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Prostaglandin F2α facilitates female mating behavior based on male performance

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Abstract Hormones play an important role in the regulation of reproductive behavior. Here, we examined the effects of the fatty acid derivative prostaglandin F2α (PGF2) on female sexual behavior as well as the interaction between PGF2-induced mating behavior with male courtship display in the lek-breeding African cichlid fish, *Astatotilapia burtoni*. In a two-way choice paradigm, we found that nonreproductive females preferred to associate with smaller, less aggressive males over larger, more aggressive males. However, PGF2-treated females dramatically reversed their preference to larger males. In a second experiment, PGF2 treatment dramatically increased sexual behavior in nonreproductive females as measured by time spent in the bower of the stimulus male, even when the female and the stimulus male were separated by a transparent divider. This effect was even more pronounced when the stimulus males were exposed to the putative female pheromone 17α,20β-progesterone (17α,20β-P). Under full-contact conditions, only PGF2-treated females visited a stimulus male's bower, where they even displayed circling behavior usually only seen during spawning. Interestingly, male performance prior to PGF2 treatment predicted female sexual response. Our study demonstrates the importance of PGF2 in the control of female reproductive behavior in interaction with male performance.

Keywords Mate choice · Sexual selection · Reproductive physiology · Prostaglandin F2 alpha · Sexual behavior · Cichlid · *Astatotilapia burtoni*

Introduction

Sexual selection by female mate choice is thought to play an important role in the evolution of male sexual traits (ornaments and displays) and the maintenance of reproductive isolation among closely related species (Andersson 1994; Kraaijveld et al. 2011). During mate choice, females assess potential mates on the basis of male courtship displays, which is a means for potential mates to advertise their quality (Candolin 2003; Byers et al. 2010). Although our understanding of the ultimate mechanisms governing female mate choice has increased in recent decades (e.g., Jones and Ratterman 2009), the physiological basis of female mating behavior, also in relation to male behavior, has received less attention.

In teleost fish where gametes are fertilized externally, it is important for males and females to coordinate the reproductive process and synchronize their mating behavior to maximize fertilization of the eggs (Munakata and Kobayashi 2010). Reproductive behavior is regulated by the complex interaction of endogenous and exogenous cues that are processed and interpreted by the brain. Courtship is not only a means for both sexes to synchronize their behavior to

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ensure successful mating, but also allows—at least in principle—potential male suitors to advertise their (genetic) quality (Johnstone 1995; Byers et al. 2010). Intense courtship displays can be costly in terms of energy expenditure and predation risk, thus contributing to the reliability of male sexual traits as quality indicators (Johnstone 1995). It is therefore not surprising that more intense male courtship displays elicit a stronger female response (Candolin 2003; Byer et al. 2010). However, the female can also influence male behavior, and carefully controlled experiments are required to determine the causal relationships and endocrine underpinnings of female sexual responses to male courtship displays (Baldauf et al. 2009; Lomborg and Toft 2009; Ramsey et al. 2012).

Synchrony of female sexual behavior with both the state of oocyte maturation and male sexual behavior is necessary to ensure fertilization (Stacey 1981; Crews 2005; Haselton et al. 2007). As a result, female sexual behavior is often dependent on ovarian state. In teleosts, the control mechanisms of female sexual behavior fall into two categories: they are either responsive to sex steroids or to the fatty acid derivative prostaglandin F2α (PGF2) (Kobayashi et al. 2002; Stacey 2003; Forlano and Bass 2011). In internally fertilizing live-bearing fishes, where fertilization and spawning are temporally dissociated, female sexual behavior is mediated by sex steroids (Stacey 2003; Ramsey et al. 2011). In contrast, mating systems that require precise synchronization of male and female gamete release utilize PGF2 as both an endogenous releaser of female spawning behavior and (after PGF2's release through the female urinary tract) as an exogenous releaser of male courtship and spawning behavior (Munakata and Kobayashi 2010). In a number of teleost species, PGF2 can stimulate spawning behavior in nonreproductive females (goldfish, *Carassius auratus*: reviewed in Munakata and Kobayashi 2010; paradise fish, *Macropodus opercularis*: Villars et al. 1985; Black Acara cichlid, *Cichlasoma bimaculatum*: Cole and Stacey, 1984; dwarf gourami, *Colisa lalia*: Yamamoto et al. 1997; silver barb, *Puntius gonionotus*: Liley and Tan 1985; and medaka, *Oryzias latipes*: Oshima et al. 2003). Although the role of PGF2 in facilitating reproductive behavior in female teleosts is well known, how the effect of this hormone on female reproductive behavior interacts with variation in male courtship behavior and mate choice cues, such as male body size, has not been examined. Examining the complex interrelationships between male courtship displays and female sexual responses is crucial for understanding the adaptive significance of courtship displays and the role of sexual selection in driving evolutionary change in female mating preference and corresponding male mating displays.

