



# 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.

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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: Identifying 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,

trials that contained putative copulation events ( $N = 4$ ) were further examined by replaying the videotape at slow speeds to accurately determine all behaviours that females displayed towards males leading up to possible copulation events. Given the aims of this study, the only data presented from this experiment are the frequency of different female behaviours in barrier-trials and barrier-free trials, and the correlation between different 'receptivity' behaviours and association time. Data concerning female preference for UV ornamentation are reported elsewhere (Cummings et al. 2003, 2006).

## Experiment 2: Repeatability and Consistency of Different Preference Measures

From April to May of 2004, we conducted a second experiment to examine the repeatability and consistency of different female preference indexes (association time versus receptivity behaviours), with a group of 15 non-virgin female *X. nigrensis*. Females averaged 29.6 mm SL (range 23–35.5 mm). These females were isolated from male contact for at least 2 weeks before the experiment. In this experiment, female preference indexes for large versus small male *X. nigrensis* were recorded in three different mate choice trials conducted with at least 2-day intervals between each trial. Each female was tested for repeatability with the same pair of large and small males, which included: Pair 1 (41.5 mm versus 24 mm SL), Pair 2 (42 mm versus 24 mm) and Pair 3 (38 mm versus 21 mm). We used the same experimental tank described above with both males placed behind UV-transparent filters in each end of the tank. Each focal female was placed in an opaque cylinder in the neutral zone for a 5-min acclimation period. Directly following the acclimation period, the opaque cylinder was lifted and female association time and the number of receptivity behaviours (identified by experiment 1) with either the large or small male were recorded for 15 min. Males were then switched to opposite sides of the experimental tank to control for side bias, and female association time and receptivity behaviour with each male were recorded for another 15 min. Each observation period was extended to 15 min (from 10 min in the previous experiment), with 30 min of total observation time to achieve comparable recorded activity time as in experiment 1 (20 min of barrier-trial observations + 20 min of barrier-free trial observations). We did not increase the observation time to 40 min because pilot trials showed reduced behavioural response in females after 30 min of nonphysical (barrier) interactions.

Female association time and number of the most frequent receptivity behaviour identified in experiment 1 ('glides') were recorded by the same observer as experiment 1 (D.M.) for all 15 control trials and 42 of the 45 repeat preference trials (15 females  $\times$  3 treatment trials). The remaining three preference trials were recorded by another observer (K.R.). A nested ANOVA was computed to determine whether behavioural measures differed by observer. A second nested ANOVA was computed to determine whether female response differed between the three male pairs used in the repeatability trials.

To assess each female's consistency, we calculated the coefficient of variation or the standard deviation expressed as a fraction of the mean ( $CV = \sigma/\text{mean}$ ) for each preference measure (proportion of association time and glides) across the three repeat trials. Both measures of preference were arcsine transformed before calculating repeatability on the repeated measures. A single factor ANOVA was used to calculate significant variation between female performances and estimate the variance components for repeatability estimates. Repeatability,  $r$ , and standard error estimates were calculated as a balanced design according to Becker (1984) and similar to other studies examining female preference (Boake 1989; Godin & Dugatkin 1995; Kodric-Brown & Nicoletto 1997; Brooks & Endler 2001), where repeatability is calculated as the ratio of between-individual variance ( $s_A^2$ ) to the total variance ( $V_t = \text{between} + \text{within} = s_A^2 + s^2$ ).

