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Orange Spots as a Visual Cue for Female Mate Choice in the Guppy (*Poecilia reticulata*)

KEVIN D. LONG & ANNE E. HOUDE

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Abstract

Previous studies have suggested that orange pigment in the color patterns of male guppies is a cue for female choice. This paper describes a manipulative experiment designed to test this hypothesis. The color patterns perceived by females were manipulated by varying the color of light used to illuminate the experimental aquaria. Orange light dramatically reduces the conspicuousness of orange spots to human observers, and probably also to female guppies. As in previous experiments, female guppies discriminated among males based on differences in the extent of orange pigment, under white, blue, and green light conditions. Under orange light, however, females no longer appeared to discriminate on the basis of orange spots. These results support the hypothesis that orange spots, rather than other correlated characteristics, are a basis for female choice under normal lighting conditions.

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Introduction

The guppy, *Poecilia reticulata,* provides a good illustration of sexual selection by female choice. Evidence suggests that color pattern is a cue for female choice in the guppy (ENDLER 1983; KODRIC-BROWN 1985; HOUDE 1987). HOUDE (1987) reported that females appear to use orange spots as a cue for mate choice in a guppy population from the Paria river of Trinidad. This population is characterized by large conspicuous orange spots, and striking variation among males in the extent of orange in the color pattern. HOUDE's experiments provided strong correlative evidence that Paria females discriminate among males on the basis of differences in the extent of orange, but could not rule out the possibility that the

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actual cue used by females was some other character correlated with orange spots rather than the spots themselves. This paper reports a manipulative experiment designed to test the hypothesis that orange pigment in male color patterns affects the mating preferences of female guppies. We manipulated the male color pattern experimentally by using orange, green and blue theatrical gels to alter the color of incident light in test aquaria.

The perception of an object's color depends upon the distribution of wavelengths reflected at the object's surface (the reflectance spectrum). Reflectance, and hence the perception of color, can be altered by varying the wavelength of incident light, as well as by altering the object itself. For a complete discussion of color physics and vision theory, see FALK et al. (1986). This experiment depends on the observation that the spots (in particular orange spots) and the background areas (unpigmented skin) of Paria guppies appear different when the wavelength of incident light is altered. When altering the wavelength composition of incident light the change in composition of light reflected from adjacent unpigmented skin areas relative to that reflected from orange spots is critical to the conspicuousness of orange spots in the overall color pattern. Under orange light, the composition of light reflected from adjacent skin areas is similar to that reflected from orange spots. When Paria guppies are observed by humans under orange light, orange spots become more difficult to distinguish from unpigmented areas, and variation between males in the extent of orange becomes less obvious. Under blue light, which is complementary to orange, orange spots reflect little light and appear almost black, while adjacent unpigmented areas reflect most of the incident blue-shifted light. When Paria guppies are observed under blue light, orange spots contrast sharply with background areas and are easily distinguished. Under green light, orange spots appear little changed to humans. If changes in light color similarly affect how female guppies perceive male color patterns, then we would expect to see corresponding changes in mating preferences.

Specifically, we expected orange light to reduce the differences in the extent of orange in male color patterns perceived by female guppies, and so we predicted that mating preferences based upon orange spots should be reduced or eliminated under orange light. Two factors could contribute to female preferences based on orange spots. First, the total amount of orange reflected by the color pattern could be important. Second, the effect of orange spots on the overall appearance of "spottedness" of the color pattern could be important. We attempted to distinguish these possibilities with the blue-light treatment. If orange reflectance itself is important, then a blue filter should reduce or eliminate the ability of females to discriminate because much or all of the incident orange light is blocked. As a result all males would appear lacking in orange reflectance. If general spottedness of the color pattern is important then females should continue to discriminate under blue light because orange spots appear, if anything, more distinct. The green-light treatment was included to control for the possibility that green-reflecting parts of the color pattern might be important to female preferences.

