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ORIGINAL ARTICLE

Social stimulation, nuptial colouration, androgens and immunocompetence in a sexual dimorphic cichlid fish

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Abstract The nature of the costs maintaining honesty of sexual signalling in inter- and intrasexual interactions remains a contentious issue. For carotenoid-based colour ornaments, it has been hypothesized that the honesty of the signal is enforced when carotenoid allocation to colour expression is traded off against carotenoid availability for immunocompetence. In addition, honesty is enforced if androgens required for colour expression are immunosuppressive. We tested whether there is a trade-off between colour expression and immunocompetence in a lek-breeding haplochromine cichlid fish, *Pundamilia nyererei*, from Lake Victoria with a carotenoid-based nuptial dress. First, we showed that expression of red nuptial colouration and social rank in a group were positively correlated. We then successfully manipulated the level of colour advertisement by socially stimulating individually housed males with a rival male: Stimulated males developed larger areas of red nuptial colouration and had higher levels of circulating 11-ketotestosterone (11-KT) than non-stimulated males. We subsequently tested the humoral immune response to a novel antigen (sheep red blood cells). There was no overall significant effect of social stimulation on antibody production, but in the stimulated males, the degree of red colouration and the antibody response were negatively correlated. This significant interaction between colour and treatment thus shows that the negative correlation between colouration and antibody production is dependent on the (manipulated) social environment. This study provides correlational and experimental evidence for a trade-off between expression of a sexual trait and one component of immune function in fish. We found no evidence for a direct effect of 11-KT on antibody production, and we discuss alternative mechanisms that could mediate the trade-off.

Keywords Immunocompetence · Sexual selection · Male–male competition · Cichlid fish · Lake Victoria

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Introduction

In many animals, males advertise extravagant characteristics that are used as cues in both female mate choice and male–male competition (Zahavi 1975; Andersson 1994). In female mate choice, females are expected to prefer males with traits that are honest indicators of quality that are passed on to their offspring (Hamilton and Zuk 1982; Johnstone 1995). Such traits may also signal social status and resource-holding potential in intrasexual communication (Parker 1974), indicating dominance and/or fighting ability, thereby preventing or reducing the costs of combats with a predictable outcome (Rohwer 1975; Maynard Smith and Harper 1988; Berglund et al. 1996). For example, a male can increase colour expression to indicate superior quality, thereby deterring rivals. Alternatively, a male can reduce the level of signalling to avoid conflict with more powerful opponents (Candolin 1999). If signalling is costly due to male–male competition and the costs decrease with an increase in the quality of the male, frequent testing through male–male competition could ensure the honesty of status-signalling (Rohwer 1975; Maynard Smith and Harper 1988). However, theory suggests that social control is not enough to maintain honest signalling and that contest-independent costs are needed to prevent the occurrence of cheating in a population (Johnstone and Norris 1993).

It has been suggested that honesty of signalling male quality is ensured if sexual signalling is compromised by immunocompetence (Hamilton and Zuk 1982). This trade-off between signalling and immune function has been extensively investigated in the context of female mate choice for high-quality males (i.e. Roberts et al. 2004), but it may also be relevant in ensuring honesty in intrasexual signalling (Johnstone and Norris 1993; Buchanan et al. 2003). This is particularly important in species with lekking behaviour where male contest competition over mating territories is severe and likely to be a primary component of sexual selection. In general, success in contest is probably associated with male condition and overall health (Borgia 1979; see also Freeland 1981; Koskimäki et al. 2004), and reduced signalling as a result of infection or immune activation has been demonstrated in several animal species (Milinski and Bakker 1990; Houde and Torio 1992; Faivre et al. 2003; Brawner et al. 2000).

Two direct trade-offs between immune function and sexual signals have been proposed: competition for carotenoids (Lozano 1994; von Schantz et al. 1999) and the dual effect of androgens stimulating sexual traits and suppressing immune function (Folstad and Karter 1992). Red, orange and yellow colourations are often carotenoid-dependent ornaments (Hill 1999). Carotenoids need to be ingested from food and are considered a limiting source in nature (e.g. Grether et al. 1999). They are also required for an effective immune system (Lozano 1994). Competition for carotenoids between ornaments and immune function may hence enforce honesty on carotenoid-based sexual signals (Lozano 1994; von Schantz et al. 1999). In accordance with this hypothesis, studies in several species have shown that carotenoid supplementation can enhance ornament colouration (e.g. Kodric-Brown 1989; Evans and Norris 1996; Hill 1992; Blount et al. 2003; but see Fenoglio et al. 2002), as well as immunocompetence (for review see Bendich 1993; Christiansen et al. 1995; Blount et al. 2003; McGraw and Ardia 2003; Grether et al. 2003; but see Navara and Hill 2003).

Folstad and Karter (1992) proposed that display might reflect an individual's immunocompetence because testosterone is not only responsible for the development of exaggerated sexual signals (Wingfield et al. 1990) but may also have detrimental effects on the immune system. Because immunosuppression leads to higher susceptibility to disease, only high-quality males could afford to display sexual characteristics fully. Studies on the immunocompetence handicap hypothesis have yielded ambiguous, if not contradictory, results (Roberts et al. 2004). This may be due to our lack of understanding of the mechanisms by which testosterone modulates immune function (e.g. Marsh 1996).

