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Strategic exploitation of fluctuating asymmetry in male Endler's guppy courtship displays is modulated by social environment

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Abstract

Lateral asymmetry in signalling traits enables males to strategically exploit their best side. In many animals, both body colouration and fluctuating asymmetry are signals of male attractiveness. We demonstrated experimentally that even sexually naïve male *Poecilia wingei* were able to identify their most attractive side (i.e. that with a higher proportion of carotenoid pigmentation) and use it preferentially during courtship. Notably, males retained their strategic signalling in a male-biased social environment, whereas they ceased to signal strategically in a female-biased environment. The degree of asymmetry in colouration did not affect overall courtship activity. Strategic lateralization in courtship displays was strongest and most repeatable in the male-biased social environment where males competed with rivals for matings. Individual asymmetry in colouration changed considerably over a period of 3 months. This suggests that colouration is a dynamic feature during adulthood and that males are capable of tracking and strategically exploiting their lateral asymmetry in accordance with their social environment.

Introduction

Females use male signalling to make mating decisions. In response, males adopt strategies to increase the efficiency of their signalling (Rodriguez *et al.*, 2012; Kahn *et al.*, 2013). This is particularly important in mating systems where the male contribution to reproduction is limited to sperm transfer. Despite growing evidence that females can attend to complex traits such as cognitive skills (Boogert *et al.*, 2011) or particular personality traits (Schuett *et al.*, 2010), ornamental traits are still considered to be the major targets of female choice across taxa (Amundsen, 2003; Kuijper *et al.*, 2012). A potential link between abstract, cognitive traits and male ornamentation is strategic modification of male signalling in response to feedback from females. This allows males to effectively emphasize their signalling effort (Patricelli *et al.*, 2002; Sullivan-Beckers & Hebets, 2014) or mask their particular deficits (Gross *et al.*, 2007).

Fluctuating asymmetry (FA) is a deviation from bilateral symmetry, most likely resulting from developmental errors or instability during ontogeny (Palmer, 1996). FA is considered to signal lower male quality in general (Møller, 1993; Watson & Thornhill, 1994) and has been demonstrated as playing a significant role in mate choice across taxa (humans: Koehler *et al.*, 2002; birds: Møller, 1992; invertebrates: Harvey & Walsh, 1993) including fish (Sheridan & Pomiankowski, 1997; Morris & Casey, 1998; Schlüter *et al.*, 1998; but see Brooks & Caithness, 1995; Gross *et al.*, 2007).

FA also enables asymmetric males to exploit their superior side via preferential lateral display (Gross *et al.*, 2007; Amcoff *et al.*, 2009). Many teleost fishes have laterally compressed bodies and can therefore benefit from strategic behavioural laterality during courtship. Even sexually naïve males of the common guppy *Poecilia reticulata* Peters adjusted their courtship to exploit their more colourful side (Gross *et al.*, 2007) and male swordtail characins *Corynopoma riisei* Gill responded to experimental manipulation of their paddle-like extension on gill cover by strategically biasing their lateral displays (Amcoff *et al.*, 2009). It remains unclear; however, how such strategic signalling is affected by individual experience and how such experience is modified

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by social environment. Laterality is widespread in fish (Bisazza *et al.*, 1998), but our understanding of whether it may be adaptive by being utilized strategically in response to FA is surprisingly weak.

There is also abundant evidence that females base their choice on relative variation among males (Hughes *et al.*, 2013), highlighting the role of social environment. Social environment has a major effect on individual behaviour, including mating behaviour (Pruett-Jones, 1992; Plath & Bierbach, 2011; Barbosa *et al.*, 2013). Indeed, male courtship and signalling are strongly influenced by experience and current social environment (Miller & Brooks, 2005; Guevara-Fiore *et al.*, 2012). It is therefore critical to relate the behaviour of an individual to its current and past social experience. Surprisingly, the question of how individual experience and social environment influence lateralized behaviour remains unexplored.

Carotenoid pigments are reliable and taxonomically widespread indicators of individual condition and social status (Sefc *et al.*, 2014). Carotenoids cannot be synthetized by animals *de novo* and must be obtained in the diet (Latscha, 1990), making them potential indicators of male foraging ability (Kodric-Brown, 1989). Females can evaluate several critical features of a male's phenotypic and genetic quality, including his condition (Olson & Owens, 1998), genomewide heterozygosity (Herdegen *et al.*, 2014) and fertility (Locatello *et al.*, 2006; Pike *et al.*, 2010; Smith *et al.*, 2014) from the amount of carotenoid pigment displayed on his body. Consequently, males are expected to preferentially display their most carotenoid-coloured side during courtship. At the same time, assumptions about the role of carotenoids as sexual signals cannot be uncritically generalized across populations of particular species. Targets of female choice are often variable across populations (Endler & Houde, 1995) due to differences in natural selection (Endler, 1980), signalling environment (Seehausen & van Alphen, 1998; Summers *et al.*, 1999; Fuller & Noa, 2010; Maan *et al.*, 2010) or reinforcement (Kirkpatrick & Servedio, 1999; Williams & Mendelson, 2013), resulting in high interpopulation variability in preference for visual signals.

We used Endler's guppy (*Poecilia wingei* Poeser, Kempkes & Isbrücker) [also reported as Cumaná guppy or Campoma guppy (Evans *et al.*, 2011)] to investigate strategic male signalling in response to FA in their colouration. We were particularly interested in the role of orange carotenoid pigmentation because it has been suggested that female *P. wingei* prefer males with more orange colouration (Lindholm & Breden, 2002; Alexander & Breden, 2004; *P. wingei* reported as Cumaná guppy). Male *P. wingei* are generally more colourful than male *P. reticulata* and possess orange-coloured vestiges of swords in the caudal fin, providing additional circumstantial evidence of the importance of carotenoid colouration in their reproductive success. Indeed, female preference for male colouration has been proposed to drive divergence between *P. wingei* and *P. reticulata* (Alexander & Breden, 2004).

Poecilia wingei is closely related to the common guppy (*P. reticulata*) (Poeser *et al.*, 2005) and their behaviour is similar (Houde, 1997; Poeser *et al.*, 2005; personal observation), allowing inferences on *P. wingei* to be drawn from studies on *P. reticulata.* Males devote a significant proportion of their time to reproductive behaviour. Males court females by sigmoid displays which provide them with the opportunity to preferentially show their more colourful side. Alternatively, males can mate coercively through gonopodial thrusts (Poeser *et al.*, 2005), but with only a limited sperm transfer (Pilastro & Bisazza, 1999). The sexual behaviour of *P. wingei* is considerably influenced by individual experience and social environment (Řežucha & Reichard, 2014).

We tested four objectives. First, we examined the association between the overall level of courtship behaviour and the extent of fluctuating asymmetry. Second, we tested laterality of courtship behaviour in relation to fluctuating asymmetry in colouration in young, sexually inexperienced (virgin) males that lacked any feedback from prior female responses to their courtship. We compared this with laterality in courtship behaviour in similarly young but sexually experienced males. Third, we tested laterality in courtship behaviour in experienced males following housing in contrasting social environments – female-biased environment (FBE, focal males housed with two females) or male-biased environment (MBE, focal male housed with a rival and a single female). Finally, we explored temporal changes in FA for individual males over a three-month period.

