Multiple Sexual Ornamentation Signals Male Quality and Predicts Female Preference in Minnows

Jukka Kekäläinen*, †, Hannele Valkama†, Hannu Huuskonen† & Jouni Taskinen*

* Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland
† Ecological Research Institute, University of Eastern Finland, Joensuu, Finland

Introduction

In many species, sexual displays are often highly complex, involving many different signal components (Candolin 2003). Different ornaments may indicate quality in different time scales or different traits may reflect different aspects of signaller quality (Møller & Pomiankowski 1993; Fitzpatrick 1998; Brooks & Coulridge 1999; Badyaev & Hill 2000; Candolin 2003). Red, orange and yellow ornaments of birds and fish usually consist of carotenoids (Aquilera & Amat 2007). Because animals are unable to synthesize these pigments, they must acquire carotenoids from food (Fox 1976). Carotenoid ornaments have been demonstrated to indicate fertilization success and parental ability as well as certain genetic benefits in various animal taxa (Lozano 1994; von Schantz et al. 1999; Preault et al. 2005; Locatello et al. 2006; see also Folstad & Karter 1992).

Instead, dark melanin pigments are synthesised from amino acids, as a by-product of metabolism
(Fox 1976), which may make them much cheaper and thus possibly less reliable signals of quality than carotenoids (Badyaev & Hill 2000). However, even these potentially unreliable signals may be important cues in female choice without being attractive to females (Brooks 1996). Melanin pigments may amplify conspicuousness of areas of carotenoid and iridescent colours, or alternatively they may have an independent signalling function, indicating male dominance (status) and fighting ability (Kodric-Brown 1998; Tarof et al. 2005). Status signals may also have a dual function both in male–male competition and female choice (Berglund et al. 1996; Alonso-Alvarez et al. 2004). Females are expected to exploit status signals in their mate choice, because the reliability of such ornamental traits is continually tested by competitors and cannot be faked without incurring high costs (Berglund et al. 1996; Tarof et al. 2005). Therefore, although melanins may be energetically inexpensive to produce, socially imposed costs may help to maintain the honesty of the ornaments.

Males of certain fish species can rapidly change their colouration even within few seconds (ephemeral colour change: Kodric-Brown 1998). These colour changes are often occurring during agonistic interactions between males and during the courtship of females (Beeching 1995), and the presence of other males can have large impact on signal expression (Morris et al. 1995; Bee et al. 1999; Mateos & Carranza 1999; Candolin 2000a,b,c). European minnow (Phoxinus phoxinus) belongs to species capable of rapid colour changes. During the breeding season, male minnows develop conspicuous breeding colouration with a bright red, carotenoid-based abdomen colour and red lips (Tack 1940; Müller & Ward 1995, 1998). Also, the colouration of the lateral skin darkens and attains a metallic green appearance. During the breeding activities, males Butt both each other and females, and frequently present their colouration even within few seconds (ephemeral colour change: Kodric-Brown 1998). These colour changes are often occurring during agonistic interactions between males and females (Beeching 1995), and the presence of other males can have large impact on signal expression (Morris et al. 1995; Bee et al. 1999; Mateos & Carranza 1999; Candolin 2000a,b,c). European minnow (Phoxinus phoxinus) belongs to species capable of rapid colour changes. During the breeding season, male minnows develop conspicuous breeding colouration with a bright red, carotenoid-based abdomen colour and red lips (Tack 1940; Müller & Ward 1995, 1998). Also, the colouration of the lateral skin darkens and attains a metallic green appearance. During the breeding activities, males Butt both each other and females, and frequently present their colouration to the females in head-down positions. Females usually release their eggs when only one large (dominant) male is present (Müller & Ward 1995; Jacob et al. 2009). Previous studies on other species have demonstrated that females that mate with highly ornamented males often produce offspring with higher viability (Norris 1993; Petrie 1994), better condition (Sheldon et al. 1997), higher growth rate (Petrie 1994; Eilertsen et al. 2009), higher first feeding success (Huuskonen et al. 2009) or better parasite resistance (Barber et al. 2001), when compared to offspring of less ornamented males.

Our main objective was to study the signalling function of male ornamentation in minnows and whether females utilize these signals in their mate choice. More specifically, we tested how male–male competition affects signalling (redness of the belly and darkness of the lateral skin) in male minnows and whether these colour changes are related to dominance status and courtship activity of the males. Furthermore, we studied how female minnows choose between males, when only visual signals are available. We predicted that male colouration indicates their success in male–male competition (dominance) and that females have a stronger behavioural response towards dominants and/or more intensively coloured males in mate preference trials.