Few studies on the trade-off between sexual signalling and immune function concern fish. Haplochromine cichlids exhibit a pronounced sexual dimorphism, with often drab females and brilliantly coloured males (Seehausen 2000). They are maternal mouth brooders, and maternal care continues for up to several weeks after the free-swimming juveniles are released. For males, territory ownership is a prerequisite to gain access to spawnings (Parker and Kornfield 1996; Maan et al. 2004). Male contest competition over spawning territories is intense. The complete lack of paternal care and intense territoriality lead to strong inter- and intrasexual selective pressure on male colour patterns (Maan et al. 2004; Seehausen and Schluter 2004; Dijkstra et al. 2005, 2006, in press).

In this study, we focus on *Pundamilia nyererei* (Witte-Maas and Witte 1985), a Lake Victoria cichlid fish with a carotenoid-based nuptial dress (Maan et al. 2004). We first established whether colouration is a signal of social dominance (Experiment I). Then we asked whether sexual advertisement is stimulated in a male–male competition context (Experiment II). In the same experiment, we analysed if honesty of signalling may be enforced by an immunological cost, and if so, whether this trade-off is caused by androgens.

Materials and methods

Fish

We studied first-generation offspring of wild-caught *P. nyererei* from Makobe Island in the Speke Gulf, Lake Victoria, Tanzania. Males of *P. nyererei* are crimson dorsally, yellow on their flanks and have a crimson dorsal fin. We used 31 adult fish (weight, mean±SE=16.6±1.1; standard length [SL], mean±SE=83.5±1.5). The animals were raised at the Department of Animal Ecology, University of Leiden (The Netherlands). They were transported to the Zoological Laboratory in Haren, The Netherlands 2 months before the commencement of the experiments.

Housing

All males were housed in a large stock aquarium (300 l) without females at least 2 months before the commencement of the experiments. Thereafter, they were kept individually in 15-l (Experiment I) or 50-l compartments (Experiment II) containing a polyvinyl chloride (PVC) tube as a hiding place. The sides and the back of the aquaria were covered with black plastic. All aquaria were connected to a central biological filter system, and water circulated continuously. Water temperature was kept at 25±2°C, and a 12:12 h light–dark cycle was maintained. All aquaria contained a gravel substrate. The fish were fed flake food (TetraMin Tropical Fish Flakes) six times per week and a mixture of ground shrimps and peas twice per week.

Experiment I

Males were housed in isolation for at least 1 week in 15-l compartments to minimize the effects of prior experience. The experiment started when a group of males, consisting of eight individuals, was released in large aquaria (760 l). Each aquarium contained ten *Melanotaenia lacustris* as background fish. These fish lack bright colouration, are nonterritorial and are standard background fish in our laboratory to reduce the level of aggression. Each male in a group, individually recognizable by a fin clip, was followed on the fourth day after release by focal sampling for 5 min. The aggression varied from displays to charges (see Baerends and Baerends-Van Roon 1950 for more details of aggressive interactions in cichlids). We scored the frequency of chases and displacements. A chase was defined as charging another fish. A displacement was defined as a fish that was displaying followed by his opponent fleeing, or when two fish were displaying to each other followed by one of them fleeing. In each aggressive interaction, we were, in most cases, able to identify a winner and a loser (the one that is fleeing after a chase or display). Thus, during focal sampling of an individual, we gathered information on which male the focal male defeated as well as which male defeated the focal male. We used the number of won interactions (sum of chases and displacements) to rank the fish in each group from dominant to subordinate.

After focal observation of a male, two judges independently scored the intensity of the red nuptial colouration on a 0- to 5-point scale, with a score of 5 representing the brightest red male. There was a general agreement between the judges: The red score did not differ between the judges (paired *t*-test: $t_{31}=-1.72$, $P=0.1$), and the red score of the two judges correlated well (Cohen's Kappa=0.64, $P<0.001$, $N=32$). Colour measurements by means of photography as described below for Experiment II was not done because moving individual fish from a group for photography would potentially lead to rapid changes in hierarchy and red scores. We observed four groups of males.

Statistics

The relationship between rank (fish with the largest number of won interactions rank 1, fish with the smallest number of won interactions rank 8) and red score (averaged over the two judges) was calculated by the Spearman rank correlation for each group separately. We used Fisher's combination test to combine significance levels over several samples (Sokal and Rohlf 1995).

Experiment II

Experimental design

At the start of the experiment, all males were housed individually and divided into two treatment groups: one group in which males were socially stimulated with a similar-sized neighbouring conspecific rival male in the same aquarium ($N=16$, weight mean±SE=17.2±1.7, SL mean±SE=84.5±2.3) and one group in which males were not stimulated ($N=15$, weight mean±SE=16.0±1.5, SL mean±SE=82.4±2). The non-stimulated males were given a small juvenile conspecific neighbour (SL<1 cm) to avoid unwanted effects of social isolation. Within aquaria, fish were separated from one another by transparent PVC sheets (thickness 1 mm).