We predicted that overall levels of male courtship would be unaffected by fluctuating asymmetry in colouration, including carotenoid colouration. We expected that the level of laterality in courtship displays would be related to the degree of individual FA (positive association). We predicted that experienced males would bias their courtship towards displaying their best side, as a result of positive feedback from females, but that virgin males would lack such bias. We expected that males in MBE would preferentially display their best side more often compared to males in FBE due to the presence of mating competition. Finally, we predicted that individual FA scores in colouration might vary slightly during the course of colouration development but would be broadly repeatable across ontogeny.

Materials and methods

Fish housing

Subject animals were taken from our breeding stock composed of outbred descendants of *P. wingei* imported from Laguna de los Patos (northern Venezuela) in

2007. The stock population was kept in a 120-L aquarium and fed twice a day with commercial flake food and frozen chironomid larvae. Water temperature was maintained at 25 °C ± 2 °C, and the aquarium was subject to a natural daylight regime via a glass rooftop (10–14 h of light a day). Water was continuously aerated and its quality maintained by air-driven foam filters and regular water exchange. Live plants were provided as refugia. Over a period of one month, all emerging juveniles were collected from the stock aquarium and isolated in a separate 72-L aquarium. Juveniles were frequently sexed, and females were removed and kept separately.

Sixty-five males (approximately 19 weeks old) were collected from a total of 146 juveniles and placed individually in 2-L plastic tanks. The tanks were visually separated from each other, experienced a natural light regime, and additional light was provided for 12 h a day (08:00–20:00) by a 40 W Sun Glo daylight spectrum fluorescent tube. Water temperature fluctuated with ambient temperature between 22 and 25 °C. Artificial plants were provided as refugia in each aquarium. Males were fed once a day with commercial flake food, and water was exchanged every two weeks.

Experimental males

The first behavioural test of male mating behaviour (Trial 1) was performed after 2 months of separation. We tested two groups of males. Virgin males ($n = 65$) had no prior experience with an adult receptive female and therefore no feedback on their courtship displays. Experienced males ($n = 32$) were randomly chosen from the social aquarium where they had lived from birth. Their age (estimated on the basis of body size and development of colouration) was approximately 6 months (matching the age of the virgin males) and they interacted fully with a group of females of various ages. The sex ratio in the social aquarium was not directly measured but fluctuated naturally over their adult period and never departed considerably from parity. All experienced males were separated from females for 3 days prior to testing to standardize their mating effort and to replenish sperm reserves (Liley, 1966).

Upon completion of all trials at the first time point, two females were added to 33 randomly assigned tanks with focal males to create a female-biased social environment (one male, two females). In the remaining 32 tanks with focal males, a single male and single female from the stock population were added to create a male-biased social environment (two males, one female). The second test of sexual behaviour of focal males (Trial 2) was completed after 5-week housing in the particular social environment for a subset of males who survived until Trial 2 (naïve males in Trial 1). Males had unrestricted access to females (and rivals in the MBE) and gained mating experience in their particular social environment. Experimental males in the MBE treatment were readily distinguished from the second male on the basis of colouration patterns (Magurran & Magellan, 2007). Despite temporal changes in colouration, the primary colour pattern enabled unambiguous distinction of the two males in all cases. Therefore, we are confident that treatment males were identified correctly, although no additional confirmation (e.g. based on genotyping) was undertaken. Upon completion of the second trial, males were placed back in their home tank. The social environment treatment was maintained by replacing any dead female or nonexperimental male. Focal males were not replaced and focal male mortality caused a minor decrease in sample size during the second trial. Experienced males from Trial 1 (i.e. from the large social aquarium) were not tested in Trial 2.

Behavioural assays

Male mating behaviour was scored in a 6-L aquarium with the back and sides covered with black fabric to minimize disturbance. Light was provided by a 25 W Sun Glo daylight spectrum bulb positioned above the aquarium, ensuring an even distribution of light. Females used in the experiment were collected from a virgin female aquarium and left with a group of three adult nonexperimental males from the stock aquarium for one day prior to the experimental trials (Liley, 1966). This standardized female sexual receptivity by ensuring that all females were nonvirgin and in the same reproductive state. Each female was tested with 4 experimental males. During Trial 2 (social environment treatments), focal males were isolated from all conspecifics for 1.5 day prior to testing to standardize their mating effort. A female was allowed to settle for 5 min in the test aquarium. After this period, a randomly chosen focal male was gently captured, added to the test aquarium and left for 10 min to settle. After settling, male mating behaviour was scored for 15 min by a single observer using JWATCHER 1.0, software for behavioural scoring (Blumstein *et al.*, 2007). We scored the number and duration of sigmoid displays (courtship) using left and right sides. The sigmoid display is inherently lateral; the male spreads his fins and arches his body into a sigmoid shape and quickly undulates, exposing the bright colouration on one side of his body. Sigmoid displays may be repeated in succession, with either the same or the opposite side of the body displayed (Houde, 1997). After 15 min, the focal male was gently captured and returned to his home tank.

Photographing and analysis of colour pattern

Focal males were photographed at the age of approximately 19 weeks (4 weeks prior to Trial 1) and at the age of 32 weeks (1 week after Trial 2). A male was gently captured and immediately released into a small photo-aquarium. No anaesthetics were used to prevent any distortion of colour pattern (Reynolds *et al.*, 1993; Pélabon *et al.*, 2014) and intensity (Gray *et al.*, 2011). Sedation with cold water was similarly not employed as any stress associated with photographing was considered less than stress associated with cold-water sedation. Each male was fixed in a stable position in the front of the aquarium using a soft sponge and quickly photographed from both sides using a Canon EOS Rebel XTi camera (Canon Inc., Tokyo, Japan) equipped with a Sigma Macro 100 mm lens. The camera was positioned on a stationary tripod to ensure a constant distance from the lens. A small ruler was placed next to each individual to provide a scale. Standardized fish colouration and constant light conditions were ensured by placing the photo-aquarium in a dark case and using flash illumination. Orange carotenoid spots do not appear to vary according to the surrounding environment or the emotional state of the fish (Brooks & Caithness, 1995). The melanophore system can respond to the surrounding environment in 7–35 min in *P. reticulata* females (Neill, 1940 in Baerends *et al.*, 1955), and it is possible that male black spots can be modified even more rapidly (Baerends *et al.*, 1955). All pictures were taken in the shortest interval possible, typically < 3–4 min. We treated all individuals equally during handling and photographing and potential artificial changes in melanophore expression should be distributed equally across individuals and treatments. Two photographs of each individual were taken from each side, and the photograph where the fish was in the best position on each particular side was used for analysis. Where both photographs were of comparable quality, the one used for analysis was chosen at random. All pictures were taken in compressed JPG format with 3888 × 2592 pixels resolution and 72 DPI.

All image analyses were performed in FIJI, an image processing package (Schindelin *et al.*, 2012) based on IMAGEJ 1.47n software (Abramoff *et al.*, 2004). We measured the area of each colour category of interest, and the number of separate dots of each defined colour. It was not possible to determine the number of iridescent patches unambiguously [as is the case with *P. reticulata* (Ruell *et al.*, 2013)], and we did not consider the number of iridescent patches in our analysis. The total area of iridescent patches was readily quantified.