Material and Methods

The guppy is a small freshwater fish found in streams and rivers of northern South America, Trinidad and adjacent islands. Males are polychromatic while the females appear whitish to dull grey. The male color pattern consists of orange, blue, green, yellow, silver and black spots distributed over much of the body surface. These spots vary from fish to fish and from population to population (ENDLER 1983). Mature males are almost always ready to mate. Prior to mating, the male courts the female by presenting himself broadside and in close view of the female. He assumes a rigid S-shaped posture and quivers vertically with spread fin rays. A responsive female usually ceases her previous activity, arches her body laterally and moves slowly toward the male in a gliding motion distinct from normal swimming patterns. The male then swims next to the female and may insert his modified anal fin, the gonopodium, into her genital pore to transfer sperm. A more detailed description of the courtship behavior is given by BAERENDS et al. (1955) and LILEY (1966). Females are receptive only when they become sexually mature and in brief periods immediately following the birth of a litter.

The guppies used in this experiment were descendants from a stock collected from the Paria River in the Northern Range, Trinidad. Females selected randomly from the stock were isolated in 10-l aquaria and their young were reared. Males and females were separated at 4 to 6 weeks of age under MS-222 anaesthesia, and were reared to maturity in 40-l aquaria (two litters of up to 12 offspring per aquarium). All fish were fed ad libitum twice daily, with liver paste in the morning and newly hatched brine shrimp nauplii in the evening. The observation and breeding rooms were kept at an air temperature of 22—27 °C, with a 12 : 12 light : dark cycle. All experimental observations were made from 08.00 to 09.00 h, before feeding. Guppies in the laboratory were sexually most active and their behavior was most consistent at that time (ENDLER 1987; HOUDE, pers. obs.).

To quantify the surface areas of each color on the experimental males drawings were made by placing the males in a narrow viewing chamber. The size, shape, position and color of each element in the color pattern were sketched on blank outline diagrams of guppy profiles. The area of each color spot was calculated using the digitizer board of an HP-85 computer. The color patterns are usually symmetric on both sides of the fish. The average of both sides for the total area of each color was used when asymmetry was present. In addition, the total body area of each fish in profile (including the caudal fin but not other fins) was calculated. The total area of orange spots divided by the total body area was used as a measure of the relative amount of orange in the color pattern. In repeated scoring of the same individuals, this method of measuring the extent of orange in color patterns gave 95 % repeatability (A. HOUDE, unpubl. data).

Sexually experienced males were selected according to the amount of orange pigment they possessed relative to their total body area. Four males matched to within one month in age were used in each trial. Males were generally similar in size within groups. Each group of 4 males consisted of one male with a small amount of orange, one with a large amount of orange, and two intermediate males. The 4 males were then acclimated for 2 days in a 189-l observation tank containing 6 females and coarse, neutral color gravel.

White light was provided by two 100-W "soft" white incandescent bulbs. The lights were positioned 30 cm apart, 50 cm over the water surface to illuminate the aquarium as uniformly as possible. Colored light was provided by filtering the white light through theatrical gels (Rosco). Transmission spectra of the filters used, redrawn from data provided by the manufacturer, are given in Fig. 1. As described above, the orange filters made the orange spots of Paria guppies appear indistinct to our eyes, and the green filter had little effect on the appearance of the color pattern. The blue filter, however, was nearly, but not completely effective in blocking red-orange wavelengths (see also Fig. 1). Under blue light, orange spots appeared to us to be perceptibly reddish, though nearly black.

We were concerned that differences in the total level of illumination under the different light regimes could affect female behavior and confound the effects of differences in wavelength. We attempted to balance the level of illumination between treatments crudely using a camera photometer and a rheostat (see below for further discussion of this adjustment). Relative voltage levels for the four treatments were as follows: blue > orange > green > white. Varying the voltage supplied to aquarium lights probably altered the spectra of incident light relative to those shown in Fig. 1 because incandescent bulbs produce cooler (bluer) light at high voltage and warmer (redder) light at low voltage. This difference in color temperature should have enhanced the difference between the blue and orange light treatments.