After housing the males in the two treatments (day 0), we followed the development of nuptial colouration by photographing males on seven occasions (days 0, 4, 6, 10, 17, 20, 25). On day 14, all males were challenged with sheep red blood cells (SRBC, see below for details). Fourteen days later (on day 28), around the time of the peak of the primary response (Dijkstra et al., unpublished data), we took a post-immunization blood sample. The primary response is the antibodies produced upon first exposure to an antigen. Weight measurements were taken on days 0, 10 and 25.

Colour measurement

We photographed all males under standard conditions always before weighing. Individual male fish were briefly confined in a Perspex cuvette with water. Fish were kept in place by gently squeezing the male between a grey PVC sheet and the front window. One flank of a male was photographed, and the left or right flank was randomly chosen. The distance between the camera lens and front window was 20 cm. We used a digital photo camera (Sony Cybershot DSC-F717) with flash lights. Both flash lights were located 40 cm to the left and right from the camera. We analysed the colours of the fish body, excluding the fins and the eyes, in Sigmascan Pro 4.0 (SPSS). For colour analysis, we followed the procedure of Maan et al. (2004, 2006) described for colour analysis in our study species *P. nyererei*. To determine the area of red, we defined criteria by a combination of hue and intensity (hue 0–26 plus 232–255, saturation 40–97%). The resulting area of red was expressed relative to the total body area, yielding a percentage of red body coverage that fulfilled these criteria (Maan et al. 2004, 2006). This method corresponds well to the colour score judged by eye on a scale of 0–5.

Haemagglutination assay

Males were immunized with SRBC (Harlan, The Netherlands). The cells were washed three times with phosphate-buffered saline (PBS) and resuspended in PBS at 25% SRBC. Each fish received an intraperitoneal injection of the SRBC suspension (0.1-ml SRBC suspension/16-g body mass). After 14 days, we collected 20- to 100-μl blood from the caudal vein with a 1-ml syringe. The blood was mixed with a drop of heparin (<1 μl) and centrifuged at 13,000 rpm for 10 min. The blood plasma was stored at −20°C until the haemagglutination test and quantification of 11-ketotestosterone (11-KT) levels. Pre-immunization blood samples for the haemagglutination test were not taken because antibody titre is 0 for unimmunized *Pundamilia* cichlids (N=11; Dijkstra, unpublished data). Antibody concentrations in 20 μl of plasma were estimated in a standard haemagglutination titration assay (Hudson and Hay 1989). To prevent lysis of SRBC by complement, the plasma was heated to 56°C for 30 min (Collazos et al. 1994). Thereafter, plasma was diluted 1:1 in PBS and then serially diluted in PBS in 96-well microtitre plates. An equal volume of 0.2% SRBC was added to these dilutions, and the plates were incubated at 37°C for 60 min. Titres were scored visually as the highest twofold dilution of plasma showing haemagglutination. Scoring was done blindly with respect to treatment and colour score of the males.

Plasma 11-KT analysis

Quantification of 11-KT plasma levels (the most important androgen in fish, Kime 1993) by radioimmunoassay (RIA) was carried out as described previously (Schulz et al. 1993), except that smaller plasma volumes were available (20–40 μl). Therefore, the effective lower limit of detection for 11-KT in individual samples was 0.48 ng/ml plasma. The intra-assay coefficient of variation of a pooled plasma sample containing 10 ng/ml was 13%. All plasma samples were analysed in a single assay.

The individual samples (20–40 μl of plasma) were diluted with a twofold volume of an aqueous sodium azide solution (0.05%, w/v), incubated at 80°C for 1 h to liberate protein-bound steroid hormones and then centrifuged at 14,000 rpm for 30 min at room temperature. The supernatant was harvested and stored at 4°C. The same day, 50 μl of the supernatant was analysed for its content in 11-KT using a RIA (Schulz 1985). The details of antibody specificity and cross-reactivities are given in Schulz (1985).

Statistics

We used a repeated measure analysis of variance (RM-ANOVA) to analyse the development of red score over the experimental period, with treatment as fixed factor and day as repeat. We removed one non-stimulated male from the RM-ANOVA because it was not photographed on the first day due to technical problems with the camera.

We collected blood from 13 males from the stimulated group and 13 from the non-stimulated group for the haemagglutination test. Of these individuals, we collected enough plasma of 9 stimulated and 11 non-stimulated males for conducting the RIA too. For the analysis of antibody production, two animals, one in both groups, were left out of the analysis because they did not show any antibody response, suggesting unsuccessful injection of SRBC.

We used a backward stepwise selection procedure to determine if the red score before immunization on day 10 (pre-immunization red score) was a predictor of the antibody response. The full model also included treatment and the interaction term of treatment and pre-immunization red score. The criterion to remove a variable was set at P=0.15 to reduce the probability of making type II errors (incorrectly failing to reject the null hypothesis).