Three colour categories – carotenoid (light orange to dark red), melanin (dark black and fuzzy black) and iridescent (mostly green, blue, purple and silver, formed by guanine crystals in iridophores) were established. First, we defined the colour scale of each category in RGB colour space on a subset of individuals. The RGB colour space is defined by the three chromaticities of the red, green and blue primaries (Pascale, 2003). This adjustment allowed us to measure precisely the specific colour areas of each particular side using the Colour Thresholder and Select tools in the FIJI package. The area of specific colour (*Ac*) was then measured as a proportion of total area of lateral body projection:

$$\forall\ N_{px}C\epsilon R\langle r_1, r_2\rangle \wedge G\langle g_1, g_2\rangle \wedge B\langle b_1, b_2\rangle :$$
$$A_c = \frac{N_{px}C}{N_{px}S} \times 100\%$$

where $N_{px}C$ is number of all pixels of colour within defined intervals of R (red), G (green) and B (blue) in RGB colour space, and $N_{px}S$ is total side area selected with the Freehand selection tool (under sufficient magnification to minimize measurement error). The dorsal fin, eyes and gonopodium were not in a fixed position during photographing, and their areas were excluded from our analysis (i.e. not included in $N_{px}S$). Some males did not have evenly spread caudal fins in the photographs, and we used mean caudal fin area for their respective body size. We did not exclude caudal fin area as it possessed a non-negligible amount of colouration. The number of clear colour spots was determined visually. Small and discontinuous fuzzy areas were not counted as separate dots but were included in the measure of overall area of the particular colour. We acknowledge that this approach is sensitive to subjective perception of colour, but the same lighting conditions and the same set of threshold values applied to all photographs and analysis performed by the same person minimized any bias. Measurement of colour characteristics was made blind with respect to behavioural data.

Data analysis

Relative asymmetry (in %) was calculated as the absolute value of asymmetry (i.e. giving left and right bias the same sign) divided by the larger value for one side (Amcoff *et al.*, 2009). Symmetry of directional fluctuation in male traits was tested using paired *t*-tests on arcsine transformed data, for Trials 1 and 2 separately. Pooled data for Trials 1 and 2 were used for visualization of FA using histograms. Note that a subsample of males (32 of 109 males) was included twice in histograms (as young and old males), resulting is some pseudo-replication. However, the analysis did not include any pseudo-replication.

The relationship between FA (measured as absolute asymmetry) and total carotenoid colouration on overall courtship activity was tested by a set of Pearson correlations, independent for each social environment treatment. This was because we knew a priori that social treatments affected overall courtship activity (Řežucha & Reichard, 2014). An alternative was the use of generalized mixed models with 'social environment' nested within 'fish age' (Trial 1 and 2) and 'male identity' as a random factor. Such a complex design is beyond the current development of the GLMM.

The effect of colouration on directional bias in male displays was analysed using a generalized linear mixed model (GLMM) with a binomial distribution applied to the counts of sigmoid displays of left and right sides of the body as the response variable. Males that performed no sexual display within the 15-min observational period were not considered in the analysis. We adopted an IT approach (Burnham & Anderson, 2002). We first selected a set of biologically plausible models (Table 1). We predicted *a priori* that carotenoid colouration would be targeted by females (Houde, 1997; Alexander & Breden, 2004). However, given the large interpopulation variability in the importance of male traits targeted by female choice in the closely related *P. reticulata*, we considered other traits and their interactions which could potentially affect female choice and directional bias (Fig. 1). Collinearity between explanatory variables was evident from an initial data exploration, with clear association between the number of patches of particular colour and its total area. We accounted for this collinearity in the choice of models, but retained models with alternative colouration measures to compare their effect on laterality of sexual displays. We compared 21 models of varying complexity (Table 1). Female identity was treated as random factor for all models. Fits of alternative models were compared using the Akaike information criterion corrected for small sample size (AICc). Model weights were calculated from relative likelihoods. For the model with the best fit, the residuals were examined to ensure model assumptions were met. We considered all models within ΔAIC < 2 to have substantial support (Burnham & Anderson, 2002). However, given parsimony and interpretation, we further explored only the best-fitting models (see further). Models for Trial 1 and Trial 2 had identical structure but were treated separately (Table 1). Statistical analyses were performed in the R environment (R Development Core Team, 2009). In Trial 1, there were 49 naïve males and 28 experienced males performing sigmoid displays. In Trial 2, 17 males in the MBE and 15 males in the FBE performed sigmoid displays during behavioural observation. The overall mean number of sigmoid displays (including males not displaying to females) was 11 (SD = 8.8, median = 9) per 15-min observational period.

Temporal change in the magnitude of FA in colouration traits and sigmoid displays was tested using the nonparametric Wilcoxon paired test on absolute values of the differences. Repeatability was tested as intraclass correlation *sensu* Lessells & Boag (1987) and visualized as a bivariate plot between individually based values at the age of 19 and 32 weeks.

Table 1 Set of candidate models for strategic signalling in response to the fluctuating asymmetry and its modulation by social environment and their relative evaluation. All models also included random factor 'female identity'.

		Time 1: naïve/ experienced			Time 2: MBE/FBE		
Model ID	Model description	d.f.	ΔAIC	*w*	d.f.	ΔAIC	*w*
T2	*Trt + %Carot + Trt* x *%Carot*	**4**	**0**	**0.28**	**4**	**0**	**0.39**
T7	*Trt + %Melan + %Carot + %Melan* x *% Carot + Trt* x *%Melan + Trt* x *%Carot*	**7**	**1**	**0.17**	7	7.5	0.01
T9	*Trt + %Irid + %Carot + %Irid* x *%Carot + Trt* x *%Irid + Trt* x *%Carot*	**13**	**1**	**0.17**	13	7.6	0.01
M2	*% Carotenoids (%Carot)*	**2**	**2**	**0.10**	2	2.2	0.13
M9	*%Carot + %Irid + %Carot* x *%Irid*	**4**	**2**	**0.10**	**4**	**1.6**	**0.17**
M4	*% Iridescence (%Irid)*	2	3	0.06	2	4.4	0.04
M7	*%Carot + %Melan + %Carot* x *%Melan*	4	4	0.04	4	7	0.01
T4	*Trt + %Irid + Trt* x *%Irid*	4	5	0.02	4	8.1	0.01
M5	*Number of carotenoid patches (NCarot)*	2	6	0.01	2	5.3	0.03
M1	*Intercept only (Null)*	1	7	0.01	1	3.4	0.07
M8	*%Irid + %Melan + %Irid* x *%Melan*	4	7	0.01	4	6	0.02
M10	*%Carot + %Melan + %Irid + %Carot* x *%Melan + %Carot* x *%Irid + %Carot* x *%Melan*	7	7	0.01	7	11	0.00
T8	*Trt + %Irid + %Melan + %Irid* x *%Melan + Trt* x *%Irid + Trt* x *%Melan*	7	7	0.01	7	11.4	0.00
T1	*Treatment (Trt) only*	2	8	0.01	2	4.4	0.04
M3	*% Melanin (%Melan)*	2	9	0.00	2	5.9	0.02
M6	*Number of melanin patches (NMelan)*	2	9	0.00	2	5.7	0.02
T3	*Trt + %Melan + Trt* x *%Melan*	4	9	0.00	4	9.4	0.00
T6	*Trt + NMelan+ Trt* x *NMelan*	7	9	0.00	7	9	0.00
M11	*NCarot + NMelan + NCarot* x *NMelan*	4	10	0.00	4	6.6	0.01
T5	*Trt + NCarot + Trt* x *NCarot*	4	10	0.00	4	8.9	0.00
T11	*Trt + NCarot + NMelan + NCarot* x *NMelan*	8	15	0.00	8	19.2	0.00

d.f., degrees of freedom; ΔAICc, the difference between the best model and evaluated model; relative model weight (*w*). Models with ΔAICc < 2 are highlighted in bold typeset.

