Chemosphere 187 (2017) 286–293

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The agricultural contaminant 17β-trenbolone disrupts male-male competition in the guppy (*Poecilia reticulata*)

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HIGHLIGHTS

- 17β-trenbolone (TB) is a widespread agricultural contaminant used in cattle farming.
- Male guppies were exposed to TB at an environmentally relevant level for 21 days.
- TB increased male aggression towards a rival and decreased courting of a female.
- Males exposed to TB performed more 'sneak' mating attempts towards females.
- First study to show disruption of male-male competition by exposure to TB.

ARTICLE INFO

Article history:
Received 14 June 2017
Received in revised form
18 August 2017
Accepted 23 August 2017
Available online 31 August 2017

Handling Editor: Jim Lazorchak

Keywords:
17β-trenbolone
Endocrine-disrupting chemical
Guppy
Hormonal growth promotant
Sexual selection

ABSTRACT

Despite a growing literature highlighting the potential impact of human-induced environmental change on mechanisms of sexual selection, relatively little is known about the effects of chemical pollutants on male-male competition. One class of environmental pollutant likely to impact male competitive interactions is the endocrine-disrupting chemicals (EDCs), a large and heterogeneous group of chemical contaminants with the potential to influence morphology, physiology and behaviour at minute concentrations. One EDC of increasing concern is the synthetic, androgenic steroid 17β-trenbolone, which is used globally to promote growth in beef cattle. Although 17β-trenbolone has been found to cause severe morphological and behavioural abnormalities in fish, its potential impact on male-male competition has yet to be investigated. To address this, we exposed wild male guppies (*Poecilia reticulata*) to an environmentally realistic concentration of 17β-trenbolone (average measured concentration: 8 ng/L) for 21 days using a flow-through system. We found that, in the presence of a competitor, 17β-trenbolone-exposed males carried out more frequent aggressive behaviours towards rival males than did unexposed males, as well as performing less courting behaviour and more sneak (i.e., coercive) mating attempts towards females. Considering that, by influencing mating outcomes, male-male competition has important consequences for population dynamics and broader evolutionary processes, this study highlights the need for greater understanding of the potential impact of EDCs on the mechanisms of sexual selection.

1. Introduction

In many species, competition between males for access to potential mates is a key mechanism of sexual selection (Darwin, 1871). Male-male competition plays a pivotal role in the maintenance and exaggeration of male traits and behaviours (Andersson, 1994; Berglund et al., 1996), and has important consequences for both male mating success (Møller and Jennions, 2001) and female fitness (Fisher et al., 2006). It is now well established that anthropogenic changes to the environment can interfere with male-male

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http://dx.doi.org/10.1016/j.chemosphere.2017.08.125

competition by compromising the transmission and/or reception of male sexual signals (reviewed in Wong and Candolin, 2015). Increased urban noise, for example, is causing male great tits (*Parus major*) to sing at a higher minimum frequency (Slabbekoorn and Peet, 2003), while anthropogenically induced water turbidity is allowing male three-spined sticklebacks (*Gasterosteus aculeatus*) to signal dishonestly, thereby increasing the likelihood of females mating with poor-quality suitors (Wong et al., 2007). However, despite a growing literature documenting the effects of human-induced environmental change on mechanisms of sexual selection, relatively little is known about the potential impacts of an altered chemical environment on male-male competition. This is surprising given the increasing prevalence of chemical pollutants in the environment and the severe impact that chemical pollution can have on morphology, physiology and behaviour (reviewed in Vos et al., 2000; Clotfelter et al., 2004; Frye et al., 2012).

Endocrine-disrupting chemicals (EDCs) are one class of chemical pollutant with the potential to interfere with male-male competition. Endocrine disruptors are a large and highly heterogeneous group of chemicals capable of altering hormonal signalling by blocking, mimicking or modulating the production, release, transport, metabolism, binding, action and/or elimination of natural hormones (Kavlock et al., 1996; Lintelmann et al., 2003; Buchanan and Partecke, 2012). This group includes both natural (e.g., phytoestrogens, Cederroth et al., 2012) and synthetic compounds (e.g., plastics, pesticides and pharmaceuticals, Diamanti-Kandarakis et al., 2009), which enter the environment from a range of sources, including industrial and domestic wastewater, as well as agricultural run-off (Johnson and Sumpter, 2001; Thorpe et al., 2009). Endocrine disruptors pose an insidious threat to wildlife, resulting from their ubiquity in the environment and tendency to bioaccumulate (WHO/UNEP, 2013), potential to act transgenerationally (Anway and Skinner, 2006; Crews et al., 2007; Walker and Gore, 2011) and ability to affect organisms at extremely low concentrations (Diamanti-Kandarakis et al., 2009). Although studies investigating the environmental impacts of EDCs have conventionally focused on their morphological and physiological effects, a growing body of research has begun to highlight the potential behavioural impacts of EDC exposure (reviewed in Clotfelter et al., 2004; Zala and Penn, 2004; Frye et al., 2012). As a result, it is becoming increasingly apparent that behavioural abnormalities induced by exposure to EDCs can often manifest at concentrations that are much lower than those required to induce morphological and physiological change, meaning that behaviour can serve as a particularly sensitive biomarker for EDC contamination (reviewed in Melvin and Wilson, 2013). For example, we now know that exposure to various EDCs at environmentally realistic levels can have severe detrimental impacts on male reproductive behaviour in fish (e.g., Salierno and Kane, 2009; Saaristo et al., 2010; Bertram et al., 2015). However, very few studies have investigated how these behavioural anomalies may manifest in a competitive setting.

