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Original Article

Female nuptial coloration and its adaptive significance in a mutual mate choice system

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Adaptive female coloration is likely to occur when males largely invest into reproduction or variance of quality between potential mating partners is high. Although recent studies have shown male choosiness of female traits, little is known about the extent to which female ornamentation signals benefits to males. Female ornamentation might signal individual quality information and thus might be sexually selected by males or play a role in female–female competition. Here, we investigate the role of the female ventral coloration in sexual selection of *Pelvicachromis taeniatus*, a biparental African cichlid with mutual mate choice. We show that the female ornament 1) is sexually selected by males, who preferred females that showed a larger extent of the nuptially colored area. Female purple coloration, which consists of blue and red color components, 2) transmits information about female quality and ripeness. The magnitude of the red area predicted female readiness to spawn, whereas the extent of the blue area female fecundity, maternal quality, and offspring fitness. Ornamentation 3) is important in female–female competition. Dominance tests conducted under different illumination conditions that maintained or abolished the differences in nuptial coloration suggest that female coloration functions as a threat signal. These results support that female ornamentation may evolve as an indicator of quality through male choice, female–female competition, or both, in a species with mutual mate choice.
Key words: cichlid fish, female—female competition, male mate choice, multicomponent signaling, *Pelvicachromis taeniatus*, sexual selection. *[Behav Ecol 22:478–485 (2011)]*

INTRODUCTION

Sexual selection is a widespread evolutionary process that imposes strong selective pressure on behavioral and morphological traits (Andersson and Simmons 2006). The theory of sexual selection was introduced by Darwin (1871) who aimed to account for conspicuous ornaments, particularly in males, that would otherwise remain unexplained by his theory of natural selection. Due to biases in the reproductive costs between males and females, the classical theory suggests that males are competitive to gain access to females who themselves are choosy and select high-quality males as mating partners (Andersson 1994). Numerous examples in the animal kingdom have shown that sexual selection may lead to the evolution of male ornaments like conspicuous visual signals that convey information about individual phenotypic or genetic quality, thereby increasing the fitness of the signaler and the receiver (see Andersson 1994; Andersson and Simmons 2006 and references therein).

In contrast, female ornamentation has long been considered as nonadaptive, solely being the result of a genetic correlation to male ornaments (Lande 1980). However, recent theoretical work and empirical studies in different taxa (e.g., Amundsen 2000; see Kraaijeveld et al. 2007 for review) stress the importance of male mate choice and female–female competition in species in which paternal investment and variance in female quality are high (Clutton-Brock and Vincent 1991; Kokko and Johnstone 2002; Parker 2006) suggesting that conspicuous female traits are sexually selected as well (e.g., Amundsen 2000; Houde 2001). Often, male choice for large highly fecund females is reported, but little is known about the extent to which female ornamentation signals benefits to males (see Bonduriansky 2001 for review).

Few studies have investigated the adaptive significance of female ornaments except in sex-role reversed species where male choice is analogous to female choice in species with conventional sex roles (Berglund et al. 1986; Rosenqvist 1990). Nevertheless, recent studies suggest sexual selection of female ornamentation by male choice in "conventional" species (e.g., Hill 1993; Bakker and Rowland 1995; Amundsen et al. 1997; Griggio et al. 2005; Rick and Bakker 2008). Clear experimental evidence for male sexual selection of female ornaments in fish with paternal care has been shown in 2-spotted gobies, *Gobiusculus flavescens*, in which more colorful females are preferred (Amundsen and Forsgren 2001). In this species, unusually dynamic sex roles lead to a female-biased operational sex ratio at the end of the breeding season, resulting in highly competitive and courting females (Forsgren et al. 2004). Recent theory argues that males might be selected to show preferences according to their own quality (Hardling and Kokko 2005; Servedio and Lande 2006; Servedio 2007). For example, the potential benefits of general male preference for brightly ornamented females might be devalued by increased costs of male–male competition for such females. This might lead to some sort of assortment, that is, when less competitive males prefer lesser ornamented females.

Female ornamentation may play also a role in intrasexual competition (LeBas 2006). Female intrasexual competition should occur when variation in male quality is high or access to males is limited (Amundsen 2000). However, female

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intrasexual competition and the function of ornamentation have received little attention in research except in sex-role reversed species like the pipefish, *Syngnathus typhle*, in which female ornamentation might act as a signal of status in mating competition (Bernet et al. 1998). Nevertheless, female competition could be promoted in species with conventional sex roles (Amundsen 2000).

Theory suggests that mating systems with mutual mate choice decisions should lead to the evolution of sexual ornaments in females as well as in males (Kokko and Johnstone 2002; Clutton-Brock 2007). Mutual mate choice is often assumed to be relatively uncommon in nature, but recent studies suggest that it might occur in many more species than expected (Kraaijeveld et al. 2007; Hooper and Miller 2008). It occurs predominantly in genetically or socially monogamous species in which both sexes show a comparable amount of parental investment in their offspring (Burley 1986; Parker 2006). Many cichlid fishes show biparental brood care and a distinct sexual dimorphism/dichromatism (Barlow 2002), thus making them a potential model system concerning questions of mutual mate choice and the evolution of sexual ornaments in females.

