COMPUTER ANIMATIONS AS A TOOL IN THE STUDY OF MATING PREFERENCES

by

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Summary

The study of multiple female mating preferences and multiple male signals requires correct and precise measurement of preferences. A review is given of existing preference test paradigms. Non-interactive preference tests using computer animations perfectly fulfil the demands for the study of multiple preferences for visual traits: exclusion of confounding variables, exclusion of variation within and between male pairs, great potential of experimental manipulation of single and combinations of visual traits including behaviour. We give a detailed description for the production of computer animation movies based on commercial software. Finally, we show how computer animations can be properly applied to the testing of mating preferences. In sticklebacks, female mating preferences that were tested in this way agreed with preferences that were measured with other test paradigms.

Keywords: preference tests, computer animation, methodology, sexual selection, multiple signals, multiple preferences.

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Introduction

The enormous interest in sexual selection during the past decades (Andersson, 1994) has stimulated the development of test paradigms with which one can measure social preferences. In the case of sexual selection through female mate choice these paradigms aim at measuring sexual (mating) preferences. Preference tests can be used in the study of both preferred male traits and female mate preferences.

The demands that must be made upon preference tests depend on whether one wants to study the causes or the consequences of female mate preferences. In studying the causes of female preferences one has to get rid of confounding variables which will often be a difficult if not an impossible task. Tests involving simultaneous choice situations are most sensitive in detecting preferences and thus preferred in causal studies. When studying the ecology of mate choice, sequential choice situations may be more appropriate (*e.g.* Milinski & Bakker, 1992). In drawing conclusions about the evolutionary consequences of mate choice one has to be cautious not to over interprete the results of standardized preference tests in the laboratory. Selection does not operate on single traits in isolation: the selection exerted on single traits in nature is unavoidably influenced by confounding variables. It may thus be desirable to allow for confounding influences when studying the evolutionary consequences of mate preferences using preference tests.

Recently, theoreticians have begun to explore the evolution of multiple ornaments (Pomiankowski & Iwasa, 1993; Schluter & Price, 1993; Iwasa & Pomiankowski, 1994; Johnstone, 1995, 1996). Empirical studies clearly lag behind theoretical developments in the case of multiple signals. This is partly the result of limitations to do genetical studies (Bakker & Pomiankowski, 1995), and partly because of limitations in the experimental quantification of female preferences. New developments in techniques to measure preferences for visual traits will stimulate the latter area. In the classical preference test, a female can choose between two confined live males. Recently the live males have been replaced by video playbacks or by computer animations displayed on a monitor (*e.g.* Clark & Uetz, 1990; Rowland *et al.*, 1995a, b; McKinnon & McPhail, 1996). This opens the possibility of experimental manipulation of single and combinations of male traits. Video tests produced more pronounced and more repeatable choices than tests with live males in a comparative study using different presentation techniques in guppies (Kodric-Brown & Nicoletto, 1997). Preference tests using computer animations will add further dimensions in the measurement of female preferences: now it will be possible to experimentally test for preferences of trait combinations and of behavioural traits. Because monitors are build for human colour vision, these techniques may not be generally applicable: with birds less good results have been obtained than with fish (*e.g.* Patterson-Kane *et al.*, 1997).

The present paper critically reviews existing test paradigms for measuring female mate preferences. We advocate the use of computer animations in studying multiple visual signals and preferences. With the aid of computer animations one can generate a fully controllable test situation in which a test individual can choose between two virtual conspecifics that only differ in the character(s) under investigation. The study of visual signals using computer animations is analogous to the use of synthetic acoustic signals in studies with for instance amphibians and insects (*e.g.* Ritchie, 1996). A step-by-step description for the construction of computer animations based on commercial software is provided together with a check-list in order to avoid possible pitfalls in applying computer animations in preference tests.

An inventory of preference tests for visual signals

In this section we will describe and compare the different test paradigms that are used for measuring mate preferences in causal studies of sexual selection through female mate choice.

1. Natural preference tests

In natural preference tests all possible interactions in the test group are allowed for (*e.g.* Houde, 1987). An advantage of this test is, that one can check whether mate preferences actually translate into mate choice. In all test paradigms this is assumed but rarely tested. Without further experimentation, which is more limited with this than with other test paradigms, only correlative evidence about preferred traits can be obtained. The test allows a quantification of the intensity of selection exerted by female mate choice on specific male traits (*e.g.* Schluter, 1988). Instead of studying mate preferences in a confined group of animals, one can study actual female mate choice in nature. One may then be able to draw conclusions about the consequences of mate choice for the evolution of male traits but the study of mating preferences cannot be decoupled from the ecological setting. The targets of female mate choice can be identified by experimental manipulation of male traits (*e.g.* Andersson, 1982).

