



Original Article

# Egg-spot pattern and body size asymmetries influence male aggression in haplochromine cichlid fishes

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Assessing an opponent's strength is an important component of attack strategies in territorial combats between males. Body size is often considered to directly influence an individual's strength, but other honest visual signals may also affect the assessment of opponents. Among such visual signals are the so-called egg-spots, a conspicuous ovoid marking on the anal fin of male haplochromine cichlid fishes, made up of carotenoid-containing and other pigment cells. It has long been assumed that egg-spots are mainly relevant in courtship and spawning behavior, and previous work has focused primarily on their function in intersexual selection. Recently, however, both body size and egg-spots have been suggested to play a role in male–male interactions. To test whether egg-spots function in female choice or whether egg-spots and/or body size function as a predictor of strength and the subsequent attack strategy in male–male interactions, we performed a series of behavioral experiments in the haplochromine cichlid *Astatotilapia calliptera*. The trials revealed a limited involvement of egg-spots in female choice, yet a much stronger influence in male interactions. Territorial males combined information from the strength assessment based on body size and egg-spots to adopt their attack strategies. They launched more attacks against the larger intruder with many egg-spots compared with the smaller intruder without or with fewer egg-spots. Our study provides evidence that egg-spots serve as honest visual signal and that the level of asymmetries in egg-spot pattern and body size determines the relative impact of each trait in strength assessment.

**Key words:** *Astatotilapia calliptera*, attack strategy, East African cichlid fishes, egg-spots, female choice, Lake Malawi, male aggression.

## INTRODUCTION

Competition over mates constitutes a key mechanism in the process of sexual selection, either through mate choice by the opposite sex or via contests for mates (Darwin 1859, 1871; Andersson 1994). In many territorial species, for example, one of the sexes—most commonly males—competes for a territory in order to gain access to mating partners. Males have thus evolved a variety of strategies to pursue own interests without investing too much energy into fighting or taking the risk of injuries (Maynard Smith and Price 1973). An important component of such male–male interactions is the evaluation of the strength of an opponent, the so-called “resource holding potential” (RHP), which serves to prevent the escalation of fights (Parker 1974). Body size is a direct predictor of the RHP in intraspecific contests because larger males are usually more likely to win combats (e.g., Fryer and Iles 1972; Tokarz 1985; Crespi

1986; Keeley and Grant 1993; Pavey and Fielder 1996; Jenssen et al. 2005; Odreitz and Sefc 2015). In case body size asymmetry is small between opponents or if body size is not a reliable indicator of strength, other factors included into strength assessment either add to or cancel out the effect of body size (Clutton-Brock and Albon 1979; Beaugrand et al. 1996; Sneddon et al. 1997). Other factors that may influence the assessment of an opponent's strength comprise a wide array of male signals, including conspicuous traits such as ornaments (Berglund et al. 1996). The production and display of ornaments that signal male quality involve costs, which in turn prevent dishonest signaling and therefore incorrect strength assessment (Zahavi 1975).

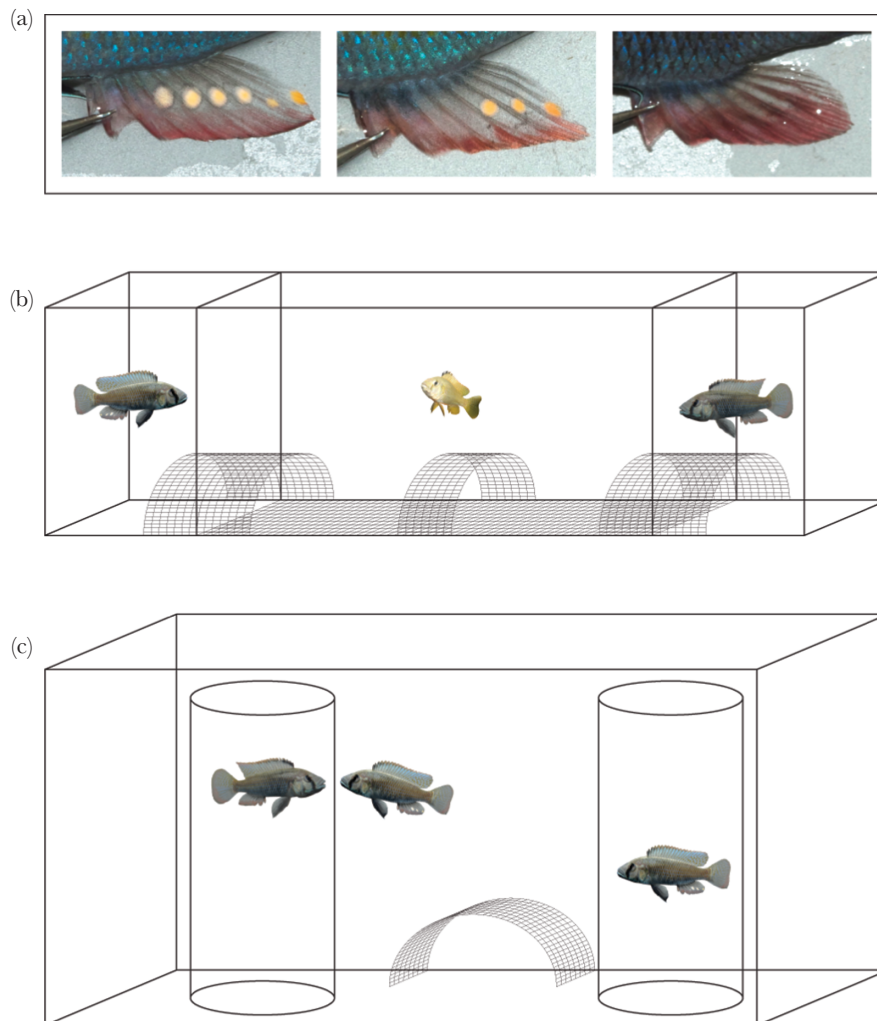
A prime example for honest visual signals are ornaments based on pigment cells containing carotenoids, which cannot be synthesized de novo by animals and, thus, have to be taken up via diet (Goodwin 1986). The costs arising from carotenoid-based visual signals can be manifold and may include competition for carotenoids in environments with carotenoid-poor food (Hill 1992), being conspicuous to predators (Endler 1978, 1980), and

