

Courtship signalling with a labile bilateral signal: males show their best side

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Abstract Asymmetries in courtship signals can result from both developmental instability during ontogeny and from temporary or permanent damage following mating, fighting, or interactions with predators. These two types of asymmetries, which can be divided into fluctuating asymmetry (FA) and damage asymmetry (DA), have both been suggested to play an important role in mate choice as potential honest indicators of phenotypic and/or genetic quality, while at the same time, DA may affect ornament asymmetry in a random manner. Interestingly, despite the massive research effort that has been devoted to the study of asymmetry during the past decades, very little is known about how an individual's behaviour relates to asymmetry. Here, we measure and characterise asymmetry in morphological courtship signals in *Corynopoma riisei*, a fish where males carry elaborate paddle-like appendices on each side of the body that they display in front of females during courtship. Moreover, we investigate whether male courtship display, employing this bilateral morphological trait, reflects trait asymmetry. Finally, we assess whether males respond to phenotypic manipulations of DA with corresponding changes in courtship behaviour. We show that male display behaviour is asymmetric in a manner that reflects asymmetry of their morphological courtship trait and that male display behaviour responds to manipulations of asymmetry of these paddles. Our results thus suggest that males preferentially use their best side and, hence, that

males respond adaptively to temporary changes in signal trait asymmetry.

Keywords Sexual signalling · Sexual selection · Lateralization · Mate choice · Sensory bias · Indicator · Self-awareness

Introduction

Courtship signalling plays an important role in mate choice in most animal taxa (e.g., Andersson 1994; Bradbury and Vehrencamp 1998; Searcy and Nowicki 2005). The study of the evolution of courtship signalling has to date mostly focused on how and what information can be transferred from a signaller (usually a male) to a receiver (usually a female; e.g., Andersson 1994; Bradbury and Vehrencamp 1998; Arnqvist and Rowe 2005; Searcy and Nowicki 2005). However, little is known about the ability of signallers to adjust their courtship behaviours according to the current state of their signal traits. In light of this, Gross et al. (2007) recently highlighted a novel and interesting question: are courting males able to adjust their courtship behaviours according to the asymmetry of morphological ornaments? Using the guppy as a model, they showed that asymmetric males (scored as amount of carotenoid pigmentation on the right vs left side) displayed their more pigmented side more often towards females (Gross et al. 2007). This finding highlights that a signaller's response to its own signal needs to be considered when one investigates signaller/receiver associations, particularly in animals with bilateral traits with high variation in asymmetry.

Fluctuating asymmetry (FA from here on) has been proposed to be highly important in a mate choice context through a mechanism where females prefer to mate with

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more symmetrical males in order to achieve superior genes for their offspring (e.g., Møller and Swaddle 1997). Although the adaptive value for females choosing more symmetrical males is currently highly debated (e.g., Tomkins and Simmons 2003), there are a number of examples in the literature showing that females often do prefer to mate with males that show a higher level of symmetry in various signal traits (Tomkins and Simmons 2003; Brown et al. 2005; Rhodes and Simmons 2007; Polak and Taylor 2007). FA in male sexual traits is thought to originate from developmental instability during ontogeny, and FA can hence be an honest signal of an individual's genetic capability to withstand stress (e.g., Klingenberg 2003). However, asymmetry of signalling traits can also originate from physical injury following mating, fighting, or interactions with predators (Höglund et al. 1993; Uetz and Smith 1999; Barbarosa et al. 2003). Such damages can be temporary if the individual is able to regenerate the damaged tissue or permanent if regeneration is incomplete (termed *regenerative asymmetry* by Uetz and Smith 1999) and thus have dramatic effects on the asymmetry of bilaterally symmetric male courtship ornaments. In line with Uetz and Taylor (2003), we define all types of courtship trait asymmetry caused by damages as damage asymmetries (DA from here on). DA can be particularly important because damage on one side can alter not only movement patterns (Møller 1991) but also cause severe asymmetry in bilateral courtship traits (Uetz and Smith 1999). Individual ability to adjust courtship behaviour following such injuries would be adaptive, particularly in organisms where ornament quality is tightly linked to reproductive success, and such damages are common. Moreover, it could set the stage for dishonest signalling since the interests of a courting male and a receiving female may be in disagreement if a highly asymmetrical male would only display his best side (sensu Gross et al. 2007; Van Doorn and Weissing 2006).

Another alternative mechanism that needs to be considered when investigating individual behavioural asymmetries is that individuals may display according to individual lateralization (i.e., handedness). Individual lateralization is a common phenomenon in many animal taxa (e.g., Vallortigara and Rogers 2005) where individuals display right- or left-handedness in common behaviours (e.g., when escaping from a predator or when catching prey). Hence, experimental data from experiments where male ornament asymmetry has been manipulated and male display behaviours quantified are required before we can say with certainty that male display is a result of morphological asymmetry.

