Male aggression and colour in divergent populations of the threespine stickleback: experiments with animations

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Abstract: We investigated the evolution of male agonistic behaviour and nuptial coloration in populations of the threespine stickleback, *Gasterosteus aculeatus*, with either monomorphic red or monomorphic black coloration. Specifically, we examined the responses of males from the two population types to computer-generated animations of males with black, red, or dull grey throats on otherwise identically coloured bodies. Males varied greatly in their aggressive responses among individuals and among trials, and did not show statistically significant discrimination towards the differently coloured animations. These results, therefore, do not suggest a role for interactions among males in the evolution of divergent male nuptial coloration. In addition, these negative findings are consistent with other studies of stickleback from western North America, suggesting that geographic variation in agonistic colour discrimination may account, in part, for the discrepancies between the results of earlier studies. Given the diverse methods of studies on this topic, however, methodological differences may also have contributed to the inconsistent results.

Résumé : Nous avons étudié l'évolution du comportement agonistique des mâles et de leur coloration nuptiale chez des populations d'Épinoches à trois épines, *Gasterosteus aculeatus*, à coloration monomorphe rouge ou noire. Plus spécifiquement, nous avons examiné les réactions des mâles de deux types de populations à des modèles animés par ordinateur de mâles à gorge noire, rouge ou gris neutre sur des corps par ailleurs identiques. Les mâles ont manifesté des réactions agressives très variées selon les individus et selon les expériences et n'ont pas fait preuve de discrimination statistiquement significative à l'égard des différents modèles animés. Ces résultats n'indiquent donc pas que les interactions entre les mâles aient pu jouer un rôle dans l'évolution de leurs différentes colorations nuptiales. Ces résultats négatifs confirment ceux d'autres études sur des épinoches dans l'ouest de l'Amérique du Nord et semblent indiquer que les variations géographiques constatées dans les comportements agonistiques face à la coloration peuvent expliquer, du moins en partie, les contradictions entre les résultats d'études antérieures. La diversité des méthodes utilisées pour étudier le phénomène permet de croire que des différences méthodologiques peuvent aussi avoir contribué à l'obtention de résultats contradictoires.
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Introduction

Male threespine stickleback are billigerent, especially towards male conspecifics, and this pugnacity has long intrigued students of animal behaviour. In one of ethology's seminal studies, Tinbergen and his co-workers (ter Pelkwijk and Tinbergen 1937; Tinbergen 1948, 1951; Collias 1990) demonstrated that colour is critical for releasing stickleback aggression but shape is relatively unimportant; stickleback attacked even a very crude model of a male conspecific if its ventral surface was painted red, the colour of a territorial male, but not if it was grey or a similar dull hue. Attempts to replicate this frequently cited result have been relatively unsuccessful, however. In some studies, for example, males

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responded less aggressively towards red-bellied models than to grey models (Table 1) (also see reviews by Baerends 1985; Rowland 1994). In the present study, we sought to reexamine and extend previous findings with a comparative investigation of male coloration and male agonism in populations with monomorphic red or monomorphic black coloration (McPhail 1969; Hagen and Moodie 1979; Hagen et al. 1980). We used a relatively new technique, computer-generated animation.

Recently, phylogenetic patterns of male trait and female preference evolution have been the focus of much theoretical (e.g., Ryan 1990; Endler 1992; Houde 1993) and empirical (e.g., Proctor 1992; Hill 1994) work, but relatively little attention has been given to how ornamentation and responses to ornaments might (or might not) coevolve through intrasexual selection (but see Marchetti 1993). In a study of female courtship preferences partly involving the same populations used here, one of us (McKinnon 1995) found that females responded preferentially to images of males with black or red coloration over males with dull coloration. In the present, somewhat exploratory study, we ask if males similarly show differential agonistic responses to black and red images regardless of their own nuptial coloration. Hagen et al.'s (1980) study of agonistic behaviour of red and black males placed together in aquaria suggests a different predic-

Table 1. Summary of the results of studies of the aggressive responses of male threespine stickleback to red or grey models or images of males.

