Androgens and Mating Systems in Fish: Intra- and Inter-specific Analysis

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Summary

One of the predictions of the 'challenge hypothesis' (Wingfield et al., 1990) is that androgen patterns during the breeding season should vary among species according to the parenting and mating system. Here we assess this prediction of the challenge hypothesis both at the intra- and at the inter-specific level. To test the hypothesis at the inter-specific level, a literature survey on published androgen patterns from teleost fish with different mating systems was carried out. The results confirm the predicted effect of mating system on androgen levels. To test the hypothesis at an intra-specific level, a species with flexible reproductive strategies (i.e. monogamy vs. polygyny), the Saint Peter's fish was studied. Polygynous males had higher 11-ketotestosterone levels. However, males implanted with methyl-testosterone did not became polygynous and the variation of the tendency to desert their pair mates was better explained by the reproductive state of the female partner. This result stresses the point that the effects of behaviour on hormones cannot be considered without respect to the social context.

Introduction

In the last two decades evidence has been accumulated showing that behaviour, in particular social experience, may feed back and affect androgen levels (Harding, 1981; Bernstein et al., 1982; Wingfield et al., 1990; Oliveira et al., 2001). This evidence suggest a two-way type of interaction between androgens and behaviour, which have been interpreted as an adaptation for the individuals to adjust their agonistic motivation to the social environment (Wingfield et al., 1990; Oliveira et al., 2001). Thus, while androgens have a permissive effect on agonistic/sexual motivation, male-male interactions also stimulate the production of androgens, and the levels of androgens are a function of the stability of the social environment in which the animal is placed ("challenge hypothesis", Wingfield et al., 1990). One of the predictions of this hypothesis is that androgen patterns during the breeding season should vary among species according to the mating system. For example, in monogamous species with high degree of parental care androgen levels should increase above the breeding baseline only when males are challenged by other males or by mating. Therefore, the two-way relationship between hormones and behaviour mentioned above may apply to the interaction between androgens and male mating strategy. Consequently, polygynous males, that face higher levels of social challenges owing to higher male-male competition regimes, are expected to have higher breeding levels of androgens than males of monogamous species. However, increased androgen levels are also expected to influence male mating strategy by promoting polygyny (Beletsky et al., 1995). This paper analyses the interaction between androgens and male mating strategies both at the inter- and intra-specific levels.

Inter-specific Analysis

In the seminal paper by Wingfield and co-workers (1990) in which the "challenge hypothesis" was formulated, it was proposed that in seasonal breeders androgen levels should increase from a non-breeding baseline (level A) to a breeding baseline (level B) throughout the breeding season. As this breeding baseline level is sufficient for the full expression of male sexual characters and behaviour and for the maturation of the gonads. Thus, any further increase of androgen levels above the breeding baseline level and towards a maximum physiological limit (level C) could not be explained in terms of a

direct advantage in reproductive output. Wingfield et al. (1990) proposed that the variation of androgen levels above the breeding baseline would be due to the androgen responsiveness to the social environment (e.g. male-male interactions). At the inter-specific level this hypothesis predicts that males of polygynous species should have higher breeding baseline levels, close to their physiological maximum than males from monogamous species and, therefore, further androgen increases in response to social stimuli would be smaller. Wingfield et al. (1990) reviewed the available androgen responsiveness data from 20 bird species), expressed as the relative ratio (C-A) / (B-A), and concluded that males from monogamous and polyandrous species had larger androgen responses to social stimuli than males of polygynous species. Recently, this conclusion has been

