Female preferences for single and combined traits in computer animated stickleback males

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In many animal species, males have more than one secondary sexual character. Apart from theoretical considerations about the evolution of multiple traits, there are almost no empirical studies on female mate choice decisions based on combinations of traits as opposed to decisions based on single traits. Because three-spined sticklebacks are exceptionally well suited to be tested with computer animation technique, which itself fills the gap of adequate test paradigms for multiple traits, we tested female sticklebacks for their preferences for both single and combined male traits. We used virtual stickleback males that differed either in red throat coloration, courtship intensity, body size, or in combinations of these traits. The virtual male with increased redness was found to be preferred by females, whereas the male courting more intensely was not. The tests for combinations of traits revealed the more pronounced female preferences, the more traits were available to the females to judge male quality. *Key words:* computer animation, courtship, redness, female mate preference, *Gasterosteus aculeatus,* multiple traits. *[Behav Ecol 12:681–685 (2001)]*

I n species with female mate choice, males often have a number of morphological and behavioral traits in order to attract potential mates (e.g., Buchanan and Catchpole, 1997; Dale and Slagsvold, 1996; Morris, 1998). These multicomponent displays often combine different sensory modalities simultaneously. The evolution of multiple secondary sexual traits is explained by several hypotheses (Johnstone, 1997; Møller and Pomiankowski, 1993; and references in both). Empirical studies clearly lag behind these developments, which may be due to the difficulties of performing genetical studies (Bakker and Pomiankowski, 1995) or to technical limitations of available test paradigms. Video editing and computer animation techniques open the possibility of precise experimental manipulation of single male traits and combinations thereof through the creation of virtual stimuli.

There are critical attitudes concerning the usage of video techniques in behavioral ecology in general (e.g., Oliveira et al., 2000). Television and computer systems are designed to imitate color and motion for the human eye. It is obvious that such images may be perceived differently by an animal species that differs in aspects of visual processing. For an animal, a screen may be a flickering, light emitting object. The frequency at which a flickering stimulus starts to appear continuous, known as the critical flicker-fusion frequency (CFF), and the number and characteristics of light-sensitive pigment cells varies greatly across species (D'Eath, 1998). However, the most important aspects of the visual system of three-spined sticklebacks (*Gasterosteus aculeatus* L.) are very similar to those of humans (see below). For the following reasons, as we have shown in Künzler and Bakker (1998), our experimental set-up using virtual stickleback males is a valid test paradigm for the measure of female preferences for single and combined male traits. We use flicker-free computer animation movies presented at 30 frames per second (fps) on a 120 Hz Trinitron computer display. Sensitivities of stickleback photoreceptors

match those of humans (see Methods section), which means that colors are correctly reproduced for sticklebacks, additional depth cues like water turbidness and shadows help in simulating a third dimension, and last but not least, ready-to-spawn female sticklebacks react with a sexual response: they court the virtual males by showing the typical head-up posture, and they try to swim towards their preferred mating partner (see Methods section below for more detailed presentation; see Künzler and Bakker [1998] for a precise description of the method).

In the present study, we focused on stickleback females' preferences in an experimental system, where mate choice decisions could be based on one isolated trait or on combinations of traits. Stickleback males have several secondary sexual characters in addition to red throat coloration, which may be of importance to the females. Some of these traits are known for sticklebacks to be under sexual selection pressure by female mate choice, for example courtship intensity (Rowland, 1995; stabilizing selection), others such as body condition, body size, and eye color still lack experimental verification. The methodological set-up we used allows the alteration of the expression of a single trait independently of other traits within a virtual stimulus individual. We expect stronger preferences towards the high-quality male when indicated by multiple traits compared to preference tests based on only one male sexual characteristic.

MATERIALS AND METHODS

First, we present the most important arguments to underline the exceptional situation concerning three-spined sticklebacks and our computer animation set-up.

Screen refresh rate, presentation rate

The usage of a personal computer with a computer display (PC) instead of television equipment (TV) has several advantages. Although many authors in the field generalize and use the term "video" for all kind of systems (but see Rosenthal, 2000), PC equipment has important technical improvements compared to TV equipment. A standard PAL TV screen uses a scheme, called interlace scanning, to refresh the screen in two top-to-bottom passes, so that the lines scanned in one pass

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are positioned between lines drawn in the previous pass. These passes are called fields, the first field containing all the odd numbered lines, and the second field containing all the even numbered lines. A field therefore contains one half of the screen resolution. Together, the first and the second field make an entire image, called a frame. Interlace scanning limits image quality, introduces artifacts and constrains visible detail, due to overlap of subsequent fields caused by a memory effect of the light-emitting elements of a screen, called phosphors. The often-mentioned 50 Hertz for TV systems (60Hz for NTSC) corresponds to 50 fields per second. Focusing on a single pixel of a PAL screen, it is in fact redrawn only 25 times per second (30 for NTSC), so this is the value to be compared with refresh rates of other systems or with the CFF of a given animal species. These limitations are also valid for higher quality video systems such as Hi8 or SuperVHS, because these systems are also based on interlaced recording/playback and scanning.