| Study | Colour most attacked | Study populations | | Distance from nest of stimulus | Red coloration |
		Habitat[a]	Region		
Wootton 1971	Not sigificantly different	Freshwater	W North America	20 cm	Unavailable
Peeke et al. 1969	Not significantly different	Freshwater	W North America	Variable	5R 4.5/10
This study	Not significantly different	Freshwater (2 populations)	W North America	31–62 cm	8.75R 6/14
Collias 1990	Red	Marine	Europe	7–13 cm	Orange–red[b]
Rowland 1982	Grey	Marine	E North America	20 cm	5R 4/14
Rowland and Sevenster 1985	Grey	Marine, freshwater	Europe, E North America	30 cm	"Bright red ... Testor's Pla enamel 1103"
Rowland et al. 1995a	Intermediate intensity	Marine	E North America	No nest in testing tank	Monitor colour adjusted

Note: Where available, Munsell designations are given. Only studies in which data were analyzed with statistical tests are included.

[a]Populations in which the entire life history occurs in fresh water are referred to as freshwater; others are referred to as marine.

[b]From Bakker and Milinski (1993); colour is reportedly more orange than in Rowland's dummies, with a less strongly contrasting body colour.

tion, that red should be a more effective threat display and hence generally elicit weaker aggressive responses. The absence of agonistic colour discrimination in two previous studies of stickleback from western North America (Table 1) suggests a third potential pattern, that males from our study populations should not discriminate between differently coloured simulated rivals, owing to geographic variation in such behaviour.

Video presentations have been used as stimuli in recent behavioral studies of diverse taxa (e.g., Clark and Uetz 1990; Evans and Marler 1991), including the threespine stickleback (McKinnon 1995; Rowland et al. 1995a, 1995b). The use of animated simulations and edited videos allows the manipulation of traits such as colour while keeping behaviour consistent and realistic. In earlier investigations with stickleback, models were either immobile (e.g., Rowland and Sevenster 1985), moved by hand (e.g., Wootton 1971), or moved in a simple and repetitive manner by an electric motor (Peeke et al. 1969).

Materials and methods

Study populations
Males with red (we refer to male coloration as red because it is commonly described as such, but the nuptial coloration of the population in question is in most cases closer to orange; see below) nuptial coloration came from Cow Pasture Creek, a small stream flowing into the Salmon River just above its juncture with the Fraser River in Langley, British Columbia (McLennan 1989). Males with black coloration came from Scatter Creek where it intersects Gibson Road in Thurston County, Washington State. Further details of collecting procedures and stock maintenance are given in McKinnon (1995).

Animations
We worked in collaboration with a professional animator (Markus Tessmann, University of British Columbia) to develop an animation of a male stickleback performing a series of agonistic behaviours and displays. The animation process began with the development of

a "three-dimensional" computer model of a male stickleback, generated using Vertigo 9.0 (Vertigo, Vancouver, B.C.) animation software running on a Silicon Graphics computer. The model was based on a series of morphological measurements from a stream-resident male stickleback. An appropriate colour pattern was added by digitizing a photograph of the same male and wrapping or "texture mapping" it onto each side of the model.

We then added an overlay onto the throat and operculum of the model, coloured either red, black, or light grey, on different versions of the animation. The red hue was matched with a Munsell (1946) colour chip (10R 6/14) that closely approximated the throat coloration of many individuals from the red population used in this study; the colour ultimately obtained on the playback monitor differed slightly from this, being approximately 8.75R. The black was a deep black, similar to that on males of the Scatter Creek population. The grey was the same as that on the very light grey (slightly lighter than colour 81 in Smithe 1975, Munsell 5.1GY 7.9/1.1; elsewhere we refer to this colour pattern as dull) ventral portions of the digitized stickleback photograph, and appeared to approximate closely the coloration of the ventral portion of a poorly coloured male stickleback or of a female. During some male agonistic behaviours, such as portions of the head-down display, the light striking the male's lateral surfaces will come principally from above; consequently, the apparent colour of the lateral surfaces will be influenced little by water coloration (McKinnon 1994).