2. Interactive preference tests using confined animals

A more standardized preference test using live males is the interactive preference test in which the sexes are separated but can interact visually. The test female is placed in a central tank with two adjacent tanks on opposing sides, each of them containing a courting male (*e.g.* Bischoff *et al.*, 1985). Because of the opposite position of the two male compartments, males can see and thus influence each other across the compartment in the middle. This effect can be excluded by the usage of two polarizing films (Houde & Torio, 1992), by two partly overlapping partitions in the middle compartment (Balshine-Earn & McAndrew, 1995) or by placing the two male compartments separated by an opaque partition side by side on the same side of the female tank (*e.g.* Milinski & Bakker, 1990). Males are chosen to differ only in the trait under study and to be as similar as possible in all other male traits. This is in most cases not possible because traits covary. It is then necessary to perform an experiment in order to exclude the influences of confounding variables. For instance, in preference tests for the red intensity of stickleback males' breeding coloration the influence of confounding variables on female preferences like male condition and the intensity of courtship could be ruled out by performing the preference tests under green light conditions (Milinski & Bakker, 1990), where differences in red intensity are difficult to assess. Even then, it is still difficult to pin female preference down to the red intensity; it may be confounded by other aspects of red coloration that covary with the red intensity like the extension of red. The method is limited to the study of preferences for male traits that are amenable to experimental manipulation. Traits that cannot directly be manipulated like body size require a different approach (*e.g.* Partridge, 1994).

The female's behaviour in the preference test will likely lead to assymetries in the amount of attention given to both males and may thus evoke asymmetries in courtship intensities which may influence the female's choice (Collins, 1994). Another concern is the variance within and between male-pairs which reduces the power of the test. Asynchrony of courtship behaviour within male-pairs, males' loss of interest over subsequent tests and differences between male pairs lead to variable experimental conditions between replicates (Kodric-Brown & Nicoletto, 1997).

3. Non-interactive preference tests using live animals

In non-interactive preference tests, one-way mirrors are placed between the test female and the males to eliminate the possibility of mutal interactions: only the test female is able to see the males (*e.g.* Kodric-Brown & Nicoletto, 1997). The choice situation is extended to more than two males in the so-called Amsterdam apparatus, where the centre compartment with the female is surrounded by several choice chambers (*e.g.* Bateson, 1982). Because the males in non-interactive preference tests do not perceive the female, they do not court and may thus be not very attractive to the choosing female. The test can be improved by making the males court to for instance an enclosed female in their compartment which is not visible to the test female and placed close to the one-way mirror (S. Zala & T.C.M. Bakker, unpubl. data). One has to realize that the specific illumination settings that are necessary in order to make a one-way mirror work in the desired way always leads to experimental conditions where the female is placed in relative darkness compared to the males. The major problems of confounding variables and variance within and between male-pairs also remain in this preference paradigm.

The great advantage of non-interactive preference tests is that preferences are more repeatable because interactions between the sexes are excluded. For the same reason, non-interactive preference test suffer from reduced female responses in comparison with interactive preference tests (Kodric-Brown & Nicoletto, 1997; R. Künzler & T.C.M. Bakker, unpubl. data).

4. Non-interactive preference tests using dummies

A rigorous standardization of preference tests is achieved by replacing live males with dummies of males that differ only in the trait under study. In this way, one gets rid of confounding variables and variance within and between male-pairs. Females' responses are likely to be reduced even more than in the case of non-interactive preference tests using live males because dummies do not react either and in addition do not behave. Although females vary in their responses towards male dummies, good results with this method can be obtained (*e.g.* Rowland, 1994). Standardized movement of dummies may improve the method (*e.g.* Baube *et al.*, 1995) but movements are necessarily simple and repetitive.

The dummy method may prove useful in field situations that cannot be otherwise standardized. An advantage of the dummy method is that the expression of male traits outside their natural ranges can be tested thus allowing for the study of the form of preferences (*e.g.* Rowland, 1989).

5. *Non-interactive preference tests using video playbacks*

Variance between male-pairs can also be eliminated by usage of a single set of video recordings for all test females. The female is placed in front of two video displays and allowed to choose between video-playbacks of two males that differ in the expression of the trait under study. Both playbacks are composites of recordings of several courting sequences of the corresponding males with the aim of reducing the influence of confounding variables; the composites can for instance be optimised to comparable levels of courtship activity. By this, the influence of confounding variables can considerably be reduced but still not eliminated. Different versions of this technique have been used for jumping spiders (Clark & Uetz, 1990), lizard conspecifics and heterospecifics (Macedonia *et al.*, 1994), swordtails (Rosenthal *et al.*, 1996) and guppies (Kodric-Brown & Nicoletto, 1997).

A different approach is to display a video-playback of one single courting male on two screens simultaneously which differ in the presence or absence of motion (Clark & Uetz, 1992) or in the colour settings of the monitor (Rowland *et al.*, 1995a, b; Bolyard & Rowland, 1996). This method is limited to tests for colour cues and basic video perception experiments like measurement of the critical flicker fusion rate, minimum contrast and brightness settings and so on. With identical monitor settings, the method can be used for a control experiment (Clark & Uetz, 1992) or for testing for female preferences for territory traits like dissolved oxygen concentration in stickleback territories (T.C.M. Bakker, unpubl. data).

By way of a simple device using a one-way mirror, male courtship can be recorded as if the male is courting to the video camera (McDonald *et al.*, 1995). The playback of the recording is then directed to the test female thereby enhancing the female's interest in the male.

6. *Non-interactive preference tests using digitally edited video playbacks*

Nowadays computer technology allows researches to go even a step further and overcome the restrictions described above. Video sequences of a selected male can be digitized and edited on a computer in a frame-by-frame manner such that copies of the video recording of the same male which differ in the trait(s) under study are produced. Professional video editing systems which feature the so-called chroma keying, even allow to exchange a given hue with another color in real time. Females are then given a simultaneous choice between two video playbacks which show two courting males that only differ in a single trait or combinations of traits.

