6) (Oliveira and Almada, 1998b). Moreover, the number of dark males in a given tank was positively correlated with the number of nests present in the tank. Since the ambient conditions were kept constant during the observation period, these results strongly suggest that the fishes synchronized their breeding activity. The synchronization of breeding males could have been a response to synchronism in the spawning cycles of the females (as shown by Bruton and Boltt, 1975) which, excluding brooding, have a periodicity of ca. 25 days (Smith, and Haley, 1988). This synchronization is possibly mediated by hormonal pheromones released by the females as demonstrated for goldfish, *Carassius auratus*, and other fish species (Dulka et al., 1987; Stacey and Sorensen, 1991; Sorensen and Scott, 1994). It has been shown that males of *O. mossambicus* with unlimited access to ripe females have higher levels of courtship activity than males with only visual access to females (Silverman, 1978). And the water of ovulated females not only is more attractive but also stimulates courtship behavior in males, to such a level that they engage in courting their own image in a mirror (Falter and Dolisy, 1989). Regardless of the causes of male breeding synchronization, the result will be an increase in the already high level of male-male competition caused by male breeding aggregations. High levels of male intra-sexual selection could lead to: (a) the existence of a substantial proportion of males that are unable to keep territories in the lek; (b) a high sexual motivation in territorial males that could result in a decrease of sex discrimination. These two

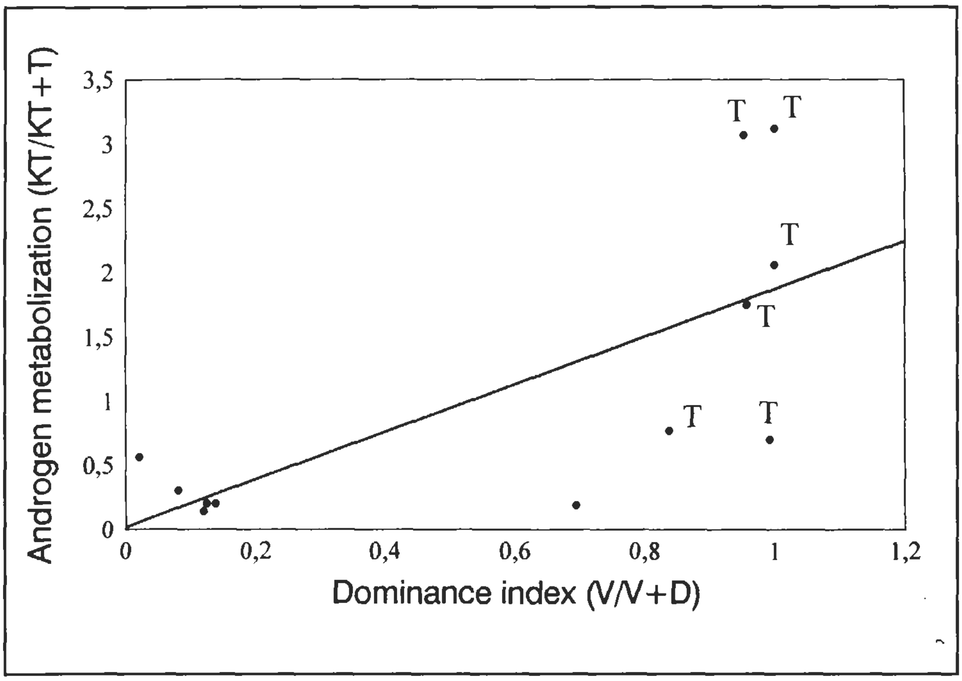

Figure 2. Influence of social status on the relative concentrations of 11-ketotestosterone to total androgen (11-ketotestosterone plus testosterone: KT/KT+T) in male *O. mossambicus*. T in the graph indicates territorial males.

consequences would have set the stage for the evolution of the observed male alternative mating tactics and male-male courtship.