## RESULTS

### Experiment 1

Although 21 females participated in experiment 1, technical difficulties prevented behavioural recording of one female; consequently, behavioural displays from 20 females were evaluated for receptivity identification. In the barrier-trials, females performed a mean  $\pm$  SE of  $2.4 \pm 0.05$  fin displays,  $3.63 \pm 0.246$  circle swims,  $8.2 \pm 0.61$  glides,  $0.87 \pm 0.14$  body jerks,  $2.95 \pm 0.19$  up-down-movements and  $0.025 \pm 0.012$  body quivers. Glides refer to a sequence of events where a female orients towards the male, then glides away from the male in a slow swim, often returning to the initial male-facing position. This sequence of events is similar to the sexual response described in guppies in an open environment as 'glide response' (Liley 1966) and 'gliding motion' (Houde 1997), whereby the female glides towards the male after initial orientation. In a barrier environment, however, glides towards the male are prevented by the Plexiglas. Similarly, the circle swim has been described by other researchers (Ryan & Causey 1989) and refers to fish swimming in a tight circle or vertical spiral. In the barrier-free trials, females performed a mean  $\pm$  SE of  $2.67 \pm 0.29$  fin displays,  $3.33 \pm 0.48$  circle swims,  $5.55 \pm 0.61$  glides,  $1.81 \pm 0.36$  full body jerks,  $2.04 \pm 0.24$  nudges,  $0.41 \pm 0.15$  body quivers,  $2.07 \pm 0.31$  pair swims (female swims with male) and  $0.69 \pm 0.18$  stationary swims below male (female swims below male and remains stationary). The only behaviour that females displayed in the barrier environment that they did not display in the barrier-free environment was the 'up-down-movement', in which the female swims vertically up and down while directly facing the filter. Glides were the most commonly displayed behaviour in both the barrier- and barrier-free trials.

From 40 recorded observation periods (20 females, 2 males each) in the open-environment conditions, physical contact between male gonopodia and female gonopore areas was observed in only four trials with four different females. This relatively low number of 'mating events' could be a function of the time of year (early winter), the

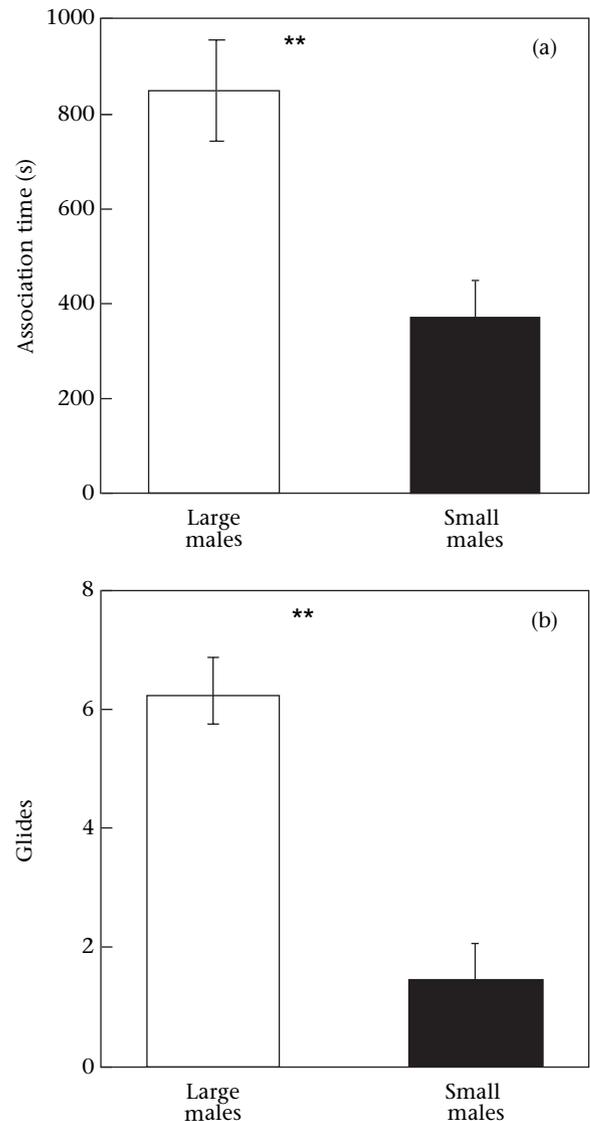
previous experience of the females (nonvirgins), or the short observation period (10 min per male). These events were considered putative copulation events, and the behaviours preceding these events were termed 'receptivity behaviours'. The following receptivity behaviours were common to all barrier-free trials associated with copulation opportunity: glide, circle swim, nudge and pair swim. In contrast, only glides and circle swims were performed by females during barrier trials, and females performed glides nearly twice as frequently as they did circle swims. There was a significant correlation between the proportions of female glide displays and association towards individual males in the barrier-trials (Pearson's correlation:  $r_{38} = 0.46$ ,  $N = 40$  10-min observation trials,  $P < 0.05$ , critical  $r_{38,05} = 0.31$ ), whereas the same comparison with circle swims showed no significant correlation ( $r = 0.24$ ). Consequently, we selected 'glides' and 'association time' as two behavioural indexes to examine repeatability estimates of female preference in *X. nigrensis*.