A species suited for studies of behavioural asymmetries in relation to courtship trait asymmetries is the swordtail characin (*Corynopoma rüsei*) within the Glandulocaudinae

tetras (Nelson 1964; Weitzman and Menezes 1998; Arnqvist and Rowe 2005). In this small freshwater fish (approximately 5 cm total length), common in river systems in Trinidad and northern Venezuela (Nelson 1964), males carry a conspicuous paddle-like extension on each of both gill covers (Kutaygil 1959; Nelson 1964; Fig. 1). The tips of these paddles are flattened and form a flag-like structure containing dark chromatophores (Nelson 1964; Arnqvist and Rowe 2005). During the complicated courtship ritual, the paddles, which are normally held close to the body, are extended one at a time, in front of the female (Fig. 1) who starts nipping and biting at the flag (Kutaygil 1959; Nelson 1964; own observations). The paddle ornament thus represents a strong candidate for having evolved as a result of sensory exploitation (Endler and Basolo 1998) by mimicking the invertebrate prey items females feed on (Wickler 1968; Arnqvist and Rowe 2005; own unpublished data). By presenting this “lure” to the female, the male apparently strives to position the female appropriately for successful sperm transfer, since insemination in this internally fertilising species (Burns et al. 1995) usually takes place after the female bites at the flag (Nelson 1964). The exact positioning of the female seems important in this species, in part because the male lacks a gonopodium (Kutaygil 1959; Nelson 1964). The biting by the females can be quite violent and often results in the flag being damaged or even lost (Kutaygil 1959; Nelson 1964; own observation), after which, regeneration follows (Kutaygil 1959). Moreover, males also bite at the flags of other males (Nelson 1964; own observations).

Here, we first assess the level of asymmetry in the paddle ornament in a laboratory population and across 17



Fig. 1 Male swordtail characin (fish to the *right*) displaying his right-hand paddle ornament in front of the female (fish to the *left*)

wild populations of the swordtail characin in order to elucidate whether asymmetry in the trait is caused by FA or DA. We then investigate if there is any relationship between asymmetry in paddle morphology and the level of asymmetry in male courtship behaviours. Finally, we build on the correlation found by Gross et al. (2007) by manipulating the level of asymmetry in paddle morphology to establish causality of male courtship asymmetries and to answer the question: do males display their best side?

Methods

Laboratory husbandry

The fish used in the experiments were tank-raised fish purchased from an aquarium fish wholesaler. They were initially kept in groups of 50 individuals in 100 l tanks and were fed daily with dry flake food ad libitum. To ensure that all fish used in the experiments were virginal before the experiment, they were isolated into smaller tanks of 50 l at first sign of sexual maturation, which in the males is easily identified by the first sign of a developing paddle ornament (Bushmann and Burns 1994). All behavioural experiments took place in 50 l tanks (46×36×30 cm), and the feeding regime was changed by complementing the standard flake food with frozen brine shrimp once per day starting 3 days prior to any experimental observations to ensure females were in good condition. All tanks were kept on a 12:12 h light–dark cycle through daylight fluorescent lighting. The water was composed of 50% de-ionised water and 50% tap water. The temperature was held at 23–25°C, and the pH ranged from 6–7.5. All tanks had motor-driven filters and were cleaned from food residues once a week. Approximately 20% of the water was changed weekly.

Field collection

For the measures of paddle asymmetry in wild *C. riisei*, we collected samples of *C. riisei* from 17 different populations located in eight distinct drainages covering much of the island of Trinidad, during May 2005, using a two-person push seine (total $n=148$ adult males; note that one of these males had intact paddles but only one flag). Whole fish were immediately preserved in 95% ethanol for subsequent analyses.

Measurements

In order to investigate the asymmetry in male paddle size within the laboratory population, 72 males were anaesthetised with benzocaine and photographed with a digital

camera (Nikon D70 equipped with an AF Micro Nikkor 60 mm 1:2.8 D macro lens) on both sides in September 2007. No fish were lost during this procedure. Male size and paddle morphology were analysed using Image J 1.38× (<http://rsb.info.nih.gov/ij/>). The standard length (snout to posterior end of last vertebra), total paddle length (operculum to flag tip), and flag area on both sides were measured. To reduce measurement error during the image analysis, all bilateral traits were measured four times (i.e., one picture for each side and four measures per picture), and the average was used. The repeatability of these measures was assessed using univariate ANOVAs, treating individual as a factor (Lessells and Boag 1987). These analyses revealed high repeatability in all image analysis measures of paddle size ($R=0.96–0.99$, $P<0.0001$ in all cases). To investigate what type of asymmetry (i.e., FA or DA) characterises the paddle ornament, we explored the nature of asymmetry of both total paddle length and flag area based on these measures.

Experiment 1: relationship between paddle asymmetry and courtship display asymmetry

To test if males showed a behavioural bias in response to their own asymmetry in paddle length or flag area, we paired 20 males individually with females. Males were chosen based on their level of paddle asymmetry in order to accurately cover the existing variation in asymmetry in the lab population. Hence, the sample of males included the full spectrum of males ranging from heavily “left-handed” males via low asymmetry males to heavily “right-handed” males (see results for an overview of paddle asymmetry in the lab population). The females were all sexually mature virgins (Standard length range=42–52.5 mm) randomly assigned to the males.