Some of the ventral region of the texture-mapped image was initially blue–black. Not all populations of stickleback exhibit this blue–black abdominal coloration, so to be more consistent with previous studies utilizing dummies (e.g., Rowland 1982) and reduce the effects of interaction among the male's different nuptial colours, we coloured this area the same grey, sampled from another part of the ventral surface, as was used in the "dull" throat overlay. All fins were translucent, with slightly darkened rays. The pelvic spines of the model were always completely erect, as they are in a displaying male, while the dorsal spines were partially erect. The size of the animated image was scaled so that it would appear, to the male stickleback watching it, to be approximately 49 mm in standard length, the size of a relatively large stream-resident male (for additional details see McKinnon 1995).

All animated behaviours, and their timing, were based on video-tapes of actual males involved in agonistic interactions. The ani-

mated stickleback "swam" on screen from the left and alternately performed head-down displays or darts towards the screen. The body of the model bent during turns, as does that of a real fish, and the pectoral fins beat regularly. The animated sequence was 20 s in length, and at the end the stickleback image exited to the left and stayed off screen for 1 s; this sequence was repeated until a 10-min composite was obtained. The composite was then transferred to Hi-8 format videotape, as a Sony TR81 Hi-8 camcorder was the source for playbacks. Animations were presented to males on a 33-cm (maximum diagonal screen diameter) Sony PVM-1340 commercial monitor.

Three versions of the animation were produced, one with each of the three throat colour patterns mentioned above. All versions appeared against a background that was matched as closely as possible to "olive-green," colour 46, Munsell 9.4Y 4.0/3.8 in Smithe (1975); after transfer to video the colour ultimately achieved was closer to No. 52, "olive-yellow," Munsell 7.5Y, 7.0/7.0 in Smithe (1975). The background coloration was chosen on the basis of observations in a natural stream and of the coloration of cryptically coloured female and juvenile stickleback (McKinnon 1995). To make the background more realistic, in the lower third of the screen this blended rapidly with a slightly darker colour (approximately No. 50, 8.2Y 4.6/6.2, "yellowish olive-green" in Smithe (1975)), as if with a substrate.

Video systems are designed for humans, and visual systems can differ greatly between species of vertebrates (Endler 1990); to the stickleback, therefore, colours on the video monitor may not mimic colours in nature. Nevertheless, available evidence suggests that the stickleback's colour vision is reasonably similar to, though certainly not identical with, human colour vision (reviewed in Frischknecht 1993; McKinnon 1995). Further, the female preference for red males observed in studies with fish that were unmanipulated or manipulated with different techniques (e.g., McLennan and McPhail 1990; Milinski and Bakker 1990) was also observed in a study using video presentations (McKinnon 1995).

Experimental protocols

During experimental trials, each male was housed in an aquarium of dimensions 61 × 30 × 30 cm, the bottom of which was covered with a 1- to 2-cm layer of limestone gravel. A nesting dish full of fine sand was placed at the centre of each tank and sprinkled with aquatic vegetation for males to use in building their nests. Each tank was filtered by a single corner filter and covered on three sides with opaque plastic partitions so that males would not see individuals in other aquaria. Lighting came from a single 40-W incandescent bulb placed over each tank, and the photoperiod was 17 h of light to 7 h of darkness, a day length slightly longer than peak day lengths in the region (Berry 1992).

To bring all males to the same stage of the reproductive cycle before testing, a gravid female in a clear plastic container was placed against each male's tank for 10 min daily, beginning on the third day post introduction. Once a male had added a roof of glued vegetation to his nest, a ripe female was placed in his tank and left there until she had tried to enter the male's nest or 10 min had elapsed. Experimental trials began on the second day after the nest possessed an obvious entrance. Males that did not possess a complete nest by day 7 were not used in experimental trials.

