The outcome of male–male encounters affects subsequent sound production during courtship in the cichlid fish *Oreochromis mossambicus*

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(Received 7 January 2004; initial acceptance 27 February 2004; final acceptance 24 June 2004; published online 7 December 2004; MS. number: 7950)

Social status in lek-breeding species is highly correlated with mating success. Reproductive failure of subordinates may be caused either by direct aggression by dominant males or by induced physiological changes. We hypothesized that recent social status (winning or losing an encounter with another male) affects the production of courtship sounds in male Mozambique tilapia, *Oreochromis mossambicus*, in the absence of dominant males. We staged dyadic encounters between males and subsequently allowed full access to a female to either the winner or the loser (one fish per dyad). We minimized possible effects of social experience by isolating the subject males before the experiments. All males courted the females but winners produced more courtship sounds than losers and showed significantly shorter courtship latencies and longer courtship durations. The sounds of winners had longer pulse durations and lower peak frequencies. Male size and condition factor, fighting latency and duration, and number of escalated agonistic acts were not correlated with number of sounds or any of the courtship parameters measured. Differences between winners and losers in courtship were probably related to transient physiological changes induced by the encounter outcome, such as changes in levels of circulating monoamines, cortisol and androgens, which can modulate courtship behaviour including sound production. We suggest that in nature the outcome of recent social interactions between males may affect the production of courtship sounds and courtship behaviour in general from the early stages of hierarchy formation.

In social species, hierarchies develop because of competition for limited resources such as food, territories or mates, and dominant individuals usually gain priority to their access (Huntingford & Turner 1987). Fighting experience is thought to influence the outcome of a later contest, known as the winner and loser effects (Chase et al. 1994), and thus may mediate the formation of dominance hierarchies (Dugatkin 1997). Most studies of the effect of fight outcome on subsequent activity have focused on agonistic performance (e.g. Chase et al. 1994; Hsu & Wolf 1999) and only a few on postfight courtship performance (de Catanzaro & Ngan 1983; Greenberg & Crews 1990; Schuett 1996).

Winners and losers usually experience different physiological changes after a staged fight. Defeated animals show a general depression of behaviour modulated by a variety of physiological stress responses such as the activation of the sympathetic-adrenomedullar (chromafin in teleosts) system, the hypothalamic–pituitary–adrenal (interrenal in teleosts) axis and the serotonergic system (Winberg et al. 1991; Overli et al. 1999). Winners and losers may also have different steroid levels (Hannes 1986; Cardwell & Liley 1991) or incur different metabolic costs (Chellapa & Huntingford 1989; Haller 1995; Neat et al. 1998), which may also affect subsequent sound production and other aspects of behaviour. The contest outcome rather than fight characteristics seems to be the main factor affecting subsequent behaviour. Schuett (1996) studied the courtship performance of male copperheads, *Agkistrodon contortrix* (Viperidae), that had had a single winning or losing experience and found that there was a complete suppression of courtship behaviour in losers shortly after the fight (30 min) and a similar or increased courtship activity in winners, which could be explained by differential levels of glucocorticoids but not by fight duration (Schuett & Grober 2000).

The Mozambique tilapia, *Oreochromis mossambicus*, is a lek-breeding mouthbrooding African cichlid fish. Males...
form dense nest aggregations in shallow water during the breeding season, where they display to attract females for mating (Bruton & Boltt 1975). Mating success in the laboratory is highly skewed towards dominant territorial males in established groups (Oliveira & Almada 1998a) and males higher in the social hierarchy are more effective at defending territories, build larger nests, have higher androgen levels and gonadosomatic indexes, and also court at a higher rate (Oliveira et al. 1996). Territorial males also produce low-frequency pulsed acoustic signals during courtship and spawning, probably to attract females and to synchronize gamete release (Amorim et al. 2003). Courtship sounds in this species may also advertise spawning readiness, as sound production rate is positively correlated with courtship rate (Amorim et al. 2003).

In *O. mossambicus*, a dominance structure appears a few hours after group formation and most agonistic interactions are concentrated in male–male dyads (Oliveira & Almada 1998b), suggesting that a male’s ability to attract females is determined early on during hierarchy establishment through dyadic contests. We hypothesized that recent male social status (winning or losing an encounter with another male) affects subsequent acoustic and visual courtship behaviour.

**METHODS**

**Study Fish**

The fish were reared in our Institute’s laboratory. They were descended from a stock kept in Aquário Vasco da Gama, Lisboa, Portugal, since the early 1970s, originating from the Incomati River, Mozambique. Fish were kept in mixed-sex groups in 200-litre aerated stock aquaria with sand substrate, on a 12:12 h light:dark cycle, and with water temperatures of 26 ± 1°C. They were fed with tropical fish commercial flakes.