The haplochromine cichlid fish *Astatotilapia burtoni* has become an important model system in social neuroscience (Hofmann 2003; Robinson et al. 2008). While much of the research in this species has taken advantage of the extreme social and behavioral plasticity of males (Hofmann et al. 1999; Hofmann and Fernald; 2001; Renn et al. 2008; Maruska et al. 2011), recent work has begun to examine maternal and female aggressive behavior as well (Renn et al. 2009, 2012; Grone et al. 2012). Circulating levels of reproductive hormones show the typical vertebrate pattern throughout the ovarian cycle, with peaks in sex steroid and PGF2 levels in the days prior to spawning (Kidd et al. 2013). During the non-breeding phase, individual *A. burtoni* females are generally associated in large schools with other females, subordinate males, and juveniles (Fernald and Hirata 1977). When gravid (egg bearing), females leave the school and visit the spawning pit (bower) of territory-holding dominant males, who attract females by vigorous courtship displays (lateral display and lead). After spawning, the female leaves and assumes sole care for the offspring held within her mouth (McElroy and Kornfield 1990; Renn et al. 2009).

Here, we first examine the role of PGF2 in mediating female reproductive behavior. We administered PGF2 to nonreproductive females and allowed them to choose between attractive and non-attractive males. Based on Kidd et al. (2013), we predicted that under normal conditions, nonreproductive females would associate preferentially with the smaller, non-attractive male, while PGF2-treated females would associate preferentially with the larger, attractive male. We then tested the effect of PGF2 on the reproductive behavior of *A. burtoni* females in relation to a male's courtship performance. We treated nonreproductive females with PGF2, but only allowed visual cues between the focal females and stimulus males, which enabled us to manipulate the males' responses by exposing each focal male to the pre-ovulatory hormone 17α,20β-progesterone, which can act as a pheromone upon release into the water (17α,20β-progesterone (17α,20β-P); DeFraipont and Sorensen 1993). We predicted that males treated with this pheromone would increase their courtship activity and (PGF2 treated) females would concomitantly increase their responses to such males. Finally, we tested the effect of PGF2 treatment in a full-contact paradigm, specifically testing the hypothesis that exogenous PGF2-induced female sexual behavior varies according to male behavior.

Materials and methods

Housing and collection of nonreproductive females

Mixed sex groups of adult *A. burtoni* were housed in 110-l aquaria on a light/dark cycle of 12/12 h and closely monitored for reproductive activity. After spawning within a community setting, brooding females were collected within 1 day of spawning, the eggs were removed, and subjects were either immediately transferred to the experimental tanks (experiments I and II: Fig. 1) or placed back in their community tank and transferred to the experimental tank 2 days after egg removal

