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A ROLE FOR RECEIVER BIAS IN ROSE BITTERLING MATE CHOICE?

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Abstract:-

The evolution of costly secondary sex traits in males may arise as a result of male-male competition or female mate choice. Female mating decisions may be associated with either direct or indirect benefits, and may evolve as a consequence of selection for signal detection in a non-mating context. We raised fish in the laboratory fed only on green coloured food and subsequently tested their foraging preferences by presenting them with plastic strips of different colours and recording the number of bites directed at each. We conducted separate tests on juveniles and sexually mature fish, and compared the responses of males and females. Male rose bitterling (Rhodeus ocellatus) display red, carotenoid-based nuptial colouration but its function in the mating system is unclear; there is little evidence that male colour plays a role in female mating decisions although there is a weak relationship between colour and dominance among males. We tested for a bias towards red in a non-mating context and found that bitterling show a strong response to red food items, irrespective of age, sex or prior experience. This correlation between a sexually selected trait and an intrinsic attraction to red appears to support the receiver bias hypothesis for the evolution of red nuptial colouration in bitterling. We discuss how this trait could be maintained under sexual selection in the absence of strong preference or any obvious fitness benefits.

Keywords: - *Sexual selection, mate choice, receiver-bias, carotenoid-based nuptial colouration, Rhodeus ocellatus*

INTRODUCTION

Sexual selection arises through variance in reproductive success, either as a result of competition between members of the same sex, generally males, and termed intra-sexual selection, or as a consequence of female mate choice, termed inter-sexual selection. In some species female mating decisions are associated with direct benefits to females; they may obtain nutrients or items of food, assistance with parental care, access to breeding sites, and protection from harassment or predation. Alternatively, females may acquire indirect benefits from mate choice, by selecting mates through producing offspring of enhanced viability reviewed by [1].

Alternative mechanisms of sexual selection have also been proposed, prominent among these is that of receiver bias [2; 3; 4; 5]. In the context of mate choice, the receiverbias hypothesis predicts that sexually selected traits are those most conspicuous to the perceptual or cognitive systems of females. Receiver-bias may have played a central role in the evolution of mate choice [6; 7]. It has been demonstrated receiver biases for specific colours in terms of mate preference and foraging in a wide range of taxa [8; 9; 10; 11]. In fish, mating preference of female guppies (*Poecilia reticulate*) for orange-coloured males and female three-spined stickle backs (*Gasterosterus aculeatus*) for male with red throat may arise from a preference for orange and red coloured food [4; 12]. The basis for preference of certain colours in food items may associate with a requirement for carotenoids [13; 14] which are essential in some physiological functions [15].

Bitterling are cyprinid fish belonging to the sub-family Acheilognathinae that use fresh water mussels as a spawning site [16; 17; 18; 19 for review see 4]. As all bitterling species *R. ocellatus* shows a striking sexual dimorphism. Usually male size is larger and more deep-bodied than female. Mature males and females have different coloration; females are brownish whereas male develops a red carotenoid-based nuptial coloration in the iris and on tail fin, a reddish pink coloration near pectoral fins, a bluish-blackish coloration on the dorsal sides and a light blue stripe along the sides [20; 21]. In the breeding season, males compete for territories around unionid mussels and lead females to spawn [18; 22]. Female bitterling have long ovipositor that they use to place their eggs onto the gills of a mussel through the exhalant siphon of mussel [4; 16; 23]. Territorial males release sperm over the inhalant siphon of mussel thus sperm enter with the drawn water into the gills of mussel to fertilize the eggs. Rival males (an adjacent territory holder or a male that does not possess a territory) also release their sperm into a rival's mussel by sneaking fertilisation [17; 18; 24; 25]. Fertilised eggs develop inside the gill of mussel and emerge from mussel as free swimming larvae in about 30 days [19; 26]. The aim of this study was to test for a sensory bias towards red in a non-mating context in the rose bitterling *Rhodeus ocellatus*.