This method is bound to technical limits in manipulating video recordings. It has been applied to editing video recordings with respect to courtship vigour in jumping spiders (Clark & Uetz, 1992), male coloration in sticklebacks (McKinnon, 1995; McDonald *et al.*, 1995), playback speed of stickleback courtship (Rowland, 1995c), and morphology of sexual traits in Siamese fighting fish (Allen & Nicoletto, 1997).

7. *Non-interactive preference tests using computer animations*

The latest method of quantifying female mate preferences makes use of computer animations and unifies the advantages of most other test paradigms. A virtual three-dimensional computer model of a male is constructed and its behaviour animated based on video-recordings of real males (McKinnon & McPhail, 1996). In the test situation, models, which only differ in the trait(s) of interest, are offered. With this powerful technique, traits that can be manipulated not only involve various aspects of breeding coloration (like hue, chroma, value, contrast, extension, patterns) and male morphology (like body size and form, expression and symmetry of extensions), but also courtship behaviour (like form, symmetry, length and speed of courtship dance, nesting activities) or any other visual cue, even of supra- or supernormal expression. Preferences for combinations of characters can be examined and questions about functions of behaviours can be addressed.

The production of computer animations exemplified by sticklebacks

In the following three sections, we will describe procedures for the construction of virtual 3D models (1), of their behaviour (2), and of animation movies for testing mating preferences (3). In order to keep these sections readable, we have put technical details into the Appendix.

We aimed at constructing realistic animation movies. It may seem superfluous to make the movies as realistic as possible, because females may respond to more stylized versions as well. We do not know how much realism is needed, but for the following reasons we favoured realistic morphology and behaviour. First, less realistic animation movies run the great risk of evoking supranormal responses. Because in non-interactive preference-tests responses will be reduced anyway, further reduction may seriously handicap the measurement of preferences. Second, even when only the traits to be tested are made realistic, preferences for these traits may be negatively influenced by interactions with less realistic aspects of the animation movies. Females may not prefer one unattractive male over another unattractive male even when the former has one trait that is more attractive. Thus, when less realistic properties are included in animation movies, the lack of preferences may be difficult to interpret. Third, we were interested in using animation movies for the study of multiple preferences. Preferences for combinations of traits may be even more sensitive to interactions of seemingly irrelevant traits than preferences for single traits. In conclusion, although it may not be necessary to make all aspects of animation movies as realistic as possible, decisions about which aspects can be stylized can only be made after having a complete inventory of preferences based on realistic animation movies.

1. How to construct a realistic 3D model

Before starting the whole procedure it is advisable to document the morphology of the specimen one wants to copy by means of standardised photographs or video recordings. The aim of the following procedure was to generate a precise model of a male three-spined stickleback *Gasterosteus aculeatus L.* on the computer display. Every single step was optimised in such a way that the original shape of the male's body would be retained. A representative male (standard body size 4.7 cm) from the Wohlensee population near Bern, Switzerland was chosen, poisened and put into fixa-

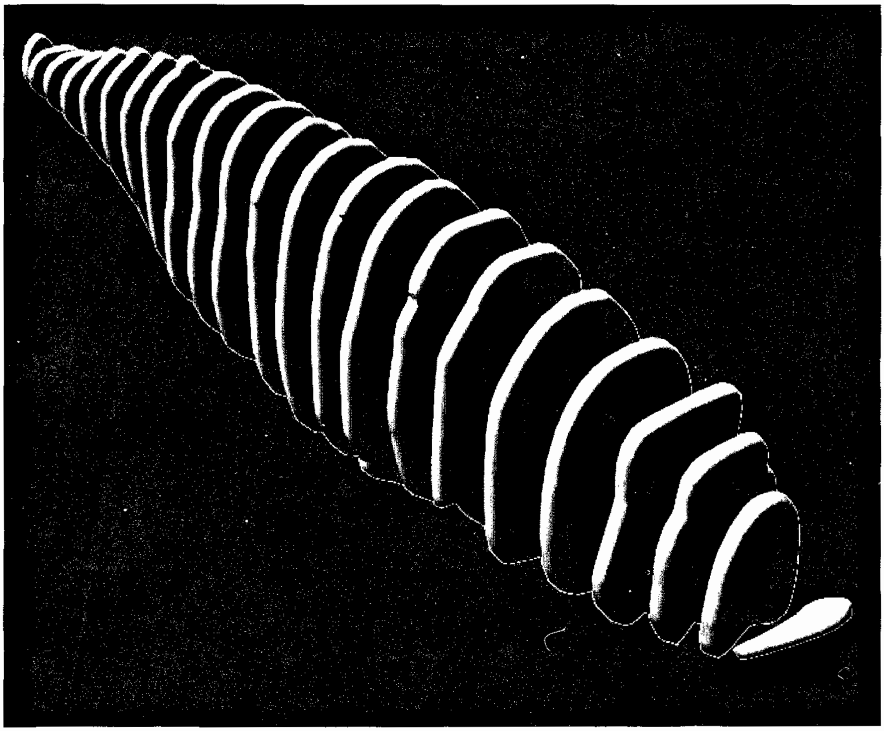

Fig. 1. 3-dimensional view of the 23 digitized outlines of a male stickleback after importing and positioning in the 3D program. See the text and the Appendix for further explanation.