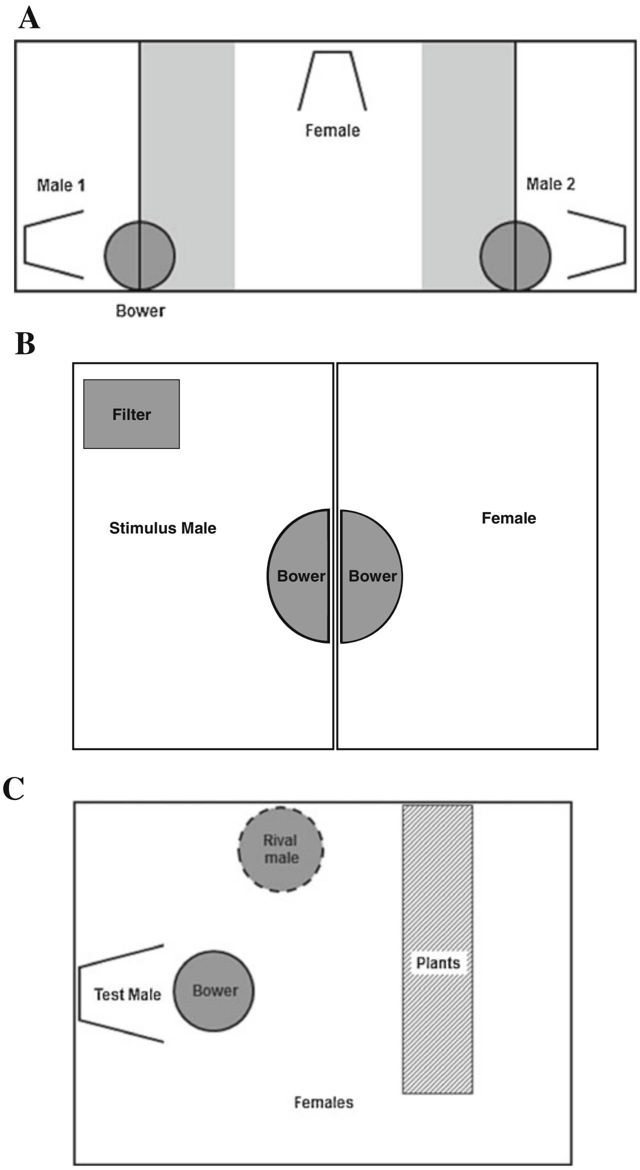

Fig. 1 Schematic drawings of the three behavioral paradigms used in the present study. **a** Setup for experiment I (prostaglandin and female mate choice). Focal females were placed in the central compartment of a 200-l tank divided into three sections and exposed to a large and a small male across perforated transparent partitions. The association zone was within 15.5 cm of the male's partition. A bisected clay saucer placed in each tank indicated the location of the male's bower. **b** Setup for experiment II (controlled sensory environment). Males and female subjects were placed in adjacent, but separate 40-l aquaria, so subjects could interact using only visual cues. **c** Setup for experiment III (full-contact paradigm). Tests were performed in a 100-l aquarium with a bower, eight schooling females, artificial plants for them to hide in, and an intruder male isolated in a transparent cylinder next to the test male's bower. The purpose of the intruder male was to measure the level of aggression of the test male

(experiment III: Fig. 1). Premature cessation of the normal 15-day oral gestation period results in an instant acceleration of vitellogenesis and subsequent ovulation within 28±3 days (Macintosh and Little 2005; Kidd et al. 2013). All test females were therefore in a nonreproductive state. The standard length of female subjects was (means ± SE) 4.67±0.07 cm. Males were dominant and reproductively active for at least 1 week before being placed into the experimental tanks. The standard length of male subjects was (means ± SE) 6.54±0.12 cm. Male and female subjects were used not more than once.

Courtship behavior in *A. burtoni* resembles that of other haplochromine cichlid species (McElroy and Kornfield 1990). A territorial male will approach a reproductive female and perform lateral display often followed by a lead. In lateral display, the male turns sideways next to the female and quivers. During leads, he swims in front of her with waving tail beats in order to lead her back to his bower. The female can follow the male and enter his bower where he will present his egg dummies on his anal fin. These bower visits can proceed to actual spawning when the female swims in tight circles with the male within the bower. During circling, the female deposits a small number of eggs and picks them up in her mouth, followed by the female mouthing the male's anal fin. Sperm is released during mouthing and the eggs are fertilized in the female's mouth (Baerends and Baerends-van Roon 1950; Renn et al. 2009).

Experiment I: prostaglandin and female mate choice

Focal females were placed in the central compartment of a 200-l tank divided into three sections by transparent, perforated dividers (Fig. 1a). Within this experimental tank, females were exposed to visual and olfactory cues from "attractive" (large, >7 cm standard length; brightly colored) and "less attractive" (small, <4.5 cm standard length; dull in coloration) males. A bisected clay saucer placed in each tank mimicked a male bower. The larger male is considered more attractive than the smaller male in this paradigm, as females will spawn with the larger male most of the time (Kidd et al. 2013). Female association with a male (scored as the amount of time spent in the "association zone" (within 15.5 cm of the male's partition)) and in the bower was recorded for 10 min at 10:00 am on days 7–10 using a multichannel digital video surveillance system (Video Insight, Video Insight, Inc., Houston, TX). On day 8, females were given an intraperitoneal (50 μl) injection of either 0.5 μg/g body weight PGF2 (Cayman chemicals, Ann Arbor, MI) (n=5) or saline control (n=8) immediately after the 10-min baseline recording. Another 10 min focal observation was performed 20 min after injection. A blind observer with respect to treatment scored the female response as the time a female spent in the association zone and the time a female spent in the bower.