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reallocation of carotenoids from antioxidant activities and/or other physiological processes to the ornament (reviewed in [Svensson and Wong 2011](#)). Therefore, only healthy and strong individuals should be able to afford the costs of carotenoid allocation to visual signals ([Lozano 1994, 2001](#)). Consistently, reddish signals in general correlate positively with winning combats throughout the animal kingdom (e.g., [Evans and Norris 1996](#); [Pryke et al. 2002](#); [Hill and Barton 2005](#); [Hamilton et al. 2013](#); [Sefc et al. 2015](#)). Moreover, there is growing evidence that red coloration may constitute a general signal of intimidation (e.g., [Dijkstra et al. 2005](#); [Pryke 2009](#)). The intimidation effect of red coloration and body size seems to be context dependent, though, and can sometimes be defeated by deploying a high-risk strategy. In male three-spined stickleback, for example, red belly coloration has been shown to intimidate opponents outside a settled territory ([Bakker and Sevenster 1983](#); [Baube 1997](#)), but to evoke attacks in territorial males toward more reddish intruders ([Ter Pelkwijk and Tinbergen 1937](#); [Tinbergen 1948](#)). Additionally, in some species smaller individuals are more aggressive or even prompt a combat ([Moretz 2003](#); [Svensson et al. 2012](#)).

The initiation and outcome of a combat can therefore not always be predicted based on the contestants' strength alone because an individual's investment often depends on factors such as the subjective value of the contested resource (see "sequential assessment game" and its extension, [Enquist and Leimar 1983, 1987](#)). In other words, individuals will fight more, if the subjective value of resource is higher. Therefore, in different contexts, the same visual signal can either inhibit or evoke aggression.

In this study, we focus on a visual signal that is characteristic to the most species-rich group of cichlid fishes and test whether this carotenoid-based ornament and/or body size function as a predictor of strength and subsequent attack strategy in male–male interactions. The visual signal under investigation is the so-called egg-spot pattern of the East African haplochromine cichlids, which are ovoid markings on the anal fins of males ([Salzburger et al. 2007](#); [Santos et al. 2014](#)) ([Figure 1a](#)) (note that they can also be found in females but are then usually less elaborate). Previous work on the function of egg-spots has primarily focused on their putative role in female choice. [Wickler \(1962\)](#), for example, suggested



**Figure 1**

Haplochromine egg-spots and a schematic view of the experimental setups. (a) Egg-spot patterns on male anal fins of *Astatotilapia calliptera* (from left to right: male with many egg-spots; male with few egg-spots; male without egg-spots). (b) The 2-way female choice setup (experiment 1) with the female in the central tank containing an egg-trap and flanked by the stimulus males' tanks (male with many egg-spots vs. male without egg-spots). (c) The setup for the male aggression experiments with the territorial focal male being able to interact with the 2 stimulus intruder males in the plastic cylinders (experiment 2.1: male with many egg-spots vs. male without egg-spots; experiment 2.2: male with egg-spots vs. male with fewer egg-spots).

that egg-spots mimic real eggs, act as releasers for egg-uptake, and maximize fertilization rates. Mate choice experiments in *Astatotilapia elegans* (Hert 1989) and *Pseudotropheus (Maylandia) aurora* (Hert 1991) revealed that females prefer males with many egg-spots over males with fewer egg-spots. Coullidge (2002), on the other hand, found that *P. (M.) lombardoi* females preferably choose males with an artificially enlarged egg-spot over males with one natural or many egg-spots. More recently, however, experiments with *Astatotilapia burtoni* demonstrated that females of this species do not show a preference for males with many egg-spots (Henning and Meyer 2012; Theis et al. 2012). Instead, it appears that egg-spots have an intimidating effect in male–male competition in *A. burtoni* (Theis et al. 2012), suggesting that this ornament serves multiple, species-specific functions in haplochromine cichlids. Interestingly, this intimidating effect of egg-spots was not found in the same species during male aggression trials with direct contact between the 2 opponents (Henning and Meyer 2012). The latter study allowed for large asymmetries in body size, though, which was in the end the only trait that determined winning a combat. Taken together, it thus seems that the egg-spot phenotype as well as body size asymmetries of opponents can influence the strength assessment and interact with each other and that the attack strategy is based on the intimidating effect of egg-spots and body size in *A. burtoni*.