The aim of this study was to investigate the role of female visual signals in a small riverine cichlid fish, *Pelvicachromis taeniatus*. Its mating system is described in detail in Thünken et al. (2007a, 2007b). In short, *P. taeniatus* is a socially monogamous cave breeding cichlid with mutual mate choice and intense biparental brood care. Males and females are sexually dimorphic and dichromatic (Baldauf, Kullmann, Winter, et al. 2009). Males are larger than females and show a yellow coloration of their ventral side, whereas females develop a purple ventral belly coloration. During the breeding season, males occupy appropriate breeding caves, which they defend against rivals. Females compete among each other for access to males. During mutual courtship, both sexes present their ventral body region by arching it toward the partner while intensely quivering the whole body. After spawning, the female cares for the eggs in the cave while the male defends the territory against predators, whereas free-swimming fry are guarded by both parents for a period of several weeks (Thünken et al. 2010). Recent studies have shown that both sexes of *P. taeniatus* are choosy during mate choice: Close kin are preferred over nonkin (Thünken et al. 2007a, 2007b) and larger mating partners over smaller ones (Baldauf, Kullmann, Schroth, et al. 2009). Furthermore, females of *P. taeniatus* prefer yellow nuptially colored males over dull males (Baldauf, Kullmann, Winter, et al. 2009).

The study had 3 objectives: first, we experimentally tested whether female coloration is sexually selected by male mate choice. In order to measure male mating preferences, we conducted a series of choice experiments using computer animations of digital images of females that differed in their area of ventral coloration. A striking advantage of computer-manipulated stimuli is a high degree of standardization between the stimuli, thus minimizing the effects of confounding variables like rapid changes in coloration or different responses in stimuli fish (Künzler and Bakker 1998; Rosenthal 2000; Baldauf et al. 2008). Second, female ornamentation may function as an honest signal of female quality, similar as in males, for example, by transferring information about female quality, fecundity, or readiness to spawn (Doutrelant et al. 2008). We therefore investigated between-female variation in purple ventral coloration in relationship to individual quality information like fecundity and maternal effort under standardized laboratory conditions. Furthermore, we examined within-female temporal changes in coloration in relationship to female reproductive activity within a breeding cycle. Third, we investigated the role of female coloration in female–female competition in which coloration might function as a signal of dominance. We examined the competitiveness of colorful and dull females under different light conditions.

MATERIALS AND METHODS

Female coloration and male mate choice

Experimental animals

All 17 male individuals used in experiments originated from 17 different families of the lab-raised F1 generation of fish that had been caught from the River Moliwe, Cameroon (lat 04°04′N, long 09°16′E), in 2007. Fish were raised in mixed-sex family tanks (80 × 30 × 30 cm). All holding aquaria were surrounded by opaque plastic sheets to avoid visual contact to other fish. The water temperature was kept at 25 ± 1 °C, and light conditions resembled natural day length (L/D 12/12). Fish were fed once a day with a mixture of defrosted *Chironomus* spp. and *Artemia* spp.

Preparation of computer stimuli

The experiment aimed to test whether males show a preference for female stimuli showing a greater extent of their nuptially colored ventral area. To achieve a high degree of standardization, we created computer stimuli. Nevertheless, computer displays are tailored to human vision and do not emit polarized light or wavelengths, such as ultraviolet (D'Eath 1998; Baldauf, Kullmann, Winter, et al. 2009). However, previous studies have shown that *P. taeniatus* reliably responds to computer stimuli concerning movement, body shape, and coloration displayed by a cathode-ray tube (CRT) monitor (Baldauf, Kullmann, Winter, et al. 2009). Additionally, in the present experiment, we controlled whether the color information emitted by the monitors corresponded to spectral data of the female nuptially colored area. To achieve this, we measured spectral data of 10 colorful females in LAB color space (Avantes USB 2000, Spectrawin 5.1) and calibrated the monitors by using a digital colorimeter (Quato Silver Haze pro) until they emitted a color information similar to the spectral readings in LAB color space (see Supplementary Figure S1 for spectral reflectance of the females and of the calibrated monitors).

We took digital photographs (Olympus Camedia Widezoom 5060) of 5 nuptially colored females to obtain source data for a 2D fish model. 2D models were successfully established for mate choice experiments in *P. taeniatus* (Baldauf, Kullmann, Schroth, et al. 2009; Baldauf, Kullmann, Winter, et al. 2009). The pictures were saved in RAW format to avoid the loss of coloration data due to algorithmic compression and were white balanced during import into Adobe Photoshop CS2 (http://www.adobe.com/). We constructed an artificial female stimulus from the source material and manipulated the extent of the ventral nuptial coloration for each treatment using Adobe Photoshop CS2 in RGB color mode.