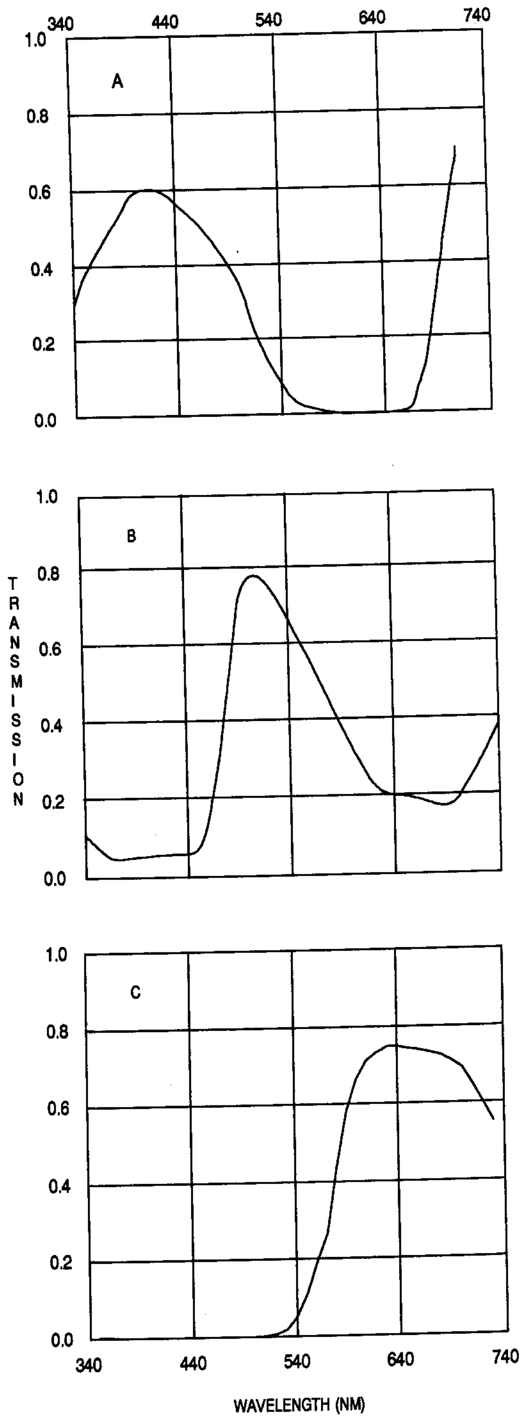

Fig. 1: Transmittance spectra of filters: A, blue light treatment (filter no. 80); B, green light treatment (filter no. 86a); C, orange light treatment (filter nos. 20 and 23 used together)

Each group of males was observed first under white light and then under orange, green and blue light in random order. A different group of females was used with each light color. Observations were conducted on two successive days for each color in the trial. Each trial was completed in two weeks. Four such trials, each involving four males, were run.

One day prior to each observation session, 6 virgin female guppies, matched for size, were selected and placed in the observation tank. The males were allowed to court and mate with the females freely for 24 h prior to the observation session. It was not possible to conduct observations immediately upon introduction of the females because most virgin females copulate initially within 30 min of introduction to males. After a copulation, males cease all courting behavior and females become unresponsive for several min to an hour, making observations difficult. After 24 h however, females remain sexually responsive to male displays but the frequency of copulations during observation sessions is reduced.

In each trial, observations were made by scanning the tank for courtship behavior for 40 min at a distance of 60 cm from the aquarium. A male courting a female was watched until the courtship ceased, then scanning continued until another pair was observed. When a male was observed courting a female, his identity, number of displays for that particular pairing, and whether or not the female responded to each display were recorded. The female's characteristic gliding motion toward the male (LILEY 1966) indicated a sexual response to a male. Each group of females was observed on two successive mornings and then was removed. The two days' observations were summed and a measure of each male's attractiveness was found by dividing the number of positive responses by the total number of displays for that male. HOUDE (1988) found that the fraction of displays that elicit responses is positively correlated with male mating success. This variable was not transformed because most values fell between 20 % and 80 % and did not deviate significantly from normality. Relative area of orange also follows a normal distribution. Analysis of covariance (SAS General Linear Models program, SAS, Inc. 1982) was used to compare the pattern of female preferences between light-color treatments.

