Original Article

The role of boundary length and adjacent patch contrast in guppy mate choice

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The presence of various combinations of adjacent colors within polymorphic species' color pattern could have a major impact on mate choice. We studied the role of pattern geometry in predicting mate choice in guppies using boundary strength analysis (BSA). BSA estimates the visual contrast intensity between two adjacent color patches (ΔS) weighted by the lengths of the boundaries between these adjacent color patches. We measured both the chromatic (hue and saturation) and achromatic (luminance) ΔS for each pair of adjacent patches. For each male's color pattern, we measured BSA as both mean ($m\Delta S$) and coefficient of variation ($cv\Delta S$) of all ΔS weighted by their corresponding boundary lengths. We also determined if specific color patch boundaries had an impact on female preferences and whether these predicted overall male contrast ($m\Delta S$). We found that males with a higher $m\Delta S$ were more attractive to females and that six boundaries containing either fuzzy black or black as one of the pair colors significantly affected female preferences, indicating that 1) females favored highly conspicuous males and 2) melanin-based patches could be used as a signal amplifier, not only for orange but for other colors.

Key words: animal color pattern, boundary strength analysis, color patch adjacency, mate choice, signal amplifier.

INTRODUCTION

Color patterns are used by vertebrates and invertebrates to make behavioral decisions linked to survival and reproduction (Endler 1978; Endler and Basolo 1998; Osorio and Vorobyev 2008; Kemp et al. 2015; Ruxton et al. 2019). The evolution of those color patterns in plants and animals are ultimately driven by the behavioral response of the viewers, which depend upon their perception of colors (see Endler and Mappes 2017 for review). Understanding color pattern perception is complex because it relies on multiple parameters such as the reflectance spectra of the color patches in the color pattern, the visual background, the ambient light environment as well as the visual abilities of the viewer (Kelber et al. 2003; Endler and Mappes 2017; Van Den Berg et al. 2020). Several new methods were developed in the last decade and allow describing and analyzing complex color patterns from the viewer's perspective (see Endler 2012 and Van Den Berg et al. 2020 for review). In order to obtain a global understanding of the mechanism underlying color pattern evolution, it is essential to include those methods in the study of color-mediated behaviors. In this study, we explored the effects of contrast geometry between adjacent color patches in a mate choice context (Endler 2012; Endler et al. 2018).

This is important because adjacent patches strongly stimulate receptor fields, which detect local changes in color and luminance (Elder and Sachs 2004; Stevens and Cuthill 2006; Endler 2012; Troscianko et al. 2017; Endler et al. 2018). Therefore, we used a novel method in the field of visual ecology, the boundary strength analysis or BSA (Endler et al. 2018) to explore the effects of color patch adjacency that influence female mate choice in guppies.

BSA describes the intensity of local contrast across the male pattern in term of mean or coefficient of variation of ΔS ($m\Delta S$ and $cv\Delta S$, respectively, Endler et al. 2018). ΔS (the distance in receptor space relative to receptor noise) is an estimation of the visual contrast intensity between two adjacent patches relative to photoreceptor noise and uses species-specific parameters. In other words, it indicates how different the two patches are. According to the receptor noise level (RNL) model, under optimal conditions, a given individual is theoretically able to discriminate between two color stimuli when $\Delta S \geq 1$–3, depending upon assumptions and assuming ΔS is not very different from 1 (Vorobyev and Osorio 1998; Olsson et al. 2018). ΔS is estimated using the RNL model (Vorobyev and Osorio 1998) and the calculation is equivalent to the Mahalanobis distance in multivariate statistics, comparing the differences between the two sets of cone captures (for the two adjacent colors; see Kelber et al. 2003; Endler et al. 2018). We weighted ΔS by the lengths of the boundaries between adjacent color patches to obtain individual mean BSA $m\Delta S$ and measured

the coefficient of variation across the male pattern $cv\Delta S = sd\Delta S/m\Delta S$. $cv\Delta$ is a measure of the relative variability of contrast across the male pattern. A high $m\Delta S$ indicates that the individual color pattern possesses highly contrasting boundaries, and a high $cv\Delta S$ indicates that the color pattern varies in boundary contrast; some boundaries have high contrast and others have low contrast across the pattern (see Figure A4 in Sibeaux et al. 2019a for illustration). We used the RNL model (Vorobyev and Osorio 1998) to estimate ΔS between all combinations of adjacent pairs in the guppy male pattern. ΔS between two given color patches is constant but males vary in which patches are adjacent and in patch boundary lengths. Therefore, the length of a boundary might be a better predictor of BSA rather than its contrast alone. We calculated chromatic and achromatic ΔS values separately (Vorobyev and Osorio 1998; see Siddiqi et al. 2004 for achromatic contrast calculation adapted to a species visual system). The color contrast of a male pattern is a combination of chromatic and achromatic cues which are largely processed by distinct pathways at the retinal level (i.e., early visual processing), each pathway having different acuity, as well as different temporal and spectral properties (Livingstone and Hubel 1988). Therefore, the chromatic and achromatic geometric patterns can be very different (see the "fort diagrams" in Endler et al. 2018). Chromatic ΔS represents the combined differences of hue and chroma between adjacent patches, whereas achromatic ΔS represents the luminance difference of adjacent patches. Both chromatic and achromatic signals are probably combined to produce a behavioral response (Lehrer and Bischof 1995; Aksoy and Camlitepe 2012; Kelber et al. 2003). Moreover, because strong edge contrast has the potential to draw and retain attention, it is likely that the strength of boundaries in males' color pattern (measured with BSA) affect females' preference (Endler et al. 2018; Sibeaux et al. 2019a).

Sibeaux et al. (2019a) showed that the BSA method was successful in predicting mate choice in guppies compared to another method looking at the Overall Pattern Contrast (OPC) of male pattern which did not consider the contrast between adjacent patches (for comparison between BSA and OPC, see the Supplementary Tables A1 and A2). In the published experiment, females preferred males showing a high $cv\Delta S$ and a low $m\Delta S$. That experiment was performed in three light environments in order to create a wide variation in male's visual appearance as a strong test of the method (Supplementary Figure A1). However, those light environments did not contain UV wavelengths, a natural light component used in guppy mate choice (Smith et al. 2002; White et al. 2003; Archard et al. 2009). Although the intensity of UV reflection in male patterns did not affect female preferences in some laboratory populations (White et al. 2003), males showing UV-deprived patterns were significantly less attractive to females compared to males displaying UV wavelengths in other populations (Smith et al. 2002). Consequently, part of the coloration information used by female guppies could have been missing in our previous experiment.