To achieve moving animations of the models, we used "The GIMP 2.2.17 with animation package" (http://www.gimp.org/). A gray background image (1024 × 400 px) was created (RGB: 238, 238, and 238) including a plant as a reference object in the middle of the image. Each animation consisted of 30 frames per second, which is an established method to present artificial stimuli to fishes (Künzler and Bakker 1998; Baldauf, Kullmann, Winter, et al. 2009). Each stimulus moved its pathway from one side of the monitor to the other for a period of 15 s, including a 2-s stop in the middle. After that, it recurred horizontally flipped and moved its pathway back in the same time frame.

We created 3 different female stimuli showing a different extent of its area of nuptial coloration. We measured the number of pixels forming the nuptially colored area of the virtual female and decolorized 10%, 40%, and 70% of the pixels that formed the nuptial colored area, respectively. Thus, we created 3 stimuli showing either 90%, 60%, or 30% of the original area of nuptial coloration (equates to 26%, 18%, and 10% of the total lateral projection area, proportion of blue/red pixels 8:1, see Supplementary Figure S2). Each combination of stimuli was presented to focal males, thus 3 experimental treatments were conducted. The display resolution was set to 1024 × 768 px (width × height). This highly standardized set of stimuli was used to investigate the response of test males to differences in the manipulated area of coloration. Theoretically, using one basic stimulus for modifications potentially tests the response of males to a single-specific female stimulus (McGregor 2000). Nevertheless, the basic female model used in this study was constructed from body parts of 5 different females to present an average female. Thus, we assume that the difference in the elicited response of test males arises because of differences in the manipulated trait.

Experimental design

Experiments were conducted between 23 June 2008 and 04 July 2008. Before the start of the trials, males were randomly chosen and individually isolated in separate tanks (30 × 20 × 20 cm) for a minimum period of 2 days. The readiness to mate was reliably determined visually on the basis of the ventral coloration of an individual (Thünken et al. 2007a; Baldauf, Kullmann, Schroth, et al. 2009). The isolation tanks were surrounded at the broad sides by printouts of the animation's background image and opaque, gray partitions at the longer sides, thus ensuring that fish did not interact with other isolated individuals and could habituate to the reference objects used in the trials. Male tanks were equipped with a breeding cave. All other conditions were similar to those of the mixed-sex tanks. Each individual was tested in the 3 experimental treatments in random order. Test fish were tested in their isolation tank, thus reducing stress by leaving the fish in its familiar habitat.

Tanks containing the test fish were placed between 2 CRT monitors (Baldauf et al. 2008) of the same model (EIZO Flex Scan F520, 85 Hz, connected to a Matrox G550 PCIe graphic board). The level of the bottom of the tank was adjusted to the lower margins of the monitor screens. An association zone of 5 cm in front of each monitor was marked on the white Styrofoam under the tank creating a 15 cm neutral zone in between. The setup was illuminated by a fluorescent tube (37 W) installed 1 m above the middle of the tank. Additionally, white Styrofoam surrounded the tank.

It was randomly determined on which monitor a stimulus type was presented. During an acclimatization period of 15 min, both screens showed the gray background with the plastic plant. After acclimatization, the stimuli appeared simultaneously (MacLaren and Rowland 2006). With a webcam installed on top of the setup, we recorded 2 min after the fish had visited the first association zone, which is a time frame that has been established in other behavioral studies of this species (Baldauf, Kullmann, Winter, et al. 2009). After recording, the background showing the plant was shown again for 5 min. The trial was then repeated showing reversed positions of the stimuli.

A naive observer analyzed the video recordings. Male mating preferences were measured as association time near the female stimulus. Association time near a female reliably predicts mating decisions in male *P. taeniatus* (Thünken et al. 2007a, 2007b). The time spent in each association zone was calculated over a period of 2 min after the fish had visited an association zone. In the analyses, relative proportion of time spent in one of the association zones was used, which is a standard procedure in fish studies (e.g., Mazzi et al. 2003) and also established in studies of *P. taeniatus* (Baldauf, Kullmann, Winter, et al. 2009). For each test fish, we averaged the time spent in front of each stimulus in the first and the second trial in all experimental trial pairs, thus controlling for potential side biases.

The ultraviolet (UV) component of the spectrum was not included in this and the subsequent experimental designs for several reasons. First, test fish were raised under laboratory conditions that did not offer UV wavelength. Second, the UV signal is present at all body regions of the females (including areas without coloration), and preliminary data suggest that there is low variation between individuals in UV at the belly region (Baldauf SA, unpublished data). Third, computer monitors do not allow emitting UV wavelengths to avoid damage to the human eye. All color information in this study thus refers to the human visible spectrum (400–700 nm).

Intersexual signal function of female coloration

An image analysis was conducted in order to investigate whether female quality relates to female ornamentation. Changes in the expression of the ornament during the breeding cycle were investigated by behavioral observations.

Experimental animals

Individuals were wild-caught fish as well as the lab-raised F1 generation of fish that had been caught in 2003 from the River Moliwe. F1 test individuals were raised in mixed-sex family tanks (80 × 30 × 30 cm), whereas wild-caught fish were kept in a single tank (80 × 30 × 30 cm). Fish were kept as described above.