Results

Observations of the four sets of males yielded average female preference curves for each colored light treatment (Fig. 2). The curves are averages because the data represent the combined responses of several females in each trial. The graphs show the effect of male orangeness on the fraction of displays eliciting a female response. The extent of orange in a male's color pattern had a significant effect on female responsiveness under white, blue and green light (Fig. 2, Table 1). Females tended to respond more frequently to males with a greater area of orange than to males with less orange. Under orange light, however, the amount of orange in male color patterns did not affect the responsiveness of females (Fig. 2, Table 1). In this treatment, all males elicited responses at about the same rate that males with little or no orange did under white light. Best-fit

Table 1: Best fit linear regression equations for the effect of orange on female responsiveness under the four light color treatments

Light color	Slope	Intercept
White	0.022*	0.34
Orange	0.000 ns	0.29
Green	0.019*	0.27
Blue	0.018*	0.24

* $p < 0.001$; ns $p > 0.5$

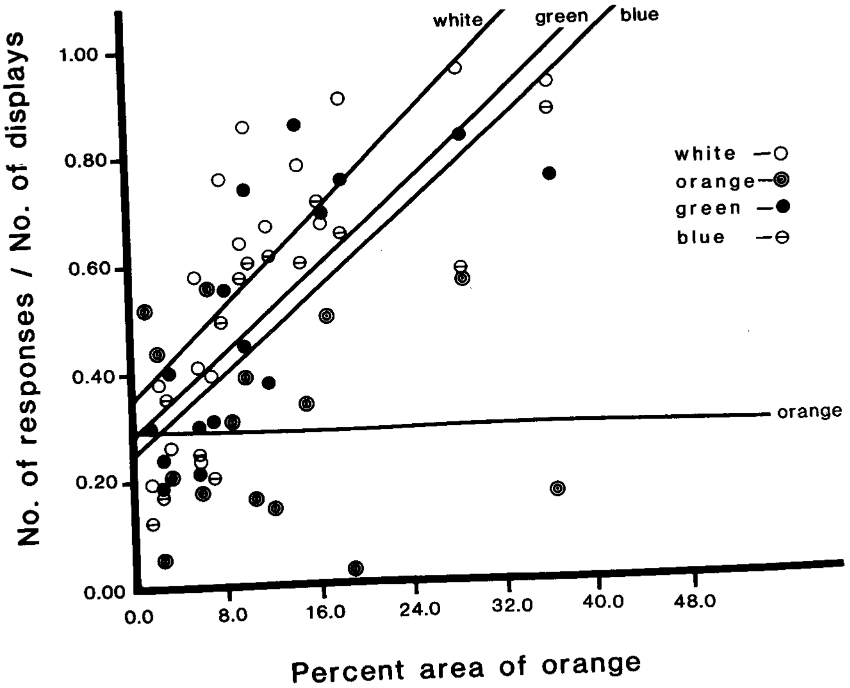

Fig. 2: Average female preference curves for the four light-color treatments. The fraction of each male's display that elicited a female response is plotted against the relative area of orange in his color pattern for each of the light-color treatments. Linear regression lines are plotted for each treatment

lines for each treatment were found by regressing female responsiveness against the relative area of orange in each male's color pattern (Table 1). The significant interaction term in the analysis of covariance (FREUND & LITTELL 1981) indicates significant differences in the linear regression coefficients (slopes) between light-color treatments (Table 2). The slope for the orange-light treatment was significantly different from the slopes for each of the other light-color treatments (t tests, $p < 0.01$ in each case). The slopes under white, blue, and green light did not differ significantly from one another (t tests, $p > 0.5$ for all comparisons).

Table 2: Analysis of covariance for the effect of amount of orange on female responsiveness. Interaction term tests for differences in slopes among light color treatments. F-tests are based on type I sums of squares. $R^2 = 0.64$

Source	V value	df	Significance
Light color	10.55	3	$p < 0.001$
Amount of orange	50.14	1	$p < 0.001$
Interaction	5.59	3	$p < 0.010$

Discussion

The orange-light treatment reduced or eliminated the ability of females to discriminate among males on the basis of differences in orange spots. The fact that the pattern of discrimination by females could be altered by manipulating the perceived color pattern implies that the orange spots themselves, rather than any correlated character, are used in mate choice by females.