To avoid potential aggression from the resident female, the male was introduced to the female’s tank in a small fish net breeder 48 h prior to observations to ensure a period of acclimatisation where the male and the female could interact visually and olfactory but not physically. Following this acclimatisation, on the day of releasing the male into the female tank, the behaviours of the male and female were observed first for a 2-h period between 10 a.m. and 12 a.m. and thereafter for seven 10-min periods every half hour until 15.30. Thereafter, the fish were observed for a 10-min period during morning and afternoon for 4 days. In total, each pair was observed for 270 min. During this time, the number of male paddle displays with the left and the right paddle were counted separately. To avoid biases in the analyses caused by males that displayed rarely, we only included males that had displayed more than five times in the analyses of courtship display asymmetry below.

Experiment 2: male response to manipulation of paddle asymmetry

To investigate if males could detect their paddle asymmetry as well as respond to DA (e.g., from females biting at the flags), we performed a second experiment on the 17 males that performed paddle courtship displays in experiment 1. These males were anaesthetized again, and the flag on each side was partly ablated using a scalpel. A larger ablation was done on the previously preferred (see “Results”) larger paddle, leaving approximately 1 mm of the flag. By not cutting off the entire flag, we ensured no damage to the sclerotized part of the paddle. The flag on the opposite side was also ablated but only with less than 0.5 mm. This procedure enabled us to reverse the previous asymmetry in paddle length and flag size but also to control for ablation per se. Yet, a potential confounding effect might arise as the more manipulated paddle could be used less frequently due to it being more damaged. To remedy this concern, we allowed a 6-week time span between the paddle manipulation and the actual behavioural experiment. This ensured that any injuries had healed properly prior to the behavioural experiment. We note here that daily visual inspections from the date of manipulation revealed no signs of infection in the manipulated areas. Further, the manipulations were within the range of natural damages, presumably caused by female biting during courtship (Nelson 1964; own observations). The paddle manipulations were successful at changing which paddle was larger: the previously longer and more frequently used paddle was much smaller at the time of experiment 2 than prior to manipulation, both with regards to paddle length and flag area (preferred paddle’s length before manipulation (mean±SD)=21.0 mm±2.0; previously preferred paddle’s length after manipulation=19.0 mm±1.7, paired *t* test: *n*=14, *t*₁₃=9.1, *P*<0.0001; preferred paddle’s flag area before manipulation (mean±SD)=4.6 mm²±1.5; previously preferred paddle’s flag area after manipulation=1.4 mm²±0.6, paired *t* test: *n*=14, *t*₁₃=6.6, *P*<0.0001). The previously less used, now less manipulated paddle, did not change significantly between the two experiments (less preferred paddle’s length before manipulation (mean±SD)=16.6 mm±6.8; previously less preferred paddle’s length after manipulation=16.4 mm±6.6, paired *t* test: *n*=14, *t*₁₃=1.2, *P*=0.25; less preferred paddle’s flag area before manipulation (mean±SD)=3.2 mm²±1.4, previously less preferred paddle’s flag area after manipulation=3.1 mm²±1.2, paired *t* test: *n*=14, *t*₁₃=0.5, *P*=0.65). We paired the manipulated males with a new, randomly chosen sexually mature virgin female, and the number of male paddle displays was again recorded for each paddle separately. Since we noticed only limited signs of aggression during the 48-h acclimatisation period in experiment 1, we paired the male and the female immedi-

ately, without acclimatisation, in experiment 2. As all comparisons across experiments were within individuals, we do not consider this procedure to be a cause of systematic error.

Since these experiments used live fish and were thus not designed to completely rule out any effect of female cues on male display, we instead collected detailed data on male display before and after a female had responded to a displayed paddle (based on the most direct cue to female paddle preference, a female bite at the paddle (Nelson 1964)) to investigate if male courtship was affected by female preference.

