A computer animation based mating preference assessment protocol

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Abstract

In this protocol, we used computer animated stimuli for two mate choice tests of *Gambusia affinis*, such that stimulus pairs differed only by (a) body size and (b) locomotor activity, but not in other morphological or behavioural traits that could affect mate choice decisions.

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Before start

We acclimated the fish to laboratory conditions for at least one month before we conducted behavioral experiments and maintained them in groups comprising both sexes at roughly even sex ratios, at densities of around 40 fish per tank, in several aerated and filtered 200-l aquaria under a 12:12 h light/dark regime. Aquaria were well equipped with plants, twigs and stones.

We fed the fish twice a day *ad libitum* with commercially available flake food, frozen blood worms (chironomid larvae), as well as *Artemia salina* nauplii and shrimps. In the stock tanks and all experimental tanks, water temperature was kept at 25 ± 1 °C. Water quality was maintained by exchanging half of the water every two weeks, while one tenth of the water was exchanged every day in case of the (smaller) isolation tanks. Aged tap water was used for water changes and throughout the entire experiment.

After acclimation period, we randomly selected adult focal fish from our stock tanks and isolated them, separated by sex, in 96-l tanks 24 hours prior to behavioral tests. To avoid aggressive interactions between individuals and to enable repeated testing of the same individuals, we kept each focal fish separately in 1.5-l transparent perforated plastic bottles, which allowed water exchange with the environment.

A web cam (KC-QB960AK, Keeper, Shenzhen, China) was fixed in a central position approximately 70 cm above the test tank during all behavioral observations, allowing us to remotely observe the focal fish from above. We introduced an air stone connected to an air pump into the test tanks between trials to guarantee well-oxygenated water, and we changed the water every day after a testing session (every 4 trials). Fish were returned to their isolation tanks between subsequent trials.

Protocol

Step 1.

Data base for the establishment of animations. In order to generate animation pairs that represent natural variation in body size and activity levels (swimming speed) of our study species, we measured the standard length (SL) of n = 268 wild-caught *G. affinis* (n = 141 females and n = 127 males) from ethanol-stored samples. Body size ranges were established as 20 – 44 mm (mean ± SD: 29.10 ± 4.67 mm) for females and 15 – 29 mm (mean ± SD: 22.30 ± 2.86 mm) for males. Moreover, we assessed activity levels of an additional n = 72 fish in the laboratory (n = 42 females and n = 30 males) using the experimental set-up for activity assessment as described in http://dx.doi.org/10.17504/protocols.io.m68c9hw. By counting the numbers of squares (5 × 5 cm) crossed within 300 s, we estimated swimming speed [cm s-1] as: number of squares crossed × 5 / 300. Activity levels were thus established as 0.95 – 7.2 cm s-1 (mean ± SD: 2.71 ± 1.22 cm s-1) for females and 1.01 – 4.53 cm s-1 (mean ± SD: 2.63 ± 0.87 cm s-1) for males. Data from those pre-trials merely served as a reference to produce the animations and were not included in later analyses, nor were fish used in these assessments retested in the main behavioral experiment.

Step 2.

Preparation of animations. To generate animations, we used high resolution photos showing wild-caught, laboratory-maintained individuals in lateral view. We placed individual fish in a small tank (20 × 15 × 15 cm) in front of a light gray background. The tank was filled with aged tap water of 25 ± 1 °C to a level of 10 cm height. Photos were taken under natural light conditions while avoiding direct sunlight using a Canon 650D digital camera (Canon, Tokyo, Japan), positioned 30 cm in front of the tank. We took photos after the fish had habituated to the new situation and resumed swimming freely. Altogether, we thus obtained n = 48 photos (24 females and 24 males) and saved them as .jpeg files. From each picture, we extracted the image of the stimulus fish from the background using the “magic extractor” function in Adobe Photoshop CS4. The resulting images were animated and converted into .flv files (resolution 1024 × 768; 30 frames s-1) using Macromedia Flash 8. Fish used for generating the animations were not used in the behavioral experiments.From each of the 48 individual images, four animations were generated (i.e., two animation pairs: large vs. small body size with the same activity level (average swimming speed for a given sex) and high vs. low activity level with the same body size (average SL for a given sex). We defined ‘large’ body size and ‘high’ activity as the empirical mean values (for a given sex) plus the respective standard deviation (see above), while ‘small’ body size and ‘low’ activity were defined as the empirical mean values minus the associated SD-values.Each computer animation showed one virtual stimulus fish swimming in a straight line from left to right and back in front of a uniformly light grey background, with an invisible turn of one body length before changing swimming direction (i.e., we let the animated fish continue swimming outside the display window for one body length and then turn around without being seen by the focal fish).

Step 3.

General testing procedure. The experimental set-up for the dichotomous association preference tests consisted of a tank (60 × 30 × 35 cm) with two computer screens (L1510A, Lenovo, Beijing, China) placed on both smaller sides. We set the two screens to the same calibration configuration to achieve uniform display properties with respect to brightness and hue. The test tank was visually

divided into three sections: two preference zones (10 cm) adjacent to the screens and a central neutral zone (40 cm). Both long sides of the tank were covered by black plastic foil to minimize outside disturbance. We filled the tank with water to a level of 25 cm, which matched the height of the screens. Illumination was provided by a 35 W LED lamp 40 cm above the tank in addition to diffuse room illumination.To initiate a trial, we introduced the focal individual into a clear Plexiglas cylinder (10 cm diameter), placed centrally into the neutral zone of the tank, and started playback of the first pair of animations. After a 5 min habituation period, during which the fish could see both animations, we gently removed the cylinder. During the following 5 min observation period we measured association times, i.e., times spent in each preference zone. Association time in this experimental situation has been demonstrated to be a good indicator of female mating preferences in related species. To avoid potential side-biases, we retransferred the focal fish into the central cylinder, interchanged side-assignments of the stimulus animations, and repeated measurement of association preferences after another 5 min for habituation. The second mate choice test was conducted on the next day.