To analyse if 11-KT was related to antibody production, we carried out backward and forward selection procedures using the subdataset that included individuals of which we measured 11-KT levels. As in the previous paragraph, the response variable was antibody production. The full model included the red score on day 25 (final red score, which is closest in time to blood collection and therefore likely to be related to 11-KT levels), 11-KT level, treatment and all two-way interaction terms. Again, the criterion to remove a variable was set at P=0.15, starting with the interaction terms.

In all models, we also analysed the effect of the weight of the test fish. We did not include weight in the starting model in the interaction terms. Weight never explained a significant part of the variation in the response variable (Ps>0.4). The same was done with the amount of plasma we collected: The amount of plasma never explained a significant part of the variation in the response variable (Ps>0.8).

All percentage variables were arcsine-square-root-transformed before use in parametric tests. All other variables did not require transformation to meet assumptions of parametric testing. Quoted probabilities are for two-tailed tests of significance. We report means±SE.

Results

Experiment I

On average, males were involved in 11.3 aggressive interactions/5 min (N=32, SE=3.9/5 min). Redder males had higher dominance (Fig. 1, P values for each group: P= 0.03, 0.07, 0.8 and 0.03; Fisher's combination test, χ^2= 19.55, *df*=8, P=0.012).

Experiment II

Nuptial colouration

At the moment of separating the males (day 0), red score did not differ between the treatment groups (Fig. 2, independent *t*-test: t_{28}=0.459, P=0.65). On day 4, stimulated males increased in red score in comparison with day 0 (Fig. 2, paired *t*-test: t_{15}=−6.833, P<0.001), whereas there was no detectable change in red score in the non-stimulated males (paired *t*-test: t_{13}=0.269, P=0.80). The change in red score over the entire experimental period was significantly higher in the stimulated group than in the non-stimulated group (RM-ANOVA: treatment $F_{1,23}$=25.276, P<0.001; day effect, repeat $F_{1,23}$=12.372, P=0.002; repeat×treatment $F_{1,23}$=6.892, P=0.015). From day 4 onwards, stimulated males maintained a substantially higher red score than the non-stimulated males (independent *t*-tests: all days Ps<0.001). Thus, socially stimulated males rapidly showed elevated sexual signalling relative to non-stimulated males.

Antibody response and nuptial colouration

Antibody titres were on average lower but not significantly so in the stimulated group (6.5±0.9, N=12) than in the non-stimulated group (7.8±0.4, N=12, independent *t*-test: t_{22}= 1.254, P=0.22).

We tested if pre-immunization red score was a predictor of antibody production. The backward selection procedure retained all variables in the final model ($F_{3,20}$= 3.612, P=0.03) with treatment, red score and the interaction between treatment and red score as significant predictors of antibody response (Fig. 3; Table 1). After removing the interaction term between treatment and red score, neither red score ($F_{1,21}$=2.727, P=0.11) nor treatment ($F_{1,21}$= 0.084, P=0.78) affected antibody response. Overall, red score and antibody response correlated negatively (Fig. 3, Pearson's correlation test: r=−0.515, P=0.01, N=12). Within the stimulated group, red score and antibody response correlated negatively (Fig. 3: r=−0.615, P= 0.033, N=12), but this correlation was absent in the non-stimulated group (Fig. 3: r=−0.077, P=0.81, N=12). These findings show that expression of red nuptial colouration correlated negatively with antibody response but that this negative correlation is dependent on the manipulated social environment.