Results

Paddle asymmetry in the laboratory population and in the wild populations

Paddle length was strongly correlated to flag area, both in the laboratory (R paddle length vs R flag area: *r*=0.78, *P*<0.0001; L paddle length vs L flag area: *r*=0.80, *P*<0.0001) and in the wild populations (R paddle length vs R flag area: *r*=0.66, *P*<0.0001; L paddle length vs L flag area: *r*=0.68, *P*<0.0001). Since there was no difference in level of asymmetry across the wild populations (ANOVA with unsigned asymmetry as dependent variable and population as factor: total paddle length: *F*_{16,131}=1.1, *P*=0.34; flag area: *F*_{16,130}=1.34, *P*=0.18), we pooled the populations for all following analyses. Both the laboratory population consisting of individual males reared in isolation and the wild collected specimens showed high levels of asymmetry in total paddle length (relative asymmetry (absolute asymmetry divided by the larger paddle) in the laboratory population: mean=5.9%, SD=7.9%, *n*=72; wild specimens: mean=4.0%, SD=4.1%, *n*=148) and particularly in the flag area (relative asymmetry in the laboratory population: mean=20.0%, SD=23.3%, *n*=72; wild specimens: mean=17.2%, SD=21.1%, *n*=147). The distributions of signed asymmetry in both total paddle length and flag area deviated significantly from a normal distribution (Palmer 1996) in both the laboratory population (total paddle length: Shapiro–Wilk *W*=0.85, *P*<0.0001, *n*=72; flag area: Shapiro–Wilk *W*=0.92, *P*=0.0002, *n*=72) and the wild specimens (total paddle length: Shapiro–Wilk *W*=0.91, *P*<0.0001, *n*=148; flag area: Shapiro–Wilk *W*=0.83, *P*<0.0001, *n*=147). Given this, we further investigated the levels of kurtosis and if there was any evidence for directional asymmetry (Palmer 1996) in either paddle length or flag area. The levels of kurtosis were high for signed asymmetry both in the lab population (total paddle length kurtosis±SE=5.6±0.6; flag area kurtosis=2.7±0.6) and in the wild (total paddle length kurtosis±SE=

6.4±0.4; flag area kurtosis=7.4±0.4). We found no evidence for directional asymmetry in total paddle length, since neither the lab population nor the wild collected specimens deviated from a mean signed asymmetry of 0 (lab population: mean=-0.14 mm, SD=1.7 mm, $t_{71}=-0.69$, $P=0.49$; wild specimens: mean=0.01 mm, SD=0.19 mm, $t_{147}=0.82$, $P=0.41$). However, the laboratory population differed significantly from 0 in mean signed asymmetry of flag area (mean=-0.27 mm², SD=0.86 mm, $t_{71}=-2.7$, $P=0.008$), although this was not the case in our larger sample of wild collected males (mean=-0.03 mm², SD=0.30 mm, $t_{146}=-1.1$, $P=0.28$). We also investigated whether there was any relationship between trait asymmetry and trait size, which is predicted by some theory (e.g., Møller and Pomiankowski 1993; see discussion in Polak and Taylor 2007). However, we found no significant relationships between trait size and trait asymmetry, neither for the laboratory population (paddle length vs paddle length asymmetry: $r=0.19$, $n=72$, $P=0.11$; flag area vs flag asymmetry: $r=0.18$, $n=72$, $P=0.27$) nor in the wild specimens (paddle length vs paddle length asymmetry: $r=0.15$, $n=148$, $P=0.065$; flag area vs flag asymmetry: $r=0.02$, $n=147$, $P=0.84$).

Experiment 1: relationship between paddle asymmetry and courtship display asymmetry

Seventeen out of the 20 males included in the experiment displayed more than five times (mean number of displays±SD=116±189.4) and were therefore included in the analyses. Out of these 17 males, one was identified as an outlier in all analyses ($>2\times\sigma$) and was therefore removed from all further analyses. Note, however, that the removal or inclusion of this individual in no case altered our ability or inability to reject null hypotheses. Following the removal of this outlier, the residual distribution of our inferential models did not differ significantly from normality for the analyses presented below (Shapiro–Wilk tests, $P>0.05$ in all cases). To control for the potential effect of variation in display rates among males, the main analyses (Table 1) were weighted for number of displays (square root transformed).

Males showed a strong behavioural asymmetry that corresponded to their paddle asymmetry, both with regards to paddle length and flag area asymmetries (Table 1). Males tended to be left-handed or right-handed in terms of their display, depending on which of the paddles that were larger (Fig. 2). There was also a strong relationship between the level of display asymmetry and trait size, both for paddle length and flag area (Table 1). Since mean paddle length was positively correlated to body size in the laboratory population ($r=0.70$, $n=72$, $P<0.0001$), this indicates that display asymmetry scales with body size such that large males are behaviourally more asymmetric.

Table 1 Multiple regression analyses of relationship between paddle asymmetry and display asymmetry

Independent variables	β	SE	$t_{(13)}$	P
(a) Right paddle–left paddle	0.64	0.16	4.1	0.001
Mean paddle length	0.54	0.16	3.4	0.004
(b) Right flag area–left flag area	1.02	0.20	5.1	<0.001
Mean flag area	0.83	0.20	4.1	0.001

The analyses are weighted for total number of displays (square root transformed), and we controlled for allometry by the inclusion of mean trait size as a covariate (Palmer 1996). Residual distributions were checked, and they all conformed to normality. (a) The result of a multiple regression model with asymmetry in displays (number of displays with right paddle out of total displays with both right and left paddles) as the dependent variable and signed asymmetry in paddle length (right paddle–left paddle) and mean paddle length as independent variables (multiple $R^2=0.68$, $F_{(2,13)}=13.6$, $P<0.001$). (b) Results of a multiple regression model with asymmetry in displays (number of displays with right paddle out of total displays with both right and left paddles) as the dependent variable and signed asymmetry in flag area (right flag–left flag) and mean flag area as independent variables (multiple $R^2=0.68$, $F_{(2,13)}=13.9$, $P<0.001$). We note that these analyses did not suffer from collinearity (all tolerances >0.61) and that these relationships remained statistically significant (at $\alpha=0.05$) regardless of whether an outlier was removed or not (see text for details).

