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Repeatability and consistency of female preference behaviours in a northern swordtail, *Xiphophorus nigrensis*

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Informative and reliable measures of mate choice are essential for accurately identifying female preference patterns across species. Here we identify female receptivity behaviours in mate choice experiments in a species of northern swordtails, *Xiphophorus nigrensis*, and quantify repeatability and consistency of different female preference indexes (association time and receptivity behaviour). We first identified receptivity behaviour by examining female behaviours towards males in both viewing-only (barrier) and free-ranging (barrier-free) trials. Receptivity behaviours were identified as behaviours that females displayed in the barrier-free environment preceding putative copulation events. Female glide response was significantly correlated with association time in the barrier trials, and was also the most frequently displayed female behaviour in both barrier and barrier-free trials. A second experiment examined the consistency and repeatability of these preference indexes in *X. nigrensis* females for large versus small males on three different days. Repeatability (intraclass correlation, r) and individual consistency (coefficient of variation) for each preference index (association time and glides) were compared across 15 females. Females showing the strongest preference for males also displayed the greatest consistency in behavioural responses across trials. Repeatability estimates were much higher for association time ($r = 0.322$) than they were for glides ($r = -0.088$). Overall, our results indicate that while both glides and association time identify preferences across females, association time represents a more consistent and repeatable estimate of preference.

Sexual selection models predict differential female response to variation in male phenotype or behaviour by different pathways, such as genetic covariation of preference and trait (runaway selection; Fisher 1930), environmentally dependent preferences (sensory drive; Endler 1992), or sensory-dependent preferences (sensory exploitation; Ryan et al. 1990a). Regardless of the different mechanistic underpinnings, all of these models share the assumption that female behaviour is not random, and that responses to males of varying phenotype will be consistent across repeated exposure to stimuli. Having consistent behaviours that predict mate choice is an important feature of sexual selection, yet it has received far less empirical attention than quantifying variation between individuals or populations, a measure known as repeatability (Widemo & Sæther 1999). In this study we focus on identifying behaviours that accurately and consistently predict mate preference in a species of northern swordtails, *Xiphophorus nigrensis*, and measure phenotypic variation in female response within individuals (consistency) and between individuals (repeatability).

To accurately assess mate preference requires identifying behavioural cues that offer informative and reliable measures of female preference. When mating is a relatively rare event or not easily observed under experimental conditions, many studies of fish rely on indirect measures of female preference such as association time or displays of receptivity behaviour to quantify preferences. In some of these systems, such as the guppy, *Poecilia reticulata*, researchers use a combination of behavioural coding (recording distinct behaviour patterns: Houde 1987, 1997; Endler & Houde 1995; Jirotkul 1999; Grether 2000; Rodd et al. 2002) and female association time (recording the amount of time a focal female spends in the vicinity of a particular male: Kodric-Brown 1985, 1993) to provide an overall measure of female preference. In another important female mate choice model system, swordtails (members of the genus *Xiphophorus*), researchers have relied solely on association time as a measure of female preference (Ryan & Wagner 1987; Basolo 1990, 1995; Morris et al. 1995; Rosenthal & Evans 1998; Rosenthal et al. 2001). Although female mate choice behaviours have been described in one species of northern swordtails

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(Ryan & Causey 1989), researchers have not yet used receptivity behaviour as a measure of preference in this poeciliid. Here we attempt to identify receptivity behaviours in *X. nigrensis* and then evaluate the repeatability and consistency of different behavioural measures of preference.

Repeatability and consistency are related but not synonymous terms describing behaviour. Repeatability or the intraclass correlation, *r*, describes the phenotypic variation among individuals (Becker 1984), and is computed as a ratio of variance components between individuals relative to the total variance (between and within). Since repeatability is computed as a ratio, its values are always expressed relative to the variation between individuals. Understanding whether individuals show consistent behaviours in repeated trials, however, is difficult to ascertain from the repeatability ratio because low repeatability values can indicate either consistent mate choice (low variation between and within females) or random mate choice (high variation between and within females; Widemo & Sæther 1999). Consistency, on the other hand, is a term we use to evaluate variation of behaviour relative to each individual's successive performances. To have a measure that evaluates an individual's consistency independent of variation across the population, we calculate a coefficient of variation for female behaviour across successive trials.