The male mating tactics observed in captivity included establishing of a breeding territory, acting as a floater, or behaving as a sneaker. Territorial males dug nests, assumed a dark coloration, defended a territory centered in the nest and actively courted females. Floater males lived in the water column, displayed a light dark coloration and temporarily occupied territories for a short period (seconds to minutes) when the owners were absent, to court females. Sneaker males intruded into nests during a spawning episode and tried to remain near the female while simultaneously exhibiting quivering behavior, a behavior pattern usually related to sperm release (Oliveira and Almada, 1998b). These different mating tactics could be adopted by the same individual at different times and were clearly linked to male social status. Only dominant males established and defended territories. Acting as a floater was especially common in males of intermediate rank that had often attempted to establish their own territories without success. Sneaking was predominantly performed by males of low rank (i.e. subordinates or non-territorial males) (Oliveira and Almada, 1998b). However, even territorial males were observed to leave their territories, to intrude into the territories of their neighbors, when spawning was occurring, and engage in sneaking attempts. The majority of spawnings observed involved dominant males and were subjected to interference from other males (Oliveira and Almada, 1998b). In Tilapiine fishes, the occurrence of sneaking fertilization attempts has been described for *Oreochromis niloticus* (Heinrich, 1967), *Oreochromis alcalicus alcalicus*, and *Oreochromis alcalicus grahami* (Albrecht, 1968) and *O. mossambicus* (Turner, 1986; Oliveira and Almada, 1998b).

Males were found to court other males. The courted males frequently responded to these attempts by adopting a female-like behavior (Oliveira and Almada, 1998b). Territorial males were observed to court other males, presenting the full courtship repertoire found in this species: tilting, signaling the nest, circling and quivering. The courted males never had

Table 3. Spearman rank correlation coefficients (r_s) between urinary androgen levels and reproductive behaviors in male *O. mossambicus* (N=13) after the introduction of receptive females in the experimental tanks. To standardize female sexual receptiveness, they were injected with a peritoneal injection of 10µg LHRH analogue 48 hrs. prior to their introduction in the experimental groups. Significance levels are: p<0.01 (**) and p<0.001 (***). Number of interactions is the total number of male-male agonistic interactions in which a prticular male participated. Courtship frequency is the total number of courtship episodes per observation time directed at females by a particular male. Nest sixe dorresponds to nest volume considering that the nest had the approximate shape of an inverted cone. (Adpated from Oliveira et al., 1996).

	Number of Interactions	Courtship Frequency	Nest Size (volume)
Baseline testosterone level	-0.17	-0.01	-0.13
Testosterone level after group formation	0.47	0.79**	0.85***
Baseline 11-ketotestosterone level	-0.15	0.34	0.17
11-ketotestosterone level after group formation	0.37	0.70**	0.91***

the dark coloration, being usually in the neutral light coloration in which they school. These courted males performed the typical female sexual behavior, including immobility when courted, following the courting male to the nest and assuming a pivot position in the nest while the other male circled them. In three cases out of 204 observed, the courted males were observed to put their mouth close to the genital papillae of the courting male and when it was quivering they performed chewing movements which are typical of females inhaling sperm to ensure the fertilization of the eggs inside the mouth. In a total of 618 observed courtship episodes 33% were directed to other males, and the remaining 77% towards females. In contrast to heterosexual spawning, intermale pseudo-spawning acts (n=3) did not suffer intrusions from other males. Again, the social status (i.e. dominant vs. intermediate vs. subordinate rank) of the males involved seems to be a very important factor determining who courts and who receives the courtship. The occurrence of inter-male courtship has already been described for *O. mossambicus* by several authors (Baerends and Baerends von Roon, 1950; Neil, 1964; Pinheiro, 1980), although no attempts have been made to discuss its possible functional significance. A functional analysis of male-male courtship in this species should consider both the perspectives of the dominant courting male and that of the courted fish. From the courting male point of view, it is suggested that courtship attempts directed towards other males may be a by-product of a very high level of sexual motivation which would make them non-discriminative. In fact in a scenario of high levels of male-male competition, as described above, it may pay for territorial males to attract all individuals looking like potential mates (i.e. in a neutral light color pattern) postponing sex discrimination to a later stage of courtship when the courted individuals are already attracted to their nests. The fact that courtship attempts towards black individuals have never been reported supports this view. But why do courted males cooperate in these courtship attempts? The first obvious reason for female mimicry is to function as a tactic to approach spawning pairs, as described for other teleost species (see Taborsky, 1994 for a review). However, this explanation is unlikely since this male-male behavior was never observed when a heterosexual pair was spawning. Most likely, it could serve as a means of prolonging the presence of the courted males in the leks, reducing the level of attacks suffered from resident males. Additional possibilities could involve: 1) indirect sperm competition, whereby courted males would force the territorial males to spend sperm and energy with them, thus depleting their sperm reserves and shortening their permanence in the territories, or 2) a direct benefit to the courted male which, by eating sperm from the courting male, would receive from the sperm the nutrients required for its own sperm production. Further experimental work is needed to investigate which of these non-exclusive mechanisms may be acting.