tion solution. Appendices were removed and kept in solution for later use. The body was placed in a PVC-trough, and casted with epoxy resin. The finished casting was cut into 23 slices of 1 mm thickness from the male's snout to his tail. All the slices were scanned and digitised. The 23 vectorised outlines were imported into the 3D program, which was used for all construction and motion animation (see next section) tasks. The outlines were lined up in the correct position to the body's length axis (Fig. 1) and combined to the fish body. Spines and eyes were constructed with the 3D program, and fins were scanned. All these parts were placed on the body at the appropriate places (Fig. 2) to finish the virtual fish model. Coloration of the 3D body of the stickleback model was achieved by using a so-called texture: Instead of directly painting onto the body, a bitmap graphics file (*e.g.* a part of a scanned photograph) is wrapped around it. Eye colours were produced by directly editing the iris' colour (for details

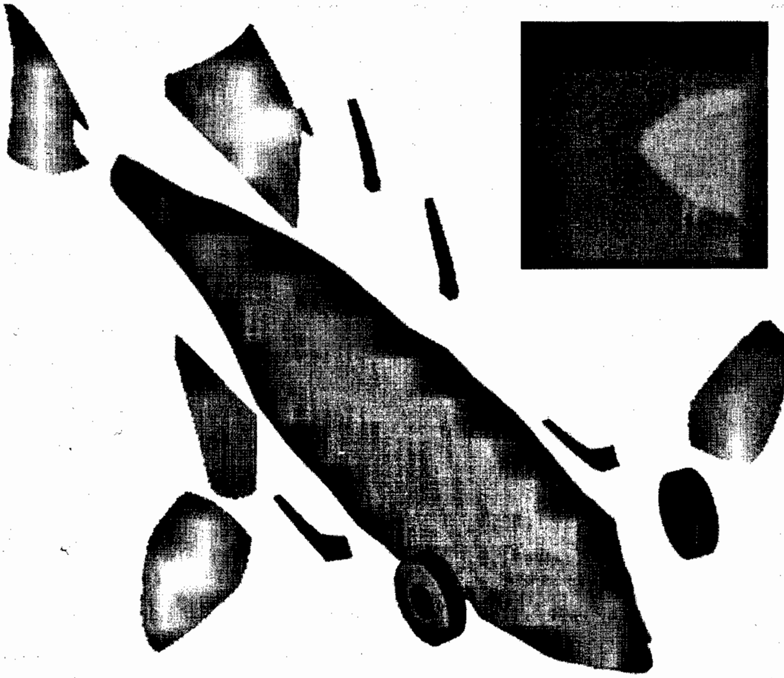

Fig. 2. The shaped body with separate parts, i.e. fins, spines, eyes, and body texture (insert) before attachment to or wrapping around the body. See the text and the Appendix for further explanation.

see Appendix). Fins were kept transparent with a slight opaqueness, and spines were body-coloured but not textured.

As alternative methods for generating realistic 3D models, one might consider the use of for instance automated localization procedures that originate from human brain research (Polhemus digitizers, see http://www.polhemus.com/3drawds.htm or 3D MRI-scanning, see http://www.neuro.com/neuroscan/; J.-P.M. Pijn, pers. comm.).

2. *How to make the model behave realistically*

The motion patterns of a courting Wohlensee male towards a ripe Wohlensee female were taken from video-recordings which were registered simultaneously from a front and top view. A representative zig-zag sequence was chosen and its path was transferred to the 3D program (Fig. 3). The 3D fish model was put onto that path and its orientation was carefully adjusted

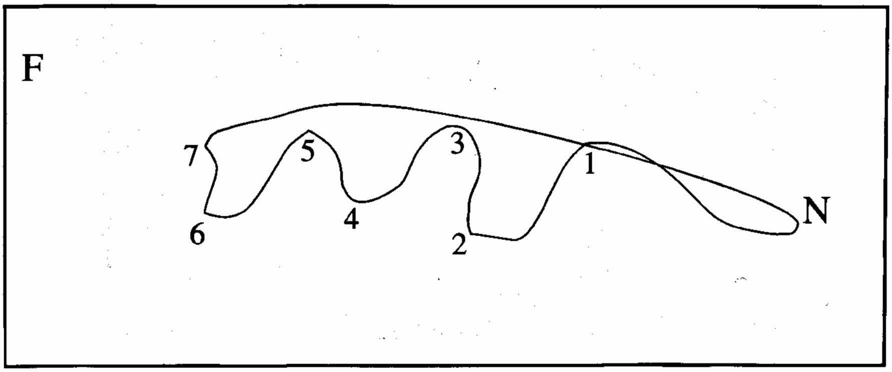

Fig. 3. Zig-zag path of a courting male stickleback viewed from the top as from the original recording. N indicates the position of his nest, F the position of the stimulating female, and the numbers indicate the turning points between zigs and zags.

by hand for the whole sequence so that it courted towards the original position of the female, where the virtual camera was placed. Focal length was adjusted so that the image obtained on the computer display used for playback was precisely life-sized when the model was in the frontmost position. The magnification effects caused by the light passing three media with different optical densities (air, plastic, and water in our setup of the preference test, see below), were determined and taken into account. The result was a courtship sequence of 8 s with correct timing, position and orientation settings (Fig. 4). A standardised sequence of fanning at the nest was added too.