## Experiment 2

A nested ANOVA showed no significant difference between observers for either association time or glides (association time:  $F_{1,15} = 2.167$ ,  $P = 0.15$ ; glides:  $F_{1,15} = 0.827$ ,  $P = 0.37$ ). A nested ANOVA on female association time with large versus small males showed no male pair effect, but a significant difference across females (male pair:  $F_{2,12} = 0.837$ ,  $P = 0.443$ ; female:  $F_{12,30} = 2.139$ ,  $P = 0.045$ ). Females differed significantly in terms of time spent with, and glides performed in front of, large relative to small males. In the first of the three repeatability trials, the 15 females spent significantly more time associating with larger males and performed significantly more glides in front of these males (paired  $t$  test: association time with large versus small males:  $t_{13} = 3.350$ ,  $N = 15$  females, two-tailed  $P = 0.0048$ ; glides with large versus small males:  $t_{13} = 3.345$ ,  $N = 15$  females, two-tailed  $P = 0.0048$ ; Fig. 1).

## Consistency and Repeatability

Figure 2 shows the relationship between measures of female preference behaviour across successive repeat trials. Individual females displayed different degrees of consistency, as measured by their coefficient of variation over three repeat trials. Females showed greater consistency (lower CVs) for association time than for glides (mean CV: association time = 0.25; glides = 0.64). Females showing the greatest strength of preference (>75% time with larger male) also showed the greatest consistency in their behavioural preference (mean CV = 0.062), whereas females showing weaker preference (<75% preference with large male) showed greater variation (mean CV = 0.416). The relationship between preference and consistency was highly significant for association time (linear regression:  $r^2 = 0.67$ ,  $Y = -1.49X + 1.28$ ;  $F_{1,13} = 25.97$ ,  $P < 0.001$ ; Fig. 3a) and for glides ( $r^2 = 0.85$ ,  $Y = -2.37X + 2.24$ ;  $F_{1,13} = 72.43$ ,  $P < 0.001$ ; Fig. 3b), but interestingly, there was no correlation between individual females' consistency in performing the two behavioural preference