Mating and brood care observations

Observations were conducted in spring 2009. We formed 24 size matched unrelated pairs (11 wild caught and 13 F1) by taking nuptially colored individuals of each sex from the holding tanks. Standard length and body mass were measured shortly before fish were released into the breeding tanks, and a digital photograph (camera: Canon D70s) of each female was taken in order to investigate the role of the visual ornamentation. Photographs were taken under standardized light conditions (2 Somikon LP001 lights illuminating the lateral side), including a Munsell white standard within the scene. The pictures were saved in RAW-format to avoid the loss of coloration data due to algorithmic compression.

The pairs were allocated randomly to breeding aquaria (30 × 40 × 43 cm), which were filled with a mixture of 2/3 parts of tap water and 1/3 parts of osmotic water, tempered at 25 ± 1 °C. Two observers (S.A.B. and T.T.) recorded behavioral patterns of each female for 10 min daily (9380 observation minutes in total). The observation order was randomized and determined daily. Unmated pairs were stimulated weekly by renewing 1/3 of the water volume with the 2:1 tap/osmotic water mix, but observations were stopped for pairs that were still unmated after a period of 7 weeks.

After spawning, the cave was shortly removed, and the number of eggs was counted. We recorded different behavioral patterns, for example, the number of female courtship displays to males or whether the female cared for the free swimming young in a 30-s interval. A behavioral pattern was scored as guarding when the distance of a parent to the young was one body length or less. In order to investigate temporal variability of female coloration, we additionally recorded the area of the nuptial ventral coloration (small:

nuptial area extent < base of anal fin, mean: nuptial area extent = base of anal fin, and large: nuptial area extent > base of anal fin). Moreover, we noted female color intensity (weak, mean, and intense) by personal estimation (see Supplementary Figure S3 for how differences in spectra correspond to individual judgments). Mating was counted as successful when a female had spawned. The number of surviving young was counted 4 weeks after the offspring had left the breeding cave. The survival rate was calculated as the proportion of surviving young relative to the number of eggs that had been spawned. The fry were fed with living *Artemia* nauplii, and after 3 weeks with a mix of defrosted *Artemia*, chironomid, and mosquito larvae.

Image analyses

All RAW images were white balanced during import into Photoshop CS2 and saved in TIF format (without compression). Analyses were performed using SigmaScan Pro 5.0. As the female nuptial purple coloration consists of a blue and a red peak in the visible spectrum, we measured the extent of the red and the blue nuptial ventral coloration independently (see Supplementary Figure S4). To achieve this, we marked the number of pixels of the respective color channel (SigmaScan hue blue: 128–192/hue red: 193–255) and calculated the number of pixels. Furthermore, we measured the number of pixels forming the body area (excluding the fins) to calculate the relative extent of the nuptially colored area with respect to the lateral body projection area.

Coloration and female–female competition

Experimental animals

This experiment was conducted in winter 2005. Females used in this experiment were the F1 generation sired by wild animals from the Moliwe population (collected in 2003) and were kept in groups of mixed sexes in tanks (70 × 35 × 40 cm) at 25 °C (±1 °C) before the experiments.

In total 60 females varying in coloration were measured (standard length and body mass) and individually isolated in smaller tanks (30 × 20 × 20 cm). Each of them was given a 2-day acclimatization period in which they were fed red chironomid larvae once a day. A dull, smaller female (loser) was presented for about 10 min in a 1-l tank to every test female in order to induce a dominance status.

Experimental design

The aim of this experiment was to examine whether female coloration functions as a dominance signal in competitive female–female interactions. Because body size plays an important role in competition, we composed 20 female test-pairs consisting of individuals similar in body size. Two independent observers judged the coloration of each female immediately before every fight resulting in 1 dull and 1 bright female within a pair. Females that could not be distinguished by individual coloration only were not tested.

The test fish were simultaneously released into the arena (30 × 20 × 20 cm), which was surrounded by black plastic sheets on 3 sides to avoid radiance of light from the outside. The test aquarium was filled to a water level of 12 cm. The water was changed after each trial, and the tank was refilled with aged water from a supply tank. The optical filters (see below) were wrapped over the front side and the top of the tank. A halogen light (Osram L36 W/11-860 daylight) illuminated the tank from above. A black curtain surrounded the tank to protect the area from possible interruptions. Observations were made through a small spyhole in the curtain.

As soon as the fish were placed in the tank the behavior during the fights was recorded. Before a fight escalates females commonly display their S-shaped body to the rival (S-behavior). This behavior does not involve any physical contact. The escalating phase of a fight includes biting and sometimes jaw locking (see Barlow 2002 for a description of aggressive cichlid behavior). We recorded the number of S-behaviors performed by each female and number of attacks, that is, biting. A fight was considered to be decided when one female fled from the other or was chased and bitten continuously. If no winner arose within 30 min, the trial was terminated.