Hormonal growth promotants (HGPs) are natural and synthetic chemicals used to stimulate growth in beef cattle by specifically targeting the endocrine system (Johnson, 2015). Hormonal growth promotants are used in many beef-producing countries worldwide, including the United States, Canada, Mexico, South Africa, Chile, Japan, New Zealand and Australia (Hunter, 2010; Kolodziej et al., 2013; Johnson, 2015), and commonly include formulations of androgens, estrogens and/or progestins (Lange et al., 2001; Hunter, 2010). The androgenic steroid most commonly administered in HGP implants is trenbolone acetate (Hunter, 2010), a highly efficient synthetic steroid with 15–50 times the androgenic and anabolic potency of testosterone (Neumann, 1976; Kolodziej et al., 2013). Trenbolone acetate is hydrolysed in the cattle to form various metabolites, including the potent androgen receptor agonist 17β-trenbolone (Khan et al., 2008; Parker et al., 2012), which is detectable in solid dung and liquid manure from implanted cattle, where it is highly persistent (half-life: ~260 days measured in animal waste, Schiffer et al., 2001). After often being allowed to enter the environment, 17β-trenbolone can accumulate in aquatic habitats and has been detected at concentrations ranging from ≤1–20 ng/L in diffuse run-off and discharge (Durhan et al., 2006), to as high as 162 ng/L in ditch networks associated with agricultural fields receiving animal waste (Gall et al., 2011).

It is now well established that exposure to 17β-trenbolone can cause severe morphological and physiological abnormalities in fish, including modified gonadal morphology (Örn et al., 2006), altered body condition (Bertram et al., 2015), reduced fecundity (Ankley et al., 2003) and even female-to-male sex reversal (Larsen and Baatrup, 2010; Morthorst et al., 2010). Exposure to 17β-trenbolone can also impact behaviour, with several studies revealing that environmentally realistic exposure levels can alter reproductive behaviour in female mosquitofish (*Gambusia holbrooki*, Saaristo et al., 2013) and disrupt female mate choice in guppies (*Poecilia reticulata*, Tomkins et al., 2016). Further, recent research has shown that exposure to 17β-trenbolone can alter coercive mating behaviour in male guppies individually exposed to females (Bertram et al., 2015). However, the response of males in the presence of a competitor remains to be investigated, despite the fact that the more common (and realistic) scenario in wild animal populations is for males to compete for mating opportunities.

Guppies are a small, viviparous, freshwater fish native to north-eastern South America that have a global distribution as a result of numerous deliberate and accidental introductions (Lindholm et al., 2005). Male guppies possess a modified anal fin known as a gonopodium, which acts as an intromittent organ. Males achieve copulations via two alternate mating strategies: elaborate courtship displays employed to solicit consensual copulations from females, and sneak attempts, which involve the male sneaking up from behind the female and thrusting his gonopodium towards the female's genital pore in an attempt to mate coercively (Luyten and Liley, 1985). Further, male guppies will actively chase and nip at rivals to monopolise potential mates (Gorlick, 1976; Magurran and Seghers, 1991). Female guppies are choosy and can favour a number of male traits, including greater orange colouration (i.e., area and chroma, Endler, 1980; Brooks and Caithness, 1995), as well as increased male body size (Reynolds and Gross, 1992) and courtship display rate (Kodric-Brown and Nicoletto, 2001). In the wild, multiple male guppies often compete for the attention of a single female (Houde, 1997), meaning that investigations into the impact of 17β-trenbolone on male reproductive behaviour in a competitive setting are ecologically meaningful. Guppies are also known to inhabit polluted waterways (e.g., López-Rojas and Bonilla-Rivero, 2000; Widianarko et al., 2000), making them an ideal candidate for investigating the impact of endocrine disruptors on mechanisms of sexual selection.

Here, we test the hypothesis that short-term exposure to an environmentally realistic concentration of 17β-trenbolone will alter male guppy competitive mating interactions by influencing male reproductive behaviour and aggression. Given that, as aforementioned, exposure to 17β-trenbolone has been shown to affect coercive mating behaviour in male guppies when a single male is presented with a single female (i.e., in a one-on-one scenario, Bertram et al., 2015), we expected that 17β-trenbolone exposure would also disrupt male reproductive behaviour in the more environmentally realistic scenario of two males competing for a single female. Further, although the impacts of water-borne exposure to 17β-trenbolone on aggressive behaviour were previously unknown, circulating levels of endogenous androgens are potent mediators of male aggressive behaviour and dominance (Taves