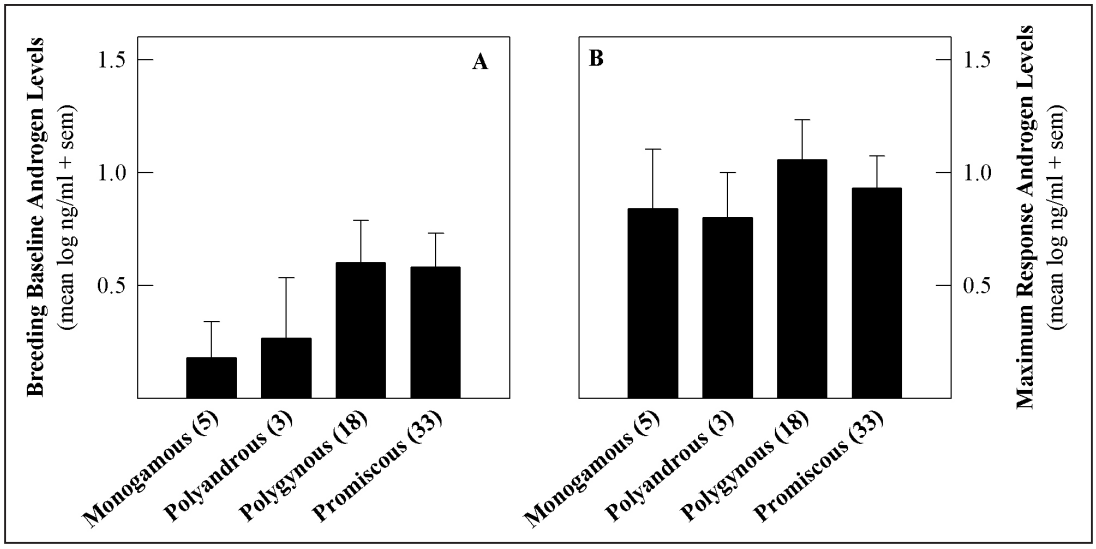

Fig. 1. Mean androgen levels (± standard errors) throughout the breeding season (A) and maximum physiological response levels (B) as determined from a literature survey on 59 teleost species. Reports of specific territorial intruder experiments as well as of seasonal androgen patterns were used. The 'breeding baseline' level B was measured as the average androgen level throughout the breeding season, including parenting phases. The 'maximum response' level C was taken from specific response measures to territorial challenges, when available, or as the peak observed during mating/spawning. When the needed data were not given in text or tables, published figures were scanned and the data were extrapolated using the UnGraph™ software package (Biosoft 1998). Numbers in parentheses represent numbers of species included per mating system category. The database is dominated by promiscuous and polygynous species which reflects the distribution of the availability of mating systems among fish (Breder & Rosen, 1966).For details about the references of the species included, please contact the authors (Katarina@ispa.pt).

confirmed with a broader database (N=83 bird species) and controlling for a phylogenetic bias with comparative methods (Hirschenhauser, Winkler, & Oliveira, unpubl. data). The 'challenge hypothesis' has also been tested among teleost species and the predicted variation of androgen responsiveness rates due to paternal care and mating system were again confirmed (Oliveira et al., in press). However, the question remained as to whether the variation of the androgen responsiveness to social stimuli in relation to the mating system is a result of varying breeding baseline levels (B) according to the mating system as proposed by Wingfield et al. (1990), or a consequence of different maximum physiological levels (C) depending on the mating strategy. To disentangle these two possibilities, we plotted the mean and standard error of levels B and C for species with different mating systems (Fig. 1). The results show that indeed, the variation of androgen responsiveness among the different mating strategies is mostly related to lower breeding baseline levels among monogamous and polyandrous male teleosts rather than to a difference in maximum physiological levels among fish with different mating strategies.

Intra-specific Analysis

Males of the Saint Peter's fish (*Sarotherodon galilaeus*, Cichlidae) display a large variation in reproductive strategies (Schwanck & Rana, 1991; Balshine-Earn, 1997) making them a good model for the study of the relationships between androgen levels, mating systems and breeding strategies within a single species. In this species males may defend territories or not, may participate in parental care by mouth-brooding part of a clutch or simply may not incubate eggs, and they may pair consecutively with the same female (monogamy) or otherwise desert the female and look for another female (polygyny).

To investigate the association between androgens and male mating strategy we have conducted two experiments. In a first experiment we tested if males that were more attached to their pair females would have lower androgen levels. To test the partner preference of males a "monogamometer" was designed (Fig. 2A). In brief, paired males were offered the access to a novel female and the time spent with each female was measured. At the end of the behavioural test