Here, we evaluate whether egg-spots function in female choice or in male–male interactions. To this end, we performed a series of behavioral experiments in *Astatotilapia calliptera* (Günther 1893), which represents the Lake Malawi “counterpart” to the previously examined *A. burtoni* from Lake Tanganyika. We first tested, using the same setup as in Theis et al. (2012), whether in *A. calliptera* females also show no preference for males with many egg-spots over males with artificially removed egg-spots (experiment 1). We then examined whether asymmetries in egg-spot pattern alone (experiment 2.1) or in combination with body size asymmetries (experiment 2.2) could be a predictor of strength and subsequent attack strategy, and therefore male aggression, in *A. calliptera*.

## METHODS

### Study species

*Astatotilapia calliptera* occurs in shallow, weedy habitats along the shoreline of Lake Malawi, but also inhabits ponds, small lakes, and rivers of its catchment (Konings 2007; Tyers and Turner 2013). With its congener *A. burtoni* from Lake Tanganyika, it shares a generalist lifestyle, the occurrence in lake and stream habitats, and a lek-like breeding system (Theis et al. 2014), in addition to the typical characteristics of haplochromines such as sexual dimorphism, female mouthbrooding, and anal fin egg-spots (Salzburger et al. 2005). The *A. calliptera* test animals used in this study were F1 individuals originating from Chizumulu Island in Lake Malawi, Malawi. Males from this locality display a blue-gray body coloration, which differs from the yellow body coloration of other *A. calliptera* populations (Tyers and Turner 2013).

Females and males were kept in separate tanks (150 × 50 × 50 cm<sup>3</sup>) providing standardized conditions with constant water temperature (26 °C) and a 12:12-h light:dark cycle. Flake food was fed twice a day, complemented with frozen *Artemia* once a day. The test animals were kept individually in mesh cylinders ( $d = 16$  cm,  $h = 40$  cm) to enable individual identification. All males were photographed (Nikon D5000, Nikon Speedlight SB-900) for later size measurements (Adobe Photoshop CS3 extended, v 10.0.1) and egg-spot counts (a complete egg-spot was counted as 1 and incomplete

egg-spots as 0.5; analogous to Albertson et al. 2014). To reduce handling stress, fish were anesthetized during the procedure (3 drops of clove oil per liter water) and were given time to recover before an experimental run (stimulus males at least 2 h, focal males 20 h of acclimation). All experiments were performed at the Zoological Institute of the University of Basel under the permission of the Cantonal Veterinary Office, Basel, Switzerland (permit numbers: 2356, 2403).

### Experiment 1: female choice

We used the same experimental setup as in Theis et al. (2012). In each experimental round, we placed a gravid female ( $n_{\text{female}} = 18$ ) in a central tank (60 × 30 × 30 cm<sup>3</sup>) and allowed visual contact with 2 males with varying egg-spot patterns presented in 2 outer tanks (40 × 25 × 25 cm<sup>3</sup>) (Figure 1b). The paired males were size matched in standard body length (SL) as precisely as possible ( $n_{\text{male pairs}} = 12$ ;  $\text{mean}_{\text{SL difference}} \pm \text{standard deviation [SD]} = 0.97 \pm 0.61$  mm;  $\text{range}_{\text{SL difference}} = 0.12\text{--}2.12$  mm) and introduced at least 20 h before the start of each experimental round to allow for acclimation and territorial behavior to develop. Egg-spots in 1 stimulus male were removed completely (“freeze branding” method; Hert 1986, 1989; see also Theis et al. 2012) but were left unaltered in the other stimulus male ( $\text{mean}_{\text{egg-spot number difference}} \pm \text{SD} = 4.22 \pm 1.06$ ;  $\text{range}_{\text{egg-spot number difference}} = 2.5\text{--}6$ ) (Figure 1a). As a treatment control, the unaltered stimulus males were also freeze branded directly above the egg-spots. All manipulations on the anal fins were performed under clove oil anesthesia (3 drops per liter water). In each experimental round, the female was able to see and to interact with both males of the stimulus pair and laid eggs within a period of few hours up to 7 days (the experiment was terminated if the female did not lay eggs within this time period). Because of the grid placed in the aquaria, eggs laid by the female would fall into this “egg-trap” before the female was able to take them up into her mouth for incubation. The egg-trap, which completely covered the floor of the female tank (see Figure 1b), made it possible to assess if the female laid the eggs in front of the male with egg-spots, the male without egg-spots, or in front of both. The position of the eggs laid was used as measure for female preference. Additionally, the interaction time of the female with each of the 2 presented stimulus males was analyzed for the first half an hour (recorded using a Sony handycam HDR-XR550VE, 12.0 mega pixels; analyzed in iMovie, v. 9.0.4) of the experiment to test if females preferably interacted with males with many or without egg-spots and if interaction time correlated with the number of eggs laid.