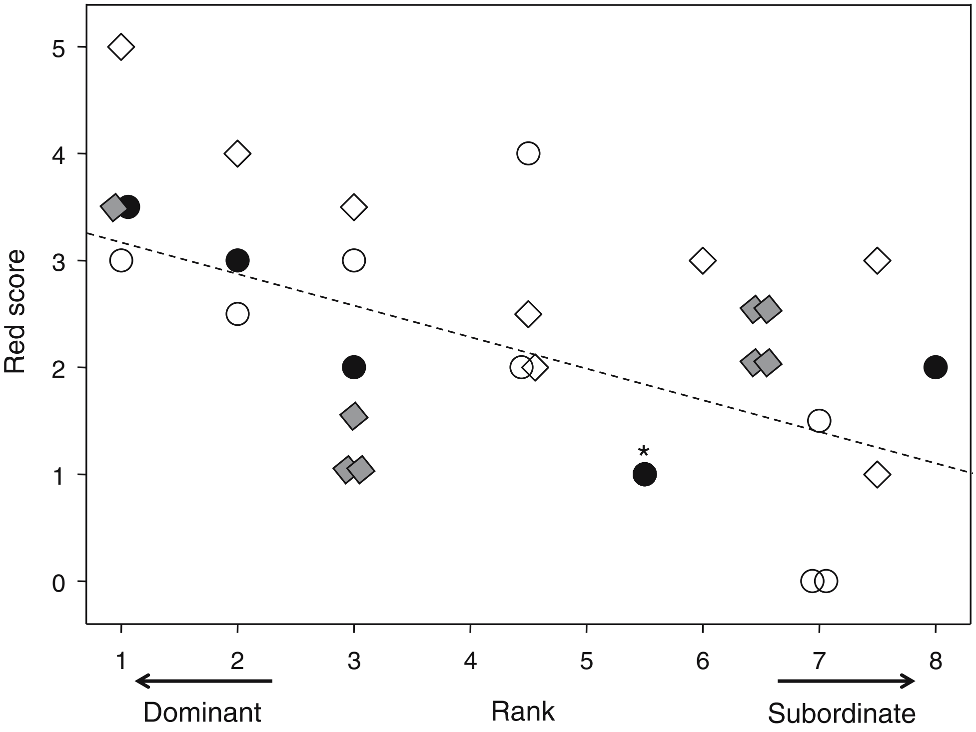
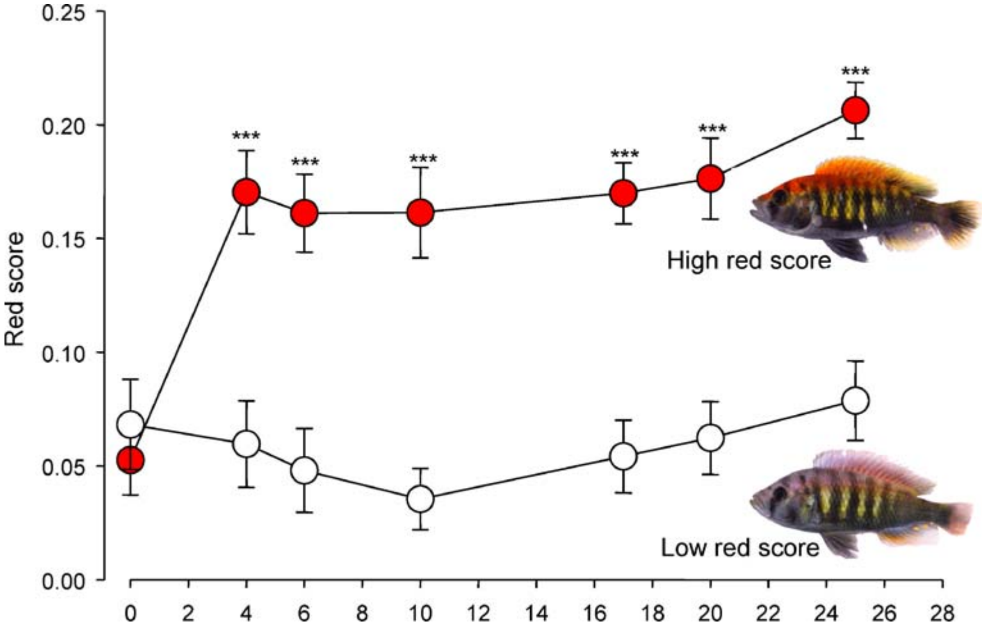


Fig. 1 Final red score (day 25) in relation to rank (1 is the most dominant, 8 the most subordinate, N=32) for four groups, each indicated by a different symbol. Overlapping points have been slightly displaced for visual clarity. *Asterisk* indicates four exactly overlapping data points. The *regression line* is fitted for all groups combined

Fig. 2 Development of red colouration of stimulated (*filled circles*, N=16) and non-stimulated males (*open circles*, N=15), expressed as the proportion of body area covered with red during the experiment. The fish were immunized with SRBC on day 14. From day 4 onwards, stimulated males maintained a substantially higher red score than the non-stimulated males (independent t-tests). Significance is indicated for each day. ***P<0.001



Hormones

Social stimulation led to a higher plasma level of 11-KT (stimulated group: mean±SE=9.7±2.0, N=10; non-stimulated group: mean±SE=3.4±0.8, N=12; independent t-test: t_{18}=−3.195, P<0.01).

When treatments were combined, the correlation between 11-KT and red score was not significant (Pearson's correlation test: r=0.416, P=0.068, N=20). This was also the case in the stimulated group (r=0.655, P=0.055, N=9) and the non-stimulated group (Fig. 4, r=−0.434, P=0.18, N=11).

To test if 11-KT is related to antibody production (Fig. 5), we carried out a stepwise selection procedure on a subset of the data, i.e. those cases with 11-KT information. The full model, with response variable antibody production, included final red score, 11-KT level, treatment and the two-way interaction terms. The procedure yielded a non-significant model (effect of 11-KT, $F_{1,18}$= 1.531, P=0.232). Because 11-KT levels showed correlated, though not significantly, with final red score, we regressed 11-KT levels against final red score for each treatment separately. These residuals of 11-KT were used in the starting model instead of 11-KT level. This analysis

Fig. 3 Antibody production in relation to pre-immunization red score for stimulated males (*filled circles*, *solid line*, N=12) and non-stimulated males (*open circles*, *dashed line*, N=12). The final model contained treatment, red score and the interaction term between red score and treatment as significant predictors of antibody response: results of this model are summarized in Table 1