A computer display uses progressive scanning for screen refresh and renders all lines in a single top-to-bottom pass. This requires that the horizontal scanning rate be doubled to scan twice the number of lines compared to interlace scanning. ''Line doubling,'' ''deinterlacing'' and ''noninterlaced refresh'' are synonyms for this scanning mode. Computer displays have even higher refresh rates. The SONY monitor we use in our experimental set-up (see below) rescans at 120 full frames per second, which is far above CFF both for humans (Fleishman and Endler, 2000) and most fish (e.g., D'Eath, 1998). Together with the computer-generated presentation rate (term from Fleishman and Endler, 2000) of 30fps—which means full frames, not fields—we can be sure that our female sticklebacks perceive a flicker-free movie of continuous and smooth motion, because the threshold for motion perception for fish is supposed to be much lower than 30 images per second (Fleishman and Endler, 2000; Oliveira et al., 2000). Note that every frame of the animation movie is refreshed exactly four times by the computer display (120Hz divided by 30fps).

Pixel spacing

A pixel of a color screen is built up by three so-called subpixels, which are phosphor elements of the three basic colors red (R), green (G), and blue (B). The grid pattern of these primaries in standard PC or TV screens (with classical Shadow Mask) can cause spatially random variation in perceived color (see Fleishman and Endler, 2000: Figure 7). This is not the case for SONY Trinitron computer displays (with Aperture Grille), because their phosphor elements are arranged much closer to each other in uninterrupted columns. Because of this, even a screen area as small as the size of a single pixel contains equal proportions of the three primaries.

Color perception

Color perception is probably the most debated issue. Animals in general are indeed not suited to be tested with any PC or TV equipment, because species differ in number and kind of photoreceptor cells and their spectral sensitivity (D'Eath, 1998; Goldsmith, 1990), and therefore the colors rendered on a screen do not reflect the natural appearance of a given object. Color reproducing devices designed for humans are capable of fooling the visual system of *Homo sapiens ssp.* because they stimulate the three cone classes R, G, and B at the appropriate ratios, that is identical to the ratios produced by a given stimulus spectrum. This is possible because for each of a screen's three primaries for example, the phosphor is selected to match the sensitivity of the corresponding human

cone class, and because the spectrum emitted by a given phosphor does almost only stimulate the receptors of the desired cone class. Because sticklebacks have three cone classes with sensitivity maxima (B 452/G 529/R 604nm; Lythgoe, 1979; B 435/G 530/R 570–610nm; Baube CL, personal communication, and cited by Rowland et al., 1995) very similar to humans (B 435/G 534/R 560nm; Boynton, 1979), a SONY Trinitron computer display (B 430–470/G 510–530/R 610–620nm; McDonald et al., 1995) can also be used for our study species. From Fleishman et al. (1998; based on Brainard, 1995), RGB values may be corrected for species with slightly different cone sensitivities compared to humans, in order to make colors appear correctly for those animals. Precalculations (Künzler, unpublished data) for sticklebacks showed that the differences between uncorrected and corrected colors would be difficult to detect for humans and therefore needless. To our knowledge, there are no indications of UV or IR vision in sticklebacks. The presence of an ultraviolet visual pigment, if it is discovered, would suggest that the blue coloration of the eye of the fish may not be rendered in natural colors by a computer image, however the red coloration, which was the ornament manipulated in this study, would likely be rendered accurately (Cuthill et al., 2000).

Depth cues

It is clear that a computer display lacks the third dimension. It is unknown whether fish do have and use depth vision at all (Zeil, 2000). Computer animation technique offers several possibilities to simulate or indicate elements of a 3rd dimension on a two-dimensional screen. Water turbidness can be simulated with a fog effect. This means that approaching virtual fish become sharper, more contrasted and easier to distinguish from the background. The virtual sun offers another key to depth perception: the distance between an object and its shadow on the ground changes relative to the movement of the object towards or away from the viewer.