Because many of the females (9 out of 18) laid their eggs exclusively next to one of the males, the data was coded into 1 and 0 to circumvent the problem of zero-inflation in statistical analyses. The data was coded as 1 if the male with egg-spots received more than or exactly 50% of the eggs and as a 0 if the male with egg-spots received fewer than 50% of the eggs. The binomial data were then analyzed with a generalized linear mixed effects model (GLMM) with a logistic link function using the package lme4 (Bates et al. 2014) in R (version 3.0.3, R Core Team 2014), which was also used for all further statistical analyses. The factor male pair was included as a random effect to account for dependence of the data, that is, the use of 6 male pairs twice. A second model was applied to test if the female spent a different amount of time interacting with one of the 2 stimulus males (note that for models with interaction time sample size is reduced by 1 due to the loss of 1 videotaping). The proportion of time (in seconds) the female interacted in front of the stimulus male with many egg-spots relative to the time in front

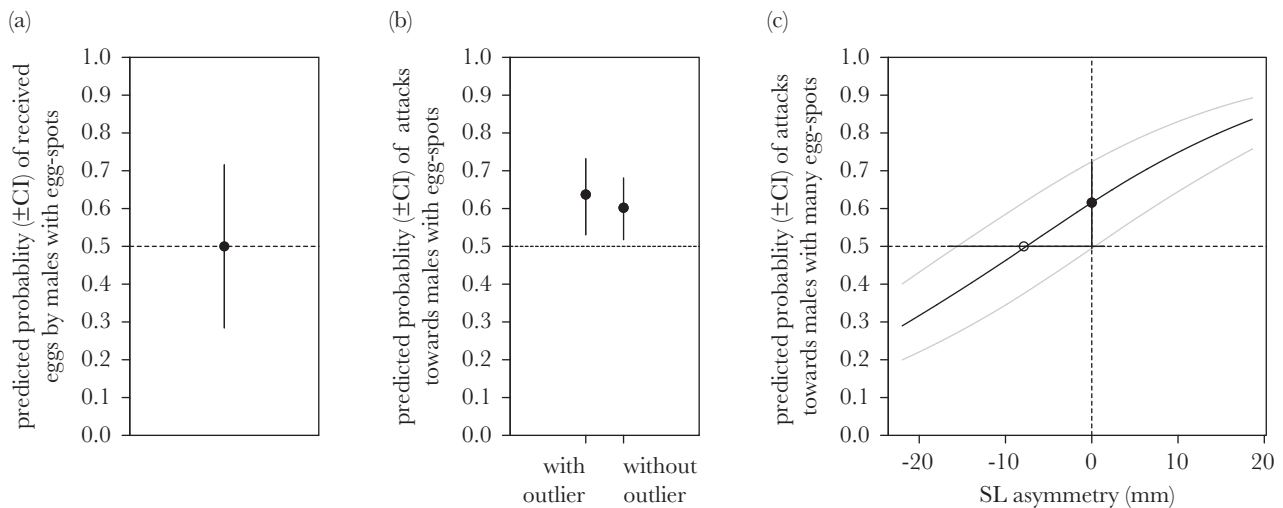
of the male without egg-spots was used as response variable in an overdispersed binomial GLMM. An observation level was included as random effect to account for the extravarience in the data. Male pair, as a second random effect, corrected for the dependence of the data due to the repeated usage of the same stimulus male pairs. Using these 2 models, we tested if the intercept on the logit scale was different from 0, which would indicate that the male with egg-spots had a probability significantly higher than 0.5 to receive more eggs (represented as a dashed line in Figure 2a), or more interaction time respectively, than the male without egg-spots. In a third model, we added interaction time as explanatory variable to the above-mentioned first model to test if the choice of egg-laying depended on interaction time.

## Experiment 2: male aggression

As described in Theis et al. (2012), the setup to test for male aggression consisted of a tank ( $60 \times 30 \times 30 \text{ cm}^3$ ) containing a shelter for the focal male and 2 transparent, perforated plastic cylinders ( $d = 12 \text{ cm}$ ,  $h = 27 \text{ cm}$ ), one for each of the 2 stimulus males (Figure 1c). The plastic cylinders were used to prevent direct contact between the males, which could lead to injuries. In addition, the plastic cylinders minimize the behavioral response of the opponent through limited space and, hence, prevent asymmetries in the expression of behaviors from opponents, which could influence the aggressive behavior of the focal males (Moore et al. 1997; Wilson et al. 2009). Stimulus males, differing in egg-spot pattern, were always presented in pairs because it is more effective to compare behavioral responses of 1 focal male toward both intruder phenotypes due to among individual differences in aggressiveness reported for many fish species (e.g., Wilson et al. 2011). All males were kept in individual mesh cylinders for at least 3 weeks before the start of the first experiment and a minimum of 24h between trials if they were used multiple times, to avoid