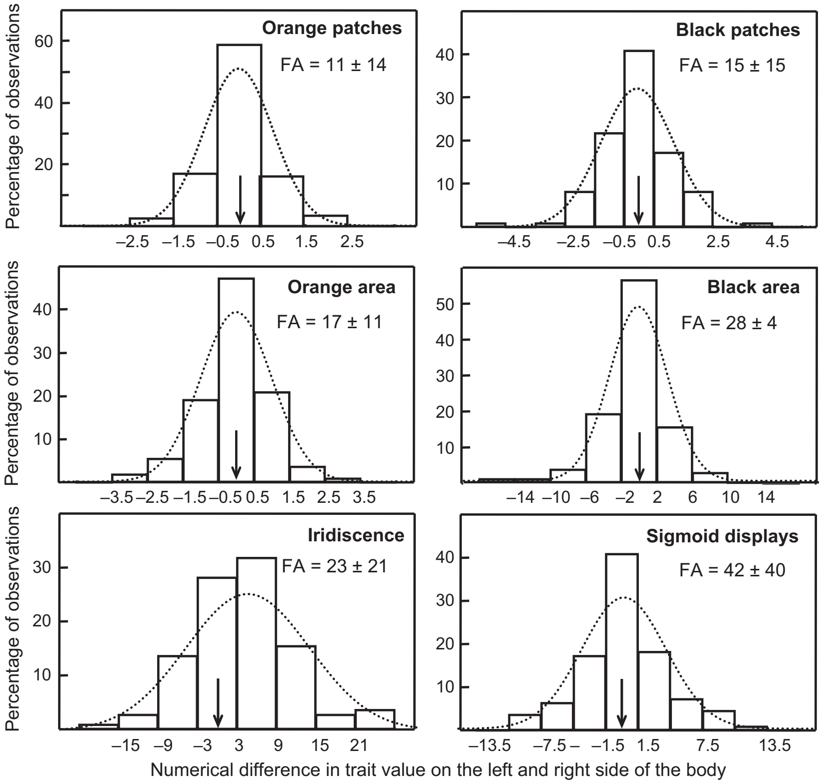

Fig. 1 Distribution of fluctuating asymmetry in five colouration traits and in sigmoid displays. An arrow indicates the perfect symmetry. Raw difference in values measured for left and right sides of the body (and numerical difference between left and right displays, respectively) is displayed. The values of relative asymmetry (in %; calculated as absolute asymmetry divided by the larger value for one side) and its standard error are also included to provide a standardized measure.

Results

Description of fluctuating asymmetry

All colouration variables and sigmoid displays demonstrated fluctuating asymmetry. The relative asymmetry in colouration ranged between 11% and 28% of the mean value, and asymmetry of sigmoid displays was even higher (42%) (Fig. 1). No directional bias was observed for any variable (paired *t*-tests on arcsine transformed data, all $P > 0.05$), except iridescence ($t_{76} = 3.47$, $P = 0.001$ and $t_{31} = 3.43$, $P = 0.002$ for young and older males, respectively; left side was more iridescent). No directional bias was found in sigmoid displays (paired *t*-test, both $P > 0.50$).

The relationship between FA and carotenoid colouration on overall courtship activity

The intensity of sigmoid displays was not related to the level of overall FA or carotenoid-based FA (Pearson correlation for each male treatment separately, $P > 0.15$ for all 8 tests) or to the overall score for total carotenoid area (Pearson correlation for each male treatment

Table 2 Evaluation of fixed effects for the best supported models of the effects of fluctuating asymmetry in colouration on laterality in male sexual displays in relation to social environment treatments.

	(a) Young males (Trial 1)			(b) Older males (Trial 2)		
Model parameter	*F*-value*	χ^2†	*P*	*F*-value*	χ^2†	*P*
Orange	6.79	6.52	0.011	4.29	5.74	0.017
Social environment	0.60	0.61	0.434	1.98	1.77	0.183
Orange x Social environment	6.34	6.43	0.011	3.99	4.21	0.040

**F*-value is based on the ANOVA table (type III) from GLMM analysis in LME4 package that included random effect ‘female identity’. Note that *P*-values cannot be computed for GLMM because the true number of degrees of freedom is not known.

†χ^2 denotes test statistics from GLM analysis of deviance for binomial distribution in the default *stats* package (random effect ‘female identity’ omitted) where *P* (statistical significance of the estimate) can be computed. Note that female identity had a negligible effect on male sigmoid displays.

separately, $P > 0.14$ for three tests). The exception was a positive correlation between intensity of sigmoid displays and total carotenoid area in males from the FBE ($r_{13} = 0.54$, $P = 0.040$), although this association was highly nonsignificant after Bonferroni correction for multiple testing.

Strategic signalling in response to FA and its modulation by social environment

Males responded to their FA by displaying their more carotenoid-coloured side preferentially and social environment modulated this response. At both time points, the models with the strongest support contained asymmetry in the percentage of carotenoid colouration, treatment (social environment) and their interaction (Tables 1 and 2). The response was strongest in naïve males (Trial 1: Fig. 2a) and males in the MBE (Trial 2: Fig. 2c). Experienced males had generally negligible FA (Fig. 2b) that may have resulted in the lack of association with the lateralized displays.

Other models with substantial empirical support also contained complex associations between percentage of carotenoid pigments and iridescence (Table 1) and their interpretation was complex. The area of carotenoid colouration explained laterality in male displays better than the number of carotenoid patches (ΔAICc = 4 and 10 for Trial 1 in models with and without social environment interaction, respectively, and ΔAICc = 3.1 and 8.9 for Trial 2 models).

Changes in strategic signalling with male age

There was no temporal change in the magnitude of FA in colouration traits (Wilcoxon paired test, $N = 33$, $P > 0.50$ for six tests and $z = 1.71$, $P = 0.088$ for carotenoid patches). The FA in sigmoid displays marginally decreased between Trial 1 and Trial 2 (Wilcoxon paired test, $z = 2.19$, $N = 32$, $P = 0.028$).