Females were tested under 2 different illumination conditions, which maintained or diminished the differences between the female color signal (violet/blue component: 400—490 nm; red component: 600—700 nm; see Supplementary Figure S5). First, full-spectrum conditions of the human visible spectrum (400–700 nm) were given (so called "full-spectrum conditions" from now on). Second, an optical filter was installed blocking medium wavelengths (from 450—600 nm) but transmitting short and long wavelength (so called "manipulated conditions" from now on). Under the altered light conditions, the interindividual differences in color expression of the blue component were removed, whereas the red component was available under both light conditions. Neutral density filters were added to provide an equal quantal flux under the respective wavelengths, thus controlling for achromatic brightness of the environment. Test pairs were used in both treatments. The order of treatments was assigned randomly. Between the tests, there was a recovery day in which females were stimulated again with a loser female to regain dominance status.

Statistics

Parametric statistics were performed when data met all assumptions of normality. Otherwise, nonparametric statistics were applied. Given test probabilities are two-tailed, and P values <0.05 were considered statistically significant. We fitted different models depending on the distribution of the data (see below for details). Likelihood ratio tests (LRT) with maximum likelihood assessed whether the removal of a variable caused a significant decrease in the model fit. Reported P values of models refer to the increase in deviance when the respective variable was removed, hence degrees of freedom (df) differ by 1 ("lm," "lme," and "lmer": F-statistics; "glm" and "polr": Pearson's chi square). Analyses were performed using R 2.9.1 (R-Development-Core-Team 2009).

Female coloration and male mate choice

Data were analyzed by fitting a linear mixed effect model ("lme," package: "nlme"; Pinheiro et al. 2009), with the relative time in front of the stimuli as dependent and stimulus type (larger or smaller area in each experimental treatment) as explanatory variable, the 3 different treatments (stimuli showing 26% vs. 10%, 18% vs. 10%, or 26% vs. 18% of female nuptial coloration in relation to total lateral projection area) as covariate, and male identity as random factor. In order to test whether male preference differed between the treatments, that is, whether male preference got stronger with a larger difference between stimuli, we tested the interaction between the stimulus types and the 3 treatment groups.

Intersexual signal function

In order to test whether female coloration is related to the number of eggs, the frequency with which females cared for their young or the survival rate of offspring, linear models ("lm") were fitted. The likelihood to mate was analyzed with a generalized linear model ("glm"), with binomial error distribution and logit link function. The number of female courtship displays was analyzed by fitting a mixed-effect model ("lmer," package "lme4"; Bates and Maechler 2009), with Poisson distribution and log link function. Observation data of the extent of nuptially colored area were transformed into an index ("small" = 1, "mean" = 2, and "large" = 3). Color intensity was transformed in a similar way ("weak" = 1, mean = 2, and "intense" = 3). The index of the nuptial area was analyzed fitting a linear mixed effect model ("lme," package: "nlme"; Pinheiro et al. 2009) with the relative time until spawning as explanatory variable and female identity as random factor. The relationship between the extent and the intensity of the nuptially colored area was analyzed with an ordinal regression model (polr). All models included a term for wild type versus F1 in subject origin to control for potential differences.

Coloration and female–female competition

Binomial tests were conducted to test whether colorful or dull females won more fights under each light condition. Wilcoxon signed-rank test were applied to test differences in behavior between both female groups, and Wilcoxon rank sum tests in order to examine differences between the 2 light environments.

RESULTS

Female coloration and male mate choice

Males clearly preferred the female stimulus showing the larger area of the nuptial coloration (Figure 1). No significant interaction between stimulus type (larger or smaller extent of area) and differences between stimuli (treatment) was found (Table 1a).

Table 1

(a) Male preference for female stimuli showing a different extent of the nuptially colored area. Data were analyzed by fitting a linear mixed effect model (lme) with male identity as random factor (see "MATERIALS AND METHODS"). Each male performed the 3 experimental treatments in random order. (b) Significance of variables explaining the number of female courtship displays by fitting a mixed effect model (lme) with female identity as random factor. Reported P values of the model refer to the increase in deviance when the respective variable was removed

Step	Simplification	df (LRT)	Change in df	Deviance change (χ^2)	*P*
(a) Maximal model	—	8	—	—	—
1	Stimulus:treatment	6	2	1.0	0.6
2	Treatment	4	2	0	1
3	Stimulus	3	1	11.5	<0.001
(b) Maximal model	—	6	—	—	—
1	Days until spawing	5	1	0.2431	0.6
2	Color intensity	3	2	40.732	<0.001