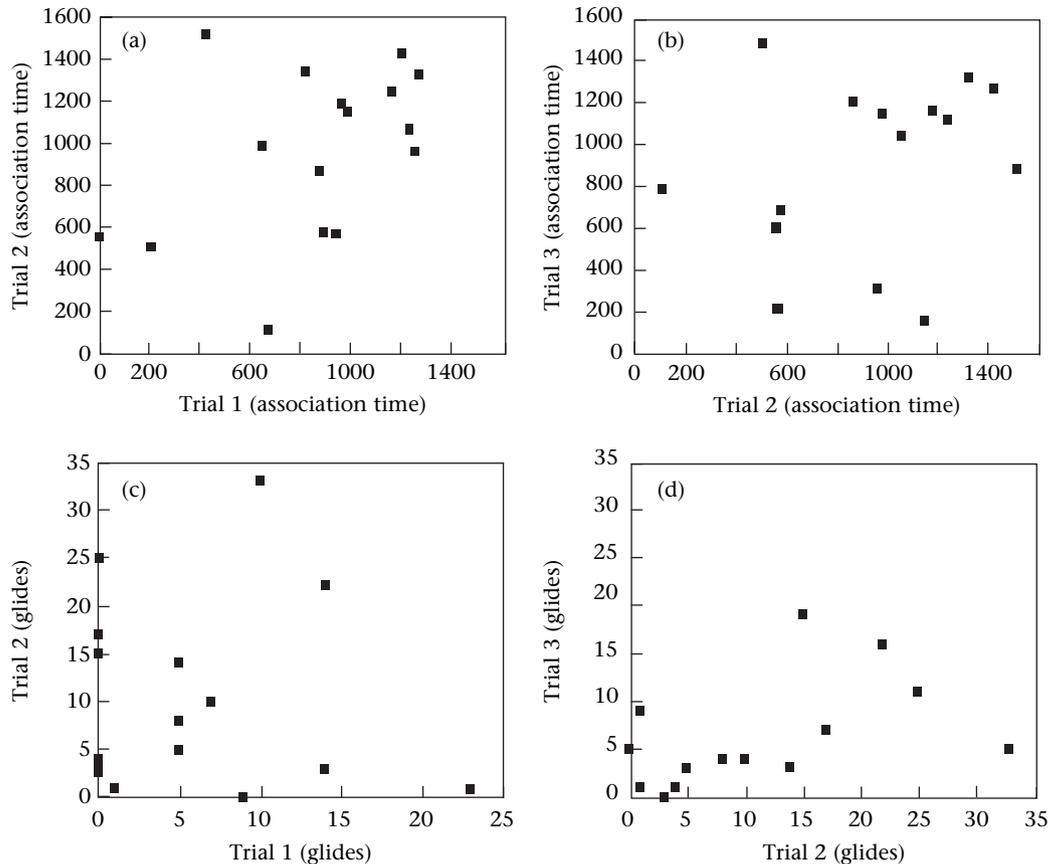


**Figure 1.** Comparison of two behavioural indexes of preference: association time (a) and glides (b) in the first repeat treatment trials (large male versus small male). Bars represent the mean value ( $\pm 1$  SE) of each behavioural measure from the 15 females in experiment 2. \*\* $P < 0.01$ .

indexes (Fig. 3c). Females that showed consistency in their association time across three repeat trials did not necessarily show the same level of consistency in performing glide displays, and vice versa. Repeatability estimates between the two behavioural indexes varied dramatically, and only association time repeatability was significantly greater than 0. The mean  $\pm$  SE intraclass correlation for the 15 females was much higher for association time (arcsine-transformed data:  $r = 0.322 \pm 0.087$ ) than it was for glide displays ( $r = -0.088 \pm 0.026$ ; Table 1).

## DISCUSSION

The most frequently performed behaviour by female *X. nigrensis* in our mate choice trials (glides) is also one of the



**Figure 2.** Relationships between female preference measures in repeat trials (experiment 2). (a) Female association time with larger male in trials 1 and 2; (b) Female association time with larger male in trials 2 and 3; (c) Number of glides performed towards larger male in trials 1 and 2; (d) Number of glides performed towards larger male in trials 2 and 3.

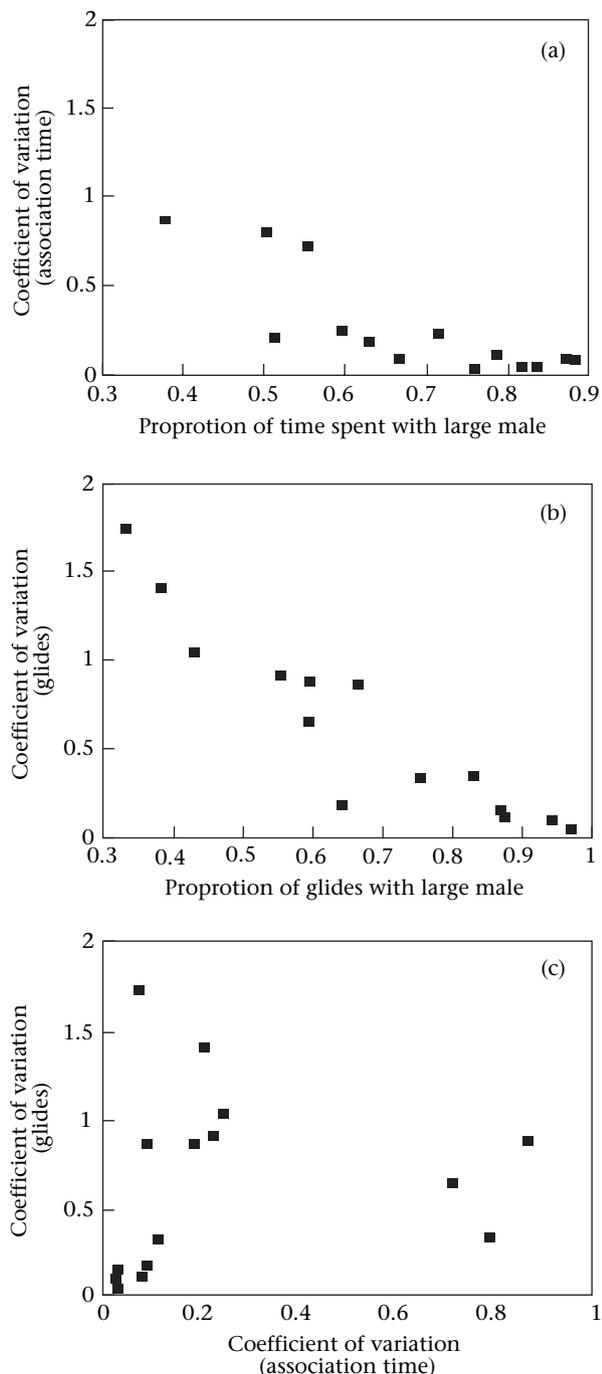
behaviours that females display prior to potential copulation events, and is consistent with other descriptions of receptivity behaviour in swordtails (Hemens 1966; 'slow-retreat': Ryan & Causey 1989). Our description of this behaviour is similar to that described in other poeciliids ('glide response': Liley 1966; 'gliding motion': Houde 1997), suggesting that behavioural receptivity may be largely conserved across species in this family. Our study is the first to use receptivity behaviour rather than simple association time as a measure of preference in swordtail mate choice experiments.