Repeatability in sigmoid displays was high ($r^2 = 0.79$) in males that were subjected to the MBE prior to Trial 2 testing, but very modest ($r^2 = 0.23$) in the FBE males (Fig. 3). In colouration traits, repeatability was modest for number of colour patches but low for areas of carotenoid, melanin and iridescent colouration and congruent between FBE and MBE treatments (Fig. 3). The FA in all traits sometimes changed from left to right bias and vice versa between the two measurements (i.e. over 13 weeks) (Fig. 3). As for repeatability, the direction of FA was relatively more stable in discrete

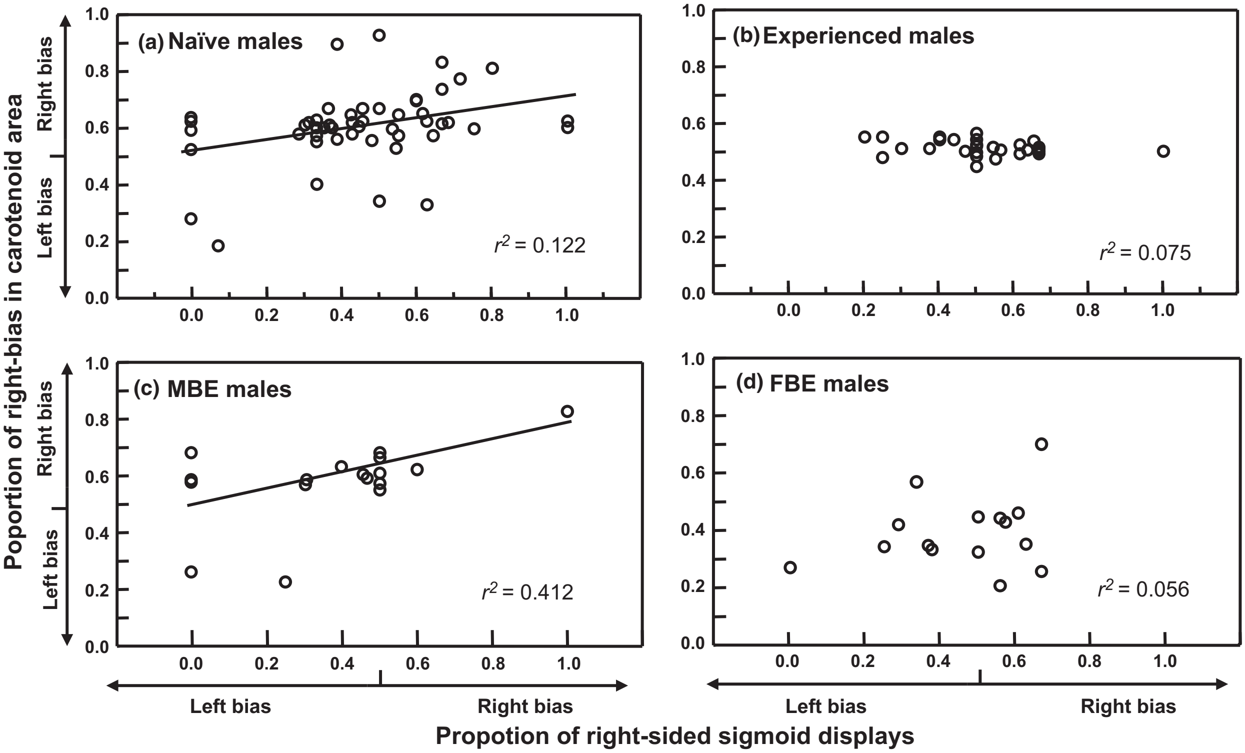

Fig. 2 Relationship between the laterality in sigmoid displays and laterality in carotenoid-based colouration in four treatment groups. Data were tested using binomial distribution, but bivariate plots on proportions were used for graphical display. The variation explained by the linear relationship between proportions (r^2 value) is included, and the trend line emphasizes significant associations.

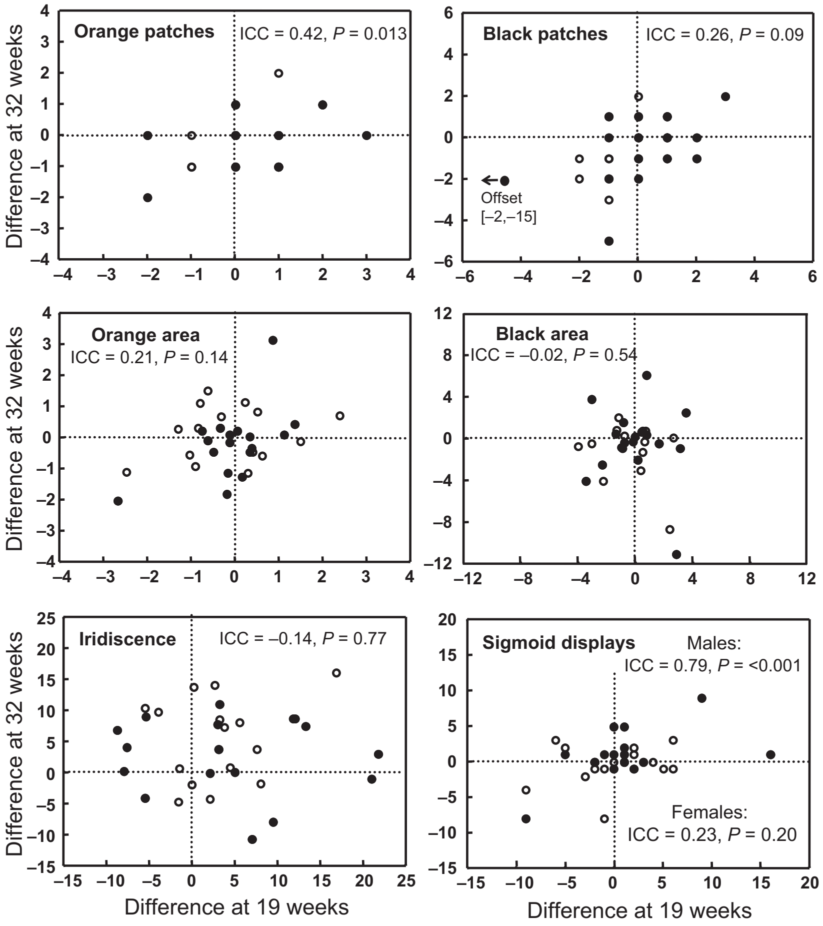

Fig. 3 Ontogenetic changes in fluctuating asymmetry for five colouration traits and for sigmoid displays visualized as bivariate association between raw individually based values from the first (age of 19 weeks) and second (age of 32 weeks) measurement. Symbols distinguish the male-biased environment (closed) and female-biased environment (open). Repeatability values (ICC) and their statistical significance are shown.

colouration patches (Fig. 3, top panels) and sigmoid displays (Fig. 3, lower right panel) than in the area of colouration (Fig. 3, mid-panels, lower left).

Discussion

We experimentally demonstrated the ability of *P. wingei* males to identify their more colourful side and to use it preferentially during courtship. Carotenoid-based orange colouration was identified as the most important source of side bias in courtship. These results are in general concordance with a previous report for the closely related *P. reticulata* (Gross *et al.*, 2007) who also found that even sexually naïve males are able to identify their more colourful side. Importantly, we demonstrated that social environment substantially modulates the magnitude of laterality in sexual display. Males from the FBE, who had not competed with any rival for access to mating for a period of 5 weeks, ceased to preferentially display their more colourful side. Young males from a mixed social aquarium also failed to display their best side – however, the FA in the percentage of carotenoids pigments was negligible in this group of males.