**Experimental Procedure**

We allowed pairs of males to interact for 20 min (starting from the first interaction) in a 200-litre aquarium. To avoid residence effects, we placed each pair of males simultaneously in a tank. Possible effects of social experience (Chase et al. 1994) were minimized by isolating the males in 45-litre aquaria for a week. Male size was 8.4–12.2 cm standard body length (SL). Differences in SL between pairs (% larger/smaller fish) varied between 6 and 29% (median = 11%).

The agonistic interactions observed consisted of displays (nescalled agonistic behaviour), which included lateral displays, frontal displays and tail beatings, and escalated agonistic behaviour such as mouth fighting, ‘carouseling’ and circling (for a description of behaviours see Baerends & Baerends van Roon 1950; Neil 1964; Oliveira & Almada 1998a). We also observed courtship behaviour between males, which is a characteristic male–male interaction, where the dominant male directs courtship acts towards a subordinate male (Oliveira & Almada 1998a).

In all trials there was a clear winner or loser. Winners were defined as the individual that after a certain stage won all agonistic interactions and showed a dark body colour characteristic of dominant territorial fish. Winners occasionally courted the loser (Oliveira & Almada 1998a), and swam freely in the aquarium. Conversely, losers were defined as the males that consistently fled or withdrew when engaged in an agonistic interaction or adopted a submissive posture, and had a pale body colour. Losers also typically stayed at the top of the water column or close to the aquarium corners.

At the end of this phase of the experiment we removed either the winner or the loser from the aquarium with a hand net and returned it to a stock aquarium. We chose the removal of either the winner or the loser before each trial and tested an approximately equal number of winners and losers.

The remaining fish (the subject male) was left to rest for approximately 15 min after which an ovulated female was introduced into the tank and courtship interactions were allowed for another 20 min (after the first courtship interaction). To increase female receptivity, we induced ovulation by an intraperitoneal injection of 200 μl of saline containing 5 μg des-Gly 10, {D-Ala6}-LHRH ethylamide (Sigma, Madrid, Spain) 48 h before the presentation to the male. We made a pit in the middle of the tank before each trial which the male usually used during the courtship interactions. The observed courtship behaviour included sound production and the acts tilt, lead, circling the nest, tail wagging and quivering, as described by Baerends & Baerends van Roon (1950), Neil (1964) and Amorim et al. (2003). Males also engaged in activities associated with the nest, such as digging, hover (Baerends & Baerends van Roon 1950; Neil 1964) and still in the nest (Amorim et al. 2003).

We tested 17 subject males: nine winners and eight losers. Winners measured on average 10.9 cm (range 8.4–12.0 cm) SL and weighed 36.4 g (19.6–46.3 g). Losers measured on average 10.8 cm (8.8–12.2 cm) and weighed 34.0 g (20.7–43.9 g). Winners and losers did not differ in SL (two-sample t test: t = −0.12, P = 0.90) or weight (t15 = 0.09, P = 0.93). We do not know the previous social history of each fish because stock tanks suffered frequent territorial male removals in the months preceding this study.

**Ethical Note**

Fights escalated in eight of 17 encounters for brief periods (X ± SE = 1.9 ± 0.5 min). In the fights that escalated, an average ± SE of 2.75 ± 0.56 aggressive interactions were observed, of which only 1.13 ± 0.13 escalated. There were no intensive aggressive interactions towards subordinates after a fight was settled that caused injuries to subordinate fish. We planned to stop fights if any serious injuries occurred or if winners harassed losers showing signs of submission, but there was never a need to intervene. Four or five males lost scales from bites and this was the only injury suffered by males; the wounds were small, did not require treatment and never became
infected. Fish always behaved normally within a few minutes of being returned to stock tanks, suggesting that they were not exposed to abnormal stressful situations. Furthermore, all losers courted the females 15 min (plus courtship latency) after male–male interactions, showing that they were in good condition. After the experiments both subject fish and opponents were monitored for at least 15 days and never showed problems either in health or behaviour. In addition, 19 females were injected to induce ovulation with a substance analogous in its physiological effects to luteinizing-hormone releasing hormone. None of the females died or showed any adverse consequences either in health or in courtship behaviour during experiments and thereafter. This procedure has been undertaken for several years in our laboratory to maintain the tilapia stock; it always produces normal ovulations and behaviour in the females and does not have any adverse side-effects.

Sound and Behaviour Recordings

During male–male interactions, we measured fighting latency and duration (min), the number of agonistic interactions, the number of escalated agonistic acts (mouth fighting and carousels) and the time taken to settle the encounter, measured from the start of the first interaction (min) to the encounter outcome (winning versus losing).