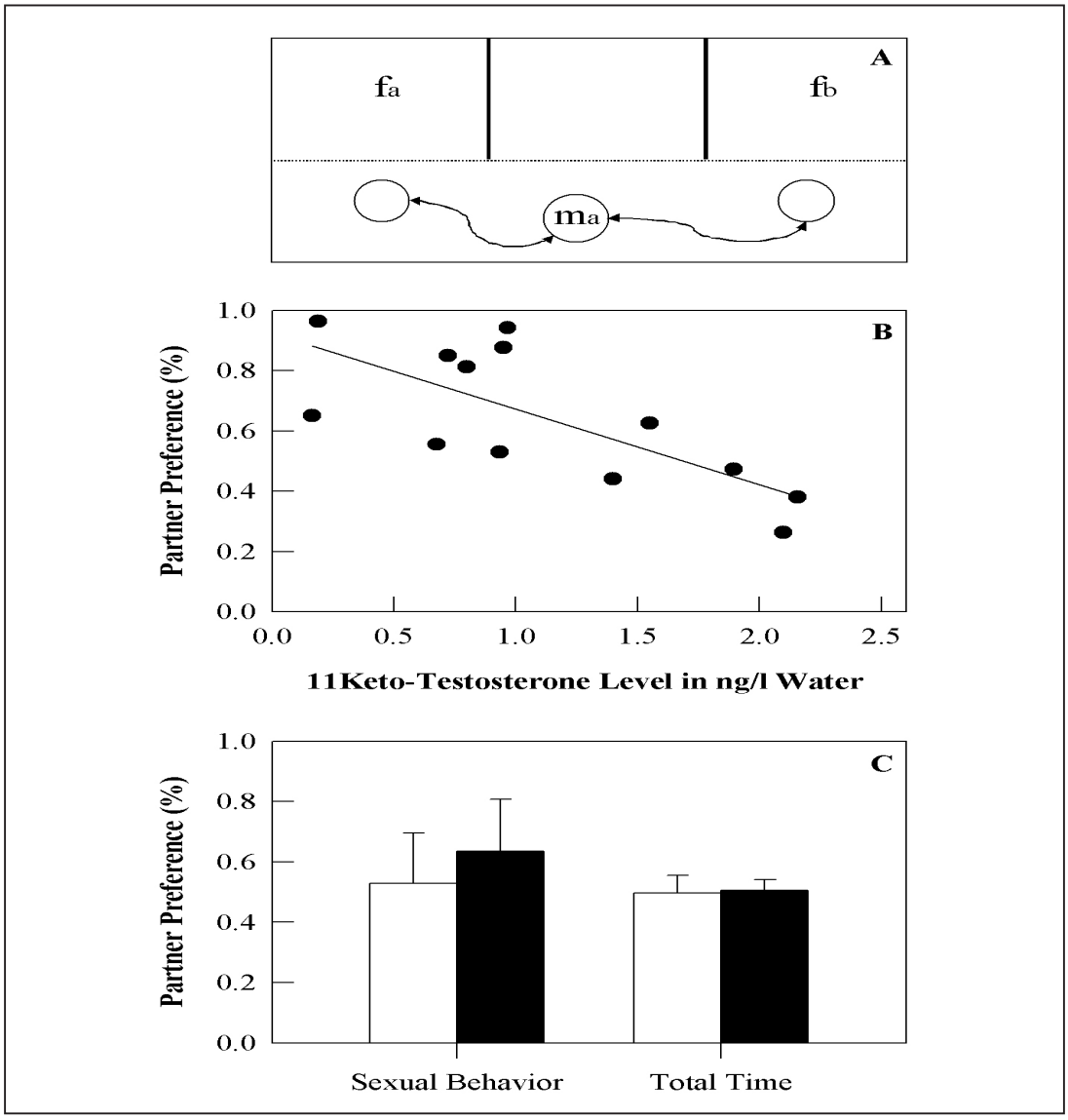

Fig. 2. Effect of androgens on mating strategies in male St. Peter's fish. (A) Experimental set-up for testing the variation in partner attachment (i.e. monogamy): f = female, m = male. The compartment with the male is at the front of the aquarium. Opaque screens separated females. A transparent screen separated the male from the females. The middle compartment was empty. In this case the female in the left compartment is the male partner and the female in the right compartment was novel. Position of the partner was randomised. Behaviour of the male was videotaped and analysed in relation to the position and behaviour of the male. (B) Effect of 1-week treatment with methyl- testosterone on choice for own partner versus novel female (expressed as percentage of time spent with the partner) during a 1-hour partner preference test. (C) Correlation between gonad weight of own partner and sexual behaviour displayed during a partner preference test. Sexual behaviour was defined as the total of quiver display, digging, skimming and spawning.