Methods

Sexually mature minnows were collected by dip nets from Uuronpuro brook in eastern Finland (62°51′N, 29°59′E) during the breeding season (May and June) in 2007 and 2008. Fish were transported to the laboratory at the University of Joensuu and housed in 45-l aquaria with continuous water flow at 10°C water temperature under natural photoperiod. Fish were automatically fed daily with commercial fish food (Biomar®; Aqualife, Aarhus, Denmark). To activate the breeding behaviour, water temperature was gradually raised to 18°C after 1 wk of maintenance and a layer of gravel (20–40 mm in diameter) was then added to the bottom of the aquaria.

We carried out three sequential (10 min) experiments for the same male pairs. Behaviour of males was continuously recorded in all experiments. In the first experiment, we observed the aggressive behaviour of two males, when only these two fish were present in the experimental aquarium (Fig. 1). In the second experiment, we added a sexually active, gravid female to the same experimental aquarium with the males and, in addition to aggressive behaviour, also the courting activity of the males was observed. In the third experiment, the same female used in experiment 2 was allowed to choose between the males when only visual cues were present. The intensity of male colouration (darkness of the lateral and redness of the ventral area) was individually ranked before the experiments and after each three experiments (four times in total). To standardize the rankings of the two independent observers, the colour-ranking procedure was trained using males that were not used in the actual experiments.
Experiment 1: Aggressiveness in the Absence of the Female

Two sexually active size-matched male minnows were captured from the maintenance aquarium and placed simultaneously in the experimental aquarium (Fig. 1). The experiment was conducted in the middle compartment of a three-part glass aquarium (length × height × width of each compartment was 300 × 300 × 225 mm). Three sides of the aquarium were covered with brown cardboard to prevent the effect of outside visual stimuli on the fish behaviour. The experimental aquarium was illuminated from above with fluorescent lamps. Otherwise, the room was dark to minimize the biasing effect of visual stimuli from the uncovered side of the aquarium. Before tests, 10 l of 18°C water and a layer of 20- to 40-mm gravel was added to the aquarium. In the experiment, we calculated aggressive attacks of each male towards the other. An aggressive attack was defined as a rapid dash towards the other male ending with a direct contact with this male. Unintentional contacts for example during side by side swimming were not calculated. Before (ranking 0) and after (ranking 1) the experiment, the darkness of the lateral and redness of the ventral areas of the males were ranked in a scale 0–4. The mean value of ranks given by two independent observers was used in the final analyses.

Experiment 2: Aggressiveness and Courting Activity in the Presence of Female

Immediately after the first experiment, one gravid female was placed in the same aquarium compartment with the two males used in experiment 1. In addition to the aggressive male–male attacks, we here also counted the touches (i.e. courtship activity) of each male to the female. The nature of these contacts often differed from male–male interactions. While the male–male attacks often comprised long and aggressive chases of the rival male, the female contacts were more often gentle pokes to the belly or other parts of the female body (Kortet et al. 2004). Again, unintentional contacts between males and between sexes were not counted. The colouration of the males was ranked (ranking 2) after the experiment as described earlier. In total, 25 male pairs were used in experiments 1 and 2.

Experiment 3: Female Preference

The female preference experiment was conducted for 16 male pairs. In nine trials, either female or one of the males was not actively swimming, which prevented the determination of female preference. Males within a male pair were placed in the left and right compartment of the same, three-part experimental aquarium and the female was left to the middle compartment (Fig. 1). All compartments had similar lighting conditions, water temperature (18°C) and bottom medium (20- to 40-mm gravel). The separating wall between compartments was solid glass, so the fish were able to see, but not to smell each other. A visible median line was drawn in the female compartment, and the active swimming time the female spent in either male’s side of her own compartment was measured. Recording of female preference was temporarily ceased if some fish was not actively swimming. The male that received more attention from the female (i.e. longer time) was interpreted as the preferred male. The colouration of the males was again ranked (ranking 3) after the experiment. After the experiments, all fish were killed with an overdose of tricaine methanesulfonate (MS-222, Sigma; Sigma Chemical Co., St. Louis, MO, USA) and frozen for further analyses. All experiments were performed according to the licence of the Finnish Animal Experiment Board (ESLH-2008-03722/Ym-23).

Controlling for the Reliability of Measurements

Between-observer reliability was controlled by conducting 10 independent dominance experiments that were otherwise identical to experiments 1 and 2, but now the two observers counted aggressive attacks and touches to the females simultaneously for the same male at a time. The correlation in the number of aggressive attacks and touches to the females between two observers was high (Pearson, $r = 0.966$ and 0.925, respectively, $n = 10$ in both cases).
was also no difference in the mean frequencies of these behaviour patterns recorded by the two observers (paired t-tests, \( t_9 = 0.447 \) and \( 0.427, p = 0.665 \) and 0.679, respectively).

