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Mate Choice in Zebrafish (*Danio rerio*) Analyzed With Video-Stimulus Techniques

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In many species, individuals of both sexes have developed a variety of visual signals and behavioral patterns with which to broadcast their quality as mating partners (1). The complexity of these signals makes it difficult to distinguish those that are most important in mate selection. Animated models offer a solution to this problem by allowing for the alteration of single parameters in the complex stimulus presented. In this study, we have tested the use of computer animated three-dimensional models to analyze mate choice criteria in zebrafish; we applied this tool to examine the roles played by two visual characteristics in mate selection.

Sexually mature wild-type zebrafish, aged at least 8 months, were kept in 9-1 aquaria under a day/night cycle of 14/10 h. Subjects were tested in a 50 cm × 32 cm tank; a vertical line drawn on the wall of the tank divided it into two equal sections, A and B. The tank was placed between two 17-inch Dell computer monitors on which animated models of swimming zebrafish were displayed. The models were created using 3D Studio Max 1.0 (Kinetix) on a Dell Optiplex GXPro computer and a Targa 1000 board for digital/analog conversion of video signals, as described by Rosenthal (2, 3).

Before each trial, an individual fish was placed in the tank and allowed to acclimate for 5 min. During each trial, the subject was simultaneously shown two different animated stimuli. Each trial consisted of four 5-min viewing periods separated by 1-min intervals during which black covers were gently slid in front of the monitors. Three pairs of stimuli were shown to the subjects: [1] male *versus* female body shape (both with natural horizontal stripes), [2] vertical *versus* horizontal stripes (both with female shape), and [3] vertical *versus* horizontal stripes (both with male shape). The vertical and horizontal stripe patterns had equal amounts of blue coloration. Female-shaped images differed from male-shaped images only in that their bellies were 10% larger in side view. Stimulus pairs [1] and [2] were presented to both male and female subjects, while stimulus pair [3] was presented to female subjects only. In addition, stimulus pair [1] was shown to females who had spawned on the day of the trial. (The females used in all other trials did not spawn on the day of the trial.) The two animated stimuli were alternated between the monitors to balance for side effects. During each viewing period, the location of the fish was recorded every 10 s. The percentage of time the subject spent in proximity to each stimulus (presence on side A or B) was calculated and compared using a Wilcoxon matched-pairs signed-ranks (WSR) test.

The results and statistical evaluations are shown in Figure 1. Males did not differentiate between the male and female-shaped images but showed a significant preference (19.6%) for the horizontal stripe pattern over the vertical stripe pattern when the images were female-shaped. Female zebrafish preferred a male-shaped stimulus over a female-shaped stimulus by 20.3%. However, females that had just spawned eggs on the morning of the trial did not show a preference for either the male or the female shape. Females also showed a significant preference (10.7%) for the horizontal stripe pattern over the vertical stripe pattern when the images were male-shaped, but did not differentiate between stripe patterns when the images were female-shaped.

The preference of females for the male-shaped stimulus over the female-shaped stimulus indicates that belly size alone allows female zebrafish to distinguish between sexes. The indifference of the females who had recently laid eggs suggests that females' interest in males correlates with their reproductive stage. The significant bias of males and females against vertical stripe pattern in the opposite-sex animation may result from selection against mating with heterospecifics.

The failure of males to distinguish between the male- and female-shaped images has at least two possible explanations: (a) the belly of the female image may not have been large enough to realistically simulate a fecund female zebrafish; or (b) male zebrafish may rely less on visual cues than on olfactory cues to select mates. Prior studies have shown that male zebrafish are attracted to the pheromones released by females (4–6).

This study shows that video-stimulus techniques can be used to further study mate choice and visual preferences in zebrafish.