Testing procedure

Female sticklebacks were hand-netted from a small water channel in Roche (Switzerland, 42°26′ N, 06°55′ E) in spring 1999 before and during the breeding season. They were kept in large group tanks in a climatized fish room (18°C) on a 16:8 h light:dark cycle and fed daily ad libitum with frozen red *Chironomus* larvae in the later afternoon. Ripe females were selected every morning and transferred to the climatized test room (18°C). Before the female entered the experiment, her standard length and wet weight were measured. Female condition was calculated as $100*$body mass in g/standard length^3 in cm (Bolger and Connolly, 1989) and included eggmass before spawning. Females were gently put into 1-l containers and allowed to acclimatize to temperature and light conditions of the test set-up for 30 min in a pre-test compartment without computer display. Shortly before the start of the experiment, the container with the female was put in front of the computer display. The following test procedure is detailed in Künzler and Bakker (1998). Briefly, we offered a simultaneous choice between two copies of a realistic model of a courting stickleback male, that was built upon a typical male differing either in courtship, redness, or in a combination of both. Male positions were alternated between tests and all females were used only once. Preference data of the choosing females were recorded on videotapes (2 min 20 s per test) taken from above to allow naïve analysis with respect to which male was on which side of the display. A preference index was calculated as the proportion of the female's total time oriented towards one male divided by the time directed to both

Throat colouration
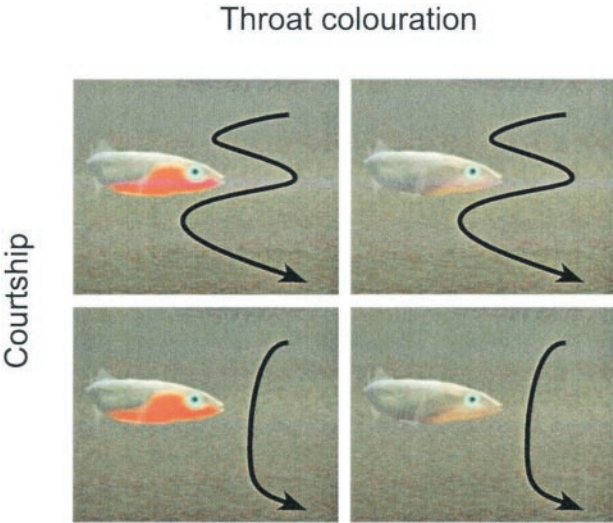
Figure 1
Possible virtual male stickleback models after combination of the two male traits throat coloration and courtship intensity.

males. Only females that courted and followed a live male to its nest shortly after the test were used.

The original bright red male model (see Künzler and Bakker, 1998) had a colored throat area which covered 16.1% of the total body area on a lateral view (measured without fins and eye). This throat area was enlarged by 191% for the a priori high-quality male, resulting in a throat area that covered 31% of the body. For the low-quality male, the original throat area was reduced in size by 57%, leaving a larger proportion of the body nonred (throat area 9% of total body area). Using standard color chips, we carefully matched the redness of the virtual males to those on standardized photographic slides taken of the throat of courting males in the field. For this study, we selected red and dull males from the tails of the distribution of throat coloration based on densitometer-analysis (Bakker, 1993). For the high-quality male, we chose a bright red (hue/saturation/brightness, HSB 18°/84%/92% as determined with Adobe Photoshop V4.0.1), and for the low-quality male a faint pale red (HSB 28°/45%/65%). With the combination of throat area and coloration, we could generate a maximum difference between the red and dull male model. Courtship behavior was modified in order to achieve a maximum difference between the two males presented for the second trait under investigation as well. While one male courted on the original zigzag path (the a priori high-quality male), the low-quality male approached the female directly on a straight path without any zigzags or stops. The swimming speed of the straightly courting male was constant and adjusted in order to have both males starting from the nest at the same time and reaching the frontmost position again at the same time. The tracks back to the nest of both males were identical.

With the four different male models (Figure 1) described above, a total of six test movies could be produced. Besides the movies for preference tests for single traits, that is either degree of redness with two levels of courtship (both males zigzag or both males court in a straight line) or courtship with two levels of redness, this design allowed two tests for combined traits: A bright red and zigzagging male was presented together with a dull and straightly approaching male, which meant that two "concordant" traits pointed to the high-quality male. The last possibility was a test between a bright red

Table 1
Pearson correlation coefficients *(r)* between preference index and potential covariates

Covariate	*r*	*p*
Body size (standard length)	.02	.83
Wet weight	−.01	.89
Condition factor	−.08	.41
Total time oriented to males	.13	.15
No. of days in laboratory	−.01	.95
Time of day	−.05	.58

Sample size is 120.

and straightly courting male versus a pale and zigzagging competitor, a situation with two "contradictory" traits.