Some of the males with negligible FA in carotenoid pigmentation still demonstrated a preferential use of one side over the other during sigmoid displays (e.g. left to right ratios of 15 : 5, 8 : 15, 8 : 2). This trend could indicate an underlying cerebral lateralization of sigmoid displays in *P. wingei* males. Cerebral lateralization can work in concert, at least to some extent, with lateralization of colouration. This would suggest that in a population there is always a set of behavioural right siders and left siders, even if colouration does not significantly differ between the two sides. Cerebral laterality has been shown to influence other aspects of behaviour, including turning left or right to detour a barrier in *P. reticulata* (Bisazza *et al.*, 1997; Irving & Brown, 2013), direction of escape in crayfish *Procambarus clarkii* (Girard) (Tobo *et al.*, 2012), preferential use of a particular side during aggressive displays in Siamese fighting fish *Betta splendens* Regan (Takeuchi *et al.*, 2010), precopulatory mating behaviour in the great pond snail *Lymnaea stagnalis* (L.) (Davison *et al.*, 2009) or visual mate choice alone in zebra finch *Taeniopygia guttata* (Vieillot) (Templeton *et al.*, 2012). Cerebral laterality can potentially explain an inherent lateral bias in *P. wingei* sigmoid displays. However, laterality in displays followed a normal distribution and was therefore congruent with the predictions of FA rather than antisymmetry (Palmer, 1996). Also, we did not record any general directional bias in male sigmoid displays, suggesting there is no species-specific or population-specific pattern in laterality of the behaviour, as for instance the right bias in lateral contest displays in male convict cichlid [*Amatitlania nigrofasciata* (Günther)] (Arnott *et al.*, 2011), preferential foot use in Australian parrots (Magat & Brown, 2009) or more efficient right-side prey handling in snail-eating specialist snakes (Hoso *et al.*, 2007).

More colourful males did not court at a higher rate than less colourful males, as suggested for *P. reticulata* (Kiritome *et al.*, 2012), regardless of social environment, age or experience. Neither did we find any association between the level of FA and overall level of courtship. In *P. reticulata*, females do not simply prefer colourful males but they prefer colourful males with the highest courtship intensity (Kodric-Brown, 1993). Colourful males can therefore increase their attractiveness further by increasing the number of sigmoid displays, but this pattern was not confirmed in our study. Courtship is energetically costly (Kotiaho, 2000) and should be used prudently. Any intensification of male courtship may be more evident in longer trials and associated with an initial female interest that we did not measure.

Importantly, social environment modulated adaptive laterality in sexual displays. Males from the MBE, who experienced constant mating competition, continued to display their best side, whereas males in the female-biased environment did not. This was in agreement with our prediction that the presence of a rival would increase the importance of male courtship quality relative to a noncompetitive environment. This was coupled with an overall decline in mating effort in the FBE males, including attempts at forced copulation (Řežucha & Reichard, 2014).

Strategic male signalling should only be possible if a male is able to recognize that the bias in his lateral displays confers higher success. This requires self-awareness to identify the best side (Amcoff *et al.*, 2009). In the swordtail characin, males promptly responded to subtle experimental changes in the FA of their signal, paddle-like extension on gill cover, that can be easily damaged and is therefore inherently labile (Amcoff *et al.*, 2009). How virgin males in our study were able to identify their best side is unknown, but the existence of lateral bias towards the best side was also demonstrated in virgin male *P. reticulata* (Gross *et al.*, 2007). Interestingly, the proportion of best side displays did not increase within and across the mating trials of virgin male *P. reticulata* (Gross *et al.*, 2007), providing no evidence that males exploit their more attractive side only after feedback from female responses. However, the contrast in strategic lateralization of sigmoid displays between social environments suggested that males used social information to modify the display of their sides in the courtship. Strategic lateralization of sigmoid displays in the MBE may have been maintained via a process of mutual reinforcement between rivals, or simply remained unaltered from its initial innate level. In the FBE, intrasexual competition among males was absent (Clutton-Brock & Parker, 1992; Kvarnemo & Ahnesjö, 1996) so males may have ceased to rely on their lateral variation in attractiveness. The strength of sexual selection therefore modulated male signalling effort both in term of courtship rate in general (Řežucha & Reichard, 2014) as well as in the strategic use of FA.

Iridescent colouration significantly affected lateral bias in sigmoid displays in addition to carotenoid-based colouration. The effect of iridescence was strongest in interaction with carotenoid colouration (Table 1). This suggests that carotenoid pigments are either not the exclusive target of female choice in *P. wingei* or their clarity may be modified via contrasts with other pigments. Female *P. reticulata* are sensitive to much subtler variations in signals of male quality (Sathyan & Couldridge, 2012) than generally suggested and the importance of iridescent pigments in female *P. reticulata* mate choice has previously been highlighted (Endler, 1983).

Temporal changes in FA have only rarely been investigated (but see e.g. Hallgrímsson, 1999; Kellner & Alford, 2003; Bartoš *et al.*, 2007). We found that the overall magnitude of FA in colouration remained unchanged between the age of 19 and 32 weeks, but at an individual level, measures of FA were relatively unrepeatable and individuals exhibited increases, decreases and even changes in the direction of FA between the two measurements (Fig. 3). This outcome was not predicted and suggests high plasticity in individual FA throughout development rather than either simple maintenance of the degree of FA throughout life, a gradual increase from its initial level (Bartoš *et al.*, 2007) or gradual corrections (Kellner & Alford, 2003). Our data are consistent with major ontogenetic changes in colouration among male *P. reticulata* (measured only on a single side of the body) (Miller & Brooks, 2005) and suggest that colouration on the left and right sides of the body can fluctuate at least partly independently.

In conclusion, we demonstrated that social environment strongly modulated strategic use of FA in male *P. wingei* courtship displays. The asymmetry in colouration and behaviour was congruent with the predictions of fluctuating asymmetry (normal distribution) rather than lateralization (bimodal distribution). The degree of FA did not affect overall courtship activity, and strategic lateralization in courtship displays was strongest and most repeatable in the social treatment where males competed with rivals. Individual variation in FA between two measurements was high and suggestive of continual changes in colouration, at least within the first 8 months of life, which constitutes much of the typical life expectancy of wild male guppies (Bryant & Reznick, 2004; López-Sepulcre *et al.*, 2013; Arendt *et al.*, 2014). We suggest future work should explore the causal role of social environment on temporal changes in male signalling traits such as colouration and their asymmetry.

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References

Abramoff, M.D., Magelhaes, P.J. & Ram, S.J. 2004. Image processing with ImageJ. *Biophotonics Int.* **11**: 36–42.

Alexander, H.J. & Breden, F. 2004. Sexual isolation and extreme morphological divergence in the Cumaná guppy: a possible case of incipient speciation. *J. Evol. Biol.* **17**: 1238–1254.

Amcoff, M., Arnqvist, G. & Kolm, N. 2009. Courtship signalling with a labile bilateral signal: males show their best side. *Behav. Ecol. Sociobiol.* **63**: 1717–1725.

Amundsen, T. 2003. Fishes as models in studies of sexual selection and parental care. *J. Fish Biol.* **63**: 17–52.

Arendt, J.D., Reznick, D.N. & López-Sepulcre, A. 2014. Replicated origin of female-biased adult sex ratio in introduced populations of the Trinidadian guppy (*Poecilia reticulata*). *Evolution* **68**: 2343–2356.