Statistics

All data were log transformed ($\ln(x+1)$) to meet assumptions of parametric testing. To test for a preference for either the smaller or larger male during control observations (i.e., without PGF2 treatment), we used a double-nested repeated measures ANOVA (RM ANOVA) with the female response to smaller versus larger male as the first repeat and observations as the second repeat (day 7, day 8 pretreatment, day 9, and day 10). We used time spent in the association zone or bower as the dependent variable. Similarly, we tested whether PGF2-treated animals reversed their preference by testing the interaction between the two repeated measures (smaller versus larger male × day 8 pretreatment versus day 8 posttreatment). All analyses were implemented in PASW statistics (version 19). All probabilities given are for two-tailed tests of significance.

Experiment II: prostaglandin effects on females exposed to male visual cues alone

Test females and stimulus males were placed in adjacent, but separate 40-l tanks (day 0, Fig. 1b). In this setting, subjects could interact only through visual cues. The male bower served as the focal point for all behavioral interactions. We recorded the behavior before and after intraperitoneal injection of the test female with either PGF2 or saline control. Prior to the trial, the stimulus males were exposed to the putative preovulatory pheromone 17α,20β-P ($n=19$) added directly to the water (0.033 mg dissolved in 33 μl 100 % ethanol, resulting in a concentration of 0.83 μg/l) or to a vehicle control (33 μl 100 % ethanol, $n=29$). We recorded female behavior for 10 min each at 10:00 am on day 2 (before 17α,20β-P exposure) and day 3 (16 h post-17α,20β-P exposure). On day 5, we recorded the behavior before and after the injection of the test female with PGF2 (total, $n=22$; tested with male saline control, $n=14$; with male treated with 17α,20β-P, $n=8$) or saline control (total, $n=26$; tested with male saline control, $n=15$; with male treated with 17α,20β-P, $n=11$). The behavior was recorded as described for experiment I.

Statistics

In this experiment, we examined the female response towards a single male in an adjacent tank. We calculated the female response by subtracting the time spent in the bower before treatment from the time spent after treatment. We tested the effect of PGF2 and "priming" of the male with 17α,20β-P on the female response using an ANOVA. We examined the residuals to confirm assumptions of parametric testing. Experimental groups were compared using independent *t* tests.

Experiment III: prostaglandin effects on females under full-contact conditions

We tested the effects of PGF2 in a full-contact paradigm (Fig. 1c) in which each experimental fish was exposed to the complete range of sensory modalities. Tests were conducted in a 110-l tank with a bower, eight schooling females, plants for them to hide in, a stimulus male, and an intruder male isolated in a transparent cylinder next to the stimulus male's bower. We added a group of females to enable the test female to school, which is a typical behavior of nonreproductive females (Fernald and Hirata 1977). To facilitate identification of the focal female, the non-focal females were marked with colored beads attached near the dorsal fin. The stimulus male along with the group of females was introduced to the experimental tank on day 0 (i.e., when eggs were removed from the test female). At the same time, we also placed an intruder male in a transparent cylinder within the stimulus male's territory to measure the level of aggression of the latter. On day 2, we placed the test female in the experimental tank. The fish were allowed to acclimate, and on day 6, we recorded baseline social behavior of the stimulus male and the test female for 10 min at 10:00 am. On day 7 (test day), we again recorded baseline social behavior for 10 min. During these baseline observations, the (nonreproductive) test female spent her time schooling with the other females, as expected. We then injected the test female with PGF2 ($n=19$) or saline ($n=6$) and immediately returned her to the experimental tank. If the female entered the test male's bower within 60 min after PGF2 treatment, we recorded behavior again for 10 min starting from the moment she entered the bower. As potential indicators of male quality, we scored the number of bites directed towards the intruder male. We recorded female behaviors as described for experiment I and also recorded the frequency of male courtship (lateral display and leads).