SOCIAL STATUS, TERRITORY ACQUISITION AND ANDROGENS

Dominance relationships have been described for several lek-breeding cichlids and shown to affect the ability of the fish to secure a territory and in some cases also the quality, in terms of attractiveness to females, of these territories (Fishelson, 1983; Chan, 1987; Mckaye et al. 1990; McKaye, 1991; Falter and Foucart 1991).

The crucial events that determine the social status of an individual in a group occur very soon (within a few hours) after group formation as shown by Nelissen (1986) for the cichlid *Melanochromis auratus*. In *O. mossambicus* the establishment and stability of hierarchies has been described in detail (Oliveira and Almada, 1998c) and can be summarized as follows. Immediately after group formation (from a few seconds to a few minutes) some individuals begin to darken, and engage in symmetrical fights involving mainly circle fights, mouth fighting, and mutual displays. Symmetrical agonistic interactions gradually decrease, while asymmetrical high intensity interactions involving attack, chase and biting emerge. This phase usually spans the first day after group formation, after which the asymmetrical agonistic interactions decrease but remain at relatively high levels. The dominance structure that emerges in the first day after group formation is a good predictor of the dominance structure on subsequent days (Oliveira et al. 1996). Pendelling, a behavioral pattern typical of encounters between territorial males in which males rush at each other alternately moving backward and forward, becomes the main element of symmetrical interactions at this stage. It is also at this point that males begin to dig nests and defend territories centered on these nests, often adopting a nuptial black coloration. When females are added to the groups a marked decrease of agonistic interactions is observed with a concomitant increase in courtship behavior (Oliveira and Almada, 1998c).

The dominance hierarchies that are established are related to territoriality and, although they are linear (Landau h= 0.94 ± 0.06), they are not stable over time (Oliveira and Almada, 1996a, 1996b). Rank reversals are less likely to occur among dominant individuals as among subordinates and are more likely among hierarchical neighbors (Oliveira and Almada, 1996b).

One of the challenging aspects of behavioral endocrinology is to try to disentangle the causal relationship between hormones and behavior. One of the objectives of our research has been to investigate the interrelationship between androgens and social status in *O. mossambicus*. Are androgen baseline concentrations before group formation good predictors of the social status that individuals will achieve after group formation? Or does social status itself modulate the individual androgen levels after group formation? To answer these questions male *O. mossambicus* were placed in social isolation for 8 days to get baseline androgen levels for each individual before group formation (time 0). At the time of group formation and 5 days later urine was collected for androgen measurements. During this time males interacted with each other and established a social hierarchy. Behavioral observations were conducted on a daily basis to allow the calculation of social ranks for each day of the experiment. According to our hypothesis we would predict that if androgens are good predictors of social status, then the baseline androgen levels measured at time 0 will be significantly correlated with the social rank achieved by the individuals. Conversely, if androgen levels were being modulated by social interactions, then we would not expect the above mentioned correlation and would predict an association between androgen levels measured after group formation (day 5) and social status. The results summarized in Table 2 support the latter

Table 4. Urinary androgen levels (ng/mL) in territorial and non-territorial male *O. mossambicus* according to social context.

	Social Isolation	All Male Groups	Mixed Sex Groups
Testosterone			
Territorial	48.0 ± 33.4	74.4 ± 9.2	45.8 ± 8.2
Non-territorial	25.6 ± 13.8	52.2 ± 0.9	16.9 ± 4.4
11-Ketotestosterone			
Territorial	48.8 ± 23.2	133.7 ± 28.2	85.0 ± 28.1
Non-territorial	18.7 ± 3.6	13.5 ± 2.9	36.9 ± 13.4

hypothesis according to which baseline androgen levels are poor predictors of social status. Rather, social status modulates circulating levels of androgens, in particular 11-ketotestosterone (Oliveira et al., 1996).