For an extra test, we added male body size as a third trait. Larger males were found to be preferred by females in another experiment (Künzler, unpublished data). A seventh movie was produced with a concordant combination of all three traits, meaning that a bright red, zigzagging large male was opposed to a dull, straightly courting small male. Body sizes were either enlarged to 120% or reduced to 80% compared to the original male model.

Variables were normally distributed according to Shapiro-Wilk W test. P-values are two-tailed unless stated otherwise. Analyses were performed using JPM-IN (SAS Institute, v3.2.1) statistical software.

RESULTS

A total of 140 females were tested. Except for one treatment, we could formulate a priori expectations about which male should be preferred (red over dull, and zigzagging over courting in a straight line). In these cases, an index greater than 0.5 indicates a preference for the high-quality male, smaller than 0.5 a preference in the non-expected direction, respectively. As for the one contradictory trait combination, we assumed coloration to be more important than courtship (see Milinski and Bakker, 1990) and defined the red, straightly courting male to be of higher quality than the dull, zigzagging one.

Although the females of the six treatments differed in mean condition and the number of days they had been kept in the laboratory prior to the test (ANOVA, $F_{5,114} = 2.69$, $p = .025$ and $F_{5,114} = 2.43$, $p = 0.039$, respectively), there was no significant linear relationship between these and other potential covariates and the measured preference index (all $p > .15$, Table 1). We therefore performed all further analyses without correcting the preference indices.

The preference index was significantly different between all six treatments (ANOVA, $F_{5,114} = 2.51$, $p = 0.034$), although all but one of the indices were higher than 0.5 (Figure 2). We performed a series of paired *t* tests within treatments for a comparison of the female's time spent oriented towards the preferred against the time toward the nonpreferred male (Table 2). In both tests with different red coloration but equal courtship behavior females significantly preferred the redder male. We found no significant preference in females of the two tests with different courtship behavior but equal redness. In the test for the concordant combination of traits females showed a highly significant preference for the red, zigzagging male. In the contradictory trait combination test, the mean preference index was slightly but not significantly below 0.5.

The comparison of preferences for one and combinations of two and three concordant traits (two levels of redness pooled, $N = 40$ females; redness and courtship, $N = 20$; red-
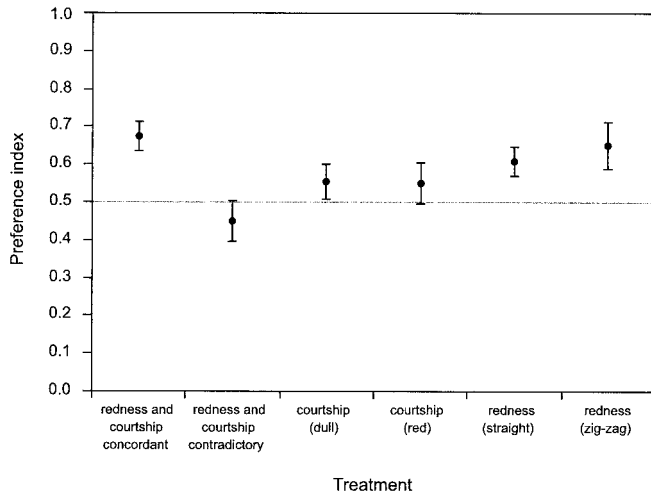
Figure 2
Preference index (mean ± SE) for single and combined male traits ($N = 20$ females per treatment). The treatments are labeled with the traits that differed for a given treatment with the level of the constant trait in brackets in the case of tests for single traits. Concordant means that both traits indicate male quality in the same direction, contradictory in opposite directions. A preference index greater than 0.5 means a choice for the high-quality male. The horizontal line indicates the index of 0.5, that is no choice.

ness, courtship and body size using the extra movie, $N = 20$) revealed a stronger preference for the high-quality male, the more traits were available for females to base their choice on (ordered heterogeneity test, Rice and Gaines [1994]; based on ANOVA, $F_{2,77} = 1.75$, $P = .18$; $r_s = 1$, $p_c = .82$, $p_{directed} = .045$; Figure 3).

Because females had to spawn with a real male immediately after the test, they could only be tested once. All females were used only once, yielding independent data across all seven treatments that do not allow tests for nonadditive, that is, interaction effects.