Each male was tested with all three colour versions of the animation. One of the six possible sequences of animation presentation (e.g., red, black, dull) was randomly assigned to each male with the condition that no sequence was reused until all had been used once (then twice, and so on). All tests for any particular male were conducted on a single day, with at least 1 h between tests. A minimum of 1 h before the start of testing, the 40-W incandescent light over each tank was turned off and the room's overhead fluorescent lighting turned on; the latter was preferable because it gave more even light for videotaping, but it was not used at other times because the fluorescent lights could not be placed on timers.

Animations were presented with the monitor screen parallel to the glass of a male's tank, 2.5 cm from the end farthest from the male's nest. Because males did not always build their nests in the same spot, the distance between the male's nest and the tank end adjacent to the monitor ranged from 28.5 to 58.5 cm. Each animation presentation began with 3 min 30 s of background before the male image appeared. Videotaping of the subject male began with his first clear orientation to the animated image, usually a swim towards the image, and continued for 4 min.

Behavioral definitions

We scored videotapes for two discrete behaviours that have frequently been examined in other studies of the aggressive behaviour of stickleback (e.g., Rowland 1982; Rowland and Sevenster 1985): (1) bite – butt: any contact of the male's head with the aquarium glass that appeared to be directed at the animated image. Butts and bites were placed in one category because they could not always be distinguished on the videotapes. (2) zigzag: a quick dart in which a male moves first away from then towards a female (Wootton 1971) in a horizontal plane. This behaviour is observed predominantly during courtship but sometimes during agonistic interactions. Each movement away from or towards the image was scored as one zigzag.

We also recorded the time spent in each of two activities: (1) within two body lengths of the animated image; during this time males were almost invariably displaying towards or attacking the image; and (2) more than two body lengths away from the image but still oriented towards it; orientation was usually obvious, but was formally defined as occurring when the image was within approximately 45° of the body axis of the male subject.

All behavioral data were recorded on a personal computer using event-recorder software. Time budgets were calculated to the nearest second.

Data analysis

The majority of the data did not meet assumptions of normality even after standard transformations (e.g., logarithmic, arcsine, square root) had been conducted, so the nonparametric Friedman test was used throughout. In the present context, this test analyzes ranks of responses, determined for each subject male, to the differently coloured animations; variation among individuals in overall response levels is thus parcelled out of the analysis. Any variation in male response due to order of presentation was dealt with in the experimental design by having order balanced, or very nearly balanced (e.g., the red animation was presented to Cow Pasture Creek males first on eight occasions, second for eight different males, and third for eight other males). Nevertheless, variation in male response due to presentation order could have added random error, and preliminary analyses indeed indicated an effect of habituation, or a decline in responses in the second and third tests (Peeke et al. 1969). Consequently, we conducted more sophisticated parametric analyses to deal with this issue for those variables that did not grossly violate normality assumptions, after the most appropriate transformation. In these univariate repeated measures ANOVAs (Winer et al. 1991), within-individual variation due to colour of animation, order of presentation, and their interaction, and between-individual variation due to individual and sequence of animation presentation were analyzed. With regard to male responses to different coloured animations, the results of these ANOVAs were in all cases identical with the results of the Friedman tests. In addition, a weak effect of presentation sequence emerged in some analyses, perhaps due to an unintended pattern in the assignment of presentation sequences to males, but this result concerns intermale variation and should not affect the interpretation of intramale response variation due to animation colour. Because the Friedman test is more robust and simpler to present and interpret, only those results are included here.