3. The production of a test sequence

Two identical (mirrored) copies of the male model and its track were placed in the virtual scenery. A green-yellowish grey background and a marbled brown mud layer were added and matched by eye to resemble the natural breeding grounds. Finally, a virtual sun was defined in order to generate appropriate lighting and shadows.

After having chosen and applied body textures and having set eye colours for both male models, a standard courtship sequence of a duration of 12 s was rendered. During the rendering process, every single frame of the sequence with both fish models is calculated from the 3D program resulting in an Apple Quick Time movie. Instead of monotonously re-

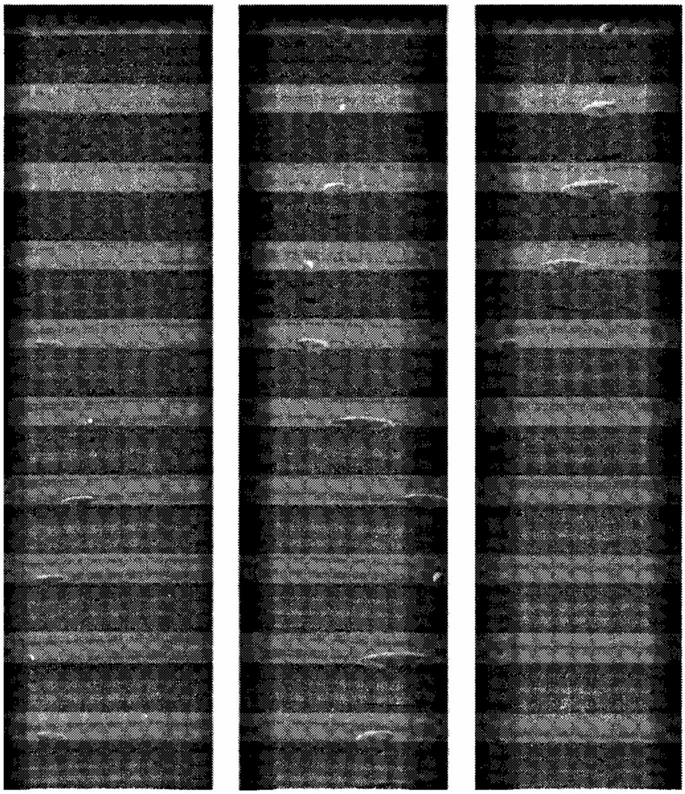

Fig. 4. Series of frames from the courtship sequence of an animation movie. A part of the originally used movie is shown in reduced resolution.

peating the standard sequence, we composed a test movie consisting of variable repeatments of the different parts of the standard sequence like *e.g.* entering the scene, courting or swimming back to the nest (for details see Appendix). Finally, the composed test movie featured 10 zig-zag bouts of three different durations, separated by 11 nest visits (5 of them with fanning) and had a total duration of 2 min. Further, two still images were copied, one featuring the empty scene without models and another one with both males in the back of the scene. Of the final 2 min-test movie, a copy with reversed male model positions was produced. The purpose of these stills and the reversed version is explained in the next section.

The application of computer animations in preference tests: set-up and testing

When developing and performing mating preference tests, one should pay attention to the following critical features (for points 5, 6 and 9 see also Milinski, 1997):

1. Symmetrical test situation
2. Distance between test female and males
3. Illumination
4. Reflections
5. Naïve measurement
6. Acclimatization
7. Awareness of the choice situation
8. Exclusion of side effects
9. Repeated testing
10. Selection of test individuals
11. Appropriateness of the set-up

These are general features for most preference tests inclusive tests that use computer animations. The following section describes how we translated these general features into our set-up. Although it is tailored to sticklebacks, it can easily be adapted to other cases.

We created a fully symmetrical set-up in a darkened and separated part of a climatized fish-room (17 ± 1°C). Illumination was provided by a fluorescent lamp (Philips TLD 18W/33) that was mounted 35 cm over the set-up and dimmed with sheets of white paper. We used one large computer display, on which both males could be shown simultaneously. The test chamber (Fig. 5) was mounted in front of the computer display. Test movies were one third of the display's height. The rest of the monitor and all walls inside the set-up were covered with recycled paper in order to prevent reflections.

Females were tested in a 1 l container (water level: 8.6 cm, equals height of test movies on the display) that was positioned exactly in the middle and in front of the screen. The rectangular containers were made of clear polyacryl plastic with all the inner walls but a window measuring 9.5 × 8 cm painted dull grey. In order to visually separate the two displaying male models, an opaque, grey PVC partition was put between the middle of the