MATERIAL AND METHODS

Experimental fish were produced from *in vitro* fertilisations in order to control for any effects of parentage. Parental fish were first generation offspring of wild-caught fish collected from the River Yangtze Basin, China; 30 males and 30 females were haphazardly selected from stock aquaria. The eggs were gently squeezed from the females in 70 mm diameter Petri dishes containing fresh water, and sperm was stripped from the males by gently pressing their abdomens and mixed in100 ml of teleost saline [27]. Sperm suspensions were pipetted over the eggs and the covered Petri dishes were left on the laboratory bench for 30 min. The fertilized eggs were washed with freshwater and incubated at 23 □C in an environmentally controlled room until they developed into freeswimming larvae, approximately a month. Larvae were reared in plastic aquaria measuring 35 (length) x 25 (width) x 20 (depth) cm with a 20 mm layer of fine gravel and artificial plants as refuges. Aquaria were on a recirculating system with water at 23 °C and fish were kept under a 16:8 h light: dark cycle. The sides of the aquaria were covered using opaque barriers in order to isolate fish from possible confounding visual stimuli. For the first 4 weeks of development, larvae were fed three times per day only green infusoria. Thereafter, they were fed a green commercial dried fish flake food twice a day.

The first colour preference tests were conducted on juvenile fish at the age of 12 weeks. Tests were conducted in plastic aquaria identical to those in which the fish were reared. Tests were conducted under natural daylight and the water temperature was 20 °C. Fish were tested in pairs, as pilot studies showed isolated bitterling did not feed freely. Two sides of each experimental aquarium were covered to prevent visual contact between adjacent pairs of fish. Fish were released in the experimental aquarium and left to settle down over the night. In the morning, fish were fed 5h before trail began to standardize their motivation to feed. During tests the fish were consecutively presented with each of 5 differently colored strips of plastic (green, blue, red, white and black) in a random order. The strips of plastic were tied to a 160 mm length of plastic line and when a strip was presented to the fish it was suspended, stationary, in the upper half of the aquarium. The total of number of bites directed at strips was recorded for 3 min. Fish were used in trials only once; a total of 15 pairs of juvenile fish were tested, each pair representing a single replicate. The remaining fish were reared until sexually mature, male and female *R. ocellatus* being easily distinguished because males develop red colouration in the iris and tail fin while females develop a long ovipositor. Pairs of each sex were tested separately for colour preference when the fish were 20 weeks old, using the same protocol as for the juveniles. Thus the adult fish had no prior experience of red, white, black and blue colours. A total of 12 pairs of males and 8 pairs of females were tested, each pair representing a single replicate.

Statistical Analysis:

Data were square-foot transformed to meet assumptions of normality and equality of variance. A one-way ANOVA was used to compare standard length among the juvenile fish while the standard length of males and females was compared with an unpaired t-test. Analysis of variance was used to analyse the colour preference data with bite rate as the 388 dependent variable, colour of plastic strips and order of presentation as independent variables. For the adult fish, sex was used as an additional independent variable. Bonferroni's test was used for *post hoc* pairwise comparisons between bite rates towards different colours.

RESULTS

The mean ± SD. body length (from the tip of the snout to the origin of the tail fin) of juvenile fish was 16.20 ± 0.564, there was no significant difference in body length among juveniles (One-way ANOVA: $F_{14,\,15} = 0.38$, $p = 0.960$). There was a significant difference in the biting rate of non-mature fish among the 5 colours of plastic strips (two factor ANOVA: $F_{4,\,66} = 7.38$, $p < 0.001$). There was no significant effect of order of colour presentation on biting rate of juveniles (two factor ANOVA: $F_{4,\,66} = 1.36$, $p = 0.257$). The highest rate of biting was directed at red strips, followed by green, black, blue and white (Fig. 1). *Post hoc* pairwise comparisons showed that there was no significant difference in bite rate between red and green (the colour of the food on which the fish had been reared) but red was preferred to all the other colours (white: $p < 0.001$, black: $p = 0.013$, blue: $p = 0.001$). Green was preferred to white ($p = 0.026$) but there was no difference in preference between green, blue and black.

The mean ± SD body lengths of males and females were 25.08 ± 0.771 and 25.19 ± 0.586 respectively, there was no significant difference in length between the two sexes (unpaired t-test: $t_{38} = 0.098$, $p = 0.922$). There was a significant effect of colour on the biting rate of mature fish (three-factor ANOVA: $F_{4,\,86} = 17.19$, $p < 0.001$). There was no effect of order of presentation (three factor ANOVA: $F_{4,\,86} = 0.79$, $p = 0.534$), or of sex (three factor ANOVA: $F_{4,86} = 1.88$, $p = 0.174$), neither was there a significant interaction between colour and sex (three factor ANOVA: $F_{4,86} = 0.12$, $p = 0.974$). The rank order of colour preference was the same as that for juveniles and did not differ between the sexes (fig. 2). *Post hoc* pairwise comparisons showed that for each sex red was preferred over all other colours (males, green: $p = 0.003$, white, black & blue: $p < 0.001$; females, green: $p = 0.005$, white, black & blue: $p < 0.001$)..