Fig. 1. Median bite–butts directed by male threespine stickleback towards three different colour versions of animations of rival males (error bars indicate upper and lower quartiles) from the "red" Cow Pasture Creek population ($n = 24$) (a) and "black" Scatter Creek population ($n = 20$) (b).
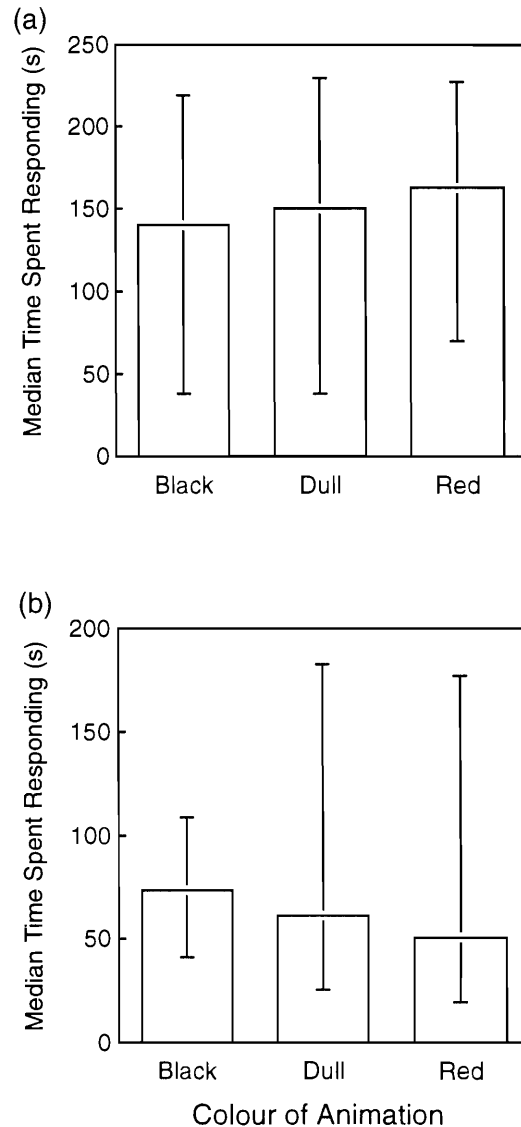

Fig. 2. Median time spent responding by male threespine stickleback towards three different colour versions of animations of rival males (error bars indicate upper and lower quartiles) from the "red" Cow Pasture Creek population ($n = 24$) (a) and "black" Scatter Creek population ($n = 20$) (b).
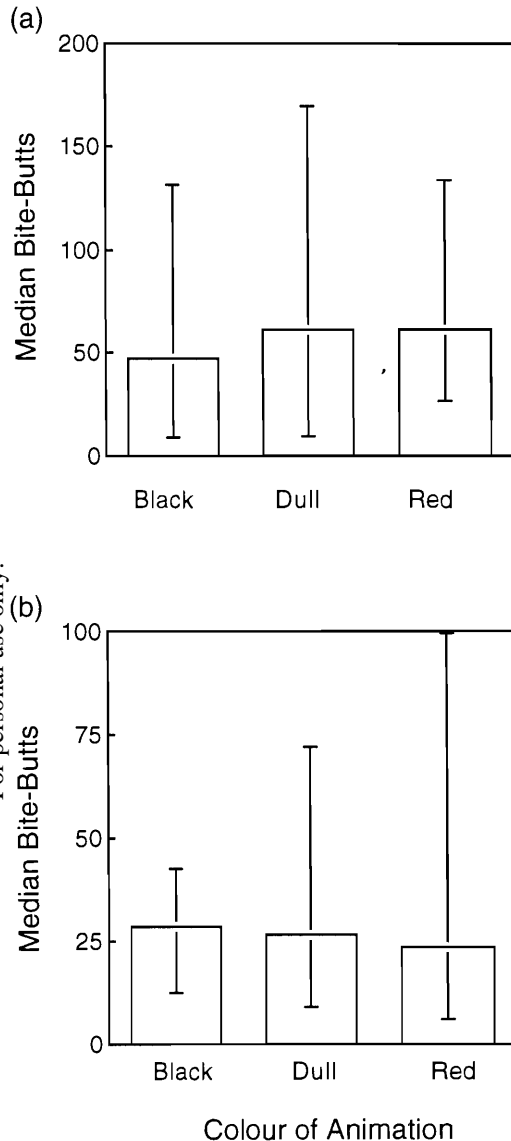

Results

Male stickleback of both populations responded to animated images much as they do to live males in glass tubes, to models (e.g., Wootton 1971; Rowland and Sevenster 1985), and to videotaped rivals (Rowland et al. 1995a). They bit at the images, often vigorously, spent much of their time following them closely or orienting towards them, and occasionally zigzagged at them (Figs. 1–3). There was great individual variation in all types of responses, as indicated by the error bars in the figures.