A similar effect of variation in naturally occurring incident light spectra on conspicuousness of color pattern has been suggested by REIMCHEN (1989; see also ENDLER 1986 for a general discussion of factors affecting color pattern conspicuousness). Threespine sticklebacks (*Gasterosteus aculeatus*) in a sample of populations from the Queen Charlotte Archipelago of British Columbia tended to have red throat patches most often in habitats with clear water and black throat patches in habitats with heavily stained water. REIMCHEN (1989) argued that heavily stained water makes incident light red-shifted, leading to a reduction in conspicuousness of red throat patches and consequent selection for more conspicuous black throat patches. This is analogous to the loss of conspicuousness of orange spots under the orange-light treatment.

The effects of the light-color treatments on our perception of male color patterns was dramatic and our results are consistent with the assumption that the treatments had similar effects on perception by female guppies. However, our photometer was probably not adequate to allow us to accurately balance the level of illumination between treatments. As a result, we cannot entirely rule out the possibility that differences in light intensity (either actual or as perceived by female guppies) affected the results. Levels of illumination between treatments appeared similar to us, so we feel that the observed differences in female preference are more likely to have resulted from effects of color of light rather than intensity. Furthermore it is not clear that balancing the light levels to equal photon flux is the appropriate adjustment, given that the spectral sensitivity of guppies varies with wavelength (see ENDLER 1986, Fig. 3a). Therefore, we believe that our relatively subjective method of adjusting light levels was adequate, though it would have been ideal to make the adjustments with corrections for the actual sensitivity of female guppies to each light color.

That females continued to discriminate among males under blue lighting could mean that orange light reflected from male color patterns may not be as important as the contrast of the orange spots with background skin or gravel color. ENDLER's (1983) experiments on the effect of background (gravel) coloration showed that males that had similar coloration to the background and therefore contrasted little had lower mating success than highly contrasting males. The orange coloration of the males contrasts with the gravel background under natural lighting, and contributes to the spotted appearance of male color patterns. Under orange light, reflection of orange by unpigmented skin areas and by background gravel of the observation tank could have reduced the conspicuousness and spottedness of male color patterns. This could contribute to the lack of female discrimination under orange light. However, it is unclear whether contrast is the only basis for discrimination because complete masking of the orange color was not possible using the blue gels available. Under blue light, orange spots appeared dark red to human observers, and possibly to female guppies. Thus it is still possible that long wavelength light reflected from orange spots is important.

Under orange light, female guppies responded to all males at a low level, comparable to response to males with little orange under normal light conditions. This has several possible explanations. Under orange light, all males should be reflecting large amounts of orange, from unpigmented body areas as well as from orange spots. One possibility is that reflecting extremely large amounts of orange made males unattractive to females. Previous experiments (HOUDE 1987) have suggested that, even under natural light, females may discriminate against males with very large amounts of orange pigment. Alternatively, large amounts of orange light reflected by aquarium gravel as well as by background skin areas could reduce the conspicuousness of all males. This could further reduce the overall level of female response.

Finally, visual adaptation and photoreceptor bleaching could explain the low level of response. There is good evidence for the presence of orange-red sensitive receptors in guppies (LEVINE & MACNICHOL 1979; ARCHER et al. 1987). The photoreceptors most sensitive to orange light would be constantly stimulated by the incoming orange rays. This overstimulation may reduce the firing of the photoreceptors by breaking down the photopigments, causing greatly reduced receptor sensitivity to orange coloration. As a result, males would appear to be uniformly lacking in orange and thus unattractive to females. Mechanisms for this visual adaptation are given in NEUMEYER (1984).

The results presented here support the hypothesis that orange spots are important in female choice and suggest that choice is unlikely to depend on a character that is merely correlated with orangeness. The results under blue light suggest that the contrast of orange spots against the background of the fish and its environment may be an important factor in female choice. Orange coloration itself may still be important, however, because the blue filter did not eliminate all wavelengths reflected by orange spots. A blue filter that more precisely complements the orange pigment of Paria guppies could clarify these results. Ultimately, it will be critical to study the ultrastructure of the guppy retina and to determine the wavelengths at which guppies are most sensitive.

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