To fight or mate? Hormonal control of sex recognition, male sexual behavior and aggression in the gecko lizard

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ABSTRACT

Squamate reptiles are a highly diversified vertebrate group with extensive variability in social behavior and sexual dimorphism. However, hormonal control of these traits has not previously been investigated in sufficient depth in many squamate lineages. Here, we studied the hormonal control of male sexual behavior, aggressiveness, copulatory organ (hemipenis) size and sex recognition in the gecko *Paroedura picta*, comparing ovariectomized females, ovariectomized females treated with exogenous dihydrotestosterone (DHT), ovariectomized females treated with exogenous testosterone (T), control females and males. The administration of both T and DHT led to the expression of male-typical sexual behavior in females. However, in contrast to T, increased circulating levels of DHT alone were not enough to initiate the full expression of male-typical offensive aggressive behavior and development of hemipenes in females. Ovariectomized females were as sexually attractive as control females, which does not support the need for the demasculinization of the cues used for sex recognition by ovarian hormones as suggested in other sauropsids. On the other hand, our results point to the masculinization of the sex recognition cues by male gonadal androgens. Previously, we also demonstrated that sexually dimorphic growth is controlled by ovarian hormones in *P. picta*. Overall, it appears that individual behavioral and morphological sexually-dimorphic traits are controlled by multiple endogenous pathways in this species. Variability in the endogenous control of particular traits could have permitted their disentangling during evolution and the occurrence of (semi)independent changes across squamate phylogeny.

1. Introduction

Animal social behavior is one of the key components of life strategies. It is influenced by endogenous processes and, in the case of sexual and agonistic displays, mostly by circulating levels of gonadal steroids (reviewed e.g. in Adkins-Regan, 2005; Nelson, 2011). In many vertebrates, including squamate reptiles (Golinski et al., 2014, 2015; Mason and Adkins, 1976; Rhen and Crews, 1999; Sakata et al., 2002), the most significant hormone controlling the expression of male typical behaviors is testosterone (T). Testosterone does not only act directly but also plays an important role as a prohormone and its effect can therefore be mediated through the action of its active metabolites. Testosterone can by aromatized to estradiol (E_2) or metabolized by 5α-reductase into the non-aromatizable androgen dihydrotestosterone (DHT). Both E_2 and DHT have been documented to control sexual and agonistic behavior in male vertebrates (e.g., Adkins and Schlesinger, 1979; Adkins-Regan, 1996; Heimovics et al., 2015; Huffman et al., 2013; Lindzey and Crews, 1986; Schlinger and Callard, 1990; Simon et al., 1998; Tokarz, 1986; Wu et al., 2009). Circulating testosterone can also be converted into its

active metabolites locally by neurons and other brain cells expressing 5α-reductase and aromatase in various brain regions, where E_2 and DHT can be the major active hormones (e.g., Celotti et al., 1992; Unger et al., 2015; Wu et al., 2009). However, the regulatory effect of both E_2 and DHT on male sexual or agonistic behaviors is not universal among vertebrates. Simon et al. (1996) proposed that there are at least three regulatory pathways promoting male aggression: androgen-sensitive, involving T and its androgenic metabolites (as DHT); estrogen-sensitive, involving aromatization of T to E_2; or synergistic, combining both these regulatory types. The fourth suggested regulatory pathway assumes that only T could directly promote male-like aggressive behavior whereas its metabolites cannot; however, as far as we know, this mechanism was supported only in a single mouse strain (Simon and Masters, 1987). Similarly, it also appears that the same regulatory pathways may occur in the control of male sexual behavior where the regulatory role of DHT or E_2 was confirmed in some (Adkins and Schlesinger, 1979; Adkins-Regan, 1996) but not all studied vertebrates (Crews et al., 1978; Rosen and Wade, 2000). One important question is whether male agonistic and sexual behaviors require organization by

gonadal androgens earlier in ontogeny and can be expressed only later when T levels are increased (e.g., at sexual maturity, Phoenix et al., 1959; Schulz et al., 2004, 2009). The induction of male-typical sexual behavior by exogenous androgens in lizard females and prepubertally castrated males (Golinski et al., 2011; Schořálková et al., 2017) suggests that earlier organization of male sexual behavior is not necessary in this group. However, it has been suggested that adult females might require longer time to perform male-typical behavior than castrated males after application of exogenous T (Schořálková et al., 2017).

Recognition of the sex and reproductive status of interacting conspecifics is a crucial aspect of social behavior. In squamate reptiles, sex recognition is based on both visual and chemosensory cues, with the former likely to be more important for diurnal lineages, at least at greater distances, but even diurnal lizards to some extent rely on sex recognition through chemosensory cues (López and Martín, 2001; López et al., 2002, 2003). Chemosensory sex discrimination is based largely on the sexually dimorphic composition of skin semiochemicals (Garstka and Crews, 1981; Mason et al., 1987, 1989, 1990; Parker and Mason, 2012). Similarly to social behavior itself, the cues used for sex recognition and assignment of partner sexual attractiveness are influenced by sex hormones (Garstka and Crews, 1981; Parker and Mason, 2012, 2014; Rhen et al., 1999; Rhen and Crews, 2000). Nonetheless, testing of their specific nature and the action of steroids has been restricted to only a few species and with equivocal results. In the leopard gecko (*Eublepharis macularius*: Eublepharidae), females treated with exogenous androgens (T or DHT) were less sexually attractive to males and were even attacked by them (Rhen et al., 1999; Rhen and Crews, 2000), while males and females with estrogen implants were attacked less (Rhen and Crews, 2000). Castrated males were courted by intact males comparably as castrated males treated with T, DHT or E$_2$, but they were mounted more frequently and attacked less often than castrated males with exogenous T or DHT (Rhen and Crews, 2000). The authors concluded that estrogens and androgens have likely an antagonistic effect on sexual attractiveness for males, probably due to an opposite effect on female sexual attractiveness pheromones, or alternatively, female pheromones can be controlled by estrogens and male-specific cues by androgens. On the other hand, the results by Mason and Gutzke (1990) suggest that in the same species males have a strategy to fight with any individual lacking female-typical scent produced by the skin. This conclusion was based on the observation that shedding females were not recognized by adult males as sexual partners but as rivals and were vigorously attacked. In the garter snake *Thamnophis sirtalis*, males with E$_2$ implants (Parker and Mason, 2012) as well as castrated males (Parker and Mason, 2014) were mated by intact males. The authors suggest that androgens inhibit the production of female sex attractiveness pheromones controlled by estrogens (Parker and Mason, 2014).