an effect of knowledge about prior fighting success, which might influence the chances of winning (it was previously shown that winner–loser effects persist for no more than 24h in many fish species, reviewed in Hsu et al. 2006). The focal male was introduced at least 20h before the experiment to acclimate and to become territorial, which resulted in aggressive attacks as soon as the stimulus males were introduced into the plastic cylinders. The aggressive behaviors as well as the interaction time of the focal males were analyzed for a time period of 30 min. Mouthlocking, bites, butts, circling, displays (frontal and lateral), and quivers (Baerends and Baerends-Van Roon 1950; Fernö 1987) were initially counted separately but added up to the category “attacks” for further analysis due to strong variations in fighting techniques among individuals.

Two different trials were conducted: in a first round (experiment 2.1), the 2 stimulus males were size matched but varied in egg-spot pattern. The egg-spots were completely removed in one of the males and were left unaltered in the other male ( $n_{\text{focal}} = 29$ ;  $n_{\text{pairs}} = 18$ ;  $\text{mean}_{\text{egg-spot number difference}} \pm \text{SD} = 3.79 \pm 0.97$ ;  $\text{range}_{\text{egg-spot number difference}} = 2-5$ ). The stimulus males of a male pair were size matched as precisely as possible among each other ( $\text{mean}_{\text{SL difference between stimulus males}} \pm \text{SD} = 0.88 \pm 0.74 \text{ mm}$ ;  $\text{range}_{\text{SL difference between stimulus males}} = 0.04-3.27 \text{ mm}$ ) and with the corresponding focal male ( $\text{mean}_{\text{SL difference between focal and stimulus males}} \pm \text{SD} = 1.64 \pm 1.24$ ;  $\text{range}_{\text{SL difference between focal and stimulus males}} = 0.02-5.13$ ). In a second step (experiment 2.2), the presented stimulus male pairs differed in body size, and to a lesser extent as compared with experiment 2.1, in egg-spot number ( $n_{\text{focal}} = 24$ ;  $n_{\text{pairs}} = 17$ ;  $\text{mean}_{\text{egg-spot number difference}} \pm \text{SD} = 2.52 \pm 0.86$ ;  $\text{range}_{\text{egg-spot number difference}} = 0.5-3.5$ ;  $\text{mean}_{\text{SL difference between stimulus males}} \pm \text{SD} = 6.06 \pm 5.88 \text{ mm}$ ;  $\text{range}_{\text{SL difference between stimulus males}} = 0.39-21.97 \text{ mm}$ ). The SL of each focal male was in between the 2 corresponding stimulus males ( $\text{mean}_{\text{SL difference between focal and average of the corresponding stimulus males}} \pm \text{SD} = 1.37 \pm 1.22 \text{ mm}$ ;  $\text{range}_{\text{SL difference between focal and average of the corresponding stimulus males}} = 0.07-4.63 \text{ mm}$ ).



**Figure 2**

Results of the female choice and male aggression experiments with *Astatotilapia calliptera*. (a) Influence of egg-spot asymmetry on female choice (experiment 1). Predicted probabilities for the stimulus male with egg-spots receiving more of the laid eggs compared with the equally sized male without egg-spots. (b) Influence of egg-spot asymmetry on male aggression (experiment 2.1). Predicted probabilities for the stimulus male with many egg-spots receiving more attacks compared with equally sized male without egg-spots (with and without the outlier). (c) Influence of egg-spots and body size asymmetries (difference between the standard length of the male with many egg-spots and the standard length of the male with fewer egg-spots) on male aggression (experiment 2.2). Predicted probabilities for the stimulus male with many egg-spots compared with the male with fewer egg-spots receiving 1) more attacks at different body size asymmetries (curve), 2) more attacks at equal body size (closed dot), and 3) the same amount of attacks at indicated negative body size asymmetry (open dot).

The recorded number of attacks was grouped into attacks against the male with many egg-spots and attacks against the males with fewer/without egg-spots; the number of attacks against the male with egg-spots relative to the number of attacks against the males without egg-spots was used as a response variable in an over-dispersed binomial GLMM. Male pair, as an additional random effect, corrected for the dependence of the data due to the repeated usage of the same stimulus male pairs (experiment 2.1: 11 pairs twice; experiment 2.2: 1 pair twice and 3 pairs 3 times). Although the males were size-matched in the first experiment, body size difference was included as an additional fixed effect in all analyses because even minor size differences are known to influence the outcome of aggressive male–male interactions in cichlid fishes (e.g., Turner and Huntingford 1986). A second model was applied to test if the interaction time of the territorial male differed between the stimulus males. To this end, the same model as described above was adjusted with “interaction time” as response variable instead of “attacks.” Using these 2 models, we tested if the intercept on the logit scale was different from 0, which indicates if the male with egg-spots had a probability significantly higher than 0.5 to receive more than 50% of the attacks (represented as a dashed line in Figure 2b,c), or more interaction time, than the male with fewer or without egg-spots. In a third model, we tested if number of attacks correlates with the interaction time. The model as described above was adjusted with interaction time difference as explanatory variable (instead of body size difference).