Our experiment here builds on the previous one and extends the use of BSA in two ways. First, it tests the value of the method under natural illumination conditions (including UV), and second, it evaluates the relative importance of the length of color boundaries and the contrast between the adjacent colors in affecting mate choice. This provides novel and critical information on the effect of adjacent contrast in guppy mate choice.

In this experiment, we tested if BSA predicts mate choice under a light environment simulating natural conditions. We investigated 1) if BSA is a good predictor of mate choice under a light mimicking a natural light environment (broad-spectrum light including UV), 2) if the length of boundaries between adjacent color pairs affect mate choice, and 3) to what extent lengths or intensities between adjacent color patches are effective predictors of BSA? We also investigated whether particular adjacent color combinations had strong or weak effects on both chromatic and achromatic BSA measures and female choice.

We used the guppy (*Poecilia reticulata*) as our biological model because males display a high color pattern polymorphism, and male coloration is assessed by females during mate choice (Endler 1980, 1983; Gamble et al. 2003; Cole and Endler 2015). Males have three types of color spots whose production is dependent upon different mechanisms: 1) carotenoid–pteridine pigments such as orange or yellow, which are partially dependent upon carotenoid intake in the diet (Kodric-Brown 1985; Grether et al. 2001); their color thus varies strongly with diet (Endler 1980; Kodric-Brown 1989; Grether et al. 2005), 2) melanin-based pigments such as black spots that can serve as signal amplifiers (Brooks 1996), and 3) structural colors, for example, blue, which are highly conspicuous to conspecifics and predators (Endler 1980). Male color patterns are the result of a trade-off between predation and sexual selection and their coloration is strongly dependent upon genetics, mostly through both X and Y linkage (Endler 1980; Brooks and Endler 2001; Eakley and Houde 2004). Numerous studies have shown that female choice depends upon orange, black, or UV components of the male pattern (Endler and Houde 1995; Brooks 1996; Rodd et al. 2002; Smith et al. 2002), but only recent studies integrated the effect of multiple color components on male attractiveness (Cole and Endler 2015; Sibeaux et al. 2019a).

We presented a total of 60 males to eight females and observed which males the females preferred. We hypothesized that 1) BSA contrast measures should predict male attractiveness, 2) the length of the boundaries which possess a higher chromatic or achromatic contrast (ΔS with respect to all cones or only double cones, respectively) might have a stronger effect on male attractiveness than boundaries with lower contrast, and 3) the length of a boundary should be a better predictor of BSA than its contrast.

MATERIAL AND METHODS
Animal husbandry

The guppies (P. reticulata) used in this experiment came from a well-established laboratory population (7 years old or 14–28 generations given generation overlap, see Figure A4 in Sibeaux et al., 2019a for pictures of individuals from the population). The population originated from a wild population of fish caught at Alligator Creek, Bowling Green Bay National Park, Queensland, Australia (19°26.79′S 146°58.65′E). To control for age, 240 mixed-sex juveniles (approximately 1-month old with a ~1:1 sex ratio), were randomly assigned to one of three 196-L glass tanks (80 juveniles per tank), 13 months before the beginning of the experiment. These control-age tanks were illuminated by high-frequency fluorescent lamps (Supplementary Figure A2) following a 12-h light–dark cycle, and the fish were fed once a day with flake food or brine shrimp.

Experimental design

Eight 13-month-old (± 1 month) females were selected randomly from the control-aged tanks to be used as focal females for the experiment. Each female was housed individually in a (22 × 35.5 × 26 cm) glass aquarium with 1 cm of white gravel at the

bottom, 14 cm deep water and continuous airflow. Temperature, pH, and kH were maintained constant at 22 ± 1 °C 7.2 and 120 ± 10 ppm, respectively. Each tank had an opaque partition including a remotely operated sliding door, which created two equally sized compartments, the home compartment, and the experimental compartment. Females could swim through it to reach the experimental compartment during the experiment (Figure 1). A mirror (12 × 12 cm, covering 75% of the left side wall of the home compartment) was placed on one wall of the home compartment to create the illusion of a companion fish to prevent social isolation of the focal female (Agrillo et al. 2016). To further prevent effects of social isolation, a liter of water from the females' initial stock tank, containing all the chemical cues from the original tank, was added to the water of their test aquarium when they were transferred from the control-age tanks at the beginning of the study.

All eight aquaria were placed long sides next to each other in a series (Figure 1). White paper sheets were placed on the sidewalls of the experimental area of each tank in order to avoid any visual cues from one fish to another during tests. A black curtain was used to separate the experimental area from the rest of the lab, thus minimizing any disturbances. Each tank was illuminated by an Exo-terra Sunray 50W reptile light which closely mimicked natural sunlight, including UV (Supplementary Figure A1a). The tanks were kept under a constant 12-h light–dark cycle and placed at 102 cm distance from the lights. We measured the irradiance (ambient light) using a cosine-corrected receptor and an ocean optics USB2000+ spectrophotometer, calibrated for photon flux (μmol photons m^{-1} sec^{-1} nm^{-1}) with a Li-Cor LI-1800–02 optical radiation calibrator (standard lamp, Supplementary Figure A1a); for details on irradiance measurements see Endler (1990). The transparency of the wall of the female tanks and male chambers was tested using a portable Ocean Optics USB2000+ spectrometer and white chalk (reflecting all wavelengths from 300 to 700 nm) placed on the other side of the transparent walls. They were both perfectly transparent from 300 to 700 nm.

Testing procedure

We tested how male patch boundaries and their visual contrast affect their attractiveness to female guppies. Sixty males were chosen randomly from four different stock tanks and were allocated to one of the 20 groups of three males ("trio," labeled A to T, Supplementary Table A3). In each trio, each male displayed a different pattern according to the human eye.

Males were transferred from their stock tanks to individual 3 L tanks 2 days before their first experimental trial for acclimation. Before each test, males were transferred from their 3 L tanks to individual chambers of 8 × 8 × 12.5 cm. These chambers had 1 cm of white gravel at the bottom and 8.5 cm of water. The side and back walls of these chambers were covered with back plastic sheets. In every chamber, 5 mL of water coming from the male's stock tank was added, to reduce stress and bring chemical cues mimicking the presence of other fish. These three chambers were placed at the experimental section of the test tank opposite the sliding door (Figure 1).