Both association time and female glides indicated a strong female preference for large males relative to small males (Fig. 1a, b), a result consistent with those of other studies of *X. nigrensis* (Ryan & Wagner 1987; Ryan et al. 1990b) as well as other species of poeciliids (Rosenthal & Evans 1998; Aspbury & Basolo 2002). Ryan et al. (1990b) found that the sexual selection pressure exerted by female preferences in the laboratory successfully predicted variation in male size-class distribution in nature. These findings were later corroborated by observations of behaviour at the same field site (Morris et al. 1992), indicating that male-male interactions could further contribute to the variance in male reproductive success.

Although consistency of female preference displays varied, overall, female association time showed much greater consistency than did glide displays (Fig. 3). This

result was also apparent when we examined the within-individual variation components between the two behavioural measures (Table 1). These results suggest that association time may be a more reliable estimate of preference than are more explicit receptivity behaviours such as glides. The difference in consistency between these two measures might reflect different motivational or hormonal states of the female. Females might consistently associate with their preferred male over a broad range of internal states, but display specific receptivity behaviours such as glides only under more narrowly or temporally constrained motivation or hormonal states. Further studies with females of known hormonal states would be useful in addressing this.

There was a significant relationship between consistency and strength of female preference for larger males. Females that showed the greatest strength of preference, measured using association time or glides, also showed the least variation in preference (Fig. 3a, b). Females with the least detectable strength of preference showed the greatest variation in preference display. Interestingly, consistency in one type of preference display did not translate to consistency in the other (Fig. 3c). Although the highest mean preference response might be expected to show the least variation, there is no inherent constraint on the variation for lower measures of preference strength. In theory, some females may consistently display moderate preference



**Figure 3.** Variation and strength of preference. (a) Coefficient of variation (CV) in proportion of female association time with the larger male across three repeat trials. (b) Coefficient of variation in proportion of female glides performed towards larger male across three repeat trials. (c) Comparison of variation in each female preference index: CV of association time with the larger male and CV of glides performed in front of larger male across three trials.

levels. However, in the present study, the relationship between strength and consistency of preference was highly significant. This result suggests that there are possible genetic, environmental, or hormonal differences across females resulting in some females showing inherently strong, consistent preferences for larger males, and other

**Table 1.** Single factor ANOVA on repeated measures

Source of variation	SS	df	MS	F	P
<b>Proportion of association time with larger male</b>					
Between females	1.978	14	0.141326	2.42	0.0205
Within females	1.7489	30	0.05829		
Relative variation among females (intraclass correlation, $r$ )					
$s_A^2/(s^2 + s_A^2)$					
Relative variation within females			0.6781		
$s^2/(s^2 + s_A^2)$					
<b>Proportion of glides with larger male</b>					
Between females	1.6815	14	0.120111	0.757	0.7034
Within females	4.7595	30	0.1586511		
Relative variation among females (intraclass correlation, $r$ )					
$s_A^2/(s^2 + s_A^2)$					
Relative variation within females			1.0881		
$s^2/(s^2 + s_A^2)$					

females showing weaker, less consistent (more relaxed) preferences.

