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Sex steroid profiles in zebra finches: Effects of reproductive state and domestication

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ABSTRACT

The zebra finch is a common model organism in neuroscience, endocrinology, and ethology. Zebra finches are generally considered opportunistic breeders, but the extent of their opportunism depends on the predictability of their habitat. This plasticity in the timing of breeding raises the question of how domestication, a process that increases environmental predictability, has affected their reproductive physiology. Here, we compared circulating steroid levels in various "strains" of zebra finches. In Study 1, using radioimmunoassay, we examined circulating testosterone levels in several strains of zebra finches (males and females). Subjects were wild or captive (Captive Wild-Caught, Wild-Derived, or Domesticated). In Study 2, using liquid chromatography–tandem mass spectrometry (LC–MS/MS), we examined circulating sex steroid profiles in wild and domesticated zebra finches (males and females). In Study 1, circulating testosterone levels in males differed across strains. In Study 2, six steroids were detectable in plasma from wild zebra finches (pregnenolone, progesterone, dehydroepiandrosterone (DHEA), testosterone, androsterone, and 5α-dihydrotestosterone (5α-DHT)). Only pregnenolone and progesterone levels changed across reproductive states in wild finches. Compared to wild zebra finches, domesticated zebra finches had elevated levels of circulating pregnenolone, progesterone, DHEA, testosterone, androstenedione, and androsterone. These data suggest that domestication has profoundly altered the endocrinology of this common model organism. These results have implications for interpreting studies of domesticated zebra finches, as well as studies of other domesticated species.

1. Introduction

Natural selection shapes both the behavior of wild animals and its underlying physiological mechanisms. The process of domestication alters natural selection and introduces artificial selection pressures. Some effects of domestication are similar across species (Zeder, 2012; Wilkins et al., 2014). For example, domesticated animals show changes to the seasonal regulation of reproduction and molt (Belyaev, 1979; Lincoln et al., 1990; Setchell, 1992). Furthermore, domesticated animals show decreased hypothalamic–pituitary–adrenal (HPA) axis reactivity (Martin, 1978; Woodward and Strange, 1987; Künzl and Sachser, 1999; Albert et al., 2008; Suzuki et al., 2012; Homberger et al., 2013; Ericsson et al., 2014). However, other effects of domestication are different across species and depend on the characteristics of the original population and the specifics of the domestication process (Zeder, 2012; Adkins-Regan, 2009).

Zebra finches are an extensively used model organism in a wide range of behavioral, evolutionary, and neuroscience research (Griffith and Buchanan, 2010). Initial breeding stocks of zebra finches were removed from the wild in Australia during the 19th century and were established into a number of independent breeding populations across the globe (Zann, 1996). Given the ease with which they can be studied in the laboratory, it is not surprising that most of the research on the physiology and neuroscience of this species has been conducted on domesticated zebra finches

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(Griffith and Buchanan, 2010). The laboratory environment itself may have caused persistent differences to arise between wild and domesticated individuals with respect to both their physiology and behavior (Calisi and Bentley, 2009; Dickens and Bentley, 2014). Furthermore, the process of domestication may have led to genetic differentiation between wild and domesticated zebra finch populations, even in the few hundred generations for which they have been separated (Forstmeier et al., 2007). It is currently unclear to what extent there may be population differences in functional traits relating to behavior and physiology, between wild and domesticated finches and also across different domesticated populations. The demonstration of such effects will provide insight into the mechanisms underlying domestication, but may also necessitate caution when comparing across populations of zebra finches that differ in their domestication history.

For some traits, wild and domesticated zebra finches are extremely similar. For example, both wild and domesticated zebra finches form long-term pair bonds, provide biparental care, and breed opportunistically (Zann, 1996). With regard to morphology, zebra finches with wild-type plumage are common in domesticated populations, and beak coloration is similar in wild and domesticated zebra finches (Burley et al., 1992). However, other traits differ between wild and domesticated zebra finches. Domesticated zebra finches are generally larger and express many different plumage colorations (Sossinka, 1970; Carr and Zann, 1986; Zann, 1996; Forstmeier et al., 2007). Furthermore, wild zebra finches have lower rates of extra-pair copulations (Griffith et al., 2010) and differ subtly in nesting behavior (Mainwaring et al., 2010; Gilby et al., 2013). In mate choice tests, captive wild-caught females are choosier than domesticated females, and domesticated males are more active courters than wild-caught males (Rutstein et al., 2007). However, it is currently unclear whether there are fundamental differences between wild and domesticated zebra finches in their endocrine profiles.