Statistics

To assess the effect of PGF2 on female sexual behavior, we calculated time spent in the male's bower, the number of circles, latency to entering the bower, and latency to first circle. All data were log transformed ($\ln(x+1)$) to meet assumptions of parametric testing. As expected, given the nonreproductive state of the females in this experiment, no female reproductive behavior was observed prior to PGF2 treatment. We therefore compared female behavior after the injection only between PGF2-treated females and saline-treated females using independent *t* tests. For *baseline male courtship* and *baseline aggression rate*, we took the mean of day 6 and day 7 (pretreatment). We then used Pearson correlation tests to explore whether female reproductive behavior was associated with the test male's behavioral performance prior to and during the trial. We corrected for multiple testing according to Benjamini and Hochberg (1995), setting the false discovery rate (FDR) at $\alpha=0.05$. We distinguished several "families" of hypotheses where FDR can be applied: those concerning the relationship between baseline male courtship rate (i.e., before PGF2 treatment) with female response (after PGF2 treatment); those concerning the relationship between male courtship rate (after PGF2 treatment) and female response (after PGF2 treatment) (eight tests each, P values <0.0125 considered significant after FDR adjustment); those concerning the relationship between baseline aggression rate (before PGF2 treatment) with female response (after PGF2 treatment); and those concerning the relationship between aggression rate (after PGF2 treatment) and female response (after PGF2 treatment) (four tests each, all P values <0.025 significant after FDR adjustment).

Results

Experiment I: prostaglandin and female mate choice

During control observations (days 7, 8 (pretreatment), 9, and 10), females spent significantly more time with the smaller male than with the larger male (Fig. 2; RM ANOVA, smaller-larger, association zone: $F=5.315$, $df=1, 12$, $P=0.040$; bower: $F=0.678$, $df=1, 12$, $P=0.426$). Within minutes of receiving PGF2 treatment (on day 8), these females exhibited a highly significant preference for the larger male over the smaller male (RM ANOVA, smaller-larger, association zone: $F=61.842$, $df=1, 4$, $P=0.001$; bower: $F=35.705$, $df=1, 4$, $P=0.004$). As predicted, this change in association preference between post- and pretreatment observation was significant (Fig. 2, RM ANOVA smaller-larger × day 8 pretreatment versus day 8 posttreatment, association zone: $F=8.085$, $df=1, 4$, $P=0.047$; bower: $F=1.596$, $df=1, 4$, $P=0.275$). Due to the transparent partition, none of these females displayed circling behavior. Saline controls did not show a change in preference from small to large males (RM ANOVA, smaller-larger × day 8 pretreatment versus day 8 posttreatment, association zone: $F=0.69$, $df=1, 7$, $P=0.434$; bower: $F=0.170$, $df=1, 7$, $P=0.692$); in fact, they showed no preference at all (RM ANOVA, smaller-larger, association zone: $F=0.985$, $df=1, 7$, $P=0.354$; bower: $F=1.187$, $df=1, 7$, $P=0.312$).

Experiment II: prostaglandin response in a controlled sensory environment

Females treated with PGF2 exhibited a significant increase in the amount of time spent in association with the male's bower when compared with saline controls (Fig. 3, independent t test on female response: $t=-3.449$, $df=46$, $P=0.001$). None of these females displayed circling behavior across the transparent partition. Priming the stimulus male with 17α,20β-P also affected female behavior in a manner contingent on whether she was treated with PGF2 or saline, consistent with our prediction (ANOVA male treatment × female treatment, $F=6.92$, $df=1, 44$, $P=0.012$). Specifically, the effect of PGF2 was enhanced by presenting the PGF2-treated female with a primed male, though this effect was only marginally significant (Fig. 3, comparing primed with non-primed males: $t=-1.996$, $df=20$, $P=0.06$). The behavior of saline-treated females was not affected by exposing the stimulus males to 17α,20β-P ($t=1.663$, $df=24$, $P=0.11$).