Male responses to the three colour variants of the animation of a simulated rival did not differ significantly (Figs. 1–3). This was true for all variables examined: response time ($\chi^2 = 2.90$, df = 2, $P > 0.20$ and $\chi^2 = 1.9$, df = 2, $P >$

0.30 for red and black populations, respectively), orientation time ($\chi^2 = 1.40$, df = 2, $P > 0.30$ and $\chi^2 = 5.43$, df = 2, $P > 0.05$ for red and black populations, respectively), bite–butts ($\chi^2 = 2.31$, df = 2, $P > 0.10$ and $\chi^2 = 0.08$, df = 2, $P > 0.50$ for red and black populations, respectively), and zigzags ($\chi^2 = 0.15$, df = 2, $P > 0.50$ and $\chi^2 = 3.33$, df = 2, $P > 0.10$ for red and black populations, respectively). Zigzags were observed only infrequently and hence are not presented in a figure. For all analyses, $n = 24$ for the red population and $n = 20$ for the black population.

Discussion

In the present study, responses of male threespine stickleback to animated images were similar to the responses of

Fig. 3. Median time spent orienting (at a distance of more than two body lengths) by male threespine stickleback towards three different colour versions of animations of rival males (error bars indicate upper and lower quartiles) from the "red" Cow Pasture Creek population ($n = 24$) (a) and "black" Scatter Creek population ($n = 20$) (b).
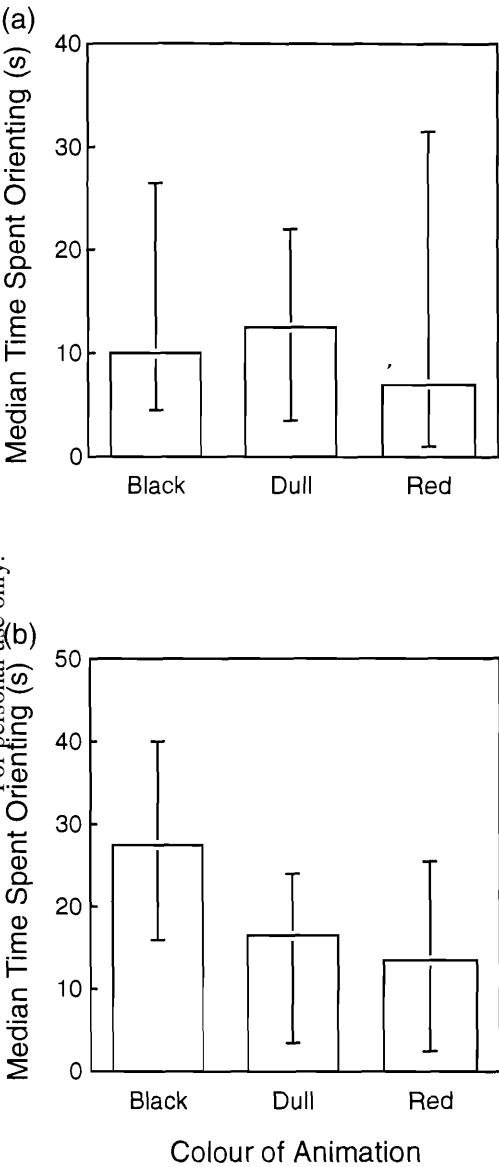
males in previous studies using different experimental stimuli (e.g., Rowland and Sevenster 1985), but males did not respond differentially to simulated rivals on the basis of the rival's coloration. This finding is consistent with some previous reports and inconsistent with others (summarized in Table 1). The literature suggests two potential explanations for these inconsistent results: variation in experimental methods or variation among study populations (also summarized for each study in Table 1).

Collias (1990; see also Rowland 1994) argues that the distance from the nest at which stimuli are presented can influence the results of experiments such as ours. In the present study, the animated image was presented at a relatively great distance from the nest, 31−62 cm (see Table 1

for comparison), and it might be argued that this led to the negative result obtained here. However, Wootton (1971) obtained similar negative results with another western North American population when models were presented only 20 cm from the nest.

