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Male-male competition ensures honest signaling of male parental ability in the three-spined stickleback (*Gasterosteus aculeatus*)

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Abstract The importance of socially imposed costs for the evolution and maintenance of honest sexual signals has received less attention than other costs. Here I show that male-male competition can increase the honesty of sexual signaling in relation to male parental ability in a species with flexible signaling. When four three-spined stickleback (*Gasterosteus aculeatus*) males were allowed to court a female first separately and then in competition, red nuptial coloration under competition reflected male parental ability more accurately than red coloration when separated. Parental ability was determined as the ability of the male to raise a clutch of eggs to the hatching stage under interaction with other breeding and non-breeding males. The increased honesty under competition was probably due to subordinate males of poor parental ability decreasing their color expression under interaction to reduce the risk of fights with superior males. However, socially imposed costs of signaling were probably not the main factors maintaining honest signaling, as red coloration reflected male parental ability also in the absence of competition, although less accurately. Nevertheless, the small-scale differences that male-male competition induced can significantly facilitate adaptive female choice and have large impacts on sexual selection.

Keywords Sexual selection · Reliable signaling · Dominance · Parental care · Nuptial coloration

Introduction

A central issue in sexual-selection theory is to explain the evolution of female preferences for exaggerated male

secondary sexual characters. For such preferences to be beneficial, the characters should reflect direct or indirect fitness benefits of mating with the male, i.e., increased female fecundity or increased offspring quality due to the inheritance of attractiveness genes (Fisher 1930) or viability genes (Zahavi 1975). This leads in turn to the question as to how the honesty of the trait can be ensured. In some instances, honesty may be ensured by the nature of the trait, for example when the exaggeration of the trait reveals the possession of attractiveness genes. However, in other cases, some constraint on signal expression is required that limits signaling to the quality of the male (Zahavi 1975). This might be, for example, the amount of energy required for the development of the signal, or increased predation risk associated with the expression of the signal. If individuals of high quality pay lower costs or receive larger benefits for a given level of signaling than individuals of low quality, then the optimal balancing of costs and benefits of exaggerations should result in a positive correlation between signal quality and male quality (Grafen 1990; Getty 1998).

In some species, the level of signaling is not fixed but can be rapidly adjusted to changes in the environment. The presence of predators, for example, has frequently been found to influence the level of signaling (see e.g., Lima and Dill 1990; Magnhagen 1991; Fuller and Berglund 1996). This may either decrease (Berglund 1993; Fuller and Berglund 1996; Reynolds 1993) or increase the reliability of the signal, depending on whether or not the susceptibility to predation is quality dependent (Candolin and Voigt 1998). Another factor that can influence signaling but which has received less attention is the quality of surrounding males. In species where male traits are used both as cues in female mate choice and in the competition among males for matings, the presence of other males can have large impacts on signal expression (e.g., Morris et al. 1995; Howard and Young 1998; Bee et al. 1999; Mateos and Carranza 1999). This opens up a possibility of increased honesty under male interactions if dominance or fighting ability correlates with male quality (Berglund et al. 1996). A male of low domi-

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nance status or fighting ability may signal at a lower level than a male of high dominance status to reduce the risk of fights with superior males that would consume more resources than he can afford or would lead to serious injuries. Socially imposed costs of signaling would then limit signal expression to the dominance status and fighting ability of the male. Some contest-independent cost may, however, be needed for reliable signaling to occur, as theoretical models indicate that socially imposed costs of signaling alone cannot maintain honesty (Johnstone and Norris 1993). On the other hand, if fighting ability and dominance do not reflect male quality (Forsgren 1997), male-male competition could reduce honesty and hinder adaptive female choice.

In the three-spined stickleback, *Gasterosteus aculeatus*, male-male competition has been found to increase male differences in red coloration (Candolin 1999a). This facilitates female choice in relation to male dominance as red coloration reflects dominance (reviewed by Bakker 1994) and females use the color as a cue in mate choice (reviewed by Rowland 1994). Male-male competition is probably not the main factor that determines signal expression, but the small-scale differences in signal intensity that it induces have large effects on the ability of females to discriminate among males. In this study, I investigated whether these small-scale changes in signaling increase the honesty of the red coloration as a signal of male parental ability. The parental ability of the male is of crucial importance for reproductive success as the male cares alone for the offspring for about 2 weeks by oxygenating the eggs and defending them against predators (Wootton 1976). The dominance status of a male may reflect his parental ability, as males usually breed in dense aggregation and the main causes of reproductive failure are the destruction of the nest and the predation or stealing of eggs by conspecific males and females (Wootton 1976). Changes in signal expression in relation to dominance status under male-male competition could therefore increase the honesty of the red coloration as an indicator of direct benefits in these breeding aggregations. Male dominance could also reflect male viability and genetic quality and thus correlate with indirect benefits, as has been found for other species (Montgomerie and Thornhill 1989; Alatalo et al. 1991).