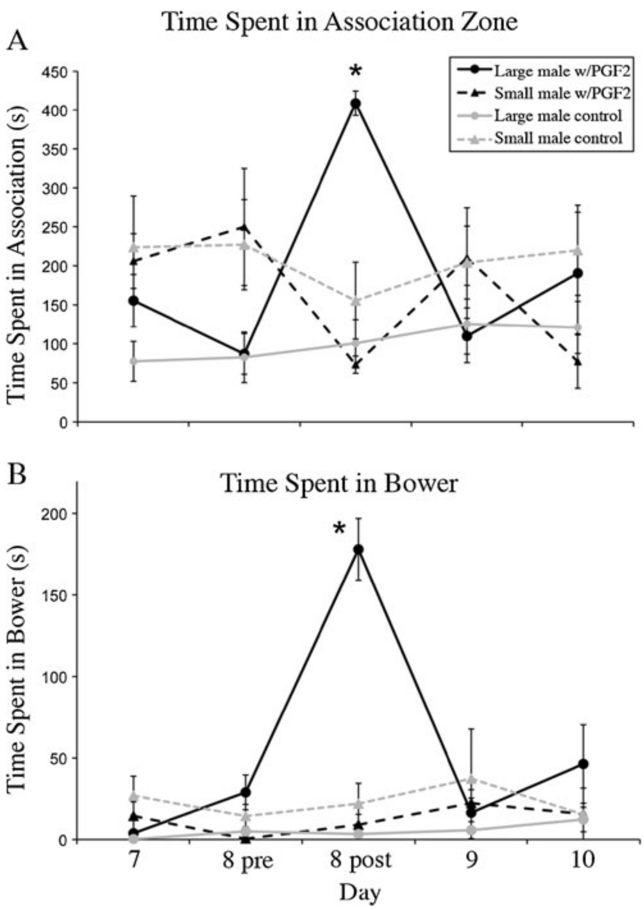

Fig. 2 Regulation of context-appropriate choice by PGF2 (experiment I). Females spend more time in the association zone (**a**) and bower (**b**) of large males when treated with PGF2 than with small males or when injected with vehicle. Days after female spawning and subsequent brood removal are indicated on the x-axis. Two observations were conducted on day 8, before (8 pre), and after (8 post) treatment with PGF2. Data are represented as means ± SE

Experiment III: prostaglandin response under full-contact conditions

Females treated with PGF2 exhibited significantly more bower visitation (independent t test $t=2.367$, $df=23$, $P=0.027$) and tended to circle more frequently ($t=1.969$, $df=23$, $P=0.061$) than saline controls. Females treated with saline exhibited none of these behaviors. In addition, PGF2-treated females did not show any reproductive behaviors before treatment (Fig. 4).

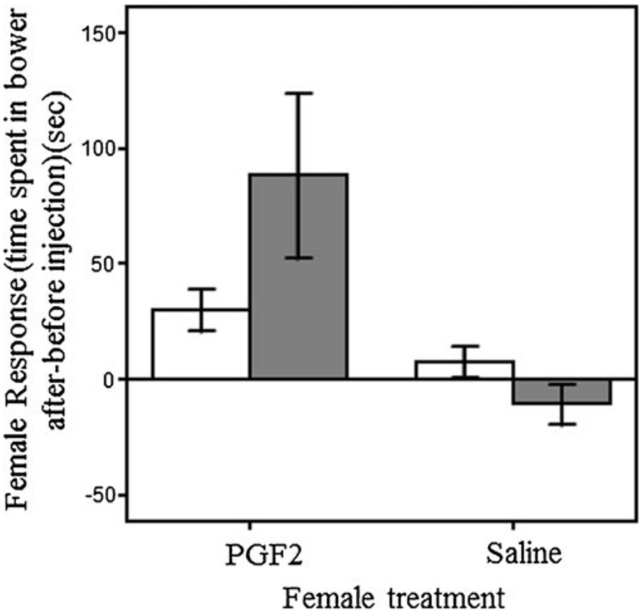

Fig. 3 Female response in a controlled sensory environment (experiment II). Female response to PGF2 depends on priming of the male with the female pheromone 17α,20β-P (*grey*), control (*white*). Female responses (mean ± SE) were calculated by subtracting the time spent in the bower before treatment from the time spent after treatment

Female response to the PGF2 treatment was correlated with male behavior during the trial, consistent with our expectations. The number of circles performed by the female was positively and significantly correlated with the number of leads performed by the male during the trial (Table 1). The amount of time the female spent in association with the male bower was also positively correlated with the number of leads and with the number of lateral displays performed by the male during the trial (Table 1).