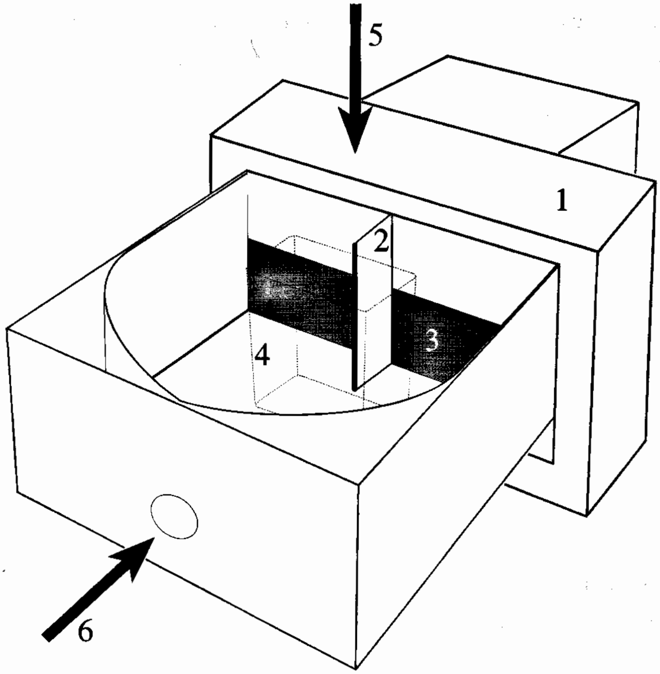

Fig. 5. Schematic view of the set-up for preference tests using computer animations with (1) computer display, (2) PVC partition, (3) movie window (filled in grey), (4) female container, (5) top view camera and (6) front view camera.

female-container and the computer display. This prevented the female from seeing the left half of the presented movie when staying in the right half of the test container and vice versa. The distance between the test female and the computer monitor was 7.6 cm. Data were collected from the top view camera. On these recordings of the choosing females, the computer display was not visible and thus naïve analysis of the preference tests was possible (Milinski, 1997). The second camera was only used for operation control during the tests (see Appendix for details).

To ensure that females were naïve with respect to the choice situation, they were housed without contact to nesting males prior to the experiment (but see Houde, 1988). Ripe female sticklebacks were selected on the basis of the extension of their bellies and the opening of the cloaca. Because female mate choice is sensitive to perceived predation risk (*e.g.* Godin & Briggs, 1996), it is important to avoid disturbances before and during the test. Females were therefore gently released into test-containers filled with water at room temperature and allowed to acclimatize for 30 min in a pre-test compartment with the same illumination as the test chamber.

Every test started with the test-container being placed in the set-up with the computer displaying a still empty scene. To allow the test individual to become familiar with the test situation the empty scene was shown for 1 min in the first test. She was then shown a still image featuring the two males in the background to make her aware of the choice situation. The test movie playback started when the female had been oriented towards each male for at least 2 s, which was achieved within one minute. After the 2 min-test, the test-container with the female was placed back in the pre-test compartment for a 1 min-break. With this procedure we hoped to achieve that the test female became disoriented and perceived the subsequent test as a new choice situation. In the second 2 min-test the positions of the males were reversed. The preference score calculated from the sum of the two tests is unbiased by side-effects. Further control of side-effects was achieved by alternating the positions of the males in first tests of successive test females. After the preference test, we checked whether the tested female was ready to spawn with a reproductively active male. We used only data of females that spawned within 24 hours after the test. Females were used for one single preference test. As to the males, animations based on one model may be sufficient as long as the morphological and behavioural traits of the model represent an average male of the population studied.

How can a set-up be proven to work correctly? How can we be sure and prove that females perceive the presented males as potential mating partners? These questions are not that simply to answer. We must judge the appropriateness of the presented stimuli from the female's reaction in clearly predictable test situations.

For example, a clear prediction can be made for a test where the female chooses between an attractive, courting male and an empty scene without male: if a ripe female recognizes the displaying male as a mating partner, one should expect that she orients more towards the male than towards the empty scene. We performed such a control test with a movie and its mirrored copy according to the test procedure described above. The male was bright red with average greenish-blue eyes and the female's time oriented towards the male and the empty scene was measured only for the time that her snout was within a 1 cm-area from the window behind which the

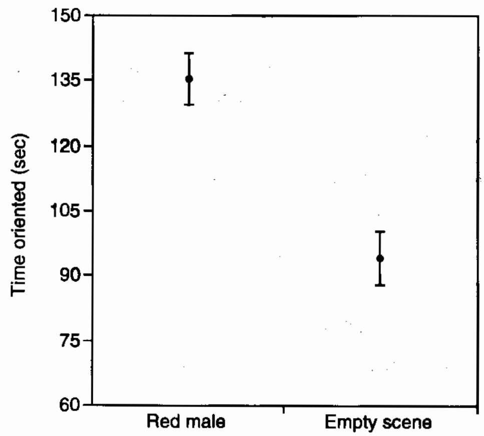

Fig. 6. Female's time (sec) oriented towards animation movies showing an empty scene or a courting male with red throat coloration (mean ± SE). $N = 10$. The total test duration was 4 min.

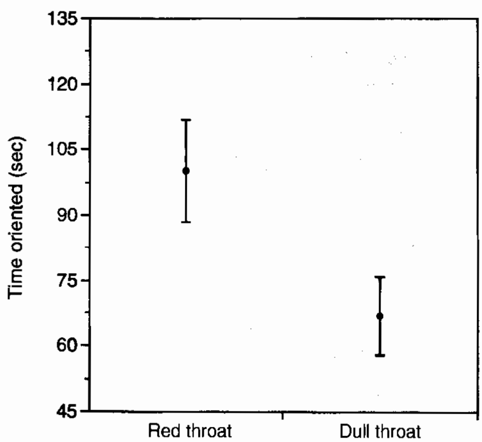

Fig. 7. Female's time (sec) oriented towards animation movies showing a male with red or a male with strongly reduced throat coloration (mean ± SE). $N = 11$. The total test duration was 4 min.

computer display was placed. The male was significantly preferred over the empty scene (Fig. 6: paired t-test, $t = 4.71$, df $= 9$, $p < 0.001$, directed; Rice & Gaines, 1994). This result shows that females were attracted by the courting male stickleback model and were thus not scared or intimidated by the model.