DISCUSSION

This study provides the first experimental evidence that female sticklebacks show stronger preferences for males that differ in concordant combinations of traits than for males that differ in only one trait or in a contradictory combination of traits. The combination of bright and extensive red throat coloration with high courtship intensity was strongly preferred by female sticklebacks. The mean preference index in this experiment was higher than in all four single-trait tests (Figure 2). As in earlier studies (e.g., Künzler and Bakker, 1998; Milinski and Bakker, 1990), the redder male was clearly preferred over the dull male, independently of whether both males zigzagged or both males courted in a straight line. In both tests for courtship intensity, no significant preference for zigzagging over courting in a straight line could be found (as in Milinski and Bakker, 1990; but see Rowland, 1995), no matter whether both males were red or both males were dull. It is of course possible that females would respond to tactile, chemical, or visual aspects of courtship other than the ones we tested, such as the zigzagging angles or the proportion of the time the male spends very close to the female in front of the scene.

In the experiment with the contradictory combination of traits, females seemed to be puzzled by the fact that one trait was advertising high quality of one male while the other trait indicated the other male to be of better quality. The individ-

Table 2
Paired comparisons (*t* test) of time spent oriented toward the high-quality and the low-quality male

High-quality male	Low-quality male	t	p
Red, zigzag	Dull, straight	4.03	<.001
Red, straight	Dull, zigzag	−0.63	.538
Dull, zigzag	Dull, straight	1.28	.217
Red, zigzag	Red, straight	1.02	.319
Red, straight	Dull, straight	2.68	.015
Red, zigzag	Dull, zigzag	2.22	.039

$N = 20$ per treatment. There were six treatments in which males differed in two traits (upper two rows) or in a single trait (lower four rows).

ual choices of the 20 females for either the redder (and not zigzagging) or zigzagging (and dull) male were not correlated to any of the additional female variables that we measured, leaving open the possibility that we did not measure the crucial variable.

Courtship intensity and red throat coloration may be correlated in real males (Bakker and Milinski, 1991). The results of our preference tests suggest that at least the interpretation of red coloration by female sticklebacks depends on male courtship: on average the redder male was preferred when courting more intensely than his rival but not so when his duller rival was courting more intensely, while more vigorous courtship was not preferred when both males had equal nuptial coloration. We suggest an additive mixture of color (stronger effect) and courtship (weaker effect) to be responsible for the presented results. With our simplification of the courtship movements for the straightly courting male, we did not only change the shape of the courtship path, but also decreased absolute swimming speed, which may be an important aspect of courtship itself.

The addition of male body size as a third concordant male trait revealed an even stronger preference toward the high-quality male in the choosing females compared to the test
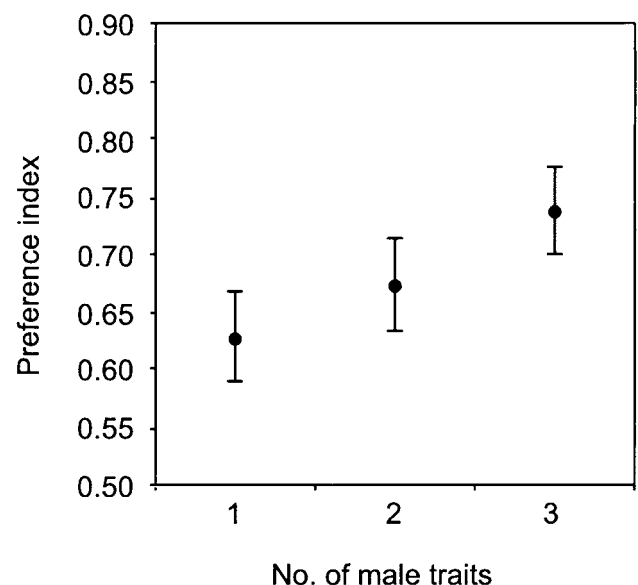
Figure 3
Preference index (mean ± SE) for one ($N = 40$ females, redness), two ($N = 20$, redness and courtship), or three ($N = 20$, redness, courtship and body size) concordant male traits.

with two concordant traits. Larger males are reported to be preferred in several species (e.g., Backwell and Passmore, 1996; Kraak et al., 1999) and differences in body size are often linked to dominance also in sticklebacks (Rowland, 1989). Because both the enlarged and the size-reduced male model had the same proportion of the throat area colored bright red or dull respectively, the absolute difference in red throat area was considerably greater than in the other experiments. We can therefore not rule out that the stronger female preference in that case was partly based on the absolute amount of red.

Given that the comparison of one, two, and three (concordant) secondary sexual traits revealed a more pronounced female preference for every additional character, preference tests using computer animations are not only able to detect qualitative choice behaviours, but also appear to be suited for assessing female mate preferences quantitatively. The seemingly linear increase in the strength of the preference with the number of traits that distinguishes the males (Figure 3) would probably become less strong with a fourth and fifth male trait being added, and asymptotically approach the maximal possible preference index of 1.0.

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