Intersexual signal function of female coloration

Female coloration and individual quality

Overall, 15 females spawned and 9 females did not spawn. The image analyses revealed that the likelihood of females to spawn was significantly explained by the extent of the red colored area (Supplementary Table S1; LRT: $\chi^2 = -4.224$, df = 1, $P = 0.04$; Figure 2), whereas the blue colored area showed no significant effect (LRT: $\chi^2 = -1.1856$, df = 1, $P = 0.27$). In contrast, the number of eggs was significantly predicted by the extent of the female's blue nuptial area (Supplementary Table S2; LRT: $n = 15$, $F_{1,13} = 11.206$, $P = 0.005$; Figure 3), but the red area showed no significant effect (Supplementary Table S2; LRT: $n = 15$, $F_{1,13} = 1.4457$, $P = 0.25$). Female standard length was neither a significant predictor for the likelihood to spawn (Supplementary Table S1; LRT: $\chi^2 = -0.6618$, df = 1, $P = 0.66$) or for the number of eggs (Supplementary Table S2; LRT: $F_{1,15} = 0.1522$, $n = 15$, $P = 0.7$) nor was it significantly related to the extent of the blue (LRT: $F_{1,23} = 1.4995$, df = 1, $n = 24$, $P = 0.23$) or red colored area (LRT: $F_{1,23} = 0.3118$, $n = 24$, $P = 0.58$). In 6 of 15 cases, the fry did not hatch or was cannibalized by the parents, thus brood care data of 9 pairs were analyzed. The extent of the blue area and female body size significantly predicted the frequency of female brood care for the free-swimming young (Supplementary Table S3; blue area: LRT: $n = 9$, $F_{1,8} = 61.425$, $P = 0.0001$; body size: LRT: $n = 9$, $F_{1,7} = 6.4581$, $P = 0.04$). Moreover, the extent of the blue area was significantly related to the survival

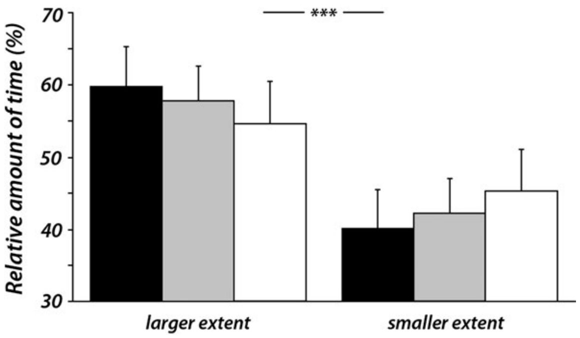

Figure 1
Male preference expressed as percentage of time spent in the association zone in front of female stimuli showing a larger or smaller extent of the nuptially colored ventral area. Plotted are means + standard error of the 3 experimental treatments (with stimuli showing 26% vs. 10% (black bars), 18% vs. 10% (gray bars), and 26% vs. 18% (white bars) of female nuptial coloration in relation to total lateral projection area). Data were analyzed by fitting a linear mixed effect model with male identity as random factor. Asterisks indicate the level of significance between all larger or smaller stimuli. ***$P < 0.001$.

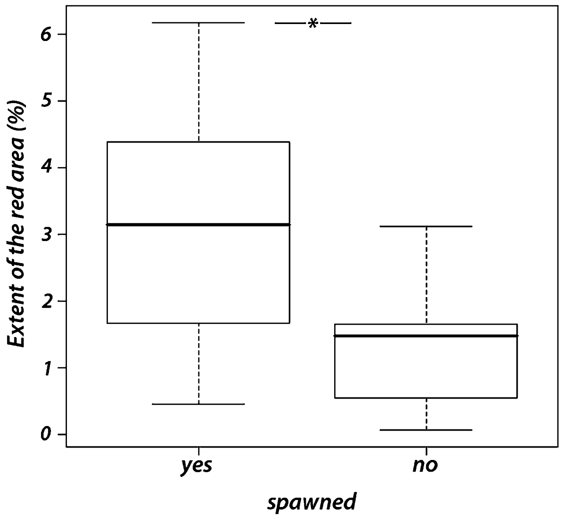

Figure 2
The likelihood to spawn is significantly related to the relative extent of the red area. Boxplots (median, quartiles, and range) show the relative extent of the area for females that spawned and females that did not spawn. *$P < 0.05$.

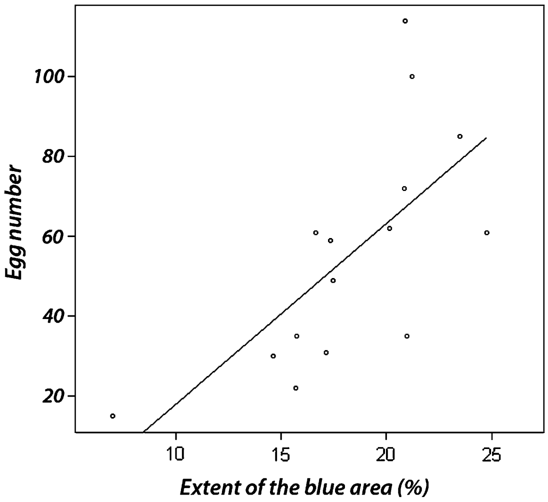

Figure 3
Relationship between the extent of the blue area relative to the lateral body projection area, and number of eggs produced by mated females.