Although our results show that association time and glide behaviours both indicate population-level preference for large males over small males (Fig. 1), only association time showed significant repeatability, allowing us to identify significant variation in preference strength across females. The difference in significance between association time and glides can be attributed to the greater within-female variation component for displaying glides across repeated trials. This result suggests that association time may be a more robust measure for studies interested in quantifying variable responses across females that share the same general preference.

Repeatability estimates of female preferences have varied across taxa: from extremely low repeatability (flour beetles, *Tribolium* sp.:  $r = 0.00$ , Boake 1989) to moderately high levels of repeatability (field crickets, *Gryllus integer*:  $r = 0.59$ , Wagner et al. 1995; barn swallows, *Hirundo rustica*:  $r = 0.57$ , Møller 1994; sticklebacks, *Gasterosteus aculeatus*:  $r = 0.65$ , Bakker 1993). Our results for female *X. nigrensis* females ( $r = 0.322$ ) for association time fall within the range for many repeatability estimates for other poeciliid mate choice behaviour (*H. formosa*:  $r = 0.722$ , Aspbury & Basolo 2002; guppies, *Poecilia reticulata*:  $r = 0.577$ , Godin & Dugatkin 1995;  $r = 0.399$ , Brooks & Endler 2001;  $r = 0.05$  (live stimuli),  $r = 0.47$  (video stimuli), Kodric-Brown & Nicoletto 1997). While our estimates of repeatability are within the low range for poeciliids, this can indicate high consistency across females. Boake (1989) and more recent work (Howard & Young 1998; Widemo & Sæther 1999) describe various sources for low repeatability. Because repeatability is calculated as a ratio, it can appear low when variation both within and between individuals is low. Under these conditions, female choice is consistent within and unanimous across females.

When all females share the same preference function, repeatability estimates are bound to be low. Given that we tested an extreme mate choice experiment between males that court and have a suite of preferred traits (large males: UV ornamentation, size, courtship, sword) versus males that do not display courtship but rather gain access to females by forced copulation, our moderate levels of repeatability might indicate that female preference functions for suites of large-male traits relative to small-male traits are similar in female *X. nigrensis*.

Our repeatability estimates may indicate low within-individual variation in display of one female choice behaviour (association time) and high variation with another (glides), but it offers little information as to the genetic basis of these behaviours. Repeatability is a measure that compares the relative variation between and within females, and while it can set the upper bound for heritability (additive genetic variance), it is not a direct substitute. In the only study that measured both repeatability and heritability of female preference in a poeciliid fish (guppies), Brooks & Endler (2001) found that female responsiveness (a preference score similar to association time) showed significant repeatability ( $r = 0.399$ ) with a heritable component ( $h_s^2 = 0.26$ ). Studies on female choice heritability in moths have found similar levels of genetic components of preference ( $h_s^2 = 0.21$ ; Jang & Greenfield 2000). Future studies of repeatability in northern swordtails should examine whether the consistent display of female association time has a genetic basis.

Phenotypic variation in female mating preferences provides the basis for selection to operate on mating preferences and male traits. Furthermore, phenotypic variation in preference without consistent female expression of this preference leaves little opportunity for nonrandom evolution of female response or the male traits themselves. We have shown that while there is consensus among females for preferences, and great consistency within females for displaying those preferences, there is also significant variation among females. It is this consistency in female preference that places selection pressures on male secondary sexual traits. Female preference for large males over small males in *X. nigrensis* was highly consistent only in females showing the strongest preference (Fig. 3). Only when both high and low strengths of preference show high consistency can divergent selection lead to incipient reproductive isolation. The combination of strong preference and high consistency in behavioural response suggests that directional selection for larger males with correlated large-male traits will continue to be selected for in this population.

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