Our study objectives were two-fold: (1) to identify female receptivity behaviours in *X. nigrensis* that are tractable in mate choice environments where physical contact is prohibited, and (2) to compare repeatability and consistency of different measures of female preference (e.g. association time and female receptivity behaviours) in order to assess their relative effectiveness for studies of sexual selection. To address these objectives, we conducted two experiments. The first experiment consisted of a traditional mate preference experiment where females observed two large males behind transparent barriers that prevented physical contact and female behaviour and association times were recorded. Immediately following each barrier trial, the barriers were removed sequentially and females were allowed to physically interact with each male. From this experiment we identified possible receptivity behaviours (behaviours preceding copulation) with particular attention to those receptivity behaviours that females also displayed during barrier trials. The second experiment evaluated the repeatability and consistency of these different behavioural measures of preference (receptivity and association time) across a group of female *X. nigrensis* for a preference between large and small males.

METHODS

Experiment 1: Identifiying Receptivity Behaviours

At least 20 days before the experiment, we removed and sexually isolated 21 gravid female and eight large-class male *X. nigrensis* from a community tank (stocked with individuals from the nacimiento of the Rio Choy, San Luis Potosi, Mexico, or their descendents raised outdoors in 800-gallon (3028-litre) tanks at Brackenridge Field Laboratories, Austin, Texas, U.S.A.). Females averaged 30.9 mm in standard length (SL) (range 19–36 mm), and their past reproductive history was unknown. We conducted these experiments in an experimental tank (120 × 30 × 48 cm) that mimicked the natural light environment of this species' native habitat in northern Mexico (Cummings et al. 2003). All behavioural trials for experiment 1 were conducted between 0830 and 1700 hours during 6 February–1 April 2003.

Experiment 1 consisted of two treatments: (1) recording female association time and behaviours towards two large males in a noncontact environment (barrier-trials), immediately followed by (2) barrier-free trials that recorded female behaviours towards individual males in the free-ranging environment where physical contact was possible. For each barrier-trial, two large male *X. nigrensis*, paired for standard length and sword length, were placed at each end of the aquarium behind a UV-transparent (UV+, Rhöm Plexiglas GS2458 with GAM 10-40) or UV-blocking (UV−, UVGG-400 Schott glass) filter. The middle region of the tank was partitioned into three (24 × 30 cm) observational zones for the focal female: left male association zone, neutral zone and right male association zone. The experiment consisted of a 5-min acclimation period, followed by a 10-min control (no males present to evaluate UV environmental preference), and two 10-min male observation periods. To start each trial, we placed a focal female in an opaque cylinder in the central region (neutral zone) of the experimental aquarium for a 5-min acclimation period. After the acclimation period, we lifted the cylinder and recorded female association time in the UV-transparent zone and UV-blocking zone. After the control, we placed two sized-matched males behind the UV-modifying filters at each end zone and recorded female association time with either male (24 cm directly in front of each male's zone) for 10 min with stopwatches. Female behaviours were recorded with a computer using event-logger software (behave.exe; designed by Chris Patton, Hopkins Marine Station, Pacific Grove, California, U.S.A.) by a single observer (D.M.). After 10 min, the UV-modifying filters were switched between the males, and another 10 min of association time and behaviours were recorded. Filter order was alternated between successive trials to prevent an order effect.

Immediately following the 20-min of barrier-trial observations, we placed an opaque covering over the Plexiglas barrier of one of the males to prevent visual interactions and removed the other male's barrier. Both male and female behaviours were recorded on videotape and by a single observer (D.M.) using the event-logger for 10 min. After 10 min, the barrier-free male was placed back behind his Plexiglas barrier with an opaque covering, and the other male was allowed to interact with the female for 10 min. Male presentation (left versus right male) was alternated between trials with successive females. Possible 'receptive' behaviours of females were identified as behaviours shown by females preceding putative copulation events (where a female allowed male contact near her gonopore). Both male and female behaviour across all barrier-free trials were tallied using the event-logger; however,