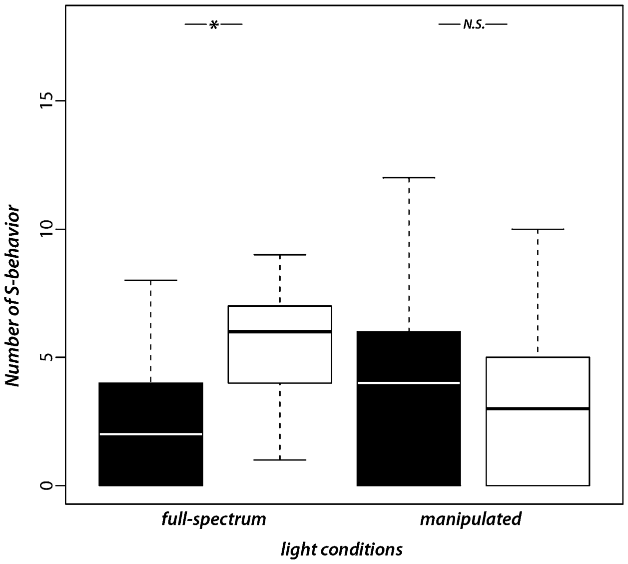

Figure 4
Number of S-behaviors dull (black bars) and bright (white bars) females performed under full-spectrum or manipulated light conditions. Under full-spectrum conditions, the color signal was visible under wavelengths from 400—700 nm, whereas under manipulated conditions, the color signal was interfered by removing medium wavelengths. Shown are medians (quartiles and ranges). $^*P < 0.05$; not significant $P > 0.05$.

rate (Supplementary Table S4; LRT: $n = 9$, $F_{1,8} = 13.992$, $P = 0.007$). Significant differences between wild caught or F1 fish were not found in all statistical models (LRT: all $P > 0.14$).

Temporal changes in female coloration

During observations, the nuptially colored area significantly expanded closer to spawning (LRT: $\chi^2 = 8.676$, df = 1, $n = 15$, $P = 0.003$). The intensity of the nuptially colored area was significantly related to the extent of the nuptially colored area (LRT: $\chi^2 = 96.1705$, $n = 15$, df = 1, $P < 0.001$). The number of female courtship displays was significantly related to the intensity of the nuptial coloration but was not significantly explained by the time until spawning (Table 1b). After spawning, the extent of the nuptially colored area (Wilcoxon signed-rank test: $V = 0$, $n = 15$, $P = 0.001$) as well as the color intensity (Wilcoxon signed-rank test: $V = 0$, $n = 15$, $P = 0.0019$) significantly decreased in comparison with the day before spawning.

Coloration and female–female competition

Only females that could be distinguished by individual coloration (dull/bright) were tested, thus sample sizes varied between treatments. Under full spectrum conditions, colorful females won significantly more contests than dull ones (binomial test, $N_{\mathrm{dull}} = 3$, $N_{\mathrm{colorful}} = 12$, $P = 0.035$). Under the manipulated conditions, this effect disappeared, that is, bright females did not win significantly more fights than dull ones (binomial test, $N_{\mathrm{dull}} = 7$, $N_{\mathrm{colorful}} = 8$, $P = 1$). Under full spectrum conditions, bright females performed significantly more S-behaviors than dull females (Wilcoxon signed-rank test: $V = 16.5$, $n = 13$, $P = 0.046$; Figure 4). Again, under manipulated conditions, the effect disappeared (Wilcoxon signed-rank test: $V = 53$, $n = 14$, $P = 1$; Figure 4). Bright and dull females did not significantly differ in attacks under both light environments (full-spectrum conditions: Wilcoxon signed-rank test: $V = 84$, $n = 19$, $P = 0.740$; manipulated conditions: Wilcoxon signed-rank test: $V = 57.5$, $n = 15$, $P = 0.909$). Total number of S-behaviors and attacks did not significantly differ between the different light conditions (Wilcoxon rank sum tests $N_{(\mathrm{full\ spectrum})} = 13$, $N_{(\mathrm{manipulated})} = 14$, $W = 83$, $P = 0.715$ and $N_{(\mathrm{full\ spectrum})} = 19$, $N_{(\mathrm{manipulated})} = 15$, $W = 145$, $P = 0.945$, respectively). Bright and dull females did not significantly differ in standard length and body mass in neither treatment (Wilcoxon signed-rank tests: all $P > 0.14$).

DISCUSSION

Usually, females are expected to be choosy due to higher reproductive costs, whereas males are expected to be indiscriminate, maximizing their reproductive success by fertilizing as many eggs as possible. Kokko and Johnstone (2002) showed that mutual mate choice decisions are especially likely when breeding is costly, and the benefits of biparental care are similar for each sex. In *P. taeniatus*, both sexes largely invest into their offspring, thus the evolution of adaptive female ornamentation by direct sexual selection can be expected. Here, we provide evidence that male mate choice and female–female competition play a decisive role in the evolution of female conspicuousness in *P. taeniatus*, a nonsex-role reversed fish with mutual mate choice.

Female coloration and male choice

The results of the male choice experiment show that a larger female nuptially colored area is preferred. When confronted with female stimuli differing in the amount of coloration males associated more with the stimulus that showed a larger extent of the nuptially colored area. By presenting virtual female stimuli, it was assured that male preference could not be confounded by other variables, for example, differences in behavior or other body variables when using live stimuli fish.