Methods

I collected three-spined sticklebacks with fish traps from the littoral of the Baltic Sea near Tvärminne Zoological Station in southern Finland. The fish were collected before the start of the breeding season (end of April) in 1998. Sexes were housed in separate flow-through aquaria (salinity 5.5 ppt) at 18°C under natural lighting conditions. Fish were fed daily on frozen chironomid larvae.

To investigate whether male-male competition increases the honesty of red nuptial coloration as an indicator of direct benefits, I subjected two groups of males to different treatments. In the first treatment, I allowed four males to court a female first when separated from other males and then while interacting with each other, and related their color expression to their ability to rear a clutch of eggs to the hatching stage in the presence of other breeding and

non-breeding males. The second treatment served as a control to determine the effect of time and earlier experience on color changes by testing single males twice.

Experiment: single and interacting males

I placed four males into an experimental aquarium (80×80 cm) divided into four equal male compartments by opaque sheets (40×40 cm) and maintained under the same conditions as the holding aquaria. The males were of the same length (± 1 mm, ranging from 46 to 55 mm across replicates) and the tip of their dorsal spines was cut so that they could be individually recognized. Spines are not used as weapons in fights and spine-clipping does not influence dominance status (personal observation). Each male compartment contained a nesting dish filled with 1 cm of sand, an artificial plant, and filamentous green algae (*Cladophora*) as nesting material. White curtains with small viewing holes reduced external disturbance. If the time it took the males to build a nest differed by more than 3 days, I replaced all males with new males to ensure that all males were in the same reproductive condition.

When all males had built a nest and crept through it, I determined the expression of their red coloration by first presenting each male to a model of a gravid female for 5 min and then photographing him under standardized conditions (Candolin 1999a). I used a dummy female to eliminate differences in female behavior that may affect male red-color expression. To photograph a male, I dip-netted the male and put him into a small glass box (6×3×6 cm) containing water and a black sponge that fixed him in place and served as a non-reflective background. I then placed the glass box in a fixed position into a large dark box containing a digital camera and photographed the male's left lateral side. The only light source was a lateral flash. This procedure took less than 1 min and the male did not have time to fade due to handling stress. To ensure that the lighting conditions were constant, three plastic strings of red, blue, and green colors on the front side of the glass box served as color references in the analyses. A tristimulus system constructed to fit the human eye was used, as color vision of sticklebacks does not differ greatly from that of humans (reviewed by Frischknecht 1993; McKinnon 1995). I determined the extent and quality of the red coloration from the digital images using image-analyzing software (MCID-M4; Imaging Research Inc.). I selected areas that ranged in color from yellow to red to purple (hue: 1–50 and 340–359; saturation: 0–0.631; intensity: 0.157–0.663), and recorded their size and mean color quality. The size of the male's red area is given as a percentage of his total lateral area.

The males were then allowed to interact by removing the partitioning sheets. After 1 day of interaction, I determined changes in red coloration by first presenting the males to a dummy female for 5 min and then photographing the males as described above. The dummy female was enclosed in a Plexiglas cylinder in the middle of the aquarium and all males were dip-netted at the same time and photographed in random order. After photographing, the aquarium was again divided into four male sections by opaque sheets and each male was allowed to mate with one female. The amount of eggs spawned was determined both by recording female wet weight before and after spawning, and by weighing the eggs 3–4 h after spawning when the egg mass had hardened. To determine egg weight, I lifted the nesting dish from the aquarium and gently pulled the nest upside down so that the eggs could be removed with forceps. I dried excess water from the egg mass on absorbent paper and weighed the eggs in a bowl of water. After weighing, I put the eggs back into the nest and returned the nesting dish to the aquarium. In all cases, the male accepted the nest and repaired the entrance and any other parts of the nest that had been damaged. This procedure has been shown to have no significant influence on egg survival (Kraak et al. 1997). The two measures of egg mass, the change in female weight and the weight of the egg mass, were highly correlated ($r=0.98$, $n=48$, $P<0.001$) and I used the latter, direct measure of egg quantity in the analyses.

When all males had repaired their nests and resumed normal parental activities, I removed the partitioning sheets and placed

two additional sexually mature males into the aquarium. The males were then allowed to care for their offspring under interaction with both the nesting and the non-nesting males. Eight days later, when the eggs were almost ready to hatch, I determined hatching success by weighing the eggs as described above and recording the percentage of eggs that had survived. Any changes in the weight of an egg during development should be the same over replicates.

To avoid pseudoreplication, test results are based on aquarium means, unless the analysis included only males before interaction when the variates were independent. Twelve replicates of the experiment were carried out. Values given are means \pm SE.