Androgen levels measured in the urine were also correlated with male courtship behavior and with nest size (i.e. nest volume; Oliveira et al., 1996; see Table 3). Since individuals that were successful in establishing breeding territories were the dominant ones (i.e. territorial individuals had higher dominance indexes than non-territorial, Oliveira, 1995) androgen levels in territorial and non-territorial males were analyzed in different social contexts. In Table 4 the data on urinary testosterone and 11-ketotestosterone levels is given for the two male classes in the different phases of the study. At the end of the social isolation period the concentrations for both androgens did not differ significantly between the individuals that became territorial and those that did not establish a territory after group formation. After group formation testosterone in the urine increased and a significant difference in hormone concentration was detected between territorial and non-territorial males. 11-ketotestosterone levels increased dramatically in territorial males during this phase while non-territorial individuals experienced a slight decrease, resulting in a significant difference between the two groups. After the introduction of receptive females into the male groups a decrease in both androgens was observed while there was an increase in 11-ketotestosterone together with a decrease in testosterone in non-territorials. Thus, at this stage the differences between territorial and non-territorial individuals were significant only for testosterone.

These data are suggestive that one of the possible physiological consequences of subordinance is the blockage of 11-ketotestosterone production (possibly through 11ß-hydroxylase inhibition) and an accumulation of the precursor testosterone. Studies on steroid metabolism in Siamese fighting fish (*Betta splendens*) further support this view, where subordinate individuals have lower expression of secondary sex characters and male display behavior, both aggressive and sexual, and the activity of 11β-hydroxylase is blocked (Leitz, 1987). This is consistent with different roles for the two androgens. Testosterone may be involved in keeping the fish in a state of behavioral readiness which will allow the individual to shift to aggressive behavior if the context is favorable, and 11-ketotestosterone may be more directly involved in gamete development and in the expression of male displaying characters both morphological and behavioral. The increase of testosterone levels in non-territorial fish can also be explained by the stimulation of the social environment to which they were exposed (e.g. territorial disputes) which they observed but did not participate directly.

To test this possibility another experiment was conducted in which one male was exposed to two neighboring conspecifics through a one-way mirror. The two conspecifics were separated by an opaque partition. After a period of acclimation the opaque partition was removed in one treatment (experimental), allowing the fish to interact for one hour, and was kept in place in another treatment (control). Thus test fish were exposed to two interacting neighbors in the experimental treatment and to two resting neighbors in the control treatment. The one-way mirror prevented the neighbors from interacting with the test fish. The relative increase in androgens levels measured from the urine of test fish exposed to fighting conspecifics showed that they respond to the social environment even when they are prevented from getting involved in the interaction (unpublished data).

The androgen response to the social environment is not exclusive to agonistic interactions. Isolated males that receive a receptive female in their tanks for an hour show a short-term increase in their urinary androgen levels after intensively courting the female (Borges et al., 1998). Interestingly, 11-ketotestosterone and testosterone responses are out of phase. 11-ketotestosterone peaks 6 hours after the interaction and testosterone peaks 24 hours later. This again suggests a differential role for these two androgens, as mentioned above.

In conclusion, results from *O. mossambicus* clearly show that in the short-term the social environment modulates androgen levels, mainly 11-ketotestosterone. As androgens are implicated in the activation of reproductive behaviors and on the expression of male secondary sex characters in cichlids (see section 1), the social environment (e.g. status) may also affect the expression of these characters.

ANDROGENIZATION OF DOMINANT MALES: ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

O. mossambicus has 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 males (Oliveira and Almada, 1995). These two sets of sexually dimorphic characters seem to be involved in male-male competition, namely the median fins in lateral displays and the jaw in mouthfighting (Oliveira and Almada 1995).