Zebra finches are opportunistic breeders; however, the extent of opportunism varies with habitat and environmental conditions (Zann, 1996; Perfito et al., 2007). Overall, the regulation of breeding in zebra finches is not as well understood as it is in seasonally-breeding species. However, studies have suggested that the hypothalamic–pituitary–gonadal (HPG) axis functions similarly in wild and domesticated zebra finches (Perfito et al., 2007, 2011; Prior and Soma, 2015). For example, the effects of natural droughts on wild finches and the effects of experimental water restriction on domesticated finches are similar (Perfito, 2010; Prior and Soma, 2015). Both cause zebra finches to stop breeding and inhibit parts of the HPG axis (Prior et al., 2013). Therefore, it is likely that (1) wild zebra finches have sex steroid profiles that change across a breeding cycle; and that (2) domestication, a process that reduces environmental variability, has affected sex steroid profiles.

We began to investigate the above hypotheses in two studies. In Study 1, we used a radioimmunoassay (RIA) to quantify circulating testosterone levels in males and females from four "strains" of zebra finches: Wild, Captive Wild-Caught, Wild-Derived (males only), and Domesticated. In Study 2, we used liquid chromatography–tandem mass spectrometry (LC–MS/MS) to quantify multiple sex steroids and precursors in wild and domesticated zebra finches. We also described circulating steroid profiles of wild zebra finches at different reproductive states across a breeding cycle.

2. Materials and methods

2.1. Ethics statement

These studies were carried out under a UBC Animal Care Committee protocol and under ethics licenses from the Animal Ethics Committee at Deakin University (B24-2012) and Macquarie University (ARA 2010/053). Experiments followed the guidelines of the Canadian Council on Animal Care and the Australian and New Zealand Council for the Care of Animals in Research and Teaching.

2.2. Study 1: radioimmunoassay (RIA) to measure testosterone levels in plasma

We used a radioimmunoassay (RIA) to compare circulating testosterone levels among four strains of zebra finches (1) Wild ($n = 20$ females and 11 males), (2) Captive Wild-Caught ($n = 4$ females and 4 males), (3) Wild-Derived ($n = 0$ females and 42 males), and (4) Domesticated ($n = 15$ females and 15 males).

Wild finches were sampled while "not nesting" or while actively nesting (Incubating or Feeding Chicks) (females: $n = 8$ Not Nesting and 12 Nesting; males: $n = 5$ Not Nesting and 6 Nesting). Subjects in other strains were not nesting at the time of blood sampling, allowing us to compare circulating testosterone levels across all four strains, in subjects that were not nesting. Wild-Derived males were housed in same-sex aviaries, and all other subjects were paired. See below for more details.

2.3. Subjects

2.3.1. Wild zebra finches

Free-roaming, wild zebra finch pairs were captured for blood sampling at Fowler's Gap Arid Zone Research Station, in the Western region of New South Wales, Australia. More specifically, pairs were from nest box colonies within Gap Hills (Griffith et al., 2008). Blood samples were collected between September–November 2012. At this location, October is often a peak breeding month for zebra finches (Griffith et al., 2008), and in this season of 2012, there was continuous breeding activity in the study area throughout the time of sampling. However, in other areas of the Fowler's Gap study site, environmental conditions were different and there was no breeding.

At the time of blood sampling, subjects were either not nesting or nesting. Breeding activity in nest box populations was assessed every 1–3 days. For active nests, the dates that eggs were laid and hatched were recorded, allowing us to determine the days of Incubation and Feeding Chicks. Breeding females and males (either Incubating or Feeding Chicks) were caught in the nest box during the early morning. Subjects that were not nesting were caught in a walk-in feeder trap from an area where no birds were nesting in nest boxes (Mariette et al., 2011; McCowan et al., 2015). While there was no nesting at these sites, because the overall environmental conditions were appropriate for breeding, it is difficult to be certain that these individuals were not maintaining a breeding nest elsewhere. Therefore, we classified these individuals as not nesting, instead of non-breeding, because they may have been physiologically capable of breeding (Prior et al., 2013; Perfito et al., 2007). Note that in zebra finches, only females develop a brood patch (Zann, 1996). Additionally, all individuals examined in these colonies had a brood patch score of 1–2, making it difficult to identify breeding state from brood patch score.

2.3.2. Captive Wild-Caught zebra finches

Wild adult female and male zebra finches were caught at the Fowler's Gap study site (in September 2012) and housed in open-air aviaries under natural photoperiod for 1–2 months prior to collecting blood samples. Subjects had access to *ad libitum* seed, water, cuttlefish bone, and grit. These subjects were housed in several aviaries, and each aviary held 1–2 pairs. All subjects were pair bonded; however, they were not nesting at the time of blood sampling (Prior et al., 2016).