As direct contact alters minnow darkness within seconds (JK and HV pers. obs.), visual colour ranking was considered to be the best available method to estimate individual colour differences as it does not require direct contact with the fish. The reliability of the colour ranking was tested in eight individual pairs of recently killed males. Both observers in the experiments 1–3 ranked independently the darkness of the lateral area and the redness of the ventral area of the males. These same fish were also presented for a third inexperienced observer who was assigned to rank the male pairs in the order with respect of their redness and darkness. Ranking procedure was always conducted blind with respect to measured behavioural traits (dominance status, courting activity and female preference). After the rankings, all fish were photographed with Sony DSC-F828 digital camera, and the colouration was further analysed with a graphical user interface developed at the InFotonics Center, University of Joensuu. Mean hue, saturation and lightness values were calculated. The colouration was measured from two standardized areas: a rectangle with the four corners defined by the origins of the pectoral and the lateral fins (ventral redness, i.e. hue) and a rectangle defined by the eye (left), lateral line (top), anus (right) and the ventral and pectoral fins (below) (lateral darkness, i.e. lightness).

The belly hue measurements gave lower hue values (=redder colouration) for those males that had been ranked redder than the other male within a pair (paired t-test, \( t_6 = 6.011, p = 0.001 \). In seven out of eight cases, also the inexperienced observer ranked the male redness similarly to the experienced ones. All three observers ranked the darkness of the males similarly: in all cases, the male that had been ranked darker had lower actual lightness values (=darker colouration) than the lighter ranked male (paired t-test, \( t_7 = 7.239, p < 0.001 \). The correlations of both colour rankings between the two experienced observers were high (Spearman \( r = 0.893 \) and 1.0, \( r = 0.893 \) and 1.0, \( n = 10 \), for redness and darkness, respectively).

Statistical Analyses

Normality was assessed using Kolmogorov–Smirnov statistics, and when needed natural-logarithm transformation was applied to satisfy the requirement of normal distribution. Non-parametric tests were used when the assumptions of the parametric tests were not met. All presented p values are from two-tailed tests with \( z = 0.05 \). All statistical analyses were performed with SPSS 16.0 statistical package (SPSS Inc., Chicago, Illinois, USA).

Results

Dominance and Courting Behaviour

The aggressiveness of the males in experiment 1 was very low: only 10% of the 50 fish tested presented aggressiveness in the absence of female. Aggressive behaviour increased to 74% of males when a female was present (experiment 2; chi-square test, \( p < 0.001 \), when also a highly significant difference in attack frequency was found between males (paired t-test: \( t_{12} = 7.155, p < 0.001 \)). The male that had been more aggressive, i.e. performed more attacks towards the other male was regarded as the dominant individual. These dominant fish had more contacts to the female than the subordinate males (paired t-test: \( t_{12} = 4.179, p < 0.001 \), Fig. 2a). The mean weight and length of the dominant and subordinate males did not differ (paired t-test: \( t_{12} = 1.338 \) and 1.640, \( p = 0.195 \) and 0.115, respectively).

Both colour rank values were higher in the dominant males than in the subordinate fish in rankings 0–2, i.e. already in the beginning of the experiments and also at the end of experiments 1 and 2 (Wilcoxon matched-pairs signed-ranks test, \( p < 0.05 \), in all cases) (Fig. 3). The darkness and redness of the males differed between colour rankings 0–2 both in the dominant and in the subordinate males (Friedman analysis of variance: \( x^2 = 27.711 \) and 9.211, \( p < 0.001 \) and \( p < 0.010 \) for dominants and \( x^2 = 28.986 \) and 8.578, \( p < 0.001 \) and \( p = 0.014 \) for subordinates, respectively). Paired t-tests revealed that darkness and redness ranks were lower after the male–male experiment (ranking 1) than at the beginning of this experiment (ranking 0) in both male groups, but that the darkness values increased when female was present (ranking 2), but only in the dominant males (Table 1).