To test the hypothesis of an effect of social status on androgen-dependent traits, males of different social status were analyzed for the expression of both male displaying behaviors and morphological secondary sex characters. Dominant fish showed highest expression of morphological (dorsal fin height, anal fin height, mandible width and premaxilla length) and behavioral (nuptial coloration, % time defending a territory, spawning pit volume, pit hovering and courtship rate) traits than subordinate fish (Oliveira and Almada, 1998a). In this study social status was also positively correlated to development of the genital papillae, considered to be a good predictor of androgen levels in cichlid fishes (Schwanck, 1980; Oliveira and Almada, 1998a). Although males of different social status did not differ in the degree of gonadal maturation they did differ however in their relative gonad size (expressed as gonadosomatic index), with dominant males having relatively larger testis (Oliveira and Almada, 1998a).

These results support a causal model according to which androgen levels are modulated by social status, which in turn modulate the expression of behavioral and morphological male traits (see Figure 1; Oliveira and Almada, 1998a). The different steps of the model were supported by path analysis to infer the most probable causal relationships between each pair of variables involved in the model (Oliveira and Almada, 1998a). The first step in the model was further supported by the observation that while castration of males from *Astatotilapia burtoni*, another mouthbrooding cichlid, decreased their aggressive behavior, it had no effect on their social status (Francis et al., 1992). 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 involving androgens as modulators of both morphological secondary sex traits and of behavioral display characters (i.e. nest size, dark coloration, and courting) is also supported by a large body of literature implicating androgens in the control of both morphological secondary sex traits and of behavioral displaying characters in teleosts (see introduction for references). The operation of this model has several consequences at the evolutionary and the ecological levels of behavioral analysis.

In terms of communication theory, the model suggests a constraint imposed by androgens on bluffing, since there is a regulation of the expression of both morphological and behavioral sex characters, according to the individual's resource holding potential.

This would support the evolution of honest signaling in *O. mossambicus* males. In fact in a system in which male-male agonistic interactions are frequent, as is the case of lek-breeding cichlids, males displaying characters that do not correspond to their resource holding potential may incur heavy costs by provoking agonistic confrontations that they will not be able to win.

The model also suggests an amplifier effect of androgens on dominance, since dominant males, by signaling their status both morphologically and behaviorally, may reinforce their social status by a positive feedback mechanism (dash arrow on Fig. 1). Thus, small initial differences in resource holding potential may result in increasingly larger differences in status.

This model further suggests that genetic models of sexual selection (see Andersson, 1994 for a review) should take into consideration that androgen dependent ornaments have a strong environmental component which cannot be ignored when looking for indirect benefits for female mate choice. This implication can be viewed as a major point to address when looking for female indirect benefits of female choice since most male vertebrate ornaments are androgen dependent characters.

Finally, if androgens indeed 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, it is well documented that the social status of a fish may affect a number of life history traits such as age/size at maturation (e.g. *Xiphophorus variatus*, Borowsky, 1973, 1978; *A. burtoni*, Fraley and Fernald, 1982), the adoption of alternative mating tactics (see Taborsky, 1994 for a review), or sex change (e.g. *Thalassoma bifasciatum*, Warner and Swearer, 1991; *Trimma okinawae*, Sunobe and Nakazono, 1993; see Shapiro, 1979 and Grober, 1998 for general reviews). All these effects can be viewed as compensatory responses so that subordinate fishes minimize the disadvantages of their status by adopting the life history pattern that makes the best of their bad situation.

BRAIN AROMATASE ACTIVITY AND THE EFFECTS OF ANDROGENS UPON BEHAVIOUR

In higher vertebrates the causal role of androgens upon behavior is in some aspects mediated by their conversion into estrogens at the target tissues (i.e. brain areas). This conversion is promoted by the aromatase enzymatic complex. A paradigmatic example of the involvement of aromatase on the expression of male behaviors is the well documented case of male copulation in quail (Balthazart and Foidart, 1993).

The function of aromatase may rely on it becoming the limiting step for the occurrence or magnitude of a neural response by regulating *in situ* the amount of estrogens available to bind to receptors. This mechanism would explain the inter-sexual, seasonal and individual variations of neural responses to circulating androgen levels (Callard et al., 1990). It should be noted at this point that brain aromatase activity has been detected in all vertebrate species tested so far including elasmobranchs, teleosts, amphibians, reptiles, birds and mammals.