We ran 160 mate choice trials. Each female saw each of the 20 trios of males in the same order (Supplementary Table A3). Each female saw four groups of males per day and each male saw four females. To control for male motivation and female interest, a gap of 48 h was allowed before a female saw another four sets of male trios. For the sake of consistency, if a set of four male groups presented at a certain time (AM or PM) to the four first females, then it was presented at the same time to the other four females 48 h later. However, the order that females were used in changed for each session. This way, all females had the chance to be in the first-time position at least once (Supplementary Table A3).
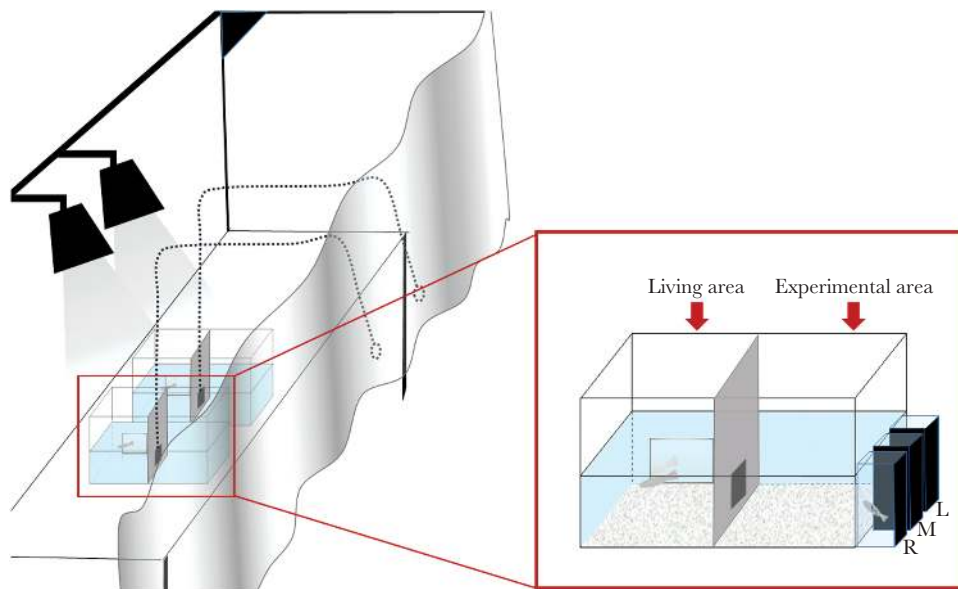

Figure 1

Overview of the experimental setup. The apparatus included eight tanks (only two shown). A curtain separating the experimenter and the tanks. The right panel shows an individual tank divided into an experimental area and a living area. The gray partition includes a sliding door covering a doorway between the living and experimental tank areas (shown here as a black rectangle located at the bottom of the gray partition). Both the partition and sliding door were made of white semitransparent plexiglass. A mirror was placed in the living area sidewall (pale rectangle) to at least partially control for social attraction. Male chambers are presented on the right (R), middle (M), and left (L) from the female point of view.

During this experiment, three males out of 60 had to be re-placed by new ones because they showed erratic swimming or constant swimming near the water surface. This increased the number of males in the experiment to 63.

We conducted trials as follows: Males were transferred to their individual chambers, which were placed in front of the experimental areas of the females' tanks (Figure 1) for 10 min of acclimatization. Males were assigned randomly to the right, middle, or left chamber. Next, the sliding door was opened, allowing the female to enter the experimental area where she could see the males; the door was closed after the female entered this compartment. Females were allowed to swim freely in the experimental area while viewing the males. After 20 min, the trial stopped, the door was opened, and the female was gently ushered back to her home compartment. The trials were recorded from above, using Panasonic HC-V100 and JVC HD Everio video cameras. Prior to all sessions, males and females were fed ad libitum. The morning sessions lasted from 8:30 AM until 12:30 PM, whereas the afternoon sessions were between 1 PM and 5 PM.

Male photography and pattern assessment

Once males finished all behavioral trials, they were photo-graphed following the methods in Cole and Endler (2015), see Supplementary Appendix A1 for details. The photographs were then analyzed with Adobe Photoshop CS5.1 and MATLAB (as in Cole and Endler 2015). Within Photoshop, we manually outlined the total fish area (i.e., from the head to the end of the caudal peduncle), the tail fin area (i.e., starting at the caudal peduncle), and the area of eleven distinct color classes: black and yellow reticulation (present on the tail of the fish), black, fuzzy black, yellow, orange, gold, silver, green, blue and violet, and saved the outlines in a multilayer PSD file. The color classes were based upon reflectance spectra (Supplementary Figure A3). In this population, we have found no colors which reflect in the UV but do not reflect in the visible, making human-visual assignment to classes and patch outlining feasible. Some structural colors can change in terms of reflectance spectra according to individual movement and hence viewing angle, while others do not. It depends upon whether the structural color is based upon interference or scattering. Only interference colors change in reflectance spectral shape (hue and chroma) with angle, and only some guppy structural colors change significantly with viewing angle. The photographic setup was designed so that the camera to male angle was approximately the same as the average female to male angle during courtship. We adjusted the lighting to maximize the contrast of the structural colors. We took multiple reflectance measurements of each male for each color classes in order to control as much as possible for reflectance variation in structural color patches. We used MATLAB to read the PSD file layers, calculate the transition Matrix and used the transition Matrix to extract the relative color area measurements, the numbers of each transition between colors, and lengths of each transition in the pattern (2 pixels sampling grid, Endler 2012; Endler et al. 2018). Because of the high degree of symmetry between the left and right sides in our population (93% of males, Cole and Endler 2015, 2016), only the right-side color pattern was analyzed.