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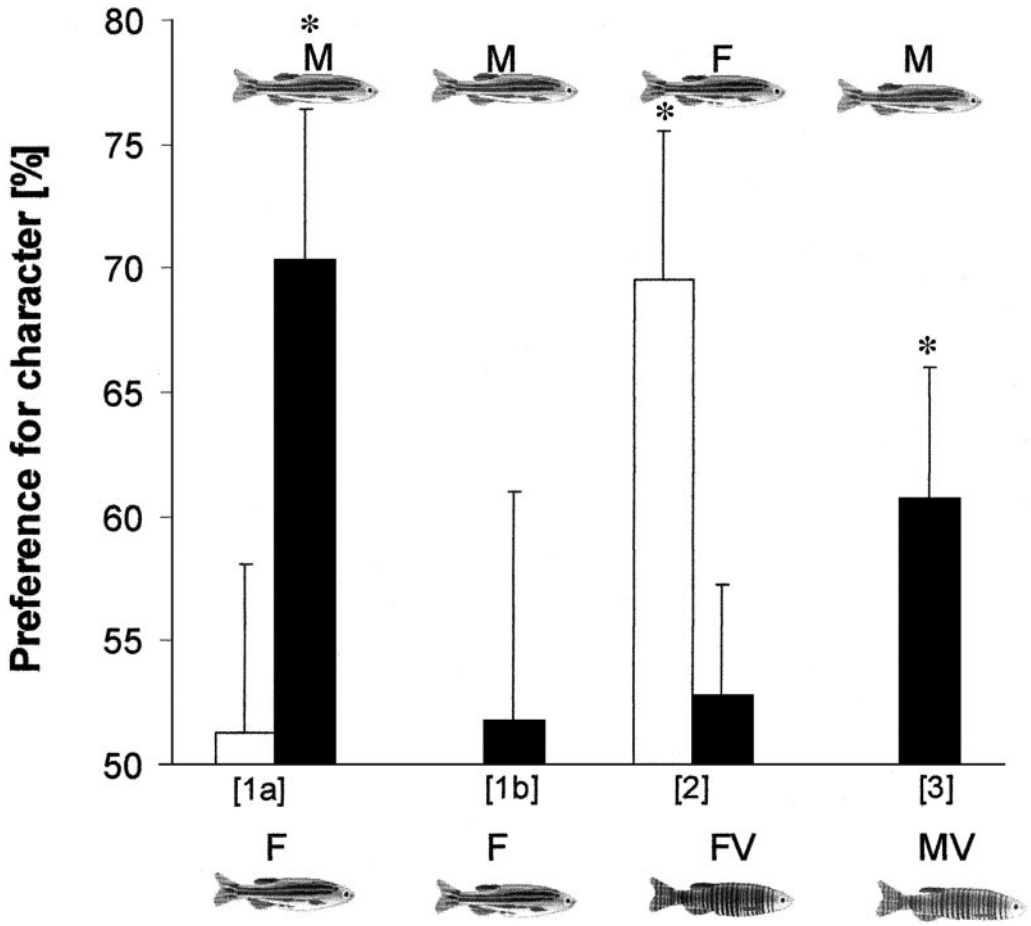

Figure 1. *Preference (% ± SEM) of males (white bars) and females (black bars) for each stimulus image, as measured by time spent in proximity to each one. [1a] Females significantly preferred the male-shaped stimulus (M) over the female-shaped stimulus (F) (WSR = 27.0,* n = *12,* P = *0.034); males showed no preference (WSR = 3.0,* n = *15,* P = *0.868). [1b] Females who had spawned the day of the trial also showed no preference between images M and F (WSR = 17.5,* n = *15,* P = *0.288). [2] Males significantly preferred the horizontal to the vertical stripe pattern when both images were female (F, FV) (WSR = 37.0,* n = *15,* P = *0.017); females showed no preference (WSR = 2.0,* n = *15,* P = *0.923). [3] Females significantly preferred the horizontal to the vertical stripe pattern when both images were male (M, MV) (WSR = 38.0,* n = *16,* P = *0.049).* * = P < *0.05.*

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