In teleosts, brain aromatase activity is significantly higher than that measured in birds or mammals in homologous brain structures (*e.g.* mammals: 1-5 fmol/ mg tissue/ h vs. teleosts: 50-200 fmol/ mg tissue/ h; Callard et al., 1981). The aromatase activity in teleosts is usually related to sex and to the sexual maturation stage of the individual. In seasonal breeding species aromatase activity peaks during the breeding season when circulating levels of sex steroids are also high, and it drops to baseline levels out of the breeding season when steroids also drop to baseline levels (e.g. *Gasterosteus aculeatus*, Borg et al., 1987; *Carassius auratus*, Pasmanik and Callard, 1988a). In the teleost species studied so far females present higher levels of brain aromatase activity than males (*Gasterosteus aculeatus*, Borg et al., 1987; *Myoxocephalus octadecimspinsus*, Callard et al., 1981; *Carassius auratus*, Pasmanik and Callard, 1985; *Opsanus tau*, Pasmanik and Callard, 1985), possibly related to the fact that in most of these species testosterone levels are similar in both sexes or even higher in females. One possibility is that aromatase activity may be blocking the expression of male reproductive behaviors in females. However, 11-ketotestosterone, which is non-aromatizable and present in most male teleosts (Kime, 1993), is more

Table 5. Aromatase activity (fmol/mg of protein/h) in different brain regins of male *O. mossambicus*.

Brain Region	Aromatase Activity
Olfactory bulb	0.24
Telencephalon	105.5
Thalamus	128.5
Cerebellum/medulla oblongata	27.5
Hypothalamus	71.4
Pituitary	14.9

effective than testosterone on the expression of male sexual behavior and male secondary sex characters (Borg, 1994). This suggests a minor role for aromatase on the activation of male behaviors in teleosts.

To assess the involvement of brain aromatase activity on the activation of male social behaviour in *O. mossambicus*, activity levels were measured in brains from males of different social status. Since there was no previous information on aromatase activity in this species, other than a report on pituitary levels (Callard et al., 1988), it was decided also to investigate the activity of brain aromatase regarding sex differences, relationship with sexual maturation and gross neuroanatomical localization.

Aromatase activity was assayed radiometrically according to the method first described by Thompson and Siiteri (1974). Three brains were homogenised in 5 mL of trout balanced salt solution (TBSS, Jalabert and Fostier, 1984). A 200 μL aliquot of brain homogenate was added to Costar tissue culture wells containing 1μCi [1μ-^{3}H] androst-4-ene-3,17-dione and 1 mmol NADPH in 800 μL TBSS. Incubations were stopped by freezing 200 μL aliquots in liquid nitrogen. Radiolabeled steroids were removed by the addition of 1 mL dextran-activated charcoal and centrifuging at 4°C. The radioactivity present in the supernatant as tritiated water provided the measure of aromatase activity. Blanks were obtained with incubations in the absence of homogenates. Protein content in homogenate samples was determined by the method of Lowry (Lowry et al., 1951). Homogenates of pooled brains and ovarian fragments from a vitellogenic female showed high levels of aromatase activity, which could be almost completely abolished by the aromatase inhibitor 4-hydroxyandrost-4-ene-3,17-dione and by unlabelled androstenedione. These results confirmed that we were measuring aromatase activity.

To compare aromatase activity of different brain regions, the encephalon was dissected into its major macroscopically recognizable parts: olfactory bulb, telencephalon/optic lobes, thalamus, cerebellum/medulla oblongata, hypothalamus and pituitary gland. Homogenates of pooled samples from three mature males for each of the aforementioned regions were incubated for 2 hours. The results presented in Table 5 show that aromatase activity in tilapia brain is higher in the thalamus, followed by the telencephalon and the hypothalamus.

To look for sex differences in brain aromatase activity, whole brain homogenates of males and females at several maturational stages were incubated for one hour as described above. Substantially higher aromatase activity was found in males (n=11, mean ± S.E.M.= 341 ± 43.8 fmol / mg of protein / h) as compared to females (n=7, mean ± S.E.M.= 124.9 ± 42.5 fmol /mg of protein / h; ANOVA one-way: $F_{1,16}=11.2$, $p<0.005$).