Female Preference

Male colouration was ranked for the fourth time (ranking 3) after the female preference experiment (experiment 3; 16 male pairs). Although there was a trend for dominant males being more colourful than subordinate individuals, this difference was not statistically significant (Wilcoxon test, \( Z_{13} = -1.925 \),
p = 0.054 for redness and $Z_{13} = -1.941$, $p = 0.052$ for darkness). The darkness and redness values of the dominant males decreased between rankings 2 and 3, but remained the same in the subordinates (Wilcoxon test, $Z_{13} = -2.226$ and $-2.070$, $p = 0.026$ and 0.038 in dominants and $Z_{13} = -1.000$ and $-0.136$, $p = 0.317$ and 0.892 in subordinates, respectively). Females spent more time on the side of the more colourful (redder and/or darker) male in 15 out of 16 cases (binomial test, $p = 0.001$, time spent: paired $t$-test, $t_{15} = 3.895$, $p = 0.001$, Fig. 2b). In eight cases, the selected male was both redder and darker, in four cases only redder and in three cases only darker than the non-selected male. In one case, female preferred the male, which was darker, but had less red colouration. Female preference was independent of the side of the aquarium where the males were located: in nine cases, left male and in seven cases right male was selected (binomial test, $p = 0.607$). Aggressive interactions (i.e. dominance hierarchy) between
males were observed in 14 out of 16 female preference trials. Female spent more time on the dominant male’s side of the aquarium in 11 out of 14 trials, but this effect was not significant (binomial test, \( p = 0.057 \)). No difference was found for female preference time between dominant and subordinate males (paired \( t \)-test, \( t_{13} = 1.310, p = 0.213 \)).

**Discussion**

In the absence of female, the aggressive behaviour of male minnows was negligible, but appeared rapidly when a gravid female was placed in the experimental aquarium. Therefore, the presence of female seems to trigger agonistic behaviour of males in this shoaling species. Dominant males courted (i.e. touched) the females more actively than the subordinate ones. The positive association between dominance and courtship activity has been demonstrated also in another cyprinid species, roach (Rutilus rutilus) (Kortet et al. 2004), and the dominance status has frequently been observed to have a strong impact on male breeding success in various animal taxa (Cox & Le Boeuf 1977; Alatalo et al. 1991; Kodric-Brown 1993; Andersson 1994; Berglund et al. 1996; Petersson & Jarvi 1997; Rantala & Kortet 2004).

In this study, dominant males were both darker and redder than their subordinate rivals in all other rankings, but not after the female choice experiment, although parallel trend was still present. In addition, when the lateral colouration had lightened up during the male–male experiment (without female), only dominant males were able to redarken significantly under the presence of a gravid female. This observation may indicate that socially imposed costs of signalling could be an important factor regulating the intensity of colour signalling during male–male competition in minnows (Candolin 1999a, 2000a). The social control hypothesis predicts that the signalling during intense male–male competition may be costly. This could increase the honesty of signalling, if only high quality (dominant) males are able to pay these costs (e.g. increased aggression from other males). Therefore, our results suggest that the male–male competition in the presence of females increases the colour differences between males, which have been demonstrated to facilitate female choice and discrimination between males (Candolin 1999a; see also Candolin 1999b).

As ephemeral colour changes of males often signal motivation and intention also in the context of courtship behaviour (Kodric-Brown 1998), it seems likely that the observed colour differences also signal differences in male motivational state, i.e. willingness to mate. For example, in guppies courting males develop large black spots and a horizontal stripe along the side of the body, thereby increasing the conspicuousness and complexity of their colour pattern (Kodric-Brown 1998). The fact that more colourful (redder and/or darker) males were both more aggressive and more active in courtship than the paler males indicates that these colours may simultaneously signal both status and motivational state of the male (Kodric-Brown 1996; Tarof et al. 2005; Griggio et al. 2007; Hoi & Griggio 2008). Thus, male colouration probably has a dual function in both male–male competition and female choice.

Both redness and darkness scores decreased during the male–male experiment (in the absence of female). This initial colour change occurred during the trapping of the males from their maintenance aquarium, and the colouration did not intensify during the whole experiment. Because in the absence of females also the aggressiveness of the males was negligible, males may not have any reason to compete or advertise their quality in male-only interactions. However, minnows did not completely reach their original signalling level either when the female was present. As our experimental trials lasted for 10 min and aggressive behaviour often appeared in the latter half of the trial, it is possible that the duration of the trials was too short for males to reach their full signalling level even in a species capable of ephemeral colour changes. In support of this hypothesis, we recently observed that when two males and one female were allowed to interact much longer (approx. 30–40 min), males achieved much darker colouration than after a 10-min period. In this study, we also found that the colour signalling level of dominant (but not subordinate) males decreased during female preference experiment, which also reduced the colour difference between male groups. Dominant males probably decreased their signalling as a response to the different social environment, i.e. to the physical absence of the rival male and the female. Therefore, exclusively visual signals may be insufficient to maintain the adequate high motivational state of the dominant males (sexual excitement and/or aggressiveness), which might be reflected to their colouration. We cannot expect similar decrease in subordinate male colouration, as their signalling (and aggressiveness) level was much lower already before female preference experiment. Thus, our results suggest that colouration signalled male dominance status also during our female
preference trials, but not as accurately as in earlier two male–male competition trials.