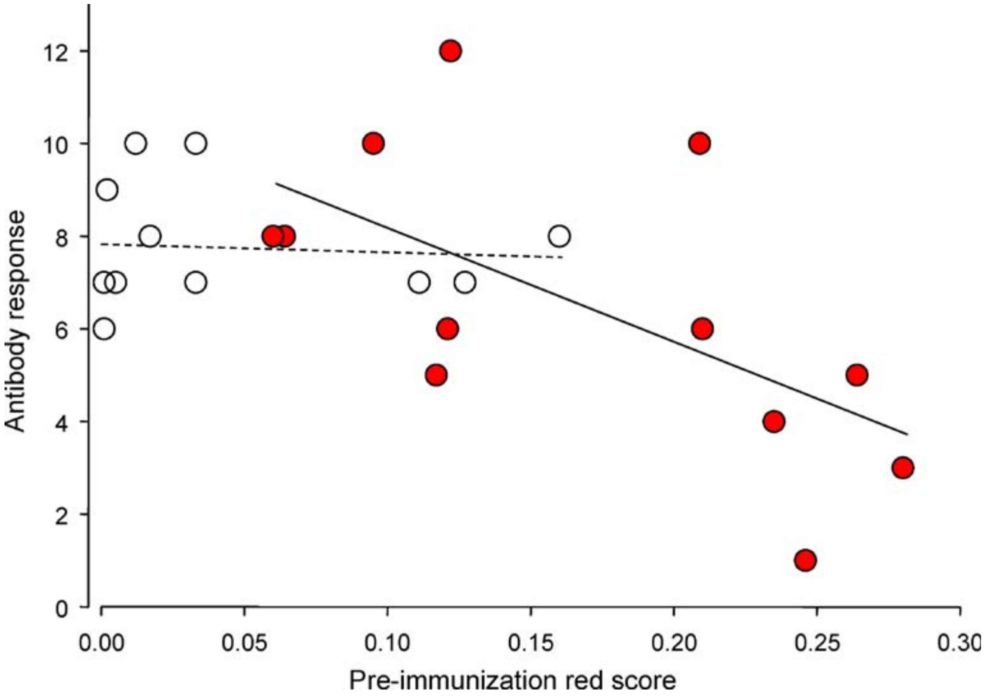


Table 1 The final ANOVA model after the backward selection procedure testing whether the pre-immunization red score was a predictor of antibody response

Source	*df*	*F*	*P* value
Treatment	1	4.875	0.039
Red score	1	5.280	0.032
Treatment×red score	1	5.471	0.030
Error	20		

For the starting model, see text. The final model retained the effect of treatment (stimulated and non-stimulated), pre-immunization red score and the interaction effect on antibody response (see also Fig. 3).

confirmed that 11-KT was not correlated with antibody response (residuals of 11-KT, $F_{1,17}=0.391$, $P=0.54$). In addition, forward selection procedures, both using either the residuals or 11-KT levels, confirmed that 11-KT did not predict antibody production (Ps>0.23).

Discussion

Male–male competition stimulates sexual advertisement

Socially dominant males expressed enhanced red nuptial colouration in a male–male competition context (Experiment I). Bright red coloration may therefore be important in intrasexual signalling, indicating social dominance, competitive ability or territory ownership (Rohwer 1975; Maynard Smith and Harper 1988). Social stimulation with a rival male resulted also in significantly larger areas of red colouration (Experiment II). This effect was sustained for the duration of the experiment. The finding concurs with other studies showing that animals modify colour expression with respect to the social environment (e.g. sticklebacks: Candolin 1999; zebra finch: McGraw and Ardia 2003). Lighting conditions differed between our study and the study of Maan et al. (2004), precluding a direct comparison between field and laboratory data. A quantitative assessment of colour scores on a 0–5 scale indicates that colour scores of the stimulated and the non-stimulated males correspond to those found in the field for territorial and non-territorial males, respectively (Maan, personal communication). Territorial *P. nyererei* males typically express bright red colouration, whereas non-territorial males appear more cryptic.

Maan et al. (2004) showed that *P. nyererei* females preferred bright red males. The present study underscores the dual role of red colouration as a signal in both mate choice and male–male competition (Berglund et al. 1996), supporting the idea that male–male competition affects mate choice (Wong and Candolin 2005).

Trade-off between sexual signalling and immunocompetence

A central prediction of the immunocompetence handicap hypothesis is that a trade-off exists between sexual ornamentation and immune function. We found an overall negative correlation between red score and humoral response in agreement with the prediction. However, the experiment failed to reveal an overall treatment effect on