No correlation was found between aromatase activity and maturational stage in females ($r = 0.15$, $n=8$, $p>0.10$) but this is not conclusive owing to the small sample size. However, a non-significant negative relationship was found in males between gonadosomatic index and aromatase activity ($r = -0.54$, $n= 10$, $p<0.10$), suggesting a potential role of aromatase in the mediation of the feedback loop of gonadal steroids to the brain.

To investigate the relationship between male social status and aromatase activity two parameters were used: 1) a correlation between a dominance index [V/(V+D), in which V is the number of victories and D is the number of defeats (Barlow and Ballin, 1976)] and aromatase activity and 2) the difference in aromatase activity between dominant and subordinate individuals. A correlation between aromatase activity and the total number of interactions in which an individual was involved was also determined to assess potential effects of social stress. None of the correlations were statistically significant: (a) aromatase activity vs. V/V+D: $r_s = 0.103$, $n=10$, $p>0.10$; (b) aromatase activity vs. number of interactions: $r_s = -0.134$, $n=10$, $p>0.10$. Furthermore, no differences in aromatase activity were found between dominant and subordinate individuals (Mann-Whitney U test: $n_{dominants} = 5$, $n_{subordinates} = 5$, $U=10$, $z=-0.52$, $p>0.10$). These results suggest that the activity of aromatase is not under social control in this species.

Since 11-ketotestosterone is the most potent androgen in a large number of teleost fishes, and because it is a non-aromatizable androgen, it is conceivable that aromatase may not be playing an important role on the integration of social stimuli on the expression of social behaviors in teleosts. As testosterone is in the biosynthetic pathway of 11-ketotestosterone, it was decided to investigate the relationship between the relative concentrations of these two steroids and social status. We have used the relative concentration of 11-ketotestosterone to testosterone [11-ketotestosterone / (11-ketotestosterone + testosterone)] as an index of the degree of metabolic conversion of testosterone into 11-ketotestosterone. Interestingly, there was a positive correlation between this index and social status ($n= 13$, $r_s = 0.76$, $p<0.01$; see Figure 2). This result supports the hypothesis presented above (section 3) according to which a raise in social status promotes the conversion of testosterone into 11-ketotestosterone by the activation of 11ß-hydroxylase. Territorial individuals also present higher relative 11-ketotestosterone concentrations than non-territorial (Mann-Whitney U test: $U= 0$, $z= -3$, $p= 0.003$; Fig. 2), with relative levels of 11-ketotestosterone never below 40%, while non-territorial males have always less than 40% (Fig. 2).

It is interesting to note in Fig. 2 a non-territorial individual that has a high dominance index. This individual despite being successful in the interactions in which it participated (approximately 70 % of victories) failed to establish a territory. Despite his high rank in the social hierarchy he presents a low relative concentration of 11-ketotestosterone, which may indicate that the success in establishing a territory, not the social status itself, is the key factor to trigger 11-ketotestosterone production in this species. This may be related to a stimulation of the neuroendocrine system and the testis, in which 11-ketotestosterone has a very well established role (Miura et al 1996) in preparation for spawning resulting from territory acquisition.

FEMALE AGGRESSION AND ANDROGENS

Androgens have also been implied in the expression of agonistic behaviors in female cichlids . Munro and Pitcher (1985), have tested the effects of different sex steroids added to the water (500 mg steroid/L) on the agonistic behavior of female *Aequidens pulcher* in a non-breeding context (i.e. mirror elicited aggression with isolated individuals and social status in an all female captive group).

Testosterone promoted the expression of agonistic behaviors in both set-ups while estradiol inhibited them. These results may reflect the hormonal modulation of behavior during female sexual cycle.