Female minnows had strong preference towards the more colourful (redder and/or darker) male’s side of the aquarium, which possibly indicated female choice (Kodric-Brown & Nicoletto 1997). It has been demonstrated that a female’s visual response when the sexes are separated by a glass partition is a good predictor of a male’s reproductive success (Kodric-Brown 1993; Lehtonen & Lindström 2008; Walling et al. 2010). As we found that the selected males can be both redder and darker (50% of trials), only redder (25%), only darker (19%) or darker, but less red (6%) it remained unclear whether the actual object of female selection was the red or dark colouration (or both). Thus, it is possible that female preferences may differ, i.e. some females have preference for the dark melanin ornaments, whereas others prefer red carotenoid ornaments. Alternatively, melanin-based colouration can act as an amplifier of carotenoid colours, even if melanin ornamentation may not be in the direct target of female choice (Brooks & Caithness 1995; Brooks 1996). Amplifiers are not energetically costly to produce and maintain, and thus amplifiers may not have independent signalling function in female choice; they only expose cues that are informative about male quality (Hasson 1990, 1997). Therefore, amplifying signals improve the accuracy of discrimination between males (Brooks 1996).

On the other hand, melanin ornaments may have an independent signalling function, because also energetically cheap ornaments can sometimes be honest (Berglund et al. 1996). In this study, all the rapid colour changes of male minnows appeared in melanin-based colours. Thus, it is possible that different ornaments may signal individual quality in different time scales (Sorenson & Derrickson 1994; Møller et al. 1998; Hill et al. 1999; Candolin 2003). Although we cannot rule out the possibility that melanin acts as an amplifier of carotenoid-based ornaments, our results suggest that melanin ornaments have an independent signalling function by indicating the present condition and/or social status. As well as rapid changes in the motivational state of the males. Instead, less flexible carotenoid-based ornaments may reveal different aspects of male quality or signal quality over longer time scales. Thus, present results suggest that breeding colouration of male minnows contains multiple cues. Depending on the species, different signals may either give information about different mate qualities (multiple message hypothesis) or they may allow more accurate mate quality assessment, if each signals reflect the same quality trait (back-up signal hypothesis) (Candolin 2003).

There was a non-significant trend for brighter males to also be the dominant males even after the female preference experiment. As our experimental conditions during female preference trial might have been unnaturally reduced the signalling level of dominant males, it seems probable that male colouration could be even more accurate signal of dominance in natural breeding conditions. Thus, it is possible that females may in fact primarily favour dominant individuals in their mate choice. The status-dependent signalling has been observed in various animal taxa (Thompson & Moore 1991; Senar et al. 1993; O’Connor et al. 1999; Setchell & Wickers 2005). Females should prefer dominant males, because dominance may reflect the superior quality of the male. Therefore, dominance per se or signals reflecting the status should be important cues in female choice (Moore 1990). Female minnows usually release their eggs when only one, dominant male is present (Müller & Ward 1995). Possibly for this reason also the fertilization success of the dominant males is higher than that of subordinate individuals (Jacob et al. 2009). These results suggest that the dominance status may be an important factor determining the reproductive success of male minnows.

In conclusion, sexual ornamentation of male minnows probably signals both dominance status and motivational state (courting activity) of the males and may have dual function, both in male–male competition and female choice. Male–male competition in the presence of female increased the colour difference between dominant and subordinate males, which may facilitate female mate choice. Furthermore, females had a clear behavioural preference towards the more colourful and/or dominant males, but it was not possible to discriminate whether the selection is based on carotenoid or melanin-based ornaments, or both. As different ornaments may reflect different aspects of quality, it is possible that females use multiple cues to reduce mate choice errors or costs of choice.

Acknowledgements

We thank Nikolaus Medgyesy, Paula Rinne, Ari Voutilainen, Kari Ratilainen, Sanna Kirvesniemi, Timo Hampinen and Kirsti Kyyrönen for advice. Heikki Simola, Leena Lindström and Mikael Puurtinen, as well as three anonymous referees are

thanked for valuable comments on the earlier version of the manuscript. Kaija Eisto and Metsähallitus are acknowledged for the permission to catch minnows. The study was financially supported by the Academy of Finland and the Kone Foundation.

Literature Cited