Experiment 2: male response to manipulation of paddle asymmetry

Two males died between the two experiments, and three other males did not display more than five times in total and were therefore omitted from the analyses, as in the previous experiment, leaving a total sample of $n=12$. The males that showed normal courtship behaviour displayed proportionately less (out of the total number of displays with both paddles) with the more manipulated paddle after manipulation than before manipulation (Fig. 3). This is unlikely to be caused by the more extensive manipulation of this paddle somehow impeding courtship display: males displayed as much with the more manipulated paddle following our treatment as they did with the smaller, less favoured, and un-treated paddle before manipulation (mean number of displays with shorter paddle before manipulation (experiment 1)±SD=37.1±42.9; mean number of displays with now shorter paddle after manipulation (experiment 2) ±SD=39.5±52.5; paired t test: $n=12$, $t=0.11$, $P=0.92$).

Our analyses of male display in relation to female paddle preference suggest that males do not use cues from females as a basis for paddle displays (experiment 1: proportion displays with bitten paddle the minute before a female bites±SD: 0.74±0.23, the minute after a female bites: 0.71±0.30, paired t test: $n=9$, $t=0.23$, $P=0.83$; experiment 2: before a female bites: 0.61±0.38, after a female bites: 0.55±0.36, paired t test: $n=7$, $t=0.48$, $P=0.65$). These results did not change if the full period of observation was included before

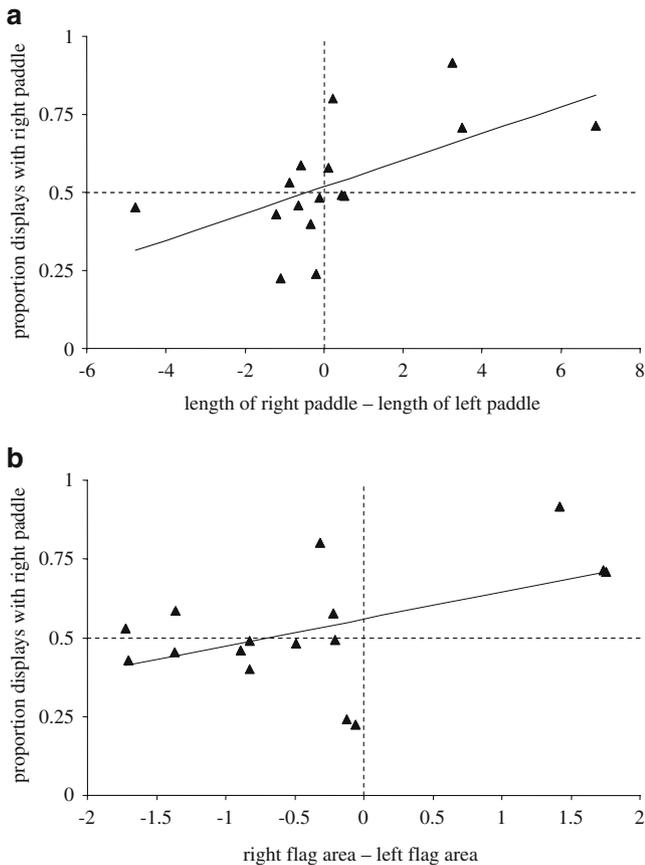


Fig. 2 Male display asymmetry in relation to signed paddle asymmetry. **a** Bivariate relationship between paddle length asymmetry and display asymmetry ($r=0.59$, $n=16$, $P=0.017$). **b** Bivariate relationship between flag area asymmetry and display asymmetry ($r=0.51$, $n=16$, $P=0.042$)

and after a bite (experiment 1: proportion displays with bitten paddle/minute of observation, before bite: 0.52 ± 0.19 , after bite: 0.53 ± 0.09 , paired t test: $n=17$, $t=0.09$, $P=0.93$; experiment 2: before bite: 0.58 ± 0.31 , after bite: 0.55 ± 0.19 , paired t test: $n=11$, $t=0.28$, $P=0.78$). The difference in sample size between the two analyses was caused by the larger number of displaying males that were included in the sample from the longer, full period of observation.

Discussion

Both the laboratory population and the wild collected males showed very high levels of asymmetry in the paddle ornament based on measures of paddle length and flag area. However, these asymmetries did not show the patterns normally expected for FA, suggesting that paddle asymmetry is not caused only by developmental instability. Males showed a positive relationship between paddle asymmetry and display asymmetry. Further, our results on display behaviour before and after paddle manipulation strongly

suggest that males indeed display according to their current level of morphological asymmetry.