DISCUSSION

The aim of the present study was to examine fish bias for red colour in a foraging context in the rose bitterling *R. ocellatus*. Male displays a red nuptial coloration in the iris and on tail fin [21]. Bitterling fish showed a significant preference for biting at red plastic strips irrespective of sex or age.

One of the ecological explanation for fish bias to red colour is that red colour may be a detectible signal for potential benefits of food with carotenoids [4; 12; 28]. Carotenoids are essential for several physiological functions such as, immune response, liver function and growth [15; 29; 30]. However, animals cannot synthesize carotenoids by themselves thus depend on diet to obtain them [31; 32]. The diet of bitterling is rich in diatoms, crustaceans, green and red algae [33; 34]. Therefore, bitterling bias for red colour may possibly relate to the natural of dietary resources. In some species, carotenoids have a role in the expression of nuptial coloration trait that delivers information in relation to male forging ability, genetic quality (good genes) or parasites resistance [35; 36; 37; 38]. The main component of the nuptial coloration on the body and fins of rose bitterling in spawning season is carotenoids pigments which are zeaxanthin, β-carotene and diatoxanthin [39].

Bitterling showed a strong response to red food items, irrespective of age, sex or prior experience. This correlation between a sexually selected trait and an intrinsic attraction to red may provide a lending support to the receiver bias model for the evolution of red nuptial colouration in bitterling. Although, the role of red carotenoid-based nuptial coloration in mate choice has been demonstrated in different species [1; 37; 40], the extent of red in the iris or on caudal fin of male *R. ocellatus* appears to have no role in female mate choice [22]. Similarity, there is no effect of red colour intensity of eye or fin on mate choice in the related species *R. amarus* [24; 41]. However, red colour may function in other contexts such as, intrasexual selection [42]. In many vertebrates, bright colour may be involved as a signal for male fighting ability [43; 44]. However, the function of red colour in male-male competition has not been demonstrated yet in bitterling [22; 41]. In *R. amarus*, the intensity of male red colour is an indicator for reproductive success and dominance status [24; 41]. However, in *R. ocellatus* male nuptial coloration has a week association with male dominance [22].

Red nuptial coloration in *R. amarus* may possibly function as an initial signal of female to inspect mussel (direct benefits) but it is not correlated with mussel quality because, males do not alter their coloration in relation to mussel quality, but female may approach and inspect a mussel because; male traits may reflect mate quality (indirect benefits) [45]. However, in *R. ocellatus*, indirect benefits are related to genetic compatibility, MHC dissimilar mates confer fitness advantages on their offspring and MHC specific odor may possibly play a role in mate choice [46]. Male may release olfactory cues from the body surface or in the urine during courtship as well as). Female may make spawning decision based on sperm smell [22]. Olfaction cue seems to be principal in oviposition choice, mussel choice may depend on the presence of the amino acid glycine (Y. Kanoh, unpublished data) and on the oxygen conditions inside the mussel gill [47; 48].

Male coloration neither significantly associated with male fertilisation success or offspring survival [46]. Thus, it may not a signal for indirect benefits [22; 24; 41]. Moreover, the red carotenoids-based coloration may not also indicate direct benefit quality [45]. Our results showed a significant male and female bias for red colour in a foraging context, males may exploit red colour to increase reproductive success via stimulating the perception system of female in order to advertise themselves and attract females to their territories before other cues such as, olfactory, occur.

It was presented that fish strongly attracted to red and green objects, but less so to black, white and blue. During the experiment, fish were only exposed to diet with green colour. Consequently, the significant attraction of bitterling to the green object may a result of having experience of green food thus learning may likely involve in colour preference. Spence and Smith (2008) were found an important effect of diet conditioning on zebra fish *D. Rerio* colour preference.

In conclusion, although, bitterling *R. ocellatus* do not utilize male red carotenoidbased nuptial coloration in mate choice, this study suggests that fish bias to red colour is an adaptive response for potential benefits in their food which provides support to receiver bias hypothesis.

Figure 1. Mean ± SE bite rate ($3min^{-1}$) at coloured plastic strips (red, green, black, blue, white) by Juveniles of R. ocellatus.

Figure 2. Mean ± SE bite rate ($3min^{-1}$) at coloured plastic strips (red, green, and black, blue, white) by mature fish (A) female R. ocellatus, (B) Male R. ocellatus.

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