Arnott, G., Ashton, C. & Elwood, R.W. 2011. Lateralization of lateral displays in convict cichlids. *Biol. Lett.* **7**: 683–685.

Baerends, G.P., Brouwer, R. & Waterbolk, H.T. 1955. Ethological studies on *Lebistes reticulatus* (Peters) 1. An analysis of the male courtship pattern. *Behaviour* **8**: 249–334.

Barbosa, M., Ojanguren, A.F. & Magurran, A.E. 2013. Courtship display persists despite early social deprivation. *Ethology* **119**: 496–502.

Bartoš, L., Bahbouh, R. & Vach, M. 2007. Repeatability of size and fluctuating asymmetry of antler characteristics in red deer (*Cervus elaphus*) during ontogeny. *Biol. J. Linn. Soc.* **91**: 215–226.

Bisazza, A., Pignatti, R. & Vallortigara, G. 1997. Laterality in detour behaviour: interspecific variation in poeciliid fish. *Anim. Behav.* **54**: 1273–1281.

Bisazza, A., Rogers, L.J. & Vallortigara, G. 1998. The origins of cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neurosci. Biobehav. Rev.* **22**: 411–426.

Blumstein, D.T., Daniel, J.C. & Evans, C.S. 2007. *Quantifying Behavior: The Jwatcher Way*. Sinauer Associates Inc, Sunderland.

Boogert, N.J., Fawcett, T.W. & Lefebvre, L. 2011. Mate choice for cognitive traits: a review of the evidence in nonhuman vertebrates. *Behav. Ecol.* **22**: 447–459.

Brooks, R. & Caithness, N. 1995. Female choice in a feral guppy population: are there multiple cues? *Anim. Behav.* **50**: 301–307.

Bryant, M.J. & Reznick, D. 2004. Comparative studies of senescence in natural populations of guppies. *Am. Nat.* **163**: 55–68.

Burnham, K.P. & Anderson, D.R. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer, New York.

Clutton-Brock, T.H. & Parker, G.A. 1992. Potential reproductive rates and the operation of sexual selection. *Q. Rev. Biol.* **67**: 437–456.

Davison, A., Frend, H.T., Moray, C., Wheatley, H., Searle, L.J. & Eichhorn, M.P. 2009. Mating behaviour in *Lymnaea stagnalis* pond snails is a maternally inherited, lateralized trait. *Biol. Lett.* **5**: 20–22.

Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**: 76–91.

Endler, J.A. 1983. Natural and sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fishes* **9**: 173–190.

Endler, J.A. & Houde, A.E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**: 456–468.

Evans, J.P., Pilastro, A. & Schlupp, I. 2011. *Ecology and Evolution of Poeciliid Fishes*. The University of Chicago Press, Chicago and London.

Fuller, R.C. & Noa, L.A. 2010. Female mating preferences, lighting environment, and a test of the sensory bias hypothesis in the bluefin killifish. *Anim. Behav.* **80**: 23–35.

Gray, S.M., Hart, F.L., Tremblay, M.E.M., Lisney, T.J. & Hawryshyn, C.W. 2011. The effects of handling time, ambient light, and anaesthetic method, on the standardized measurement of fish colouration. *Can. J. Fish Aquat. Sci.* **68**: 330–342.

Gross, M.R., Suk, H.Y. & Robertson, C.T. 2007. Courtship and genetic quality: asymmetric males show their best side. *Proc. R. Soc. B* **274**: 2115–2122.

Guevara-Fiore, P., Svensson, P.A. & Endler, J.A. 2012. Sex as moderator of early life experience: interaction between rearing environment and sexual experience in male guppies. *Anim. Behav.* **84**: 1023–1029.

Hallgrímsson, B. 1999. Ontogenetic patterning of skeletal fluctuating asymmetry in rhesus macaques and humans: evolutionary and developmental implications. *Int. J. Primatol.* **20**: 121–151.

Harvey, I.F. & Walsh, K.J. 1993. Fluctuating asymmetry and lifetime mating success are correlated in males of the damselfly *Coenagrion puella* (Odonata: Coenagrionidae). *Ecol. Entomol.* **18**: 198–202.

Herdegen, M., Dudka, K. & Radwan, J. 2014. Heterozygosity and orange coloration are associated in the guppy (*Poecilia reticulata*). *J. Evol. Biol.* **27**: 220–225.

Hoso, M., Asami, T. & Hori, M. 2007. Right-handed snakes: convergent evolution of asymmetry for functional specialization. *Biol. Lett.* **3**: 169–173.

Houde, A.E. 1997. *Sex, Color, and Mate Choice in Guppies*. Princeton University Press, New Jersey.

Hughes, K.A., Houde, A.E., Price, A.C. & Rodd, F.H. 2013. Mating advantage for rare males in wild guppy populations. *Nature* **503**: 108–110.

Irving, E. & Brown, C. 2013. Examining the link between personality and laterality in a feral guppy *Poecilia reticulata* population. *J. Fish Biol.* **83**: 311–325.

Kahn, A.T., Dolstra, T., Jennions, M.D. & Backwell, P.R.Y. 2013. Strategic male courtship effort varies in concert with adaptive shifts in female mating preferences. *Behav. Ecol.* **24**: 906–913.

Kellner, J.R. & Alford, R.A. 2003. The ontogeny of fluctuating asymmetry. *Am. Nat.* **161**: 931–947.

Kiritome, A., Sato, A. & Karino, K. 2012. Influences of orange spot patterns and operational sex ratio on male mating behaviors in the guppy *Poecilia reticulata*. *Ichthyol. Res.* **59**: 304–313.

Kirkpatrick, M. & Servedio, M.R. 1999. The reinforcement of mating preferences on an island. *Genetics* **151**: 865–884.

Kodric-Brown, A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav. Ecol. Sociobiol.* **25**: 393–401.

Kodric-Brown, A. 1993. Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration and courtship. *Behav. Ecol. Sociobiol.* **32**: 415–420.

Koehler, N., Rhodes, G. & Simmons, L.W. 2002. Are human female preferences for symmetrical male faces enhanced when conception is likely? *Anim. Behav.* **64**: 233–238.

Kotiaho, J.S. 2000. Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. *Behav. Ecol. Sociobiol.* **48**: 188–194.

Kuijper, B., Pen, I. & Weissing, F.J. 2012. A guide to sexual selection theory. *Annu. Rev. Ecol. Evol. Syst.* **43**: 287–311.

Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends Ecol. Evol.* **11**: 404–408.

Latscha, T. 1990. *Carotenoids - Their Nature and Significance in Animal Feeds/Thierry Latscha*. F. Hoffmann-La Roche, Animal Nutrition and Health, Basel.

Lessells, C.M. & Boag, P.T. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* **104**: 116–121.

Liley, N.R. 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour* **13**: 1–197.

Lindholm, A. & Breden, F. 2002. Sex chromosomes and sexual selection in poeciliid fishes. *Am. Nat.* **160**: S214–S224.

Locatello, L., Rasotto, M.B., Evans, J.P. & Pilastro, A. 2006. Colourful male guppies produce faster and more viable sperm. *J. Evol. Biol.* **19**: 1595–1602.