The particular colours of models, live fish, and animations, as well as lighting and backgrounds, have been suggested as critical for explaining the inconsistent findings in the literature (e.g., Bakker and Milinski 1993; Rowland 1994). In some previous studies, little information is provided on the backgrounds utilized or the exact nature of the lighting (e.g., Wootton 1971), but Rowland (1982) and Peeke et al. (1969) do provide Munsell (1946) notations for the "red" portions of their models. In both cases they used a colour slightly more red and less orange than we utilized, but as previously noted, Rowland (1982) found that males bit less frequently at red-throated models, while Peeke et al. (1969) did not observe a difference in males' responses to models of different colours. Rowland et al. (1995a), however, found that intensity of coloration could affect agonistic colour discrimination in paired presentations: a video image with moderately intense colour was attacked more than images with either dull or bright coloration, whereas the brightly coloured and dull images were attacked at similar levels when presented together. But it is not possible to infer from this study which aspect of male coloration was critical for moderating agonistic responses. It continues to be unclear, therefore, whether some aspect of the simulated male's throat coloration is in itself the source of the variation in results among studies. It may play a role, but the contrast between body, throat, and background coloration might well be more important (Bakker and Milinski 1993; Rowland 1994; see also Baube et al. 1995; McDonald et al. 1995). Indeed, the dull image may have been attacked as much as black and red in the present study because it differed from the green background.

Rowland et al.'s (1995a) study raises an additional issue. In paired presentations, a given stimulus may elicit different relative responses depending upon the stimulus with which it is paired. Thus, it is possible that paired presentations might produce different results from the independent presentations used here. However, this was not the case in a study using both paired and independent presentations of red and grey dummies (Rowland 1982); similar results were obtained with each method.

Examination of Table 1 suggests that geographic variation may have contributed to the inconsistent findings. The three studies in which significant colour effects were not detected were all conducted with freshwater resident populations from the Pacific coast of North America, while studies in which colour effects were significant were conducted with Atlantic populations in Europe and on the east coast of North America. But if interpopulation differences are important, they are clearly not a function simply of distance, since Rowland and Sevenster (1985) obtained very similar results for Dutch and eastern U.S. populations. Based on mitochondrial DNA sequences, however, Atlantic basin populations do form an almost distinct clade relative to Pacific North American populations, but the separation of the two lineages is incomplete and probably occurred relatively recently (Orti et al. 1994). It is also apparent from Table 1 that habitat does

not consistently predict a population's pattern of agonistic colour discrimination.

In contrast to the inconsistent results obtained in studies of male agonistic colour discrimination, studies of females' mating preferences have yielded remarkably consistent results: females prefer males possessing intense red coloration over males with less intense red, grey, or otherwise dull coloration (reviewed by Bakker and Milinski 1993; Rowland 1994; see also Milinski and Bakker 1992; Bakker 1993; Bakker and Mundwiler 1994; Rowland et al. 1995b), although other conspicuous colour patterns can also be attractive (Baube et al. 1995; McKinnon 1995; McDonald et al. 1995). Thus, while there were several differences between the male images presented in our study of male agonistic colour discrimination and those used in a sister study of female choice (McKinnon 1995), the disparate results for males and females may reflect real differences. This general pattern may indicate a greater, more consistent role for female preferences in the evolution of male nuptial coloration (McLennan 1991). Alternatively, it may simply suggest that understanding the role of male interactions in nuptial colour evolution requires more sophisticated experiments (e.g., Baube 1996).

In conclusion, the present study suggests that male responses to the coloration of rival males are not coevolving closely with male coloration in divergent populations of stickleback. Moreover, males do not show the differential response to black- and red-throated images over dull-throated images observed in a study of female choice. The absence of male discrimination has been observed in other studies of stickleback from western North America, and thus may indicate geographic variation in such behaviour. However, methodological subtleties can affect the results of studies such as the present one, and further work is called for.

Acknowledgements

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