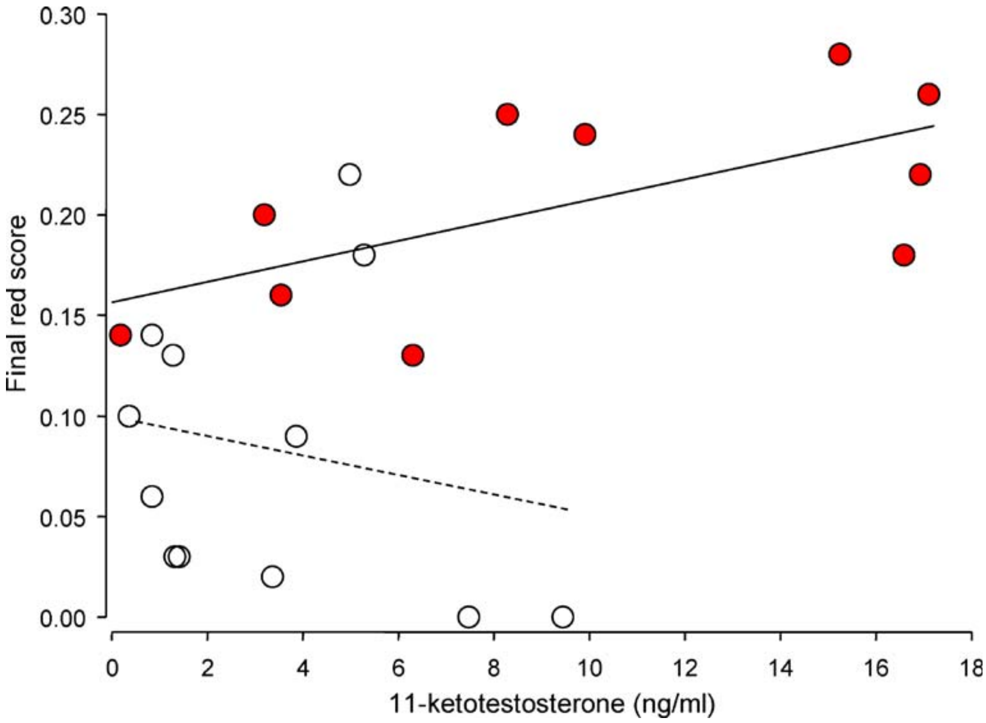


Fig. 4 Final red score in relation to 11-KT (ng/ml) for stimulated males (*filled circles*, *solid line*, *N*=9) and non-stimulated males (*open circles*, *dashed line*, *N*=12)

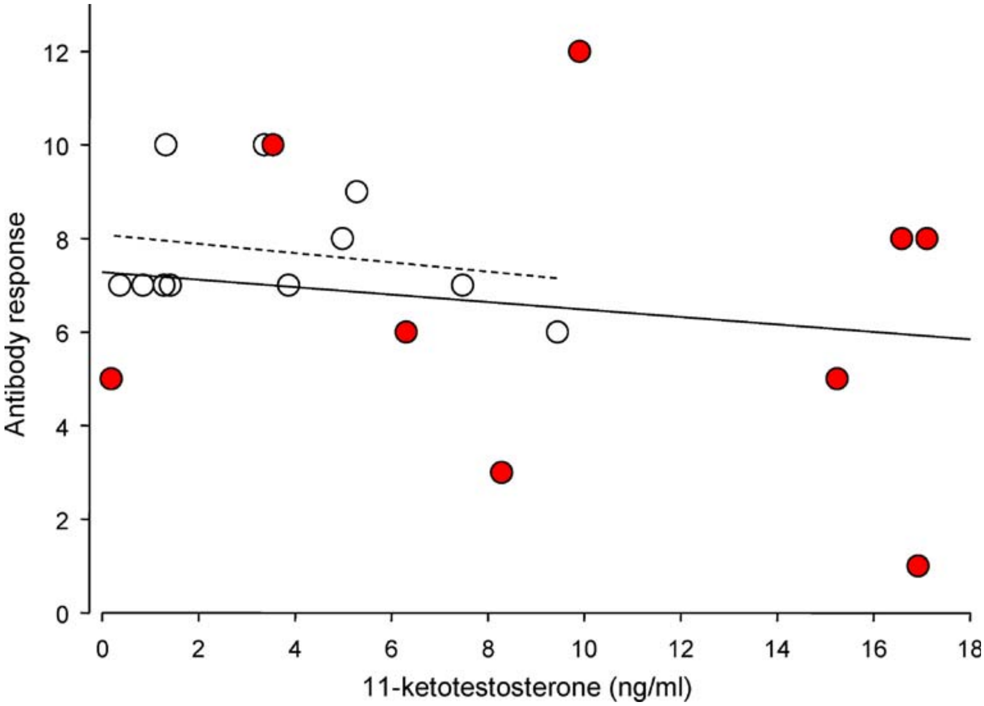


Fig. 5 Antibody production in relation to 11-KT (ng/ml) for stimulated males (*filled circles*, *solid line*, N=10) and non-stimulated males (*open circles*, *dashed line*, N=11)

antibody production, although it did affect colour display. Nonetheless, the manipulation interacted significantly with red score: Only in the stimulated group did red score correlate negatively with antibody production. This lack of a negative correlation in the non-stimulated group was probably attributable to the fact that males in this group did not reach relatively high red scores. Thus, we showed that the negative correlation between colouration and antibody production is dependent on the manipulated social environment, lending experimental support to the hypothesis that colour expression goes at the expense of a humoral immune response to a foreign antigen. The research on this trade-off has been skewed towards birds (Roberts et al. 2004). This study provides some of the first experimental evidence for a trade-off between expression of a sexual trait and one component of immune function in fish (see also Grether et al. 2003). It is possible that this mechanism reinforces honest status-signalling in *P. nyererei*, important in both mate choice and rival assessment.