During male–female interactions, we recorded sounds with a High Tech 94 SSQ hydrophone (sensitivity –165 dB re 1 V/µPa, frequency response within ±1 dB from 30 Hz to 6 kHz) placed just above the rim of the male’s (artificial) nest, and connected to a DAT recorder (Sony TCD-D10 Pro). Water temperature in the experimental tank was 26 ± 1°C (range). We stopped the aeration approximately 15 min before starting recording. We considered only the sounds that showed a good signal-to-noise ratio and a clear structure for analysis. Sounds were digitized at a rate of 22 kHz (16 bit resolution) and analysed with Canary 1.2.4 for Macintosh (Cornell Laboratory of Ornithology, Ithaca, New York). We measured the number of sounds produced during courtship and the following sound parameters (Fig. 1) as described in Amorim et al. (2003): sound duration (ms); number of pulses (equals number of pulse units in Amorim et al. 2003); pulse period (ms); pulse duration (ms); peak frequency (of the pulsed component of the sound in Hz, see Amorim et al. 2003); and maximum frequency (Hz). We also registered courtship latency and duration (min), and the number of courtship interactions.

Figure 1. Acoustic parameters measured in the courtship sounds of male O. mossambicus: sound duration (ms, bar 1); pulse period (ms, bar 2); pulse duration (ms, bar 3); number of pulses; peak frequency (Hz, thick arrow); maximum frequency (Hz, narrow arrow). (a) Power spectrum; (b) sonagram; (c) oscillogram.
Data Analysis

We tested male SL, weight and condition factor (weight/SL^3 \times 1000) and the parameters measured for male–male interactions for possible correlations with the courtship parameters (male–female interactions) using the Spearman rank test. The number of courtship sounds, courtship latency and duration, and the number of courtship interactions were compared between winners and losers with a Mann–Whitney U test. The acoustic features of sounds of winners and losers were compared with a balanced mixed-model nested ANOVA. Because a different number of sounds were analysed for different males, we considered only seven sounds chosen at random per fish to balance the ANOVA design. One male winner for which we had only four sounds analysed was not considered. The variable ‘males’ (different individuals) was nested within the variable ‘recent social status’ (winners or losers), the first being a random and the second a fixed variable. As sound frequency parameters are correlated with male size (Amorim et al. 2003), we used the residuals of a linear regression between sound frequency and fish SL, instead of the raw data.

RESULTS

In male–male interactions, dominance was settled a mean ± SD of 1.92 ± 4.10 min (range 0.00–16.50 min) after the first agonistic interaction was observed. Fight latency of male–male interactions averaged 2.25 ± 0.90 min (range 0.53–3.33 min). In eight of 17 male–male encounters, dominance was settled without retaliation from one of the males, i.e. the loser was submissive or fled from the first approach of the dominant male. The subject male size (SL and weight) and condition factor, and the characteristics of male–male interactions (the fighting latency and duration, the number of agonistic interactions, the time taken to settle the encounter outcome, and the number of escalated agonistic acts) were not correlated with either the number of sounds produced during male–female courtship, or with the courtship latency and duration and the number of courtship interactions (Spearman rank correlation: \( r_s = -0.004 \) to \(-0.67, N = 17, P = 0.004-0.99, \) all NS after Bonferroni correction).

The encounter outcome (winner versus loser), however, had an impact on sound production during subsequent courtship. All males courted the females but winners (W) produced more sounds during courtship than losers (L: Mann–Whitney U test: \( U = 14.0, N_W = 9, N_L = 8, P = 0.03; \) Fig. 2a). Courtship sounds of winners had longer pulse durations (nested ANOVA: \( F_{1,96} = 4.82, P = 0.03; \) Fig. 2b) and lower peak frequencies (\( F_{1,96} = 6.97, P = 0.02; \) Fig. 2c) than those of losers. On average, winners produced sounds 14.2 Hz lower in peak frequency than losers for any given length. All other sound variables were not affected by the encounter outcome (\( F_{1,96} = 0.41–1.32, P = 0.27–0.53).\)

Winners also showed significantly shorter courtship latencies (Mann–Whitney U test: \( U = 10.0, N_W = 9, N_L = 8, P = 0.01; \) Fig. 3b) than losers.

DISCUSSION

We have shown that recent social experience affects male sound production and associated courtship behaviour in O. mossambicus. Shortly after an encounter with another
Oliveira et al. (1996) have shown that during hierarchy formation in O. mossambicus, emerging dominant males have higher levels of androgens and subsequently show more courtship behaviour towards females. Thus, androgen responsiveness to winning could explain increased rates of sound production and courtship displays in winners.