Male BSA pattern contrast measurement

We determined males' color pattern contrast using the BSA method, which gives an estimate of the local contrast in the

male color pattern (Endler et al. 2018). The strength (intensity of visual contrast) of a boundary is estimated by ΔS (distance in receptor space) between adjacent color patches using the receptor noise model (Vorobyev and Osorio 1998; see Siddiqi et al. 2004 for the model utilized for achromatic contrast). ΔS is an estimate of the likelihood of color discrimination between two stimuli; when $\Delta S = 1$, two visual stimuli are just noticeably different (JND, Vorobyev and Osorio 1998; Endler and Mielke 2005; Kemp et al. 2015). An individual can potentially discriminate one stimulus from the other if $\Delta S \geq 1$. Under this threshold ($\Delta S < 1$), the viewer is unlikely to discriminate between two stimuli. In guppies, the behavioral discrimination threshold is close to the RNL model prediction when 0.2 is used as the standard deviation of the noise in photoreceptor (Sibeaux et al. 2019b). We used the formulae in Vorobyev and Osorio (1998) and Kelber et al. (2003) to calculate chromatic ΔS (Equation 5 and B4 respectively, see Supplementary Appendix A2 for formulae); we used the adaptation of the formula in Siddiqi et al. (2004) to calculate achromatic ΔS. To calculate ΔS, we used 0.2 as the numerator of the Weber fraction (Sibeaux et al. 2019b). In guppies, the photoreceptor relative abundance and their spectral sensitivity are 1;1;2;2 and λ_{max} = 359 nm; 408 nm; 465 nm; 560 nm for UVS; SWS; MWS and LWS cones, respectively (Long 1993; Kawamura et al. 2016; see Supplementary Appendix A2 for the details of the calculation). BSA takes into account the light environment irradiance spectra, the reflectance spectra, and relative area of each color pattern component, the spectral sensitivity functions of the guppy photoreceptors, and the geometry of the color pattern by considering which colors are adjacent (i.e., having a common boundary). It uses the intensity (i.e., magnitude of ΔS for a given patch contact zone) as well as the length of the boundary between the patches and ignores nonadjacent color pairs (see Supplementary Appendix A2 for all formulae).

For a given color pattern, we calculated ΔS for each pair of color classes which shared a common boundary (e.g., orange-black, blue-green). We also calculated their common boundary lengths. For each male color pattern, we calculated the mean ΔS of all kinds of adjacent pairs weighted by their total boundary length and will refer to this as $m\Delta S$. We also calculated the weighted coefficient of variation of ΔS ($cv\Delta S$) across the male pattern (Supplementary Appendix A2; Endler et al. 2018).

Chromatic and achromatic ΔS values were calculated separately (Supplementary Appendix A2; Supplementary Table A4), to be able to measure chromatic and achromatic $m\Delta S$ and $cv\Delta S$ for each male. Chromatic cues are detected by all four visual cone classes while achromatic cues are detected only by the double cones. We calculated ΔS for chromatic visual channels, using all four guppy cone classes, and achromatic ΔS using the guppy double cones (see Supplementary Appendix A2 for cone and eye details, see Supplementary Figure A4 for the representation of the color patches in the tetrahedron of guppy color vision).

Video analysis

To be blind to male color patterns with respect to behavior, male BSA contrast and videos were analyzed independently by different coauthors (A.S. and T.C., respectively).

Video recordings of the tests were analyzed using JWatcherTM 0.9 software (Blumstein et al. 2006). Assuming that a female was attracted by a male if she chose to be near him (Baerends et al. 1955), rather than swimming elsewhere, we set up an "area of

interest." An area of female interest was defined using a black nylon thread clipped at the top of the tank 3 cm away from the front wall of the male chambers. Because the cameras filmed from above, this thread delimitated a 3-cm wide and 23-cm-long zone in the experimental chamber in front of the male chambers. Females were recorded as being interested in a male when they were in the area of interest, in front of the male, directly facing his chamber. If a female was swimming in the area of interest but was facing away from the male, or if she was foraging on gravel in this area, she was not considered to be interested. Males were said to be interested in a female when they swam toward her and when they kept swimming at or very close to the wall facing her aquarium (Baerends et al. 1955), but only if the female showed interest first.

We measured a male's attractiveness as the time that a female spent with him (during the 20 min of trial). Because males and females were not able to contact each other in the experiment, the male's visual attractiveness measured here is used as a proxy of male overall sexual attractiveness, and nonvisual cues are excluded by the clear partitions. There is a possible confusion with social attractiveness, although the mirror image of the female in her home compartment of the tank mitigates this. We are examining relative attractiveness here, where, if a female spends most time with a particular male, he is the relatively most attractive either sexually, socially or both. Male responsiveness was measured as the time that this particular male was responsive to a given female divided by the time that the female spent interested in him.

Statistical analysis

All statistical analyses were performed in *R* (R Core team 2013) with *R Studio* (R Studio 2016, Version 0.99.8). Normality and homogeneity of the residuals were successfully verified for each model before further analysis. All tests were conducted with α = 0.05.

If a female did not visit all three males at least once during the trial, this trial was removed from the data set before analysis (six trials removed out of 160).

Effect of BSA on male attractiveness

To assess the relationship between male attractiveness and the weighted mean and CV of boundary contrast ΔS (hereafter $m\Delta S$ and $cv\Delta S$, respectively), we performed a linear mixed model (lmer package lme4, Bates et al. 2014). The response variable was the log-transformed male attractiveness. The initial models included these fixed factors: male response, position in the tank (from the female point of view: left, middle, and right, Figure 1), the BSA contrast measure (either chromatic and achromatic $m\Delta S$ in a first model, or chromatic and achromatic $cv\Delta S$, in the second model). We also included the second-order interactions between male response, position, day number, and each of the BSA variables (Supplementary Appendix A3, formula 1 and 2; see Supplementary Appendix A4 for a summary of all the variables present in the model and calculation). We tested 10 different models with different random effect structures integrating females (to control for individual motivation), BSA measure (to control for the possibility that females had differences in BSA preference), day and trial number within each session (to control for the effect of time on female motivation). These variables were either included as random intercepts or random slopes (Supplementary Appendix A3). We compared models using the

AIC criteria and performed analyses of variance (Anova) between each model pairs to check differences between models as factors were removed (Zuur et al. 2009). We did not use AICc because sample sizes were equal or very close to being equal. The model structure that best fitted our data included "females" and "day" and as random slopes and intercepts (model m3, Supplementary Appendix A3).

We then performed a backward elimination of nonsignificant interaction terms and compared models using AIC and Anova at each model reduction. Note that the model reduction was performed on the interaction only; all single variables were kept in the model. We kept the model with the smallest AIC value. We ran a post hoc analysis to control for multiple comparisons using the glht function and Holm–Bonferroni adjustment (package multcomp, Hothorn et al. 2017).