The temporal variation of sex steroids during the mouthbrooding cycle has been described for *O. mossambicus* (Smith and Haley, 1988). Plasma testosterone levels have two peaks during the sexual cycle: (a) the first at the end of the ovarian cycle, 3 to 5 days before spawning (mean testosterone plasma concentration = 17 ng/mL); (b) the second at day 15 of mouthbrooding (mean testosterone plasma concentration = 19 ng/mL), with high levels of testosterone being kept until day 25 when a marked drop to baseline levels is observed (mean testosterone plasma concentration = 3 ng/mL). It is interesting to note that testosterone levels increase in phases of higher social activity: (a) just before spawning, when receptive females are actively being courted by males and are also exposed to agonistic interactions resulting from male-male competition; (b) at the final stage of mouthbrooding, when females become more aggressive (Oliveira and Almada, 1998d), defending a mobile territory in which the fry can remain safely, returning to the maternal mouth only in cases of perceived danger (Fryer and Iles, 1972). It is interesting to note that females with a low social rank in their groups during the final stage of mouthbrooding, i.e. when they are incubating free-swimming fry without yolk sac, may experience a substantial rise in social hierarchy during those phases of high testosterone levels, managing in some cases to become the dominant individual (Oliveira and Almada, 1998d).

Although females generally lack 11-ketotestosterone, including *O. mossambicus* (unpublished data) the temporal association between testosterone and female aggression in the final phase of mouthbrooding suggests that androgens may play a major role on the expression of female social agonistic behaviors. On the other hand, the occurrence of high testosterone concentrations in the pre-spawning period would suggest a potential social modulation of androgen levels operating also in females.

The association between female aggression and androgens has also been identified in other non-cichlid species, as in the case of sex-changing protogynous species in which female-male sex change is preceded by a period of elevated aggression, immediately followed by increased levels of androgens in the sex-changing individual (see Grober 1998). Thus, a two way relationship between androgens and social status may also be acting in female teleosts.

PROSPECTS FOR FUTURE RESEARCH: CICHLID FISH AS A MODEL FOR A COMPARATIVE STUDY OF THE SOCIAL MODULATION OF ANDROGEN LEVELS

We have presented evidence, using *O. mossambicus* as a study model, that the relationship between androgens and social behavior is a two-way interaction. In addition to their classic role in the control of male reproductive function, androgen levels and metabolism can also be affected by social interactions among conspecifics. This has been interpreted as an adaptation of individuals to adjust their agonistic motivation to changes in their social environment Thus, male-male interactions stimulate the production of androgens and androgen levels are a function of the stability of the social environment in which the animal is placed - the "challenge hypothesis" (Wingfield, 1984). According to this hypothesis, androgen patterns during the breeding season are predicted to vary between species according to the amount of social interactions to which individuals are exposed. In monogamous species with high levels of parental care androgen levels should increase above the breeding baseline only when males are challenged by other males or by mating. At other times, androgens should remain at the breeding baseline so that they would not interfere with paternal care. Conversely, androgen levels in polygynous males should be near physiological maximum throughout the breeding season due to high levels of male-male competition in this type of breeding systems.

The challenge hypothesis generates a number of predictions, among which, that the breeding system of a given species influences endocrine responsiveness. Polygynous males should have higher levels of androgens for longer periods during the breeding season than monogamous males (i.e. males from monogamous or polyandrous species). This would reflect the fact that polygynous males do not participate in parental care to offspring and compete for access to mates during a longer period of time (Wingfield et al., 1990). In the only comparative studied published so far to test this hypothesis 20 species of Passeriformes, for which the breeding system and the androgen levels were known, were surveyed. Males of polyandrous and monogamous species show a higher endocrine responsiveness (difference between breeding baseline and maximum response) to social interactions than males of polygynous species. This relationship between breeding systems, patterns of parental care and androgen levels is strengthened by the fact that implanted males of a monogamous species became polygynic deserting their mates (Wingfield, 1984).

Cichlid fishes are an excellent model to further test this hypothesis since a large variation in breeding strategies and degrees of male parental investment are present within the family. The results of such comparative survey would potentially allow a further assessment of Wingfield's challenge hypothesis across different vertebrate classes and test it as an emerging general principle in vertebrate behavioral endocrinology.

ACKNOWLEDGEMENTS

The preparation of this paper was supported by PRAXIS XXI and FEDER (Praxis/2/2.1/BIA/370/94). The authors thank Vitor Almada, Rita Borges, Marco Lopes and Luís Carneiro, who contributed to the original studies and Ron Coleman for reviewing the manuscript.

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