PGF2-treated females responded more strongly to males that were inherently more vigorous. Specifically, the latency to circle was inversely correlated with both the number of lateral displays and leads the male performed prior to the trial, although these associations were no longer significant after FDR adjustment (Table 1). Finally, a female's latency to circle with the male was dependent on the male's aggressiveness (i.e., number of bites directed towards the intruder male) prior to the female treatment, although this association also was no longer significant after FDR adjustment (Table 1).

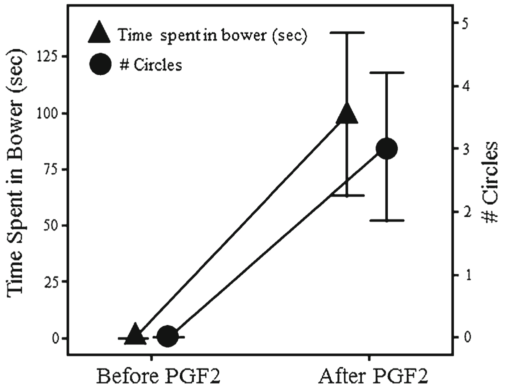

Fig. 4 Female response under full-contact conditions (experiment III). Time spent in bower and number of circles performed by females before and after PGF2 treatment (means ± SE). Control females did now show any of these behaviors

Discussion

In the present study, we have shown that female sexual behavior was rapidly induced even in nonreproductive individuals by exogenous administration of the female reproductive hormone PGF2. Exogenous PGF2 dramatically shifts female preference from smaller males to larger and presumably more attractive males, with whom females typically prefer to mate (see Kidd et al. 2013). The female sexual response was strongest in the full-contact paradigm as females in this situation were able to not only visit the bower but also proceeded to circling behavior usually only seen during spawning (Baerends and Baerends-van Roon 1950). Finally, we found that PGF2-induced sexual behavior in *A. burtoni* females was influenced by male performance in both the controlled sensory environment and full-contact paradigm.

In experiment I, we showed that exogenous PGF2 can induce female receptivity as measured by a preference for the more attractive male even in nonreproductive females. During control observations, females preferred to associate with the smaller males, presumably reflecting a propensity of nonreproductive females to school with smaller fish and stay away from aggressive territorial males. Our findings are consistent with the observation that reproductive females preferentially associate with larger, more attractive males in *A. burtoni* (Clement et al. 2005; Kidd et al. 2013). In fact, Kidd et al. (2013) showed that *A. burtoni* females prefer to associate with more attractive males only on the day of spawning, which is preceded by surges in steroid hormones and PGF2. Our study shows that a rise in PGF2 alone (and thus without priming with steroid hormones) is sufficient to induce sexual behavior and context-appropriate mate choice in nonreproductive females.

In experiment II (controlled sensory environment), PGF2-treated females spent more time in the male's bower, indicating sexual interest since under normal circumstances these bower visits often proceed to actual spawning (Baerends and Baerends-van Roon 1950; Renn et al. 2009). In addition, female sexual behavior was stronger when the males were "primed" with the putative female pheromone (17α,20β-P) compared to the control situation, most likely due to 17α,20β-P eliciting appropriate behavioral response in males that in turn facilitated increased

Table 1 The relationship between female behavior after PGF2 treatment (rows) with male behavior (columns) before and after PGF2 treatment. Pearson correlation coefficients and *P* values are shown. Included are only females that visited the bower within ≤60 min after treatment. Significant correlations are in bold; an asterisk indicates those correlations that remain significant after correcting for multiple hypothesis testing (Benjamini and Hochberg 1995)

Male behavior		Female behavior			
		Latency to bower (s)	Time spent in bower (s)	Latency to circle (s)	# Circles
Lateral displays (pre-PGF2 treatment)	Pearson	−.283	.367	−.658	.654
	P value	.399	.267	.028	.029
Lateral displays (post-PGF2 treatment)	Pearson	−.316	.732	−.436	.631
	P value	.344	.010*	.181	.037
Leads (pre-PGF2 treatment)	Pearson	−.340	.362	−.752	.579
	P value	.307	.274	.008	.062
Leads (post-PGF2 treatment)	Pearson	−.165	.816	−.303	.858
	P value	.629	.002*	.366	.001*
Bites (pre-PGF2 treatment)	Pearson	.478	−.256	.658	−.058
	P value	.137	.448	.028	.866
Bites (post-PGF2 treatment)	Pearson	−.036	.248	−.121	.303
	P value	.916	.462	.723	.366