the males were placed isolated in the dark for 2 hours in 1l of water in a glass container. Afterwards the male-holding water was extracted for steroids (for the extraction protocol see Oliveira et al., 1999) and the levels of 11-ketotestosterone (KT) were measured using radio-immunoassay (described by Kime & Manning, 1982). There was a significant negative correlation between KT and partner preference (N = 13, R = - 0.65, P = 0.02), suggesting that the more polygynous males had higher androgen levels (Fig. 2B). In a second experiment we tested the causal link between androgens and mating strategies by implanting malesintraperitoneally with silastic implants filled with methyl-testosterone (MT) or with the vehicle alone (castor oil, control grup). Since we had found in the first experiment that animals with higher levels of KT had a higher propensity to become polygynous, it was predicted that MT-treated males would have a lower partner preference than controls (measured as in exp.1). Fish were allowed to pair spontaneously in stock aquaria, the pairs were then removed and isolated for half a week. Each male in a pair was assigned at random to either the control or the MT-treated group. A week later they were tested to allow time for fish to recover from surgery. After the behavioural test males and females were sacrificed and morphological measurements were taken, including the length of the genital papillae as a bioassay for androgen levels in male cichlids (Oliveira & Almada, 1998) and treatment effectiveness, as well as the weight of the gonads. In the MT-treated group the papillae increased in size (Mann-Whithey U-test: U=6, N_{MT} = 7, N_C = 7, $p < 0.05$), confirming the release of MT from the implants. In total 14 pairs were tested. To measure the effectiveness of the treatment the length of the genital papillae was measured, as a bioassay for androgen levels in the plasma since it is considered to be a good predictor of androgen levels in cichlid fish (Oliveira & Almada, 1998). In the MT-treated group the papillae increased in size (Mann-Whithey U-test: U=6, N_{MT} = 7, N_C = 7, $p < 0.05$), confirming the release of MT from the implants. Controls did not show the expected preference for their own partner. MT did not affect this partner preference significantly (Fig. 2C, Mann-Whitney U-tests: sexual behaviour score, U=7.5, N_{MT} = 5, N_C = 3, N.S.; preference score, U=20,, N_{MT} = 7, N_C = 7, N.S.). Additionally, we tested wether the variation in female partner quality might be a factor influencing the males' decision to become polygynous. Indeed males showed a large variation in sexual behav-

iour during the partner preference test that was correlated with the weight of the gonads of their partners (correlation between sexual behaviour and female partner gonad weight, N = 10: sex to partner, R=0.30, N.S.; sex to novel female, R=0.81, $p<0.01$; total sexual behaviour, R=0.74, $p<0.01$). This result suggests that males paired with more mature partners are more sexually motivated and thus, respond more to the novel female. The variation of male sexual behaviour was not explained by the sexual maturity of the males since no correlation was found between sexual behaviour and testis weight (R_s = 0.13, N = 11, N.S.). Furthermore, no correlation was found between testes weight and ovaries weight between partners (R_s = 0.13, N = 13, N.S.).

The results from the two experiments taken together suggest that the association between partner preference and androgen levels is not due to a causal effect of androgens on partner preference. As the androgen levels were measured at the end of the experiment it can be argued that they probably reflect the variation in partner preference behaviour observed among males, rather than being its cause. The fact that MT had no effect on male mating strategy results is also apparently in contradiction with the reported effects of T on mating strategy in wild male birds (Beletsky et al., 1995). One possible explanation for this difference may be the fact that St. Peter's fish males are sequentially polygynous, while in other tested species T-treatments increases home range/territory size and as a consequence the number of females present in the area used by the males is larger, thus, promoting simultaneous polygyny.

Conclusions

The results presented here show that at the inter-specific level monogamous/polyandrous male teleosts had lower breeding baseline levels than polygynous/promiscuous ones, which might reflect the different social environments that these different mating systems convey. At the intra-specific level, it was shown that males with weaker partner preference higher KT levels. However, males implanted with MT did not behave in a more polygynous fashion when a novel female is available, and the variation of the tendency to desert their pair mates was better explained by the sexual state of the female partner. Thus, the results from both levels of analyses reveal an

association between mating system /strategy and androgen levels, which may be due to a feedback of social context on the endocrine system.

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