A second demand is that females discriminate between two males that differ in quality, *e.g.* intensity of red coloration. We performed a simultaneous preference test between two virtual males with different throat coloration similar to Milinski & Bakker (1990). Females clearly preferred the brightly red-throated male over the male with strongly reduced red throat colouration (Fig. 7: paired t-test, $t = 2.26$, df $= 10$, $p < 0.03$, directed). This shows that females do discriminate between different computer animated males and that our set-up works properly. This result confirms findings of earlier studies using other test paradigms. Thus females' preference for redder males can causally be ascribed to red throat coloration.

Computer animations — the ultimate solution?

The study of the evolution of multiple ornaments and preferences require an appropriate experimental set-up in order to quantify female preferences for any combination of male traits. This especially expresses the need for a tool which allows for precise manipulation of male traits. Computer animations offer the most advanced and flexible tool for causal studies of preferences, and they appear to be the ultimate tool for multiple preference tests. Computer animation technique even opens the possibility of controlled interactions: it will be possible to feed the computer during playback with information about the female's movements (*e.g.* via a camera, sensors). Depending on the female's behaviour, different parts of a composite animation movie may be presented to the female. This would introduce a standardized interactive component, and may help to improve the female's interest during the tests.

If male traits covary, then decoupling or reversing the relationships between male traits may have consequences for female preferences of single male traits. One has to be aware of this mutual influence of male traits when interpreting female preferences of males with experimentally manipulated traits as is the case in computer animations. We encountered such a situation when we wanted to test female sticklebacks' preference for dissolved oxygen level in the male's territory. Females were offered a choice between two identical video-playbacks of a courting male that only differed in the levels of dissolved oxygen in front of the monitors. To our surprise, females significantly preferred the territory with less dissolved oxygen (T.C.M. Bakker, unpubl. results). Females may have judged the male which was able to court at a similar rate at low oxygen level to be of higher quality.

Whether computer animations are suitable for a given species or not depends on the extent to which its visual system differs from that of humans. Colour reproduction with devices such as television, video, computer displays, photographs and colour-printers may be difficult because they are made for the human photopic visual system, which consists of three types of colour-sensitive cones in the retina with characteristic absorbing spectra (maximal absorption at 420 nm, 535 nm and 560 nm; Kaiser & Boyton, 1996). Any colour is simulated by subtractive mixture of cyan, magenta, yellow and black or additive mixture of the three primary colours red, green and blue, which corresponds to the three cone types of hymans. A limited part of the spectrum between 350 nm and 700 nm is reproduced and any colour calibration mechanism aims at matching reproduced colours with natural perception of humans as closely as possible. Additionally, we point out that all systems lack IR and UV reproduction and that some colours can not be simulated with any combination of Red, Green and Blue. As many species have different colour vision, for example more or less than three cone types and/or different absorbing spectra (reviewed by Goldsmith, 1990), colours may not be reproduced correctly for those species. The characteristics of three-spined stickleback absorbance spectra are very similar to those of humans (relative adsorbance > 0.9 for blue 410-455 nm, green 505-555 nm, red 575-645 nm; C. Baube, pers. comm.).

Although animations displayed on a computer display have a higher quality compared to video playbacks on a TV monitor (*e.g.* smaller grill aperture, much higher refresh rates) they still lack the third dimension. Females may interpret a male model swimming towards the background of the virtual scenery as a male becoming smaller and smaller, with the image still on the screen at the same physical distance to her. But there are other ways of simulating the third dimension with 3D software: size and shape of shadows change as an object moves relative to the stationary virtual sun, water turbidness can be stimulated meaning that approaching objects become more contrasted and more clear, and complex reflecting patterns e.g. from an eye's iris are properly calculated and displayed.

In addition to female and male mate preferences, social interactions in general that are based on visual signals as they are manifested in for example kin recognition, schooling or competition can be tackled with computer animations. Non-interactive preference tests using computer animations may therefore be applied in all situations that involve visual signalling.

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Appendix

1. How to construct a realistic 3D model

We killed the male stickleback (2% Phenoxy-Ethanol in tap water) and used a fixation solution (1.5% Formaline and 0.9% NaCl in tap water, daily renewed) in which the fish was hung on a thin wire (glued into the snout with cyano-acrylate instant bond). We casted the fish body in a PVC-trough with the fish's length-axis exactly aligned with the trough's edge. The 2-component resin (R & G Epoxy Resin L) was chosen not to compress or inflate the fish's body. The internal temperature remained under the boiling point during the chemical process of the resin. The resulting block was cut with a wheel of 1 mm thickness. Slices were digitised (Apple Scanner, Model No. A9M0337Z; Apple Scan Version D-1.0.2, 300 dpi, 256 greys) and processed (NIH Image, Version 1.61/ppc; see http://rsb.info.nih.gov/nih-image/): a standardised selection area of each slice was made binary (using the 'Density slice' function) and converted into a single outline (using 'Find edges' and 'Skeletonise' functions). Bitmap graphics were converted to outlines (using the 'Vectorise' function of Macromedia Freehand Version 7.0). These outlines were closed paths consisting of up to 10 Bézier-curves. To improve precision, all outlines were controlled and corrected by hand. A surface was stretched over the series of outlines (using the 'Skinning tool' of Macromedia Extreme 3D, Version 2.0). Fins were spread carefully on a slide, scanned, and their outlines extruded to very thin 3D objects. Eyes were modelled (lens, iris, glass body) in order to make them look realistic.