On the other side, losers could suffer from stress resulting in increased levels of monoamines (such as serotonin and cortisol (e.g. Overli et al. 1999)) that could directly or indirectly inhibit courtship behaviour (e.g. Meyerson & Malmnäs 1978; Morgan et al. 1999). Serotonin is thought to have a stimulatory effect on ACTH (the adrenocorticotrophic hormone), which is considered to be the main factor for pituitary control of the interrenal release of cortisol (Winberg et al. 1997), as well as a depressive effect on reproductive hormones including androgens (Sapolsky 1993). The negative effect of cortisol on reproduction including courtship has been extensively studied in fish (Pankhurst & Van Der Kraak 1997; Morgan et al. 1999). Losers may also incur higher metabolic costs from fighting (e.g. Neat et al. 1998), although in our experiment fights were usually short and mostly based on displays, and probably did not sufficiently deplete body reserves to affect subsequent investment in courtship.

Other possible factors that could have influenced both the outcome of male–male interactions and subsequently the males’ courtship behaviour were the males’ social history or other factors such as health. However, when looking at all fish used in the present experiment (both subject males and opponents) we found an approximately equal number of territorial and nonterritorial winners (9/17 were previously territorial males immediately before trials). Recent social history is also thought to be ‘erased’ by social isolation if it is long enough (Chase et al. 1994; also see Oliveira et al. 1996). Furthermore, only apparently healthy fish were used and they remained healthy for at least 15 days after trials, suggesting that neither of the above factors influenced the results of the experiment. Future work should, however, test courtship behaviour both before and after male–male encounters.

Mozambique tilapia winners produced sounds with lower peak frequencies than losers for any given length. Two hypotheses for the mechanism underlying peak frequency of discrete pulsed sounds in fish with swimbladders are found in the literature. The ‘resonance hypothesis’ proposes that the peak frequency of pulsed sounds is imparted by the resonance characteristics of the swimbladder causing this feature to be size dependent (Harris 1964; Myrberg et al. 1993). More recently, Connnaughton et al. (2000) and Fine et al. (2001) have put forward the ‘forced response’ hypothesis, which suggests that the dominant frequency of fish pulsed sounds involves a swimbladder results from the forced response of the sonic muscles. In this case, the peak frequency of sounds depends on the duration of muscle contraction, which is dependent on body scaling (e.g. Wainwright & Barton 1995): a larger fish would take longer to complete a muscle twitch, resulting in longer pulse durations and lower peak frequencies. In either case, there is a dependence of peak frequencies on body size either directly imparted by the swimbladder or caused by contraction parameters dependent on muscle size and therefore body scaling. An inverse correlation between sound peak frequency and body size has indeed been observed in...
many fish species including *O. mossambicus* (Amorim et al. 2003; Ladich 2004).

The mechanism of sound production of cichlids is not known, but it has been proposed that the jaw apparatus is involved in the production of sounds, which are then amplified by the swimbladder (Lobel 2001; Rice & Lobel 2002). If the forced response hypothesis is correct we could interpret lower peak frequencies and longer pulse durations of *O. mossambicus* winners as a result of increased mass of the muscles involved in sound production. Such differences in musculature mass could have been driven by higher androgen levels (Fine 1997) that winners may have experienced before experimentation. Oliveira (1995) has found that dominant male *O. mossambicus* from established groups have significantly heavier jaw muscles than do subordinates as well as higher androgen levels. The 8 days of isolation to which males were subject before the start of the experiments would have reduced androgen levels to baseline (Oliveira et al. 1996) but were probably not enough to erase other physiological or physical differences such as in sonic muscle mass. Yet another possible and perhaps more straightforward mechanism to explain lower peak frequencies and longer pulse durations of sounds produced by winners is that increased androgens in winners compared to losers after male–male interactions could cause a higher amplitude muscle twitch, thereby increasing twitch duration and resulting in a sound with a lower peak frequency.

Winners produced significantly more sounds than losers, which may give information about the male’s spawning readiness. The small differences in acoustic parameters between winners and losers would have a biological meaning only if they could be perceived by conspecifics, however, and this is unlikely since cichlids are hearing generalists (i.e. they do not have morphological specializations that enhance the detection of the sound pressure component of the acoustic signals) and do not have enhanced hearing abilities (Fay & Simmons 1999). However, peak frequency differences might be detected, as other hearing generalists may show very fine frequency resolution in a limited (but relevant) frequency range (McKibben & Bass 1999).

We suggest that in nature the outcome of male–male competition affects subsequent performance in acoustic courtship behaviour from the early stages of hierarchy formation. We further suggest that the higher sound production rate, and perhaps the lower peak frequency of sounds of winners, could be used to signal successful recent social experience and could increase mate attraction and influence mate choice (also see Amorim et al. 2003).

**Acknowledgments**

We thank Luis Carneiro and Rui Oliveira for comments on early stages of the project and on the manuscript. We are also thankful to Michael Fine and an anonymous referee for commenting on the manuscript. This study was supported by a grant (PRAXIS XXI/BPD/11806/97) and by the pluriannual programme (UI&D 331/94) of FCT – Portugal.

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