Control: single and single males

Single males (mean standard body length \pm SE=52.6 \pm 0.7 mm) were put into individual aquaria (35 \times 45 cm) maintained under the same conditions as the aquaria with the interacting males. When a male had built a complete nest, I determined his red coloration over 2 consecutive days using the same methods as described above. Twenty replicates were carried out.

Results

Effect of interaction

Variation among the four males in the size of their red areas tended to increase under competition (Table 1; the coefficient of variation is calculated to account for differences in means), due to some males decreasing whereas others maintained their areas. The mean size of the areas of the four males therefore decreased under interaction (Table 1). Color quality did not change under interaction (Table 1). Male interactions also caused some changes in the ranking order of the four males when the males were ranked according to the size of their red areas. This is demonstrated by the imperfect Pearson product-moment correlation coefficients between the ranking order of the four fish before and after interaction for the 12 replicates (mean $r=0.62$, SE=0.12).

The change in red coloration under interaction increased the honesty of the red coloration as an indicator of male parental ability under the prevailing conditions. This is demonstrated by an increase in Pearson product-moment correlation coefficients between hatching success and red area ($r=0.54\pm0.08$ before interaction, 0.79 ± 0.06 after interaction, paired $t_{11}=2.51$, $P=0.029$; Fig. 1). A significant difference is also obtained if the analysis is based on regression coefficients ($r^2=$

0.37 ± 0.10 before interaction, 0.66 ± 0.08 after interaction, paired $t_{11}=2.40$, $P=0.035$). Males with large red areas under interaction also appeared to steal the eggs of other males more often than males with smaller areas, since in the four replicates where a male had stolen another male's eggs, two of the stealing males had the largest red area of the four males, and two were the second-reddest males. The stolen eggs were easily identified as they were not clumped with the male's own eggs but usually buried outside the nest. They were often less developed than the male's own eggs and frequently included dead eggs, which suggests that the male spent less effort on caring for the stolen than for his own eggs. The stolen eggs were not included in the measure of hatching success.

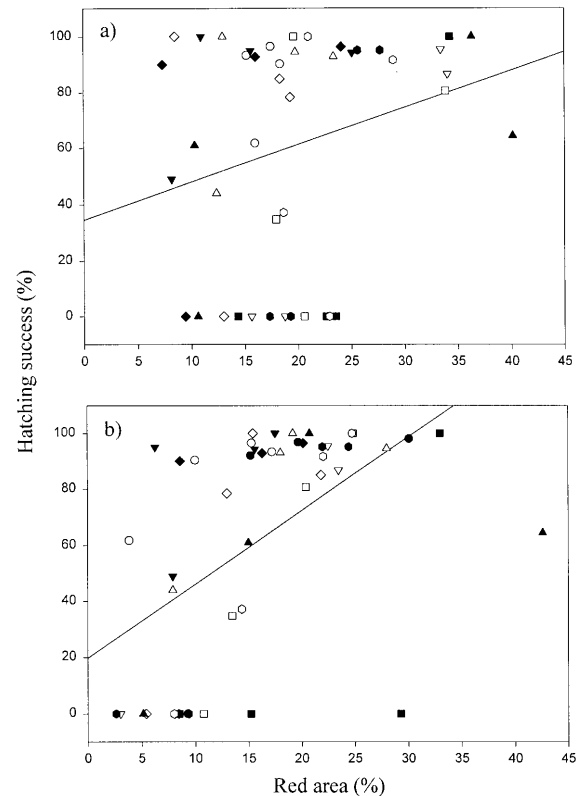


Fig. 1 Relationship between hatching success and red area before (a) and after (b) interaction for the 12 replicates. Mean regression lines are shown

Table 1 Mean red coloration and the coefficient of variation (CV) for the four males before and after interacting with each other

Red coloration		Before interaction	After interaction	Paired t_{11}	P
Area (%)	Mean	19.9 \pm 1.2	16.1 \pm 1.0	3.69	0.004
	CV	39.1 \pm 5.9	53.1 \pm 3.7	2.11	0.058
Hue	Mean	38.5 \pm 1.1	39.4 \pm 1.2	0.66	0.52
	CV	16.8 \pm 2.6	15.3 \pm 2.5	0.40	0.70
Saturation	Mean	0.19 \pm 0.00	0.18 \pm 0.00	1.54	0.15
	CV	6.3 \pm 1.0	6.6 \pm 0.4	0.32	0.75
Intensity	Mean	0.58 \pm 0.01	0.56 \pm 0.02	1.47	0.17
	CV	9.2 \pm 1.3	11.5 \pm 1.9	0.99	0.34

Table 2 Mean red coloration and the coefficient of variation (CV) of the single control males on day 1 and day 2