## RESULTS

### Experiment 1: female choice

Females showed high variability in total number of eggs laid into the 2 egg-traps (see Supplementary Table 1A for detailed information on egg counts), and 9 out of 18 females laid their eggs exclusively next to one of the males. The male with egg-spots received 50% or more of the eggs in 10 out of 18 trials, resulting in a modeled probability of receiving more eggs in exactly half of the clutches laid (average probability = 0.50, lower confidence interval [CI] = 0.28, upper CI = 0.72) (Figure 2a). Thus, females were not more likely to lay their eggs in front of the male with egg-spots compared with the male without egg-spots (GLMM,  $n_{\text{females}} = 18$ ,  $n_{\text{male pairs}} = 12$ ,  $z = 0$ ,  $P = 1$ ). In the first half an hour of the experiment, females spent more time interacting with the stimulus male without egg-spots compared with the male with egg-spots (GLMM,  $n_{\text{females}} = 17$ ,  $n_{\text{male pairs}} = 11$ ,  $z = -2.12$ ,  $P = 0.034$ ), but this interaction time did not correlate with egg-laying (GLMM,  $n_{\text{females}} = 17$ ,  $n_{\text{male pairs}} = 11$ ,  $z = -0.09$ ,  $P = 0.928$ ).

### Experiment 2: male aggression

Focal males were highly active and directed a large amount of attacks toward the stimuli males in all male aggression experiments (see Supplementary Table 1B for detailed information on attack counts). In experiment 2.1, focal *A. calliptera* males allocated significantly more attacks toward the size-matched stimulus male with many egg-spots (experiment 2.1: GLMM,  $n_{\text{focal males}} = 29$ ,  $n_{\text{stimulus male pairs}} = 18$ ,  $z_{\text{egg-spots}} = 2.50$ ,  $P_{\text{egg-spots}} = 0.012$ ,  $z_{\text{SL}} = 1.17$ ,  $P_{\text{SL}} = 0.242$ ). Residual analyses revealed one outlier, in which the focal male directed nearly all attacks against the male with egg-spots (compared with the one without). When the outlier was removed from the analysis, the model revealed additionally that larger males received significantly more attacks (experiment 2.1

without outlier: GLMM,  $n_{\text{focal males}} = 28$ ,  $n_{\text{stimulus male pairs}} = 18$ ,  $z_{\text{egg-spots}} = 2.37$ ,  $P_{\text{egg-spots}} = 0.018$ ,  $z_{\text{SL}} = 2.11$ ,  $P_{\text{SL}} = 0.035$ ). The results of experiment 2.2 support the finding that males with larger body size receive significantly more attacks and indicate a trend that males with many egg-spots receive more attacks by the focal male (GLMM,  $n_{\text{focal males}} = 24$ ,  $n_{\text{stimulus male pairs}} = 17$ ,  $z_{\text{egg-spots}} = 1.88$ ,  $P_{\text{egg-spots}} = 0.061$ ,  $z_{\text{SL}} = 2.16$ ,  $P_{\text{SL}} = 0.031$ ). Thus, the stimulus male with many egg-spots had a modeled average probability of over 0.6 to receive more than 50% of the attacks in all analyses, if the stimulus males were of equal body size (experiment 2.1: average probability = 0.64, lower CI = 0.53, upper CI = 0.73; experiment 2.1 without outlier: average probability = 0.60, lower CI = 0.52, upper CI = 0.68; experiment 2.2: average probability = 0.62, lower CI = 0.49, upper CI = 0.72) (Figure 2b,c). In experiment 2.2, the probability of males with many egg-spots receiving more attacks increased with a larger positive body size asymmetry until these males received all attacks. Complementarily, the probability of males with many egg-spots receiving more attacks decreased with a larger negative body size asymmetry. Stimulus males had an equal attack probability if the male with many egg-spots was on average 7.6 mm smaller than the male without or with fewer egg-spots (experiment 2.2: average SL asymmetry =  $-7.6$  mm, lower CI = 1.5 mm, upper CI =  $-16.7$  mm) (indicated by an open dot in Figure 2c).