We probed only one component of the immune system, namely, the humoral response to a foreign antigen. Antibody responsiveness to SRBC is known to correspond with resistance to several important parasites in some vertebrates (e.g. Parmentier et al. 1996). Yet, future studies on the trade-off between sexual display and immune function should examine other immune variables, such as antioxidant activity, phagocytosis and response to naturally occurring parasites (Adamo 2004).

Similar data have been obtained in the Azorean rock-pool blenny (*Parablennius parvicornis*) where males adopt either a parasitic or a bourgeois mating tactic (Ros et al. 2006). Parasitic males are non-territorial and have low levels of 11-KT, whereas bourgeois males are territorial and have elevated levels of 11-KT. The bourgeois mating tactic is associated with more intense sexual ornamentation, and in agreement with the prediction, antibody response to SRBC was lower in parasitic males than in bourgeois males (Ros et al. 2006).

Interestingly, in a field study on *P. nyererei*, intensity of male red colouration correlated negatively with parasite load (Maan et al. 2006). Their study shows that bright red males are less parasitized, which is unexpected in view of the immunological cost to expressing red nuptial colouration. A similar contrast has been found previously in a bird species (Peters 2000). The contrast can adequately be explained by assuming that, in unmanipulated animals, only good quality individuals are able to advertise full colouration, whereas in the experimental study all individuals are forced to enhance colouration.

The haplochromine cichlids represent one of the best test cases of sympatric speciation by sexual selection through divergent female mate choice (Seehausen 2000; Kocher 2004). Theory suggests that ‘good-genes’ processes are less likely to drive sympatric speciation than divergent Fisherian runaway selection (Van Doorn et al. 2004; Kirkpatrick and Nuismer 2004). This is because Fisher runaway process may unfold in any arbitrary direction, whereas in ‘good-genes’ models of sexual selection, female mate choice is less arbitrary and should be directed to traits that reliably signal genetic quality of the male. Our study indicates an immunological cost to expression of red nuptial dress in *P. nyererei*. This may implicate that only males with high genetic quality can afford to express colour at a high level, adding experimental support to the hypothesis that haplochromine males advertise ‘good genes’. Our study represents another possible example of numerous studies describing sexually selected traits that reliably indicate genetic quality (for reviews see Andersson 1994; Neff and Pitcher 2005). It thus appears that purely Fisherian traits are an unrealistic assumption in many speciation models (Turelli et al. 2001). Because the trait under study, male nuptial coloration, has been implicated in the rapid haplochromine species radiation (Seehausen 2000; Kocher 2004), it is necessary to improve our understanding of the mechanisms that may lead to sympatric divergence in the face of ‘good-genes’ sexual selection (see e.g. Edelaar et al. 2004).

Androgens

Social stimulation led to higher plasma levels of 11-KT. This is consistent with the observation that, in a number of cichlid genera, male–male interaction leads to a physiological maximum of 11-KT (Hirschenhauser et al. 2004). The levels of 11-KT of the stimulated males and the non-stimulated males correspond to those documented in *Haplochromis burtoni* of dominant and subordinate males, respectively (Parikh et al. 2006). Sexual advertisement is stimulated by androgens (Andersson 1994) as has also been demonstrated in cichlids (Fernald 1976, Groothuis and Ros, unpublished data). The results are consistent with this notion. Firstly, social stimulation led to a parallel increase in androgens and sexual signalling. Secondly, the relationship between androgens and red score was positive, although this was not significant.

The evidence for a causal link between elevated androgen levels and immunosuppression is ambiguous in fish. In salmonids, several studies suggest that there is a direct pathway for androgen-induced immunosuppression (Slater and Schreck 1993, 1998; Slater et al. 1995a,b). Studies in other teleosts have been unsupportive (Uglem et al. 2001; Law et al. 2001). Evidence is accumulating that the immunomodulating effects of androgens are mediated by indirect effects of androgens via regulation of resource allocation of, for example, carotenoids (Blount et al. 2003) or via its effect on glucocorticoid hormones (e.g. Hillgarth and Wingfield 1997). Law et al. (2001) found a suppressive effect of cortisol on leukocyte phagocytosis, but androgens had no effect. Experimental elevation of androgen levels is necessary to address this question further. The interaction effect of treatment and colour expression on antibody production, with the former affecting both colour expression and 11-KT levels, indicates that androgens, colour expression and antibody production are interdependent.

Conclusion

Our experiment illustrates the importance of male–male competition in sexual signalling, suggesting that red nuptial colouration is a signal to both prospective partners and rivals. We found both correlational and experimental evidence for the hypothesis that males face a trade-off between colour expression and one component of immunological defence. Carotenoid-dependent pigmentation in *P. nyererei* is therefore likely to be a costly trait, enforcing honest advertising of a male’s quality. This has implications for the involvement of colouration and sexual selection in speciation.

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