Effect of color patch transition length on male attractiveness

We used a linear mixed model (lmer package lme4, Bates et al. 2014) to assess the effect of color patch combinations on male attractiveness. For each male, we extracted the lengths of transitions between all observed combinations of color patches from the Photoshop pictures via MATLAB (e.g., length of the transition between black-orange, green-silver, and body-fuzzy black). We found 21 transitions between the colors black, fuzzy black, body, orange, silver, violet, and green. We did not include transitions containing yellow and black reticulations, yellow, blue, and gold because those colors were rare among males and found only on the fins when present at all. Adding these rare transitions as fixed factors would increase the chance to obtain false-positive results from our model. For the linear mixed model, the response variable was log-transformed male attractiveness. The initial models included all 21 transitions (i.e., black-fuzzy black, black-body, black-orange, etc.) as fixed factors, and "day" and "females" as the random intercept and random slope, respectively. We selected the random effects that suit the best model as previously (see m1 to m8, Supplementary Appendix A3).

Effects of particular color patches transition length and ΔS on mean BSA metrics

To assess the strength of the effect of each kind of color adjacent pair on the BSA measure $m\Delta S$, we performed a linear model. We used the same transition lengths and ΔS between pairs of color patches as in the previous analysis. 63 Chromatic $m\Delta S$ and 63 Achromatic $m\Delta S$, corresponding to the measures performed on the 63 males in the experiment, were used in the model. The response variable was either chromatic $m\Delta S$ or achromatic $m\Delta S$ measured for each male. The fixed variables were either the length of each of the 21 transition types or the ΔS between each of them.

Ethical note

The methods adhered to the ASAB/ABS Guidelines for the Use of Animals in Research. All individuals were handled with the highest care to minimize stress. This experiment was conducted under Deakin University's Animal Ethics Committee approval number G11-2015. Time under anesthesia for photography was been minimized (less than a minute) and the fish total recovery was assured within 2 min in a tank by itself to avoid the risk of attack from conspecific while recovering.

RESULTS

Effect of BSA on male attractiveness

Males were significantly more attractive when they possessed higher $m\Delta S$, both chromatic (Table 1) and achromatic (Table 1). The correlation between chromatic and achromatic $m\Delta S$ was negative $R = -0.12$, $IC_{95\%}$ $(-0.21; -0.03)$. Moreover, a male's response significantly positively affected his attractiveness (Tables 1 and 2, Supplementary Figure A5). In addition, we found a significant effect of tank position where females spent significantly less time with the males in the middle chamber compare to the right and left chambers (Tables 1 and 2, Supplementary Figure A6), and less time with the males in the right chamber compared to the left chamber (Table 1, Supplementary Figure A6). We did not find any significant effect of the experimental day on the time that females spent with males.

There was no effect of either chromatic or achromatic $cv\Delta S$ on male attractiveness (Table 2).

Effect of color patches transition length on male attractiveness

We found significant effects of particular transition lengths on male attractiveness when the transitions were between fuzzy black and three colors: body, green, and violet, and between black and three colors: orange, silver, and violet; see Table 3. Note how some transition lengths increase and others decrease male attractiveness.

Effects of particular color patches transition length and ΔS on mean BSA metrics

We found a significant effect of the transition length on males' chromatic $m\Delta S$ when the transitions were between fuzzy black and three colors: body, green, and violet; between body and violet; and between green and violet (Table 4a). We found a significant effect of ΔS between body color and silver on chromatic $m\Delta S$ (Table 4b).

We found a significant effect of the transition length on males' achromatic $m\Delta S$ when the transitions were between body and four colors: black, fuzzy black, orange, and silver; or between black and orange; or between fuzzy black and three colors: orange, green, and violet, (Table 5a). We found a significant effect of ΔS on achromatic $m\Delta S$ of transitions between body and violet and between black and green on the males' achromatic mean ΔS (Table 5b).

DISCUSSION

We evaluated the effect of color pattern geometry using the sensed intensity (ΔS) and lengths of the boundaries between all adjacent

Table 1

Effect of male chromatic and achromatic $m\Delta S$, position, and male response, on his attractiveness

Fixed effects	β coefficient	SE	df	t	P
Intercept	−1.74	0.55	104.0	−3.19	**0.002**
Chromatic $m\Delta S$	0.24	0.10	441.3	2.46	**0.014**
Achromatic $m\Delta S$	0.10	0.04	441.4	2.72	**0.007**
Male response	1.35	0.17	442.2	8.06	**<0.001**
Position(middle-left)	−0.45	0.08	441.1	−5.85	**<0.001**
Position (right-left)	−0.19	0.08	441.1	−2.43	**0.015**
Position (right-middle)	0.27	0.08	441.1	3.42	**0.001**
Day	−0.10	0.07	7.2	−1.40	0.204
Random effects	Variance	SD	R		
Females (intercept)	0.52	0.72			
Day	0.04	0.19	−0.89		
Residuals	0.45	0.67			

Results from the linear mixed model with females and day as random slope and random intercept. Significant results are in bold.
df = degrees of freedom; P = P value; SD = standard deviation; SE = standard error; t = t value.

Table 2

Effect of male chromatic and achromatic $cv\Delta S$, position and male response, on his attractiveness

Fixed effects	β coefficient	SE	df	t	P
Intercept	0.72	0.75	297.0	0.96	0.340
Chromatic $cv\Delta S$	−1.04	0.71	446.6	−1.47	0.143
Achromatic $cv\Delta S$	−0.40	0.60	446.2	−0.67	0.501
Male response	1.24	0.17	447.3	7.49	**<0.001**
Position(middle-left)	−0.42	0.08	446.2	−5.21	**<0.001**
Position (right-left)	−0.16	0.08	446.1	−2.08	**0.038**
Position (right-middle)	0.26	0.08	446.2	3.29	**0.001**
Day	−0.09	0.07	8.4	−1.38	0.205
Random effects	Variance	SD	R		
females (intercept)	0.45	0.67			
Day	0.03	0.18	−0.89		
Residuals	0.46	0.68			

Results from the linear mixed model with females and day as random slope and random intercept (see Supplementary Appendix A3). Significant results are in bold.
df = degrees of freedom; P = P value; SD = standard deviation; SE = standard error; t = t value.