Asymmetries of a labile trait

The levels of relative asymmetry in the paddle ornament were very high ($\geq 4\%$ for paddle length and $\geq 20\%$ for flag area) both in the laboratory and the wild populations. These levels are higher than what is normally reported for traits showing FA, which often exhibit mean asymmetries of $< 1\%$ of the total trait size (e.g., Palmer 1996; Tomkins and Simmons 2003). Moreover, we found no negative relationship between ornament asymmetry and total ornament size, which would be expected for a true FA trait in line with the prediction that more symmetric individuals also should excel in other fitness related traits such as growth (Møller and Pomiankowski 1993; Polak and Starmer 2005). Together with the fact that asymmetry deviated from normality, these results suggests that the asymmetry in the paddle ornament of the swordtail characin does not represent conventional FA. Levels of asymmetry were higher for flag area than for paddle length both in the laboratory population and in the wild. Since both males and females bite on the paddle flags of other males (Kutaygil 1959; Nelson 1964; own observations), the high levels of asymmetry in the wild may be caused by such biting damages and thus represent true DA

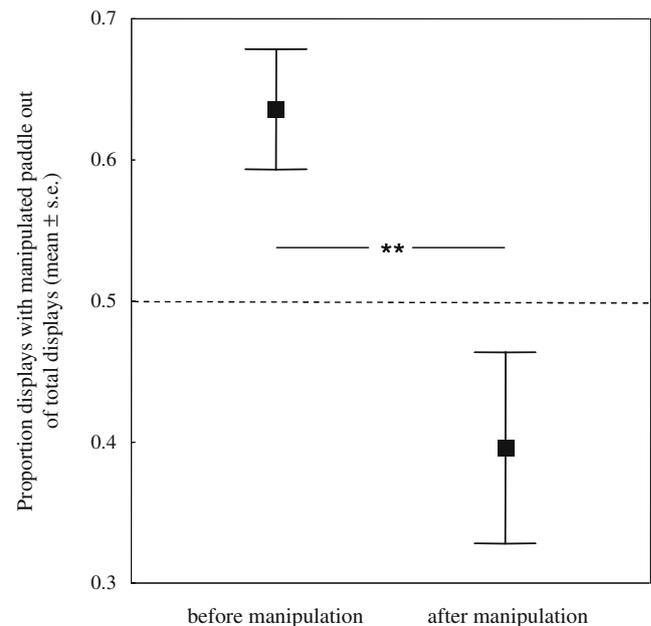


Fig. 3 Proportion of displays with the more manipulated paddle before and after manipulation. The previously larger and more used paddle in experiment 1 was significantly less used in experiment 2 following manipulation (proportion with larger paddle before manipulation (experiment 1): $\text{mean} \pm \text{SD} = 0.64 \pm 0.15$; proportion with previously larger, now more manipulated paddle after manipulation (experiment 2): $\text{mean} \pm \text{SD} = 0.40 \pm 0.23$; paired t test: $n=12$, $t=4.1$, $P=0.002$). $**P < 0.01$

(Uetz and Smith 1999; Uetz and Taylor 2003). This interpretation is also supported by the high kurtosis of the symmetry distributions, indicative of an excess of very asymmetric individuals.

Although our data clearly speaks against the paddle ornament in this species evolving through a true FA mechanism, we must point out that the laboratory males had been kept in isolation during sexual maturation, when the paddles fully develop (Bushmann and Burns 1994), and had therefore received no or very little damage to the paddles. Hence, developmental instability (Van Valen 1962; Klingenberg 2003) may contribute, at least to some extent, to ornament asymmetry in this species. The potential interaction between the effects of developmental instability and DA on ornament asymmetry may be an interesting avenue for future research on this species.

Behavioural response to ornament asymmetry

The combined results from experiments 1 and 2 suggest that there is a causal link between the status of the paddle ornament and male display asymmetry: males appear to be aware of their best side and favour this side in courtship displays. Before we discuss the importance of this result in light of the evolution of display signals, we consider some alternative explanations. Firstly, lateralization could have generated the results in experiment 1 if it caused a male to display more with one side, and this side, for some physiological reason, also grew larger ornaments. We feel that we can rule out this alternative, since males responded to the paddle manipulations in experiment 2. We cannot rule out, however, that lateralization contributes to the pattern seen in non-damaged males, given that males responded to the asymmetry of their paddles also before manipulations. Such individual lateralization in courtship behaviours has been described, for example in the Siamese fighting fish (Cantalupo et al. 1996), and could affect courtship display also in the swordtail characin. Secondly, one concern is that males may display less with a more manipulated paddle following our manipulation protocol in experiment 2. However, because both paddles were ablated in experiment 2 and because we allowed for a long time period (6 weeks) to pass between the manipulation and the actual behavioural experiment, we regard this as highly unlikely. This is also supported by the facts that (1) males showed no visible signs of suffering from our manipulations at the time of the behavioural experiment and (2) males displayed as much with their more manipulated paddles as they had done prior to the manipulation with their least preferred paddle. Hence, the level of display exhibited was within the natural range of non-manipulated fish. Thirdly, Gross et al. (2007) found that males displayed more with their more ornamented side, but only when they

displayed towards a living female, suggesting that female cues may drive display asymmetries in the guppy. Based on the present data set, we cannot rule out that males first test the reaction of females on both paddles and thereafter modify display behaviour according to female paddle preference. However, we believe this is unlikely in the swordtail characin since the display signal is likely to represent a trait resulting from sensory exploitation (Nelson 1964; Wickler 1968; Arnqvist and Rowe 2005; own unpublished data). In fact, behavioural observations suggest females are not even aware of the males' paddles until the males actively display their paddles right in front of the females (Nelson 1964; own observations). Moreover, our analyses of male display rates in relation to female paddle preference suggest that males do not increase display rate in response to female cues, at least not to the direct cue of actual bites at the displayed paddle.