The result confirms earlier studies showing that male *P. taeniatus* are choosy concerning mate choice and female quality (Thünken et al. 2007a, 2007b; Baldauf, Kullmann, Schroth, et al. 2009; Baldauf, Kullmann, Winter, et al. 2009; Baldauf et al. 2010). Furthermore, similar to mating preferences concerning body size (Baldauf, Kullmann, Schroth, et al. 2009), males generally appear to have preferences for high-quality females, that is, preferences independent from their own quality. Because male body coloration also plays an important role in female mate choice (Baldauf, Kullmann, Winter, et al. 2009), body coloration might be of importance in mutual mate choice and lead to some sort of assortative mating. However, in contrast to assortative mating patterns in other cichlids (e.g., in a Lake Victoria cichlid, *Neochromis omnicaeruleus*; Pierotti and Seehausen 2007), the assortment may not be based on preferences for a similar trait but are the result of mutual mating decisions between males and females. As preferences coincide only between high-quality mates, high-quality individuals reject individuals of lower quality, who in turn have to accept mates that do not meet their preference or fail to mate at all (see Baldauf, Kullmann, Schroth, et al. 2009).

Male choosiness has now been shown in a variety of taxa (see Kraaijeveld et al. 2007 and citations therein), however, in many studies, the adaptive significance of the male choice was often not clear. For example, Hill (1993) was one of the first to show that male preferences in the house finch, *Carpodacus mexicanus*, are present, but female ornamentation was not correlated to presumed measures of individual quality. In *P. taeniatus*, the female's ventral ornamentation signals information about the individual quality in terms of fecundity, maternal care, the number of surviving offspring, and readiness to spawn. Thus, males can expect direct fitness benefits when choosing a greatly ornamented female. The evolution of female ornamentation of *P. taeniatus* may be driven, at least in part, by male choice.

Intersexual signal function of female coloration

The purple nuptial ventral coloration of female *P. taeniatus* is a dyadic visual signal composed of the 2 colors, blue and red. The extent of the blue area predicts female quality in terms of fecundity, female brood care intensity, and the number of surviving young, whereas the red area reveals whether a female is ready to spawn. Hence, female *P. taeniatus* visually signal their individual quality to a male thus allowing male to maximize reproductive success by choosing the most highly ornamented females available.

The female purple ornamentation is not present in males of *P. taeniatus*, which develop a yellow ventral coloration instead. This corresponds to previous findings that male and female ornaments might be independent from each other (e.g., Funk and Tallamy 2000; Amundsen and Forsgren 2001). Thus, the purple coloration represents a female specific signal in *P. taeniatus*, which may not be explained by genetic correlation to male ornamentation (Kraaijeveld et al. 2007). Furthermore, the visual signal is variable between females, and the ornament was amplified closer to spawning and related to the number of female courtship displays. This also supports that the signal is actively used by females during courtship.

Although males could gain direct fitness benefits when selecting for the female ornament, the signal might be used by females to exploit male preference under natural conditions: When the expression of the signal implies low costs, it might be developed by low-quality individuals. However, it should be expected that bias in female access to resources in the natural environment should lead to greater variance in the expression of the ornament than in the laboratory. The visual signal may be not exploitable when it is linked to individual quality or costly to express (Maynard Smith and Harper 2003). Nevertheless, future studies should investigate whether the expression of the ornament is connected to costs.

Coloration and female–female competition

Female *P. taeniatus* interact aggressively when confronted with an individual of the same sex. It has been suggested that female coloration might affect female competition in cichlids (Beeching et al. 1998). When full spectrum light information was available, intensively colored individuals won fights significantly more often than dull rivals. This difference between colorful and dull females disappeared under manipulated light conditions in which the blue component of the color signal was interfered. Furthermore, dull females performed significantly less aggression (measured as S-behaviors) than bright females under full-spectrum light conditions only. Because the general aggression level of the females did not significantly differ between the 2 light conditions, the results suggest that the blue component of the nuptial coloration is involved in signaling dominance. The results are similar to studies of male–male competition in which the coloration acts as threat signal. For example, in sticklebacks brightly red colored males have advantages during intrasexual competition (Bakker and Sevenster 1983; Baube 1997). Under manipulated light conditions, the blue component was removed, whereas the red component remained unchanged (interestingly, the spectra suggest that dull females reflected more in the red component under both light conditions than colorful ones). Because colorful females won fights significantly more often only under full-spectrum light conditions, the blue component seems to play a decisive role in female–female competition of *P. taeniatus*, whereas the red component of the color signal seems to be less important.

In conclusion, inter- and intrasexual selection may favor different aspects of the purple color signal and its components. On the one hand, female–female competition might select for the expression of the blue color component. On the other hand, male choice may influence the evolution of the extent of both red and blue components of the signal. Additionally, the intensity of the signal might be influenced by intersexual selection as well: Color intensity was significantly related to the extent of the nuptially colored area during courtship.

In summary, this study shows that a conspicuous female visual signal is used in inter- and intrasexual communication of a species with conventional sex roles. The female purple nuptial ventral coloration of *P. taeniatus* is a visual signal that consists of a red and a blue component, which 1) is sexually selected by males, 2) transmits information about individual quality, and 3) plays a role in female–female competition.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/.

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