López-Sepulcre, A., Gordon, S.P., Paterson, I.G., Bentzen, P. & Reznick, D.N. 2013. Beyond lifetime reproductive success: the posthumous reproductive dynamics of male Trinidadian guppies. *Proc. R. Soc. B* **280**: 20131116.

Maan, M.E., Seehausen, O. & Van Alphen, J.J.M. 2010. Female mating preferences and male coloration covary with water transparency in a Lake Victoria cichlid fish. *Biol. J. Linn. Soc.* **99**: 398–406.

Magat, M. & Brown, C. 2009. Laterality enhances cognition in Australian parrots. *Proc. R. Soc. B* **276**: 4155–4162.

Magurran, A.E. & Magellan, K. 2007. Behavioural profiles: individual consistency in male mating behaviour under varying sex ratios. *Anim. Behav.* **74**: 1545–1550.

Miller, L.K. & Brooks, R. 2005. The effects of genotype, age, and social environment on male ornamentation, mating behavior, and attractiveness. *Evolution* **59**: 2414–2425.

Møller, A.P. 1992. Female swallow preference for symmetrical male sexual ornaments. *Nature* **357**: 238–240.

Møller, A.P. 1993. Developmental stability, sexual selection and speciation. *J. Evol. Biol.* **6**: 493–509.

Morris, M.R. & Casey, K. 1998. Female swordtail fish prefer symmetrical sexual signal. *Anim. Behav.* **55**: 33–39.

Neill, H.M. 1940. On the existence of two types of chromatin behaviour in teleostean fishes. *J. Exp. Biol.* **17**: 74–98.

Olson, V.A. & Owens, I.P.F. 1998. Costly sexual signals: are carotenoids rare, risky or required? *Trends Ecol. Evol.* **13**: 510–514.

Palmer, A.R. 1996. Waltzing with asymmetry. *Bioscience* **46**: 518–532.

Pascale, D. 2003. *A Review of RGB Color Spaces... from xyY to R'G'B'*. The BabelColor Company, Montreal.

Patricelli, G.L., Uy, J.A.C., Walsh, G. & Borgia, G. 2002. Male displays adjusted to female's response. *Nature* **415**: 279–280.

Pélabon, C., Larsen, L.-K., Bolstad, G.H., Viken, Å., Fleming, I.A. & Rosenqvist, G. 2014. The effects of sexual selection on life-history traits: an experimental study on guppies. *J. Evol. Biol.* **27**: 404–416.

Pike, T.W., Blount, J.D., Lindström, J. & Metcalfe, N.B. 2010. Dietary carotenoid availability, sexual signalling and functional fertility in sticklebacks. *Biol. Lett.* **6**: 191–193.

Pilastro, A. & Bisazza, A. 1999. Insemination efficiency of two alternative male mating tactics in the guppy *Poecilia reticulata*. *Proc. R. Soc. B* **266**: 1887–1891.

Plath, M. & Bierbach, D. 2011. Sex and the public: social eavesdropping, sperm competition risk and male mate choice. *Commun. Integr. Biol.* **4**: 276–280.

Poeser, F.N., Kempkes, M. & Isbrücker, I.J.H. 2005. Description of *Poecilia* (*Acanthophacelus*) *wingei* n. sp. from the Paría Peninsula, Venezuela, including notes on *Acanthophacelus* Eigenmann, 1907 and other subgenera of *Poecilia* Bloch and Schneider, 1801 (Teleostei, Cyprinodontiformes, Poeciliidae). *Contrib. Zool.* **74**: 97–115.

Pruett-Jones, S. 1992. Independent vs. non-independent mate-choice: do females copy each other? *Am. Nat.* **140**: 1000–1009.

R Development Core Team 2009. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Reynolds, J.D., Gross, M.R. & Coombs, M.J. 1993. Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. *Anim. Behav.* **45**: 145–152.

Řežucha, R. & Reichard, M. 2014. The effect of social environment on alternative mating tactics in male Endler's guppy, *Poecilia wingei*. *Anim. Behav.* **88**: 195–202.

Rodriguez, R.L., Haen, C., Cocroft, R.B. & Fowler-Finn, K.D. 2012. Males adjust signaling effort based on female mate-preference cues. *Behav. Ecol.* **23**: 1218–1225.

Ruell, E.W., Handelsman, C.A., Hawkins, C.L., Sofaer, H.R., Ghalambor, C.K. & Angeloni, L. 2013. Fear, food and sexual ornamentation: plasticity of colour development in Trinidadian guppies. *Proc. R. Soc. B* **280**: 20122019.

Sathyan, R. & Couldridge, V.C.K. 2012. Female preference for blue in Japan blue guppies (*Poecilia reticulata*). *Environ. Biol. Fishes* **96**: 953–959.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M. & Pietzsch, T. *et al.* 2012. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**: 676–682.

Schlüter, A., Parzefall, J. & Schlupp, I. 1998. Female preference for symmetrical vertical bars in male sailfin mollies. *Anim. Behav.* **56**: 147–153.

Schuett, W., Tregenza, T. & Dall, S.R.X. 2010. Sexual selection and animal personality. *Biol. Rev.* **85**: 217–246.

Seehausen, O. & van Alphen, J.J.M. 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behav. Ecol. Sociobiol.* **42**: 1–8.

Sefc, K.M., Brown, A.C. & Clotfelter, E.D. 2014. Carotenoid-based coloration in cichlid fishes. *Comp. Biochem. Physiol. Part A* **173**: 42–51.

Sheridan, L. & Pomiankowski, A. 1997. Female choice for spot asymmetry in the Trinidadian guppy. *Anim. Behav.* **54**: 1523–1530.

Smith, C., Phillips, A., Polačik, M. & Reichard, M. 2014. Male coloration signals direct benefits in the European bitterling (*Rhodeus amarus*). *Environ. Biol. Fishes* **97**: 335–341.

Sullivan-Beckers, L. & Hebets, E.A. 2014. Tactical adjustment of signalling leads to increased mating success and survival. *Anim. Behav.* **93**: 111–117.

Summers, K., Symula, R., Clough, M. & Cronin, T. 1999. Visual mate choice in poison frogs. *Proc. R. Soc. B* **266**: 2141–2145.

Takeuchi, Y., Hori, M., Myint, O. & Kohda, M. 2010. Lateral bias of agonistic responses to mirror images and morphological asymmetry in the Siamese fighting fish (*Betta splendens*). *Behav. Brain Res.* **208**: 106–111.

Templeton, J.J., Mountjoy, D.J., Pryke, S.R. & Griffith, S.C. 2012. In the eye of the beholder: visual mate choice lateralization in a polymorphic songbird. *Biol. Lett.* **8**: 924–927.

Tobo, S., Takeuchi, Y. & Hori, M. 2012. Morphological asymmetry and behavioral laterality in the crayfish, *Procambarus clarkii*. *Ecol. Res.* **27**: 53–59.

Watson, P.J. & Thornhill, R. 1994. Fluctuating asymmetry and sexual selection. *Trends Ecol. Evol.* **9**: 21–25.

Williams, T.H. & Mendelson, T.C. 2013. Male and female responses to species-specific coloration in darters (Percidae: *Etheostoma*). *Anim. Behav.* **85**: 1251–1259.

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