Table 3

Effect of lengths of pairs of adjacent colors on male attractiveness

Fixed effects	β coefficient	SE	df	t	P
Intercept	−0.6701	0.4026	31.1	−1.66	0.106
Body: Black	<0.0001	0.0002	426.0	−0.07	0.947
Body: Fuzzy Black	**0.0003**	0.0001	425.6	2.95	**0.003**
Body: Orange	0.0002	0.0001	427.0	1.26	0.207
Body:Green	0.0001	0.0001	425.6	0.68	0.497
Body:Silver	<0.0001	0.0002	427.1	<0.01	1.000
Body:Violet	<0.0001	0.0001	425.4	−0.18	0.858
Black:FuzzyBlack	0.0010	0.0006	426.0	1.59	0.112
Black: Orange	**0.0022**	0.0008	426.2	2.61	**0.009**
Black: Green	−0.0008	0.0005	425.2	−1.69	0.092
Black: Silver	**0.0055**	0.0025	425.2	2.26	**0.024**
Black: Violet	**−0.0019**	0.0008	425.3	−2.45	**0.015**
Fuzzy Black: Orange	−0.0002	0.0002	425.8	−1.06	0.290
Fuzzy Black: Green	**−0.0005**	0.0002	425.9	−2.27	**0.024**
Fuzzy Black:Silver	−0.0003	0.0009	425.6	−0.35	0.729
Fuzzy Black: Violet	**0.0004**	0.0002	425.7	2.02	**0.044**
Orange:Green	−0.0002	0.0003	425.2	−0.61	0.542
Orange: Silver	0.0010	0.0019	426.7	0.55	0.584
Orange:Violet	−0.0001	0.0002	425.8	−0.71	0.481
Green: Silver	−0.0006	0.0006	425.9	−0.95	0.341
Green:Violet	0.0004	0.0003	425.6	1.28	0.200
Silver:Violet	0.0008	0.0007	426.7	1.21	0.228
Day	−0.0874	0.0803	8.8	−1.09	0.306

Random effects	Variance	SD	R		
females (intercept)	0.53	0.73			
Day	0.04	0.20	−0.90		
Residuals	0.54	0.74			

Results from the linear mixed model with females as a random intercept. Significant results are in bold.
df = degrees of freedom; P = P value; SD = standard deviation; SE = standard error; t = t value.

Table 4

Effect of boundary lengths (4a) and ΔS (4b) on Chromatic mΔS

Fixed effects	(4a) Length of the transition				(4b) ΔS of the transition			
	β coef	SE	t	P	β coef	SE	t	P
Intercept	2.6030	0.1737	14.99	**<0.001**	2.33	0.41	5.72	**<0.001**
Body: Black	−0.0001	0.0002	−0.32	0.749	0.05	0.05	0.96	0.345
Body: Fuzzy Black	−0.0002	0.0001	−3.16	**0.003**	NA	NA	NA	NA
Body: Orange	0.0001	0.0001	1.02	0.316	0.11	0.11	0.94	0.354
Body:Green	−0.0001	0.0001	−1.42	0.163	−0.11	0.23	−0.47	0.644
Body:Silver	0.0000	0.0001	−0.44	0.665	−0.14	0.07	−2.14	**0.038**
Body:Violet	0.0003	0.0001	3.91	**<0.001**	0.12	0.09	1.39	0.173
Black:FuzzyBlack	−0.0001	0.0004	−0.37	0.710	−0.04	0.06	−0.72	0.477
Black: Orange	0.0008	0.0006	1.37	0.179	0.01	0.04	0.17	0.870
Black: Green	0.0000	0.0003	0.13	0.900	0.02	0.04	0.50	0.617
Black: Silver	−0.0026	0.0018	−1.49	0.145	0.07	0.20	0.35	0.731
Black: Violet	−0.0001	0.0005	−0.20	0.842	0.02	0.04	0.39	0.698
Fuzzy Black: Orange	0.0000	0.0001	0.31	0.756	−0.17	0.09	−1.99	0.053
Fuzzy Black: Green	−0.0004	0.0002	−2.22	**0.032**	−0.07	0.10	−0.68	0.498
Fuzzy Black:Silver	0.0001	0.0006	0.18	0.858	−0.18	0.11	−1.55	0.129
Fuzzy Black: Violet	0.0003	0.0001	2.30	**0.027**	−0.01	0.10	−0.06	0.957
Orange:Green	0.0001	0.0002	0.57	0.575	0.01	0.03	0.52	0.610
Orange: Silver	0.0019	0.0013	1.49	0.145	0.09	0.07	1.45	0.155
Orange:Violet	−0.0001	0.0001	−0.39	0.700	0.03	0.03	1.02	0.314
Green: Silver	−0.0006	0.0004	−1.40	0.169	0.09	0.08	1.13	0.265
Green:Violet	0.0006	0.0002	2.75	**0.009**	−0.03	0.04	−0.70	0.489
Silver:Violet	0.0001	0.0004	0.20	0.847	0.05	0.04	1.40	0.169
	Resid SE	df	Adjusted R^2			Resid SE	df	Adjusted R^2
	0.19	41	0.66			0.27	42	0.34

Significant results are shown in bold.
Coef = coefficient; df = degree freedom; P = P value; Resid = residuals; SE = standard error; t = t value.