2. How to make the model behave realistically

The female used for stimulation and the front view camera were put side by side on one smaller side of the male tank (80 × 30 × 35 cm). The front view tape was later used as a reference for the resulting animation movie. The top view tape was used to acquire the path of the courtship display. A representative zig-zag sequence was chosen and the position of the male's body centre was marked on a transparent sheet that was attached to the video screen. In order to achieve a path close to the original, not only the stop points for every zig and zag, but also additional ones had to be considered e.g. where acceleration or deceleration occurred. Once all points were defined and marked, the precise timing of each of them was registered using the frame counter of the video player (PAL 25 fps), which allowed an accuracy of 0.04 s. The whole dataset was then used to rebuild the motion pattern of a courting male using Macromedia Extreme 3D animation software. The single points along the path on the transparent sheet were converted into x- and z coordinates, where the z-axis was the line between the male's nest and the position of the imaginary female in the front left corner of the tank, the x-axis indicated whether the point was left (negative) or right of the z-axis (positive x-values). In order to be able to smoothen the angular turns where necessary, the path was converted into a series of Bézier-curves. For the last two turns, closest to the front and on return to the nest, the model's body was bent to make it look more realistic. A naturally multi-coloured standard texture was produced based on images of Wohlensee female and male sticklebacks (Adobe Photoshop, Version 4.0.1). An additional mask layer allowed to edit male throat coloration without affecting the rest of the body (Fig. 2: lighter part of the insert). It contained a naturally shaped throat area, which is more or less intensely red in stickleback males during the breeding season. This mask layer was uniformly coloured and was assigned an RGB value corresponding to intense red coloration of Wohlensee males. Standardized slides from previous Wohlensee studies (T.C.M. Bakker & B. Mundwiler, 1994, unpubl. data) were used to choose males with a high intensity of red throat coloration. The throat coloration on the slides was matched with a colour chip (Mecanorma: normacolor spatial system). On the Trinitron monitor used in the preference tests, an RGB value for the mask layer that matched the colour chip was chosen.

3. The production of a test sequence

The standard sequence (200 × 800 pixels Apple Quick Time Version 2.5 movie; rendered with Macromedia Extreme 3D at 30fps, thousands of colours, animation compressor/decompressor) was split into entering (models enter from outer borders and move directly to their nest positions, staying always in the back of the scene), fanning (both males perform synchronous fanning movements), back-front (both males zig-zagging synchronously from the back of the scene to the front, courting towards the middle where the female is, Fig. 3), front-front (both males return in the direction of their nest positions but turn around before they reach their nests (turning point at two-thirds of the distance between front and nest) and enter the zig-zag path to come to the front again) and front-back (both males return to their nest positions). To all parts, an audio track was added (Adobe Premiere Version 4.2.1), which contained a spoken neutral information (directly harddisk-recorded with an Apple microphone, edited with Sound Effects Version 0.9.2) about the model's position in the scene (e.g. at the nest, in front, returning). A courtship sequence always started at the nest, followed by a zig-zag sequence to the front (back-front part), zero, one or two front-front part(s), and ended at the nest again after the front-back part. By this, the courtship sequences had durations of 249, 405 or 561 frames respectively. The courtship sequences were separated by either a short nest visit without fanning (10 or 20 frames) or a nest visit including a short fanning sequence (84 frames). The mirrored copy of the test movie was produced using the 'Horizontal Flip' filter in Adobe Premiere.

Computer equipment

For the first steps under methods 1 and 2, we used a PowerMac 7200 (PowerPC 601/90MHz processor) equipped with 48MB RAM, which we recommend as an absolute minimum needed for the tasks involved in producing animations. Later, as tasks became more demanding especially with the 3D- (rendering, mostly done overnight) and video software (composite production), we switched to a PowerMac 8600 (PowerPC 604e/200 MHz processor) equipped with 128MB RAM and an additional 8MB graphics card for the primary 17inch Monitor. A second computer monitor was connected to the built-in onboard graphic adaptor on which all the palettes and editing windows were displayed.

An alternative method would be to use a powerful workstation computer system (*e.g.* Silicon Graphics) which offers much more computing performance. The major difference to the PowerMacintosh systems used in this study would be that such a machine is able to render a complete scene in real time. This means that movies do not have to be calculated over night, but can instantly be played back based on the current setting (throat colour, eye coloration *etc.*).

For the playback of the test movies, we used a PowerMacintosh 4400 (PowerPC 603e/200MHz) equipped with 64MB RAM and an additional 4MB graphics card for the primary 17inch SONY Trinitron Computer display (Model CPD-200SFT, set to 800×600 pixels at 120Hz, millions of colours, colour temperature 84%, contrast 87%, brightness dimmed down to 13%). A second monitor was used to control the test movie playback (Adobe Premiere movie sequence), because the primary monitor was not visible from the operator's place. Compared to many other Quick Time movie players, Adobe Premiere allows smooth and uninterrupted playback without retardation or single frames to be dropped.