Here, we report the results of our experiments with the Madagascar ground gecko, *Paroedura picta*, a member of the family Gekkonidae, aimed to shed light on the hormonal control of male sexual behavior, aggressiveness and cues used for sex recognition. In a previous study we uncovered that castration suppresses sexual behavior in males but exogenous T masculinizes sexual behavior in both males and females of this species, while offensive aggression occurs rarely and only in individuals with elevated T levels (Golinski et al., 2014). Nonetheless, at the time we were not able to distinguish whether T caused these effects directly or via its aromatization or conversion to DHT, which we now address in the current study. Moreover, our present experiment was designed to uncover cues used for sex recognition in this species and their hormonal control. We tested how ovariectomized females and ovariectomized females treated with exogenous DHT and T are perceived by intact males to investigate whether ovarian hormones are required for female sexual attractiveness and if cues used for sex recognition can be masculinized by androgens.

2. Materials and methods

2.1. Studied animals

The Madagascar ground gecko, *Paroedura picta*, is a ground-dwelling nocturnal species of the family Gekkonidae inhabiting the diverse environments of the arid lowlands of Madagascar (Schönecker, 2008). It is easily kept and bred in captivity, grows rapidly and matures at an early age (approximately three to four months). Among reptiles it is known for having extremely short intervals between clutches of one or two eggs (Kubička and Kratochvíl, 2009; Kubička et al., 2012; Starostová et al., 2012; Weiser et al., 2012). This species exhibits male-larger dimorphism in body size (Starostová et al., 2010, 2013) and males are mutually aggressive (Brillet, 1991) but do not perform elaborated courtship displays (Brillet, 1991, 1993). Moreover, male-typical aggressive and sexual behavior can be provoked by increased levels of T in both sexes (Golinski et al., 2014).

Our experimental animals were the progeny of our founding population consisting of animals imported from the wild along with their first-generation offspring born in captivity. The experiment was based on the scoring of mutual social behavior between manipulated individuals and non-manipulated males and females. For this purpose, we established two male groups and five female groups of 12 individuals. Two male groups consisted of surgically intact males with previous sexual experience (assigned as "Males A" and "Males B"). The "Unmanipulated females" group consisted of surgically intact females also with previous sexual experience. Four groups of surgically treated females included a group of reproductively active sham operated females ("Sham females"), ovariectomized females ("OVX females"), OVX females treated with exogenous DHT ("DHT-OVX females"), and OVX females treated with exogenous T ("T-OVX females"). All animals were housed individually in standardized plastic boxes ($20 \times 20 \times 10$ cm) or glass cages ($15 \times 40 \times 15$ cm) with sand substrate, shelter and water dish, and were fed crickets (*Gryllus assimilis*) dusted with vitamins (Roboran, Univit, Czech Republic) twice weekly to satiety. Water enriched with calcium (Vitacalcin, Zentiva, Czech Republic) was replaced once every two weeks with water supplemented with vitamins A, D$_3$ and E (Hydrovit, Pharmagal, Slovakia).

In the surgically and hormonally treated females we also followed growth; these results are covered in another paper (Kubička et al., 2017) where the experimental procedures are recorded in more detail, here we present only their brief description. The surgery was performed at approximately four months of age. Females were anesthetized using a combination of an intramuscular injection of ketamine and cold anaesthesia. Ovariectomy was performed by ligating the ovary blood supply with surgical silk prior to its ablation. For the Sham females, "sham" surgeries were performed, in which ventral incisions were made to expose and manipulate the ovaries while leaving them unharmed. The incision was closed using surgical sutures and covered with surgical glue. After the wound healed sufficiently (within 14 days after surgery) the stitches were removed and hormonal treatment commenced. In order to increase the circulating levels of DHT and T in the two groups of OVX females, we used a method adapted from the cutaneous application of oil-diluted steroid hormones in lizards (Meylan et al., 2003; Schořálková et al., 2017; Trompeter and Langkilde, 2011). A crystalline steroid hormone (DHT or T; Sigma-Aldrich) at the dose 2.4 μg per gram of body mass dissolved in sunflower oil was applied to the skin between the shoulders of each experimental individual twice a week at regular intervals (every 3–4 days). The mixture was absorbed into the skin within several hours. At the end of the growth experiment these animals were used for behavioral trials by which time their hormonal treatment had been carried out for 38 to 43 weeks. The longer period of hormonal treatment before behavioral testing is necessary, as the effect of androgens on social behavior can be substantially delayed in geckos (Schořálková et al., 2017).