What selective pressures have led to the evolution of the apparent self-awareness of ornament quality in the swordtail characin males? And how do males solve the conundrum of having to display a courtship ornament to achieve a mating when this means placing the ornament at risk of being damaged? As mentioned earlier, the paddles, and particularly the paddle flags, are highly labile traits since females bite at them during courtship and can damage them substantially (Kutaygil 1959; Nelson 1964; own observations). By offering the larger flag to the female during courtship, males should be able to maximise the effectiveness of his courtship although it is presently not known whether females respond more strongly towards longer paddles or larger flags. Moreover, males will effectively get another chance of displaying with an intact ornament if they only offer one flag at a time to the females they court. Theory suggest that males may often benefit from dishonest sexual signalling (e.g., Krebs and Dawkins 1984; Grafen 1990; Johnstone 1998; Rowell et al. 2006), and some empirical evidence show that this may be true for asymmetric displays (Gross et al. 2007). However, again, the paddle ornament in the swordtail characin is most likely primarily a sensory exploitation device rather than an honest signal of genetic quality (Arnqvist and Rowe 2005; own unpublished data), and offering as large a "lure" as possible to the female can therefore not be regarded as dishonest. An earlier study suggested rapid regeneration of paddles in the swordtail characin (complete regeneration of experimentally removed paddles within a few weeks; Kutaygil 1959), but our results show that this was not necessarily true: paddle flags had not grown back completely 6 weeks subsequent to the experimental manipulation. Although there may be a more rapid regeneration of the paddle itself than the flag of the paddle, our results certainly suggest there is a long-term cost associated with damages to the paddles.

In order to display according to ornament asymmetry, males have to be able to accurately assess the current state of their bilateral ornaments. Such a mechanism is difficult to imagine in the guppy or any other species carrying ornaments where the right side cannot be directly compared to the left side. However, the unique ornament structure of the swordtail characin potentially allows for direct comparison of the right paddle in relation to the left paddle simply by extending both paddles at the same time. This suggests that swordtail characin males do have the potential to accurately assess the present state of their paddles in spite of the paddle ornament being a labile trait. In light of the high level of cognitive ability of the fish brain that recent research has demonstrated (Braithwaite 2006), it is perhaps not surprising that the swordtail characin shows at least a primitive sense of self-awareness. It would be interesting for future studies to investigate if the self-awareness that has been demonstrated in certain mammals (humans, apes, dolphins, and elephants: e.g., Plotnik et al. 2006 and references therein) at least to some extent is existing also in other taxa.

Together with the previous findings by Gross et al. (2007) in the guppy, our analyses suggest males have the capacity to maximise their display success by showing their best side. Our results thus show that not only individual behavioural laterality (Vallortigara and Rogers 2005) but also within-individual variation needs to be considered in order to fully understand the evolution of courtship signals and courtship behaviours, especially in species where damages to display ornaments occur. We suggest future studies should focus not only on the empirical generality of these findings but also on the development of a novel theoretical framework regarding the evolution of courtship display with labile bilateral signals.