Focal males also spent more time interacting with the stimulus male with many egg-spots in experiment 2.1. There was a tendency of focal males to interact more with larger males in experiment 2.2 (experiment 2.1: GLMM,  $n_{\text{focal males}} = 29$ ,  $n_{\text{stimulus male pairs}} = 18$ ,  $z_{\text{egg-spots}} = 2.60$ ,  $P_{\text{egg-spots}} = 0.009$ ,  $z_{\text{SL}} = 0.74$ ,  $P_{\text{SL}} = 0.460$ ; experiment 2.1 without outlier: GLMM,  $n_{\text{focal males}} = 28$ ,  $n_{\text{stimulus male pairs}} = 18$ ,  $z_{\text{egg-spots}} = 2.06$ ,  $P_{\text{egg-spots}} = 0.042$ ,  $z_{\text{SL}} = 1.019$ ,  $P_{\text{SL}} = 0.306$ ; experiment 2.2: GLMM,  $n_{\text{focal males}} = 24$ ,  $n_{\text{stimulus male pairs}} = 17$ ,  $z_{\text{egg-spots}} = 2.19$ ,  $P_{\text{egg-spots}} = 0.029$ ,  $z_{\text{SL}} = 1.77$ ,  $P_{\text{SL}} = 0.077$ ). The attacks received by stimulus males correlated with the difference in interaction time of the focal male with the 2 different stimulus males in all experiments (experiment 2.1: GLMM,  $n_{\text{focal males}} = 29$ ,  $n_{\text{stimulus male pairs}} = 18$ ,  $z = 8.79$ ,  $P < 0.0001$ ; experiment 2.1 without outlier: GLMM,  $n_{\text{focal males}} = 28$ ,  $n_{\text{stimulus male pairs}} = 18$ ,  $z = 8.65$ ,  $P < 0.0001$ ; experiment 2.2: GLMM,  $n_{\text{focal males}} = 24$ ,  $n_{\text{stimulus male pairs}} = 17$ ,  $z = 16.85$ ,  $P < 0.0001$ ).

## DISCUSSION

The assessment of an opponent's strength is an important mechanism to determine the subsequent attack strategy (Parker 1974). In male–male competitions, the strength of an opponent is often evaluated based on body size, but can also be based on other traits, for example, ornaments (Berglund et al. 1996). In this study, we examined if the carotenoid-based ornament egg-spot and/or body size function as predictors of strength and the subsequent attack strategy in male–male interactions in haplochromine cichlid fish. Additionally, female choice experiments were conducted on the same males because previous work on the function of egg-spots has primarily focused on a role of egg-spots in female choice (e.g., Hert 1989, 1991).

The experiments with the East African cichlid *A. calliptera* presented here revealed limited involvement of egg-spots in female choice when males were size matched, but rather an influence in male interactions, which is in line with our previous results in *A. burtoni* (Theis et al. 2012). However, whereas territorial *A. burtoni* males preferably attacked the presumably weaker stimulus males with fewer egg-spots, *A. calliptera* males adopted an attack strategy spending more time and,

hence, launching more attacks against the male with many egg-spots. In addition, we found that there were more attacks against males with larger body sizes in stimulus pairs with body size asymmetries. The different attack strategies deployed by the 2 *Astatotilapia* species when presented stimulus males with asymmetries in egg-spot pattern, and body size might be explained by different resource values. According to the extension of the “sequential assessment game” theory, weaker or smaller individuals tend to attack stronger or larger competitors if the resource value is higher (Enquist and Leimar 1987)—especially if there are few or no other opportunities to obtain new resources (the “desperado effect,” Grafen 1987). Alternatively, the reason leading to the subsequent difference in attack strategy might already be based in the process of strength assessment as such. For example, other color patterns or behaviors could represent additional factors influencing strength assessment in the 2 species, which were not examined in this study. Moreover, the strength assessment based on egg-spots could differ between the species. The seemingly lower intimidation effect of egg-spots in *A. calliptera* compared with *A. burtoni* is most probably connected to the invested costs. *A. calliptera* males have fewer egg-spots (Supplementary Figure A1B in Supplementary Appendix 1), and their egg-spots are less pronounced (Supplementary Figure A1C in Supplementary Appendix 1), suggesting that they might invest less into egg-spots than *A. burtoni* males do. The costliness of egg-spot conspicuousness could also be environmentally induced by being more conspicuous to predators (Goldschmidt 1991) or physiologically, for example, through differences in type and/or density of pigments or different metabolic pathways to produce these pigments (Sefc et al. 2014). However, the possible lower investment costs in *A. calliptera* compared with *A. burtoni* seem to be high enough for egg-spots to constitute a signal of strength evoking attacks. Intruder *A. calliptera* males showing egg-spots always received more attacks if they were larger, similar sized or even slightly smaller than the males with no egg-spots. The effect of egg-spot pattern asymmetry was only overcome by the effect of body size asymmetry if the male with many egg-spots was around 10% smaller than the male with fewer egg-spots (e.g., by approximately 8 mm in experiment 2.2, see Figure 2c). Note, however, that these values should be taken with caution because freeze branding has artificially induced egg-spot variation in our experiment, which might therefore deviate from a setting involving natural variation of egg-spots. Generally, the more similar the contestants are in body size and weight, or the less those traits are used to estimate strength in a species, the more important are asymmetries of other factors (see e.g., Beauprand et al. 1996). Previous experiments in green swordtail fish (*Xiphophorus hellerii*) showed that body size asymmetries of 20–30% are necessary to eliminate other advantages such as prior social experience and prior residency (Beauprand et al. 1996). Nevertheless, also minor differences, for example, 1 mm in body size (Turner and Huntingford 1986) and few percentages of weight (Barlow et al. 1986; Enquist and Jakobsson 1986) were shown to influence the outcome of combats in cichlids.