female sexual behavior. Previous studies suggested the importance of male performance during female reproductive behavior, often resulting in a positive relationship between male courtship rate and female response (e.g., Collins 1994; Santangelo 2005; Lomborg and Toft 2009; for reviews see Candolin 2003; Byers et al. 2010). While this relationship makes intuitive sense, relatively few studies have been able to establish causality. For example, Lomborg and Toft (2009) found that in the wolf spider *Pardosa prativaga* nutrient-enriched males performed more active courtship displays and experienced higher mating success than nutrient-deficient males. However, nutritional status covaried with body size, and mate choice was likely influenced by male–male competition. Previous reports in cichlid fish have found that more male courtship displays are positively related to female responses (Maan et al. 2004; Clement et al. 2005). Our results provide experimental evidence that in cichlid fish, a female's response to a male is indeed influenced by his performance per se.

In experiment III (full-contact paradigm), nonreproductive females treated with PGF2 exhibited both bower visitations and circling, while control females never displayed any of these behavior patterns. The female response to the PGF2 treatment correlated positively with male courtship display, confirming the fact that courtship is a two-way interaction (e.g., Collins 1994).

Our findings are consistent with previous studies showing that PGF2 administration can induce female reproductive behavior in teleost fish. For example, in the Black Acara cichlid, *C. bimaculatum*, PGF2 can induce spawning behavior at any time during the reproductive cycle (Cole and Stacey 1984). Relatively few studies have investigated the role of PGF2 in regulating reproductive behavior in tetrapods, even though its role in ovarian function and parturition is well known (Slater et al. 2002). PGF2 is associated with egg laying in many lizards and birds (Hertelendy et al. 1974; Guillette et al. 1991). In the Green Anole lizard, *Anolis carolinensis* (Tokarz and Crews 1981) and the garter snake, *Thamnophis sirtalis parietalis* (Whittier and Crews 1986), PGF2 reduces receptivity, yet in female anurans, PGF2 increases receptivity and phonotaxis (Weintraub et al. 1985; Diakow and Nemiroff 1981), though in some instances only in combination with progesterone (Schmidt 1985; Gordon and Gerhardt 2009; reviewed in Wilczynski and Lynch 2011). In mammals, PGF2 has been studied in the context of maternal care, where this hormone induces nest building in pregnant and nonpregnant pigs (Burne et al. 2001) and prevents infanticide in naïve female rats (McCarthy et al. 1986). Interestingly, PGF2 appears to affect female receptivity differently in reptiles compared with other vertebrates. To fully understand the evolution of PGF2 function across vertebrates, more comparative work, also including birds, is clearly needed.

It should also be noted that under full-contact conditions females might release some of the exogenous PGF2 into the water (as they do when PGF2 surges naturally before spawning: Kidd et al. 2013), which in turn could alter male behavior, similar to the situation in goldfish (Munakata and Kobayashi 2010). Because the *A. burtoni* olfactory epithelium is not sensitive to PGF2 (at least not to the unconjugated form, see Cole and Stacey 2006), this scenario seems unlikely. However, exogenous PGF2 could also induce the release into the water of progestin metabolites, which likely act as female pheromones (see "Experiment II: prostaglandin effects on females exposed to male visual cues alone"), and thus could influence male behavior indirectly (Munakata and Kobayashi 2010). Many more studies are needed to understand these complex chemical communication systems in *A. burtoni* and other cichlids in the context of reproductive behavior (Stacey 2003).

Our study highlights the intricacies of hormonal control of female reproductive behavior and context-appropriate mate choice and the critical role male courtship performance plays in this context. Given that its effects are almost immediate, exogenous PGF2 likely regulates behavior directly via the brain instead of priming the production or release of other hormones (Kobayashi and Stacey 1993). Future studies should shed more light on how the female brain integrates internal cues such as PGF2 with cues indicating male performance. In addition, female mate choice can drive the evolution of sexually selected characters and contribute to speciation and the maintenance of reproductive isolation (Andersson 1994; Jennions and Petrie 1997; Kokko et al. 2003; Kraaijveld et al. 2011). A better understanding of the hormonal and neuronal modulation of female and male reproductive behavior may help elucidate the mechanisms by which sexual selection can drive the evolution of female choosiness and male characters.

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