Fixed effects	(5a) Length of the transition				(5b) ΔS of the transition			
	β coef	SE	t	P	β coef	SE	t	P
Intercept	9.3064	0.4374	21.28	**<0.001**	12.27	1.03	11.86	**<0.001**
Body: Black	0.0026	0.0004	6.11	**<0.001**	0.004	0.02	0.23	0.821
Body: Fuzzy Black	0.0004	0.0002	2.41	**0.021**	_NA_	_NA_	_NA_	_NA_
Body: Orange	−0.0008	0.0002	−4.10	**<0.001**	−0.29	0.27	−1.10	0.280
Body:Green	−0.0004	0.0002	−1.83	0.075	−0.08	0.07	−1.10	0.280
Body:Silver	−0.0009	0.0003	−3.59	**0.001**	−0.12	0.08	−1.47	0.150
Body:Violet	−0.0003	0.0002	−1.66	0.105	−0.54	0.23	−2.40	**0.021**
Black:FuzzyBlack	−0.0008	0.0010	−0.85	0.401	0.01	0.03	0.31	0.756
Black: Orange	0.0038	0.0015	2.56	**0.014**	−0.01	0.01	−0.77	0.444
Black: Green	0.0017	0.0009	1.96	0.056	0.02	0.01	2.82	**0.007**
Black: Silver	0.0050	0.0045	1.13	0.266	0.01	0.01	0.80	0.428
Black: Violet	−0.0007	0.0014	−0.51	0.612	0.01	0.01	0.68	0.502
Fuzzy Black: Orange	0.0007	0.0003	2.35	**0.024**	0.02	0.04	0.47	0.639
Fuzzy Black: Green	0.0020	0.0004	4.94	**<0.001**	0.01	0.01	0.57	0.571
Fuzzy Black:Silver	−0.0012	0.0016	−0.77	0.448	−0.02	0.02	−1.03	0.310
Fuzzy Black: Violet	0.0008	0.0004	2.15	**0.038**	0.09	0.06	1.52	0.137
Orange:Green	−0.0006	0.0005	−1.21	0.234	−0.05	0.06	−0.71	0.481
Orange: Silver	0.0057	0.0032	1.76	0.085	0.73	0.90	0.82	0.420
Orange:Violet	−0.0006	0.0003	−1.65	0.108	0.43	0.32	1.34	0.188
Green: Silver	−0.0015	0.0011	−1.37	0.179	−0.03	0.09	−0.34	0.735
Green:Violet	−0.0006	0.0005	−1.24	0.221	−0.23	0.14	−1.57	0.125
Silver:Violet	−0.0006	0.0011	−0.58	0.564	−0.33	0.44	−0.75	0.455
	Resid SE	df	Adjusted _R_²		Resid SE	df	Adjusted _R_²	
	0.49	41	0.7		0.68	42	0.41	

Significant results are shown in bold.
Coef = coefficient; df = degree freedom; _P_ = _P_ value; Resid = residuals; SE = standard error.

color patches found in male guppies, in order to explain male attractiveness. We also evaluated the effect of all combinations of color pairs to explain males' BSA metrics and males' attractiveness.

Effect of BSA on male attractiveness

We found a significant effect of a male's BSA contrast measure on his attractiveness. This finding was expected, due to the efficiency of strongly contrasting adjacent colors in signal transmission. However, contrary to previous findings (Sibeaux et al. 2019a), we found that males showing higher chromatic and achromatic contrasts between adjacent patches (_mΔS_) were more attractive to females (chromatic _mΔS_: β = 0.24, df = 441.4, _P_ = 0.014; achromatic _mΔS_: β = 0.10, df = 441.4, _P_ = 0.007), while the variation of boundary contrast across the pattern (_cvΔS_) did not affect male attractiveness in this study (chromatic _cvΔS_: β = −1.04, df = 446.6, _P_ = 0.14; achromatic _cvΔS_: β = −0.40, df = 446.2, _P_ = 0.50). There are several possible reasons for this difference from the previous work. First, the light environment was different between the two studies; the addition of UV wavelengths in the present study allows further information contained in UV reflecting patches to be available to females. The spectra composition of the light environment was shown to have a significant impact on mating behavior in the guppy (e.g., courtship display rate, time chasing females, etc., Archard et al. 2009). In their experiment, White et al. (2003) showed that females were less attracted to males when UV blocking filters where placed in front of the males. Not only in fish but also in birds and lizards, UV wavelengths have critical importance in various behaviors such as mate choice, foraging, aggression, or conspecifics recognition (Whiting et al. 2006; Rajchard 2009; Siebeck et al. 2010). It is

therefore essential to take UV into consideration when studying tetrachromatic species that can detect and process UV wavelengths. Second, the differences in color patch reflectance (due to difference in matching or mismatching the light environment) directly affected the range of values of _mΔS_ and _cvΔS_; consequently, male patterns were variable and would be even if the same males were used in both sets of conditions. Third, males were seen by females against a black background in the present experiment but on a white background in the previous study. Even though both downwelling and sidewelling light (from black or white backgrounds) were taken into consideration in the measures of _ΔS_ to allow for sensory adaptation, they can still have strong effects on _mΔS_ and _cvΔS_ and hence on female choice. In guppies, it has been shown that the environmental background can influence mate choice based on color cues (Endler 1983; Lynn and Cole 2019). Fourth, males presented against a black background might mimic natural and safer environment for the females. In the wild, males display to the females in front of a natural background which can vary from light to dark gray (from different rocks and gravel in the stream) or could be dark brown (dead leaves or soil) or green (algae). Consequently, there may be a range of contrasts for the same male seen against different objects. Moreover, females viewing males against a white background could relate this environment to an open water area where predation risk is higher; generating perceived higher predation risk (Templeton and Shriner 2004). In the current experiment, even though the overall light irradiance was higher, the fact that males were seen against a black background could give the perception of males being under a sheltered place or next to the edge of the stream. It is thus possible that this led to a lower perceived predation risk may and therefore conspicuous males were naturally more attractive. Thus,

males with a higher overall contrast were preferred in this study, compared to the previous one (Sibeaux et al. 2019a).

The $cv\Delta S$ did not affect male attractiveness in this experiment. If there was no need for a trade-off between being conspicuous to attract mates and being inconspicuous to decrease the predator pressure, one hypothesis is that females prefer to rely on stronger cues (higher $m\Delta S$ is more attractive) than on the $cv\Delta S$ which is more cryptic at a distance and useful only in certain environments where there is predator risk.

Effects of male behavior and location

Females were more interested in males when the males were responsive ($\beta = 1.35$, df = 442.2, $P < 0.001$). A responsive male may indicate better health and his movements may increase the conspicuousness (and therefore attractiveness) of his color signal (Farr 1980). Guppies are indeed social and gregarious animals that live in big shoals where social interaction occurs frequently.

As in a previous study (Sibeaux et al. 2019a), females preferred males when they were presented on one of the side chambers rather than the middle chamber (40.1, 25.4, and 34.5% on the left, middle, and right chambers, respectively, Tables 1 and 2). This could be explained by the general dislike of open water area. Indeed, predation risk is higher in the middle of a stream than on the sides (Templeton and Shriner 2004).