Our findings and the above-mentioned examples show that strength assessment and attack strategy can differ greatly between species and, in addition, depend on the experimental setup. The latter could also explain the different outcomes in aggression trials with *A. burtoni* by Henning and Meyer (2012) and Theis et al. (2012). First, the 2 studies differed in the combat setup, with 1 territorial male and 2 intruders (Theis et al. 2012) versus 2 males interacting in a direct combat (Henning and Meyer 2012). Second, one study combined large egg-spot asymmetries with small body size asymmetries (Theis et al. 2012), whereas the other study combined small differences in egg-spot number with larger body size

and especially weight differences (see Supplementary Figure S2 in Henning and Meyer 2012). The large body mass asymmetries together with the direct interaction in the study of Henning and Meyer (2012) could possibly explain the fact that body size alone determined the outcome of a combat rather than egg-spot number.

Taken together, these studies suggest that both egg-spot pattern and body size asymmetries influence the strength assessment in *A. burtoni* (Henning and Meyer 2012; Theis et al. 2012) as well as in *A. calliptera* (this study) and that egg-spot asymmetries become more important as the difference in body size between contestants becomes smaller. However, despite the high similarity in lifestyle, the 2 species use different attack strategies. The causes leading to the observed attack strategy in *A. calliptera* could be higher resource value and/or lower intimidating effect of egg-spots compared with *A. burtoni*.

Further support for the hypothesis that the level of intimidation induced by egg-spots could be lower in *A. calliptera* than in *A. burtoni* is provided by the results of the female choice experiments. Females of *A. burtoni* tended to lay eggs in front of males without egg-spots, which could have been due to avoidance of males with egg-spots, which were perceived as more aggressive. In *A. calliptera*, this effect of intimidation seems to be lower because females indeed preferred to interact more with the males without egg-spot at the beginning of the experiment, but showed random mating with respect to the number of eggs laid during the experiment. As several studies have shown, interaction time or time spent does not necessarily predict mate choice (e.g., Kidd et al. 2006), and females may not reveal their mating preferences until the day on which spawning takes place (Kidd et al. 2013). The random mate choice of *A. calliptera* females based on egg-spots might be explained by the lek-like mating system. In this situation, females might choose males indirectly because they either prefer to mate with clustered males (“female preference model”; Bradbury 1981), or with the most superior males in the lek (“hotshot model,” Beehler and Foster 1988), or males just formed the lek in areas with high concentration of females (“hot-spot model,” Bradbury and Gibson 1983). Alternatively, females might choose directly by assessing males based on other characteristics apart from egg-spots, which were not examined or were excluded in our experimental setup. In our experiments, for example, females were not given the choice between differences of the stimulus males in territory quality, body size, and nonvisual cues. In *A. burtoni*, body size and chemical cues are more likely to affect female choice (Kidd et al. 2013) than egg-spots, for which no preference was found (Henning and Meyer 2012; Theis et al. 2012). In addition, we cannot rule out a putative importance of egg-spots in female choice in our tested species under different conditions. Further tests should be conducted to see if egg-spots could become important in the fertilization process in case of sperm limited males and/or under different environmental conditions, for example, turbid water conditions or strong water current. Under such scenarios, egg-spots could become crucial to ensure close proximity of females with unfertilized eggs next to the male genital papilla during sperm release.

In contrast to the results presented here, the females of some haplochromine species base their mating preference on egg-spot number (Hert 1989, 1991) or egg-spot size (Coultridge 2002). Supposedly, cichlid egg-spots evolved via a female sensory bias (Egger et al. 2011), which suggests an ancestral function in female choice, with a subsequent evolution to multiple functions, for example, species recognition (Axelrod and Burgess 1973) and/or in male interactions (Theis et al. 2012; this study). Until now, no species is known in which egg-spots have a dual function as was shown for other carotenoid-based male ornaments (e.g., Candolin 1999; Griggio et al. 2007). Of course, this

might reflect the situation that, so far, only very few haplochromine species have been subjected to experiments testing for both (and either factor). Nevertheless, our findings together with the previously suggested functions of egg-spots in other cichlid species and the high diversity in egg-spot number, shape, and coloration within and among species (personal observation) show the high flexibility of this trait with respect to function, persistence, and appearance. Furthermore, the observed function of egg-spots in male aggression supports the hypothesis that the process of intrasexual selection on male coloration has played a role in the astonishing radiation of haplochromine cichlids (reviewed in Dijkstra and Groothuis 2011).

In summary, egg-spots constitute an extraordinary example of a color ornament, which evolved in manifold directions with regard to functions in female choice and male–male competition. We have shown that egg-spots are used in quality assessment of competitors, with egg-spots becoming more important as the difference in body size between contestants becomes smaller. Further knowledge on the function of egg-spots over a broader range of haplochromine species could reveal links between their function, pattern, coloration, the species' mating behavior, and their environment.

## SUPPLEMENTARY MATERIAL

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

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