Red coloration		Day 1	Day 2	Paired t_{19}	P
Area (%)	Mean	22.5±2.2	24.3±2.0	1.99	0.061
	CV	44.2	36.2		
Hue	Mean	34.9±1.1	34.7±1.0	1.05	0.31
	CV	14.5	13.1		
Saturation	Mean	0.20±0.00	0.20±0.00	0.69	0.50
	CV	9.3	7.9		
Intensity	Mean	0.56±0.01	0.55±0.01	0.92	0.37
	CV	8.8	8.1		

Control of time and experience

The red area of single males showed a tendency to increase from day 1 to day 2, but color quality did not change (Table 2). This differs significantly from the results gained when males were brought into interaction on day 2 (see above) when the area of red (averaged over the four males) tended to decrease (repeated-measures ANOVA with the two treatments as factor: time×treatment: $F_{1,30}=15.5$, $P<0.001$). Moreover, coloration on day 1 correlated with coloration on day 2 (Pearson's correlation; red area: $r=0.91$, $n=20$, $P<0.0001$; hue: $r=0.94$, $P<0.0001$; saturation: $r=0.87$, $P<0.0001$; intensity: $r=0.95$, $P<0.0001$) and the variation among the males in color variables decreased with time (Table 2). This contrasts with the results under interaction when the variation among males increased (see above). Thus, the reduction in the area of red under male interaction and the subsequent increase in the variation among males were due to the interaction with other males and not an effect of time or earlier experience.

Discussion

Red coloration is a flexible trait that can be rapidly adjusted to changes in the environment. This study showed that this property can increase the honesty of the signal. Male-male interactions were found to induce changes in the expression of the red coloration that increased the honesty of the color as a signal of male parental ability in the presence of other males. Male-male interactions caused males of low parental ability to decrease their red area in relation to males of higher parental ability. The effect of male interactions on signal expression was probably due to red coloration working as a badge of status (Rohwer 1975), with males of low dominance status decreasing their red areas to escape fights with superior males. As dominance correlated with male ability to raise their offspring to the hatching stage, a social control of deception increased the honesty of the signal as an indicator of direct benefits. This points to the importance of the social environment in maintaining honest sexual signaling in species with flexible traits. By adjusting red coloration to the dominance status of surrounding males, the coloration may accurately reflect the relative quality of a male under the prevailing conditions. This may have large impacts on female mate choice and

sexual selection, as an earlier study found even small-scale differences in signal expression to substantially increase the ability of females to discriminate among males and even to change mate choice (Candolin 1999a).

Socially imposed costs of signaling are probably not the only factor that maintains honest sexual signaling in this system. Theoretical studies suggest that contest-dependent costs alone cannot prevent cheaters from invading the system (Johnstone and Norris 1993). In support of this, red coloration also reflected male quality in the absence of interaction, although less accurately. This suggests that some contest-independent factor is responsible for the maintenance of honesty. This could be, for instance, reduced immunocompetence, as carotenoids are essential for proper immune system functioning (Folstad and Karter 1992; Wedekind 1992; Lozano 1994; Wedekind et al. 1998) and parasite infection has been found to reduce the expression of red color (Milinski and Bakker 1990), or it could be increased oxidative stress, as carotenoids are important antioxidants (von Schantz et al. 1999). Red coloration could also reflect the ability of males to escape predators, as red coloration increases the risk of predation (Moodie 1972; Whoriskey and FitzGerald 1985) and males decrease their color expression in the presence of predators (Candolin 1998). The physical capacity of the males to gather carotenoids seems not to maintain honest signaling alone, as males can adjust the amount of stored carotenoids they invest into signaling depending on environmental conditions and intrinsic properties of the male (Candolin 1999b). The intake of carotenoids is possibly more important in ensuring honesty in species where the signal is fixed during development and cannot quickly be adjusted to alterations in the environment, as is the case for the plumage coloration of some bird species (Hill 1992; Hill and Montgomerie 1994).

How commonly male-male competition increases the honesty of signaling is not clear. This study contrasts with several earlier studies that found male-male competition to obscure or alter female mate preferences (Trail 1985; Greenfield 1994; Jennions and Petrie 1997; Petersson et al. 1999). Whether male-male competition hinders or facilitates adaptive female choice depends, however, on whether it increases or decreases the probability that females will mate with high-quality males. If competition increases the honesty of signaling or prevents low-quality males from mating with females, it

may increase the intensity of sexual selection. This is possibly more common than has so far been reported, as male dominance has frequently been found to correlate with direct and/or indirect benefits of mating with a male (Bisazza et al. 1989; Montgomerie and Thornhill 1989; Alatalo et al. 1991; but see Forsgren 1997).

In conclusion, this study showed that male-male competition increases the honesty of signaling in relation to male parental quality under the prevailing conditions in the three-spined stickleback. Although male-male competition seems not to be the only factor maintaining honest signaling, it induces small-scale differences in signal intensity that can significantly facilitate adaptive female choice.

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