Effect of particular color patch transition lengths on male attractiveness

The boundary lengths of six different color patch combinations predicted male attractiveness but with different signs. The boundary lengths between fuzzy-black-body, fuzzy-black-violet, black-orange, and black-silver were positively correlated with male attractiveness (respectively: $\beta = 0.0003$, df = 425.6, $P = 0.003$; $\beta = 0.0004$, df = 425.7, $P = 0.044$; $\beta = 0.0022$, df = 426.2, $P = 0.009$; and $\beta = 0.0055$, df = 425.2, $P = 0.024$), whereas fuzzy black-green and black-violet were negatively correlated with male attractiveness (respectively: $\beta = -0.0005$, df = 425.9, $P = 0.024$; $\beta = -0.0019$, df = 425.3, $P = 0.015$). Interestingly, these combinations possess either black or fuzzy black as one member of the color pair. All these transitions possessed a high achromatic ΔS between patches but different chromatic ΔS (only black-orange, black-violet, and fuzzy black-violet possessed a high chromatic ΔS). This suggests that achromatic cues could be preferably used in the assessment of boundaries' lengths compared to chromatic and that they could be used in mate choice or species recognition. Double cones could be used preferentially to evaluate pattern when individuals are in motion (Schaerer and Neumeyer 1996), which is likely to happen when guppies swim nearby conspecifics. For example, the chromatic ΔS between black and silver was much less than 1 which means that those patches were not likely to be discriminable based on their chromatic contrast. Given that these transitions had a significant effect despite their low chromatic contrast, this means that luminance (achromatic cues) is the cue assessed by females to differentiate between those two color patches. The transition between fuzzy black and body was the most common among the males used in the experiment and was the only transition that was present in all males. Males with a longer boundary length between body and fuzzy black had bigger or more fuzzy black patches. The positive effect of the boundary between black and orange on male attractiveness support previous studies that showed that black patches were acting as signal amplifiers of orange patches (Brooks 1996). Interestingly,

our finding suggests that melanin could also be a signal amplifier of structural color patches as violet and silver as it was suggested by theoretical work by Grether et al. (2004).

In addition to acting as a signal amplifier (Brooks 1996), melanin-based coloration has been shown to be linked to fitness (e.g., allowing thermoregulation and protecting against oxidative damage, McGraw 2005; Trullas et al. 2007) and to be inherited (Griffith et al. 2006). Those advantages could lead to female preference for melanic patches. However, it is important to notice that results on melanin-based coloration linked to fitness can be heterogeneous across species (e.g., in a meta-analysis on birds; Meunier et al. 2011).

Effects of particular color patches transition length and ΔS on mean BSA metrics

As expected from the way $m\Delta S$ is calculated (combining length and ΔS for each boundary class; see Supplementary Appendix A2 for details) and the fact that all males display color patches of different size and contact length with neighboring patches, boundary lengths explained chromatic and achromatic $m\Delta S$ better than boundary intensity (ΔS).

Chromatic $m\Delta S$ was predicted by boundary lengths for body-fuzzy black, body-violet, fuzzy black-green, fuzzy black-violet, and green-violet (respectively $\beta = -0.0002$, $P = 0.003$; $\beta = 0.0003$, $P < 0.001$; $\beta = -0.0004$, $P = 0.032$; $\beta = 0.0003$, $P = 0.027$; and $\beta = 0.0006$, $P = 0.009$) combinations, whereas it was predicted by ΔS for the body-silver pair ($\beta = -0.14$, $P = 0.038$).

Achromatic $m\Delta S$ was predicted by the boundary length between body and four colors: black, fuzzy black, orange, and silver (respectively $\beta = 0.0026$, $P < 0.001$; $\beta = 0.0004$, $P = 0.021$; $\beta = -0.0008$, $P < 0.001$; and $\beta = -0.0009$, $P = 0.001$), between black-orange, fuzzy black-orange, fuzzy black-green, and fuzzy black-violet (respectively $\beta = 0.0038$, $P = 0.014$; $\beta = 0.0007$, $P = 0.024$; $\beta = 0.002$, $P < 0.001$; and $\beta = 0.0008$, $P = 0.038$). The boundary ΔS between body-violet and black-green significantly explained males' achromatic $m\Delta S$ ($\beta = -0.54$, $P = 0.021$ and $\beta = 0.02$, $P = 0.007$, respectively).

The effect of boundary length compared to boundary ΔS in predicting BSA metrics might be different if individual patch reflectances were taken from each individual male. However, this would have required repeated reflectance measures for each male's varied color patches and therefore longer or repeated anesthesia, which would not be good for the male's health. Moreover, color reflectance can change according to individual movements, health status or behaviors (Heathcote et al. 2018). Although individual health was carefully monitored during the experiment, the possible changes of color patches reflectance provoked by courtship behaviors and males' undulatory movements were impossible to measure because males needed to be anesthetized to perform reflectance measurements. However, a high number of reflectance measurements performed on males from our population were used to calculate ΔS, allowing us to obtain reliable population average reflectance spectra for each color patch class (see Supplementary Figure A3 for the chromatic variation within each color class).

The high number of melanin-based patches explaining the BSA metrics highlights, as shown earlier, the particular importance of those patches in mate choice. It is clear that different color combinations contribute to overall contrast (i.e., contrast across the entire male pattern) in different ways, and that black has a strong effect on the entire pattern contrast with respect to both chromatic and achromatic components of the pattern.

CONCLUSION

Our study has demonstrated that the BSA metrics can successfully predict mate choice in guppies. The difference of BSA metrics' significance (mean vs. CV BSA metrics explaining male attractiveness) between this and our previous study (Sibeaux et al. 2019a), and the large differences in experimental environments between them, demonstrates that careful control of the light environment and the visual background is essential to a meaningful result. Natural populations see potential mates against a variety of different backgrounds and each background may have a very different effect on the male's appearance and hence his fitness. Given this and the effect of different light environments on visual contrast, this suggests that males should display in light (Cole and Endler 2016) and against backgrounds which maximize their conspicuousness to females (Endler 1983). We also showed that melanin-based pigments in the guppy's patterns can be used as signal amplifiers not only for orange color patches but also for multiple colors including structural color patches. Therefore, multiple combinations of color pairs can be selected in a mate choice context, and thus participate to maintain color pattern polymorphism within populations (along with male novelty and Y linkage; Farr 1977; Charlesworth 2018). All of our results emphasize the importance of including pattern geometry information in the evaluation of color-mediated behaviors and being aware of the interaction between color patterns, visual backgrounds and choice behavior. We recommend that light and visual backgrounds should mimic natural environments in behavioral experiment, in order to obtain realistic conclusions about the processes of natural selection, sexual selection and the evolution of color patterns in the wild.

SUPPLEMENTAL MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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