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References

- Andersson M (1994) Sexual selection. Princeton University Press, Princeton
- Arnqvist G, Rowe L (2005) Sexual conflict. Princeton University Press, Princeton
- Barbarosa A, Merino S, Cuervo JJ, de Lope F, Møller AP (2003) Feather damage of long tails in barn swallows *Hirundo rustica*. *Ardea* 91:85–90
- Bradbury J, Vehrencamp S (1998) Principles of animal communication. Sinauer Press, Mass, Sunderland
- Braithwaite VA (2006) Cognitive ability in fish. *Fish physiology*, Vol. 24. Elsevier, Amsterdam
- Brown WM, Conk L, Grochow K, Jacobson A, Liu CK, Popovic Z, Trivers R (2005) Dance reveals symmetry especially in young men. *Nature* 438:1148–1150. doi:10.1038/nature04344
- Burns JR, Weitzman SH, Grier HJ, Menezes NA (1995) Internal fertilization, testis and sperm morphology in Glandulocaudine fishes (Teleostei: Characidae: Glandulocaudinae). *J Morph* 224:131–145
- Bushmann PJ, Burns JR (1994) Social control of male sexual maturation in the swordtail characin, *Corynopoma riisei*. *J Fish Biol* 44:263–272
- Cantalupo C, Bisazza A, Vallortigara G (1996) Lateralization of displays during aggressive and courtship behaviour in the Siamese fighting fish (*Betta splendens*). *Phys Behav* 60:249–252
- Endler JA, Basolo AL (1998) Sensory ecology, receiver biases and sexual selection. *Trends Ecol Evol* 13:415–420
- Grafen A (1990) Biological signals as handicaps. *J Theor Biol* 144:517–546
- Gross MR, Suk HY, Robertson CT (2007) Courtship and genetic quality: asymmetric males show their best side. *Proc R Soc Lond B Biol Sci* 274:2115–2122. doi:10.1098/rspb.2007.0432
- Höglund J, Alatalo RV, Lundberg A, Rätti O (1993) Context-dependent effects of tail-ornament damage on mating success in black grouse. *Behav Ecol* 5:182–187
- Johnstone RA (1998) Game theory and communication. In: Dugatkin LA, Reeve HK (eds) *Game theory and animal behavior*. Oxford University Press, New York, pp 94–117
- Klingenberg CP (2003) A developmental perspective on developmental instability: theory, models, and mechanisms. In: Polak M (ed) *Developmental instability: causes and consequences*. Oxford University Press, New York, pp 14–34
- Krebs JR, Dawkins R (1984) Animal signals: mind reading and manipulation. In: Krebs JR, Davies NB (eds) *Behavioural ecology: an evolutionary approach*. Blackwell, Oxford, pp 380–402
- Kutaygil N (1959) Insemination, sexual differentiation, and secondary sex characters in *Stevardia albipinnis* Gill. *Istanbul Univ Fen Fak Mecm, Seri B* 24:93–128
- Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. *Auk* 104:116–121
- Møller AP (1991) Sexual ornament size and the cost of fluctuating asymmetry. *Proc R Soc Lond B Biol Sci* 243:59–62
- Møller AP, Pomiankowski A (1993) Fluctuating asymmetry and sexual selection. *Genetica* 89:267–279
- Møller AP, Swaddle JP (1997) *Asymmetry, developmental stability, and evolution*. Oxford University Press, Oxford
- Nelson K (1964) Behavior and morphology in the glandulocaudine fishes (Ostariophysi, Characidae). *Univ Calif Publ Zool* 75:59–152
- Palmer AR (1996) Waltzing with asymmetry. *Bioscience* 46:518–532
- Plotnik JM, de Waal FBM, Reiss D (2006) Self recognition in an Asian elephant. *Proc Natl Acad Sci U S A* 103:17053–17057. doi:10.1073/pnas.0608062103
- Polak M, Starmer WT (2005) Environmental origins of sexually selected variation and a critique of the fluctuating asymmetry-sexual selection hypothesis. *Evolution* 59:577–585
- Polak M, Taylor PW (2007) A primary role of developmental instability in sexual selection. *Proc R Soc Lond B Biol Sci* 274:3133–3140. doi:10.1098/rspb.2007.1272
- Rhodes G, Simmons LW (2007) Symmetry, attractiveness and sexual selection. In: Baret L, Dunbar R (eds) *Oxford handbook of evolutionary psychology*. Oxford University Press, Oxford, pp 333–364

- Rowell JT, Ellner SP, Reeve HK (2006) Why animals lie: how dishonesty and belief can coexist in a signalling system. *Am Nat* 168:E180–E204. doi:10.1086/508809
- Searcy WA, Nowicki S (2005) The evolution of animal communication. Princeton University Press, Princeton
- Tomkins JL, Simmons LW (2003) Fluctuating asymmetry and sexual selection: paradigm shifts, publication bias and observer expectation. In: Polak M (ed) *Developmental instability: causes and consequences*. Oxford University Press, New York, pp 231–261
- Uetz GW, Smith EI (1999) Asymmetry in a visual signalling character and sexual selection in a wolf spider. *Behav Ecol Sociobiol* 45:87–93
- Uetz GW, Taylor PW (2003) Developmental instability and animal communication: fluctuating asymmetry as a signal and as an influence on the signaling process. In: Polak M (ed) *Developmental instability: causes and consequences*. Oxford University Press, New York, pp 213–230
- Vallortigara G, Rogers LJ (2005) Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav Brain Sci* 28:575–633. doi:10.1017/S0140525X05000105
- Van Doorn GS, Weissing FJ (2006) Sexual conflict and the evolution of female preferences for indicators of male quality. *Am Nat* 168:742–757. doi:10.1086/508634
- Van Valen L (1962) A study of fluctuating asymmetry. *Evolution* 16:125–142. doi:10.2307/2406192
- Weitzman SH, Menezes NA (1998) Relationships of the tribes and genera of the Glandulocaudinae (Ostariophysi: Characiformes: Characidae) with description of a new genus, *Chrysobrycon*. In: Malabarba L, Reis RE, Vari RP, Lucena ZMS, Lucena CAS (eds) *Phylogeny and classification of neotropical fishes*. Edipurus, Porto Alegre, pp 171–192
- Wickler W (1968) *Mimicry in plants and animals*. McGraw-Hill, New York